STREAM ECOSYSTEM RESPONSE TO MINING-INDUCED SALINIZATION IN CENTRAL APPALACHIA

OSMRE Cooperative Agreement S15AC20028

Final Performance Report

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EXECUTIVE SUMMARY

Extensive surface mining of coal has affected water resources throughout the central Appalachian coalfield. Elevated levels of salinity and trace elements associated with mining can be particularly detrimental to aquatic macroinvertebrates. Prior studies of central Appalachian mining effects have not accounted explicitly for seasonal patterns and temporal trends in water quality in headwater streams influenced by mining, and few prior studies have addressed mining effects on aquatic ecosystem functions and on bioaccumulation of trace elements.

Purpose and Objectives

The research reported here was conducted for the purpose of advancing scientific understanding of influences by mining-origin major ions/total dissolved solids (TDS), selenium (Se), and other trace elements on aquatic ecosystem structure and function in central Appalachian headwater streams.

In order to achieve the above goals, we established three overall research objectives:

Objective 1: Assess long-term temporal patterns of chemical and biological changes in central Appalachian headwater streams salinized by coal mining (Section II).

Objective 2: Determine influences of mining-induced salinity on leaf breakdown, a key carbon-processing function in headwater streams (Section III).

Objective 3: Investigate trophic transfer and bioaccumulation of trace elements, especially Se, as a potential mechanism for aquatic-community effects that are commonly observed in headwater streams with high specific conductance (SC) and total dissolved solids (TDS) (Sections IV and V).

Methods

In order to achieve these goals, we conducted research activities over a period extending from July 2015 through December 2016 in 24 headwater streams: five reference streams with watersheds that are predominantly forested, and 19 mining-influenced streams (test streams). The test streams were selected for study because of their location in watersheds that contain minimal anthropogenic disturbances other than mining. All water monitoring points were on stream segments with high-quality forested habitats, and with no evidence of excessive sedimentation, influence by acidic discharges, or other ecosystem stressors other than elevated SC and, in some cases at test sites, elevated concentrations of trace elements associated with mining and SC.

At each study location, water was monitored at 30-minute intervals with *in-situ* data loggers for SC, and quarterly water samples were obtained and analyzed for major ions, trace elements, and TDS over the study period; while benthic macroinvertebrate samples were obtained and analyzed in Fall 2015 and Spring 2016. These methods extended data series acquired with prior OSMRE support at the same study sites. We also placed multiple leaf-litter bags at all study sites, retrieved them sequentially over a 270-day period; dried and weighed each retrieved litter bag so as to determine mass loss; and calculated leaf breakdown rates for each study site. Three macroinvertebrate taxa were collected from 23 study sites and analyzed for tissue-Se concentrations. Based on those results, nine study sites (three reference sites, three test sites with relatively low Se concentrations, and three test sites with relatively high Se concentrations) were selected for further study of trace element bioaccumulation. At these sites, water, particulate environmental media (biofilm, sediments, and leaf detritus), and benthic macroinvertebrates (prey taxa, predator taxa, and crayfish [Cambaridae]) were collected and analyzed for concentrations of the trace elements Al, As, Cd, Cu, Ni, Se, Sr, V, and Zn in Fall 2015 and Spring 2016.

Specific conductance was interpreted as an indicator of TDS/major ion concentrations, in accord with well-established practice and prior studies. Continuous SC data were analyzed across all sites by modeling seasonal patterns of SC variability over the full OSMRE-supported study period (2011-2016). Eight benthic macroinvertebrate structural metrics found sensitive to SC by our prior studies were selected for focus: Richness (total number of taxa), Evenness (degree of equality of abundance among taxa), Ephemeroptera richness (number of mayfly taxa), Plecoptera richness (number of stonefly taxa), EPT richness (number of mayfly, stonefly, and caddisfly taxa combined), Ephemeroptera relative abundance (percent of total individuals that are mayflies), Predator relative abundance (percent of individuals that are predators), and Shredder relative abundance (percent of individuals that feed by shredding organic matter). Relationships of the selected metrics to measured SC values were analyzed for consistency over the 2011-2016 study period. Continuous SC, selected benthic macroinvertebrate metrics, and selected ion ratios that are indicative of TDS ionic composition were analyzed for temporal trend over the 2011-2016 study period at each of the 24 study sites. Leaf-litter breakdown rates for individual sites were regressed against SC mean values and against both relative abundance of shredders and taxonomic richness of shredders in an effort to understand factors influencing variations in breakdown rate. Trace-element concentrations of environmental-media from mining-influenced streams and reference streams were compared to one another; and Se concentrations of media were also compared among reference, low-Se, and high-Se streams. Selenium concentrations in environmental media were also analyzed by calculating enrichment factors, which are ratios of particulate media to water concentrations; by calculating trophic transfer factors, which are ratios of benthic macroinvertebrate tissue to particulate media concentrations; and by comparing mean values of enrichment and trophic transfer factors among reference, low-Se, and high-Se streams. Environmental-media concentrations for other trace elements were analyzed using techniques similar to those applied for Se, but by comparing mean values for the six mining-influenced streams to the three reference streams.

Results

Objective 1: Assess long-term chemical and biological patterns in central Appalachian headwater streams salinized by coal mining

Both reference streams and test streams exhibited significant seasonal SC patterns, which were modeled using a sinusoidal function. All reference streams, and most, but not all, test streams followed this general pattern. Day-to-day and hour-to-hour SC, at individual sites and collectively, often diverged from the modeled seasonal pattern for reasons that include rainfall dilution and apparent variability of rainfall timing over the study period. The seasonal pattern appears to be driven by hydrology and evapotranspiration, as modeled mean-SC minima occur in the late February – early March period of generally high streamflow; whereas maxima tend to occur in the late August – early September period that is generally characterized by low flows.

Relationships of SC to the eight benthic macroinvertebrate community metrics selected for focus were generally consistent over the study period within sampling seasons. Collectively, the metrics were more sensitive to SC during the Spring than during the Fall seasons. Six of the eight metrics exhibited significant negative relationships with SC for all four Spring sampling events, whereas Evenness exhibited significant negative relationships for three of the four Spring sampling events and Shredder percent exhibited a significant positive relationship with SC for all four Spring sampling events. Within the Fall season, metric relationships with SC were also generally consistent over the study period but not as consistent as in Spring. Richness, Ephemeroptera richness, EPT richness, and Ephemeroptera percent exhibited significant negative relationships to SC during all four Fall seasons; whereas the Shredder percent relationship with SC was not statistically significant for any Fall-season sample. The other three metrics selected for focus (Evenness, Plecoptera richness, and Shredder percent) exhibited negative relationships with SC during one or more but not all of the four Fall seasons sampled during the 5-year study period.

Across our 19 test sites, rapid or significant declines of water salinity over the 2011-2016 study period are not apparent but we observed gradual SC declines at some sites, which suggests recovery from mining disturbance is occurring at those sites. Long-term decreasing trends in salinity were observed at seven test sites; whereas increasing trends were observed at three sites, two of which had additional mining during the 2011-2016 period. In contrast, two of the five reference sites exhibited increasing SC trends and no declining trends were detected. Hence, it appears that gradual decline of SC is occurring at some the test sites. However, the magnitude of SC change (i.e., trend slope) was small when long-term trends were present.

Furthermore, we found no indication of a consistent pattern of biological recovery at test sites over the five-year study period. Although long-term trends were found in biological metrics at some individual sites, those trends were not consistent across sites that had either decreasing or increasing trends in SC. The lack of strong, consistent trends in the biological metrics supports our finding that there appears to be no indication of recovering biological condition in these study streams over the period of study.

Objective 2: Determine influence of mining-induced salinity on leaf litter breakdown

Mean values for leaf breakdown rate coefficients at test sites did not differ significantly from those at reference sites for 90-day (0.030 ± 0.005 day^{-1} and 0.031 ± 0.007 day^{-1}, respectively), 150 day (0.031 ± 0.003 day^{-1} and 0.027 ± 0.002 day^{-1}, respectively), and 270 day (0.028 ± 0.002 day^{-1} and 0.024 ± 0.002 day^{-1} , respectively) periods. The relationship of 90-day leaf breakdown rate coefficients to mean SC for individual sites was not statistically significant. We found no measurable effect of SC on shredder taxa richness or on percent shredder abundance. Hence, the lack of responsiveness by the shredder community to elevated SC may be an explanation for our finding that leaf breakdown rates did not respond to salinity at our 24 study sites.

Objective 3: Investigate trophic transfer and bioaccumulation of selenium and other trace elements

Selenium (Se) concentrations in all media were elevated in mining-influenced streams compared with reference streams and in high-Se streams compared with low-Se streams. Selenium bioaccumulation processes (enrichment, trophic transfer) did not exhibit major differences among stream types or seasons. Particulate media Se-concentrations in high-Se streams exceeded those found to cause fecundity impairments of benthic macroinvertebrates in laboratory studies described by scientific literature, and Se tissue concentrations in benthic macroinvertebrates of high-Se streams exceeded those reported in mining-influenced central Appalachian streams with Se-related fish deformities by other studies. However, fish were present in few of the headwater streams selected for our study, and we draw no conclusions concerning potentials for toxicity by the Se levels observed in our study streams.

Selenium was also included in the general trace element study to provide a basis for comparison for the other elements. Of the studied elements, only Se exhibited water concentrations approaching or exceeding US EPA recommended water quality criteria. All studied elements exhibited substantial enrichment in the particulate phase relative to water concentrations. Concentrations from mining-influenced streams exceeded concentrations in reference streams for all of the collected media only for Se. Particulate/water concentration ratios (which we interpret as enrichment factors for Se) were generally higher for the other trace elements relative to those calculated for Se, but prey/particulate and predator/prey concentration ratios for Se were generally high relative to those calculated for other elements. Of the elements studied, only Se and Ni exhibited elevated concentrations in mining-influenced streams, relative to reference streams, for all three of the studied benthic macroinvertebrate media (predators, prey, and Cambaridae).

Conclusions

Seasonality should be considered when monitoring mining-influenced streams for water quality and for benthic macroinvertebrate community structural measures. Specific conductance tends to vary seasonally in a predictable cyclic manner, whereas certain benthic macroinvertebrate structural measures tend to respond more directly and consistently to elevated SC in the Spring season than in the Fall season. However, richness of total taxa, EPT taxa, and Ephemeroptera taxa, and relative abundance (percent) of Ephemeroptera exhibited consistent negative relationships with SC across all study years and in both Spring and Fall seasons, indicating that those metrics are robust measures of community response to elevated salinity.

We found no measurable effect of salinization on rates of leaf litter breakdown. We interpret this result as occurring because the benthic macroinvertebrates that perform leaf-breakdown functions (i.e., shredders) appeared tolerant of salinity at the levels we observed. Taxa of the group most affected by salinity (mayflies) do not shred leaves but do perform other roles in the processing of carbon in headwater streams. Therefore, although leaf-breakdown – an important component of the carbon cycle in these streams – appears unaffected by salinity at levels we observed, effects of mayfly loss on other ecosystem functions in our study streams remain unknown.

Findings indicate that headwater streams influenced by coal-mining play a significant role in the introduction of elevated Se concentrations into the aquatic food-chain. Bioaccumulation tendencies for Se appear as unique among the other trace elements studied, as Se concentrations in all studied particulate and macroinvertebrate media were elevated in mining-influenced streams relative to reference streams. Of the other trace elements studied, results for Ni provided some concern because macroinvertebrate tissue concentrations were consistently elevated in mining-influenced streams relative to reference streams. However, we did not seek or become aware of information to indicate that elevated concentrations of Ni may be causing toxicity or ecosystem impairments.

The results we observed regarding salinity, leaf litter breakdown, and selenium are specific to the region and systems studied, but our approach is broadly transferrable. As all of our study sites were in small first-order forested headwater streams, we would expect to find similar results in other streams with comparable conditions and land uses. Our approach can be adapted to a variety of riverine systems, allowing region-specific assessment of stream ecosystem response to mining influence.

I. INTRODUCTION

Report Scope

This final technical report presents detailed methods and findings of the study "Stream Ecosystem Response to Mining-Induced Salinization in Appalachia", U.S. Office of Surface Mining Reclamation and Enforcement (OSMRE) Cooperative Agreement S15AC20028 (study period July 1, 2015 through December 31, 2016). In order to achieve Objective 1, additional data from our prior study ("Effective Monitoring and Assessment of Total Dissolved Solids as a Biotic Stressor in Mining-Influenced Streams", OSMRE Cooperative Agreements S11AC20004 and S12AC20023; study period February 1, 2011 through Dec 31, 2014) were incorporated into data analyses as appropriate.

Introduction

Mining-induced impairment of water quality is a major stressor to aquatic life in central Appalachian streams (e.g., Green et al. 2000; Pond 2004; Pond et al. 2008; Lindberg et al. 2011; Bernhardt et al. 2012; Griffith et al. 2012; Pond et al. 2014; Timpano et al. 2015b; Boehme et al. 2016; and other studies). Elevated levels of salinity and concentrations of trace elements associated with mining can be particularly detrimental to aquatic macroinvertebrates, a diverse group of organisms playing an integral role in ecosystem functions and aquatic food webs.

Despite strong negative associations between mining-induced water-quality effects and aquatic macroinvertebrate community structural measures, prior studies of central Appalachian mining effects have not accounted for seasonal patterns and inter-annual trends, due in part to the lack of long-term datasets. Furthermore, it remains unclear if water-quality effects of mining and associated changes in macroinvertebrate communities result in alterations of ecosystem functions. A related issue is the potential for bioaccumulation of toxic elements, including selenium (Se), in aquatic food webs, which has received limited study in central Appalachian headwater streams. Therefore, studies that assess long-term changes in macroinvertebrate communities, rates of specific ecosystem functions, and bioaccumulation of potentially toxic elements in central Appalachian streams will improve our understanding of the potential cumulative effects of mining on aquatic ecosystems beyond the loss of aquatic species.

Study Goals and Objectives

The research reported here was conducted for the purpose of advancing scientific understanding of aquatic ecosystem structure and functional impacts from major ions/total dissolved solids (TDS), selenium (Se), and other trace elements in mining-influenced headwater streams. Improved understanding of these stream ecosystem responses will enhance the efforts of OSMRE to manage mining-induced water salinization and trace-element release and their impacts in central Appalachian headwater streams.

In order to achieve the above goals, we established three overall research objectives:

> **Objective 1**: Assess long-term temporal patterns of chemical and biological changes in central Appalachian headwater streams salinized by coal mining.
>
> **Objective 2**: Determine influences of mining-induced salinity on leaf breakdown, a key carbon-processing function in headwater streams.
>
> **Objective 3**: Investigate trophic transfer and bioaccumulation of trace elements, especially Se, as a potential mechanism for aquatic-community effects that are commonly observed in headwater streams with high specific conductance (SC) and total dissolved solids (TDS).

Results of research to assess these objectives is described in Sections II, III, IV, and V. A summary and synthesis of findings is provided in Section VI.

Study Stream Characteristics

We have been conducting studies to assess relationships of salinity and benthic macroinvertebrate communities in mining-influenced streams of central Appalachia since 2008. In 2008-09, A. J. Timpano surveyed more than 180 headwater streams in Virginia's central Appalachian coalfield for the purpose of identify mining-influenced headwater streams with elevated salinity, but with other water chemistry and habitat conditions similar to regional headwater streams with minimal anthropogenic influence (reference streams) (Timpano 2011; Timpano et al. 2015b;
Table I-1). The study-stream selection criteria were applied for the purpose of isolating the mining-released major ions, measured as TDS and by proxy as SC, as a water-quality stressor. In 2011 with OSMRE funding, we surveyed additional streams in eastern Kentucky and southern West Virginia; and identified additional streams in southern West Virginia that conform with those same criteria. Also in 2011, we placed continuous conductivity loggers in the Virginia and West Virginia study streams. Of the 27 streams studied during the 2011-2014 period with OSMRE support (Timpano et al. 2015a), the 24 streams monitored through 2016 are the focus for this report (Figure I-1, Table I-2).

Both reference and mining-influenced (test) stream reaches selected for study are headwater streams (first-order) with high-quality habitat (Table I-3), including intact forest canopy and absence of excessive sedimentation, straight-pipe discharges, or major anthropogenic watershed disturbances other than mining. Reference sites are reaches of streams draining watersheds that are predominantly forested; some reference-site watersheds have gas wells and roads but these disturbances occupy small percentages of watershed areas. Test sites are forested stream reaches that receive waters discharged by or draining from active or completed surface coal mining operations; most contain valley fills. Test-site waters are generally alkaline, with sulfate (SO_4^{2-}) and bicarbonate (HCO_3^-) as the dominant anions and calcium (Ca^{2+}) and magnesium (Mg^{2+}) as the dominant cations (Table I-4).

Our study is unique in the central Appalachian coalfields because we have been monitoring these streams continuously for multiple years. We have applied consistent methods for characterizing water chemistry and benthic macroinvertebrate community structure during the 2011-2016 study period.

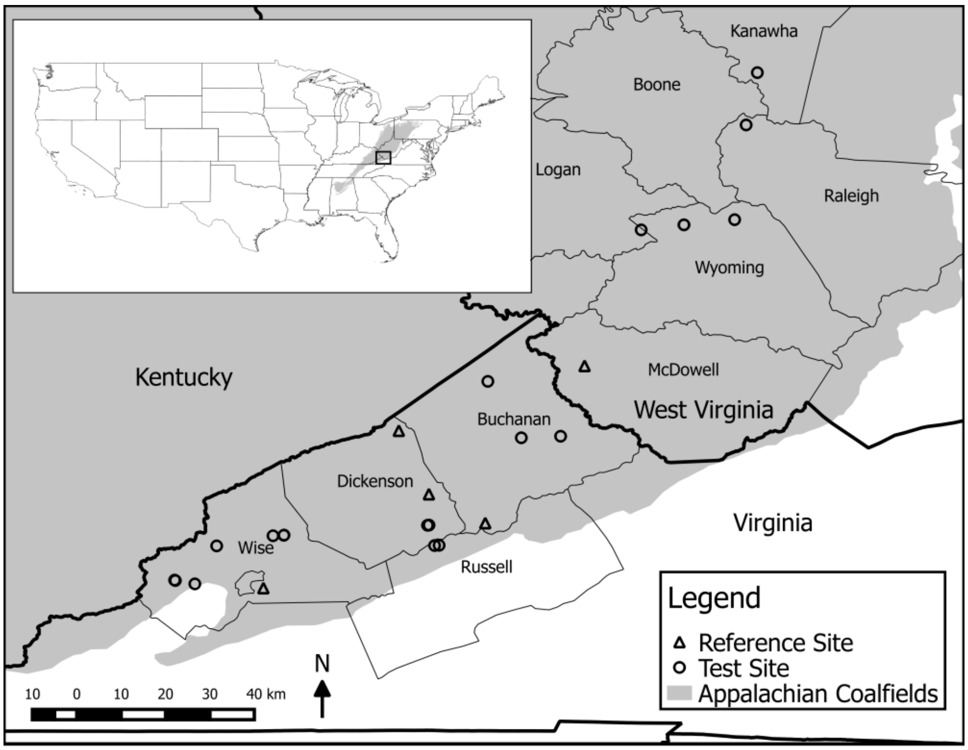

Figure I-1. Map of 24 study streams surveyed during 2015-2016.

Table I-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

[1]reference-stream criteria from Burton and Gerritsen (2003)
[2]Rapid Bioassessment Protocols habitat assessment, high-gradient streams (Barbour et al. 1999)

Table I-2. Study stream 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
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
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 Branch (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[2]	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

[1]determined using data from NHDPlus database (USEPA 2012b).
[2]UT – unnamed tributary.

Table I-3. RBP habitat assessment summary statistics for Fall 2015 and Spring 2016.

Site Type	Statistic	Epifaunal Substrate	Substrate Embeddedness	Velocity/Depth Regime	Sediment Deposition	Channel Flow Status	Channel Alteration	Riffle Frequency	Bank Stability Left	Bank Stability Right	Vegetative Protection Left	Vegetative Protection Right	Riparian Vegetative Zone Left	Riparian Vegetative Zone Right	Total Habitat Score
Fall 2015															
Ref	Min	18	9	15	6	6	20	18	8	9	10	10	9	9	159
	Max	18	16	17	15	16	20	18	10	10	10	10	10	10	177
	Mean	18	13	16	11.6	10	20	18	9	9.6	10	10	9.6	9.8	164.6
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Test	Min	16	11	15	10	6	20	16	6	8	8	8	8	8	154
	Max	18	16	17	15	17	20	18	10	10	10	10	10	10	178
	Mean	17.6	13.3	16.4	13.1	14.7	20.0	17.5	8.6	8.9	9.9	9.9	9.8	9.8	169.4
	n	19	19	19	19	19	19	19	19	19	19	19	19	19	19
Spring 2016															
Ref	Min	16	11	14	12	10	20	17	9	10	10	10	9	9	164
	Max	18	15	17	16	16	20	18	10	10	10	10	10	10	177
	Mean	17	13.6	15.2	14	13.2	20	17.6	9.6	10	10	10	9.8	9.8	169.8
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Test	Min	15	11	12	10	10	20	15	6	8	7	8	8	8	151
	Max	18	16	16	17	16	20	19	10	10	10	10	10	10	178
	Mean	16.4	13.7	14.9	13.8	14.6	20.0	17.1	8.8	9.3	9.8	9.9	9.8	9.8	168.1
	n	19	19	19	19	19	19	19	19	19	19	19	19	19	19

Table I-4. Summary of water chemistry in reference and test streams, calculated from mean values of quarterly samples for each stream (2011-2016).

Type	n	pH	SC	SO_4^{2-}	HCO_3^-	CO_3^{2-}	Cl^-	Ca^{2+}	Mg^{2+}	Na^+	K^+	Sum
		(S.U.)	(µS/cm)			- - - - - - - -	- - - - - - - -	(mg/L)	- - - - - - - -	- - - - - - - -		
Reference												
Mean	15.8	7.6	73	9	27	0.0	2	6	3	4	1	52
Min	15	7.3	26	3	9	0.0	1	3	1	1	0.4	17
Max	16	7.7	143	18	61	0.1	4	15	5	7	2	108
Test												
Mean	16.7	8.0	765	310	125	0.3	5	78	50	21	4	594
Min	16	7.7	264	71	28	0.0	1	23	9	6	2	190
Max	17	8.2	1660	844	225	1	22	165	160	62	12	1422

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II. LONG-TERM CHEMICAL AND BIOLOGICAL PATTERNS IN CENTRAL APPALACHIAN HEADWATER STREAMS

Introduction

Landscape disturbances, such as those associated with surface mining and urbanization, have directly affected > 50% of Earth's land area (Hooke et al. 2012), leading to global degradation of freshwater ecosystems (Strayer and Dudgeon 2010). Biodiversity loss in freshwater ecosystems resulting from freshwater degradation now ranks among the highest of any ecosystem type. Remediation to minimize biodiversity losses often follows most large-scale landscape disturbance, but the success of this remediation is rarely assessed over long terms. Long-term observational studies of chemical and biological responses to disturbance are critical to understand long-term dynamics in disturbed ecosystems.

Mining disturbances can be particularly detrimental to freshwater ecosystems because of the scale of disturbance. central Appalachian surface mining typically disturbs geologic materials across extensive areas, often hundreds or even thousands of contiguous hectares, and to depths extending multiple 10s of meters below the surface. When exposed to environmental weathering by the extensive fracturing that accompanies mining, these disturbed geologic materials release soluble ions and particulate pollutants to environmental waters. Mine drainage waters and streams receiving such drainage typically contain concentrations of major ions and trace elements that are elevated above natural background. Streams thus affected have also commonly have aquatic communities with different structures from those of unmined reference streams (Pond et al. 2008, Lindberg et al. 2011, Bernhardt et al. 2012, Griffith et al. 2012, Pond et al. 2014, Timpano et al. 2015, Boehme et al. 2016). The most significant alterations of water chemistry typically occur in streams receiving effluent from valley fills (Cormier et al. 2013b, Evans et al. 2014).

Reclamation of surface coal mines is regulated by OSMRE and by state agencies operating with OSMRE oversight under federal authority established by the Surface Mining Control and Reclamation Act (SMCRA). The SMCRA requires that central Appalachian mine sites have vegetation established that is adequate to control erosion, to manage acid-producing materials in a manner that prevents water-quality degradation, and to comply with Clean Water Act standards. Perhaps because concerns with aquatic ecosystem effects by major ions comprising total dissolved solids (TDS) and specific conductance (SC) in mining-influenced watersheds are of relatively recent vintage, these constituents are not regulated directly under either SMCRA or the Clean Water Act.

Influence by mining disturbances on water chemistry of receiving streams, as indicated by elevated concentrations of total dissolved solids (TDS) and elevated specific conductance (SC) often extends for decades beyond reclamation and mine-site closure (Evans et al. 2014, Pond et al. 2014). Given the strong associations of TDS and SC with aquatic community alterations (Pond et al. 2008, Cormier et al. 2013a, Pond et al. 2014, Timpano et al. 2015, and numerous other studies), there is much concern with effects of central Appalachian coal mining on aquatic ecosystems. Our study focuses on temporal patterns of water-chemistry and biology in headwater streams influenced by mining.

Objectives

This report section addresses Project Objective 1: Assess long-term temporal patterns of chemical and biological changes in central Appalachian headwater streams salinized by coal mining. To accomplish this goal, we completed the following specific objectives:

1. Extend our continuous sampling of SC for an additional 18 months through December 2016.
2. Continue sampling of aquatic macroinvertebrate community structure during Fall 2015 and Spring 2016 sampling seasons.
3. Characterize long-term salinity patterns (using continuous SC as a surrogate) as they vary over annual cycles.
4. Validate consistency of salinity-biota relationships observed to date.
5. Determine if any of our monitored streams are exhibiting a trend of SC decline or increase over the study period; if so, determine if biological recovery or degradation is evident in those streams over our multiple-year study period.

Objectives 1 and 2 entailed collecting data, which we completed. We then used those data to conduct analyses to achieve Objectives 3, 4, and 5, the details of which we describe in this section.

Methods

Site Selection

Twenty-four study sites in the central Appalachian coalfields of Virginia and West Virginia were selected for long-term pattern and trend assessment of SC and benthic macroinvertebrates. As noted in Section I, sites were selected to minimize influence by non-TDS stressors on benthic macroinvertebrate communities. See Section I for details regarding site selection criteria and site attributes.

Incorporating Data from Prior Study

Long-term patterns and trends of SC and benthic macroinvertebrates were evaluated by combining data collected during the present study (2015-2016) with data collected during our prior study (2011-2014; *Effective monitoring and assessment of total dissolved solids as a biotic stressor in mining-influenced streams*; OSMRE Cooperative Agreements S11AC20004 and S12AC20023). We report here details field and laboratory methods used for samples collected during the 2015-2016 study (hereafter "present study"); see the Final Report for the 2011-2014 study (hereafter "prior study") for detailed methods used for samples collected during that period.

Continuous Conductivity

Long-term, continuous measurement of SC was achieved using automated dataloggers (HOBO Freshwater Conductivity Data Logger, model U24-001, Onset Computer Corp., Bourne, Massachusetts). The dataloggers recorded SC and temperature at 30-minute intervals (barring malfunction/loss) from July 2015 through December 2016 (n = 5 reference, 19 test sites). These data were combined for analysis with prior data, collected at 15-minute intervals from July 2011 through July 2015 (n = 5 reference, 20 test sites).

Water Chemistry

Water grab-samples were collected quarterly at each site from July 2015 through November 2016 (n = 7). 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 μm and stored in 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.

An inductively coupled plasma-mass spectrometer (Thermo Electron X-Series ICP-MS, Thermo Fisher Scientific, Waltham, Massachusetts USA) was used to measure Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, and dissolved Al, Cu, Fe, Mn, Se, and Zn (APHA 2005). 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).

Benthic Macroinvertebrates

Biological condition was characterized by measurements of benthic macroinvertebrate community structure during Fall 2015 (October) and Spring 2016 (April). We followed the single-habitat method for high-gradient streams found in U.S. EPA's Rapid Bioassessment Protocols (Barbour et al 1999). Using a 0.3-m D-frame kicknet with 500-μm mesh, a single composite sample (approximately 2 m^2) 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.

Biological samples were sub-sampled randomly to obtain a 200 (± 10%) organism count following Virginia Department of Environmental Quality methods (VDEQ 2008), which are adapted from RBP methods (Barbour et al. 1999) and are comparable to methods used by West Virginia Department of Environmental Protection (WVDEP 2015). 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.

Data Analysis

Objective 3: Modeling Salinity Patterns

We used HOBOWare software (Onset Computer Corp., Bourne, Massachusetts) to compute specific conductance as a linear function of actual conductance and temperature as recorded concurrently by the dataloggers. The dataset was then censored to exclude false SC readings observed as a result of datalogger burial by sediment, excessive water aeration at the sensor, or extreme cold. The cleaned dataset was then constrained to the period Oct 1, 2011 through Sep 30, 2016. Modeling proceeded with constrained data, using the observed SC at 12:00 pm each day to represent daily SC.

To facilitate comparison of SC patterns among streams that span a wide range of salinity, a standardized SC metric was needed. For each site, we computed the SC relative deviation from mean (SCRDM) for each daily observation of SC:

$$SCRDM_d = \frac{SC_d - \overline{SC}}{\overline{SC}}$$

where *d* = date, and $\overline{SC}$ = mean of daily SC for the four-year study period (n ≤ 1,461).

We then computed the mean daily SCRDM across sites (n = 25) and years (n = 4) to yield a single SCRDM value that represents the deviation from long-term mean SC that is expected on a given Julian day.

We fit a first-harmonic sinusoidal linear model (after Stolwijk et al. 1999) describing SCRDM as a function of Julian day:

$$SCRDM_t = \beta_0 + \beta_1 \sin\left(\frac{2\pi t}{T}\right) + \beta_2 \cos\left(\frac{2\pi t}{T}\right) + \varepsilon$$

where

t = Julian day and *T* = length of period in days. In this case we used a period of *T* = 366 days because 2012 and 2016 were leap years.

We used the model to estimate SCRDM extrema and to gauge the ability of the model to predict the timing and magnitude of salinity extremes during the year.

Timing of minimum and maximum values of SCRDM were calculated as:

$$t_{min} = \tan^{-1}\left(\frac{\beta_1}{\beta_2}\right) \times \frac{T}{2\pi}$$

and

$$t_{max} = t_{min} + \frac{T}{2}$$

where

t_{min}/t_{max} = Julian day of minimum/maximum SCRDM and T = length of period in days

The expected SCRDM value for any Julian day, $SCRDM_t$, can be calculated as:

$$SCRDM_t = A \times \cos\left[\left(\frac{2\pi t}{T}\right) - \theta\right]$$

where

A = $\sqrt{\beta_1^2 + \beta_2^2}$, the amplitude of the sinusoidal function,
t = Julian day, T = length of period in days, and
θ = $\frac{2\pi t_{max}}{T}$, the phase shift of the cosine function in radians

We modeled reference and test sites separately to determine if a seasonal SC pattern exists in the absence of mining influence. We aggregated and modeled daily SCRDM data across sites and years using only test (n = 20) and only reference (n = 5) streams as above. We compared amplitude and SCRDM extrema timing between site type models to evaluate differences between reference and test site SC patterns.

Objective 4: Validate Consistency of Salinity-Biota Relationships

For all biological samples (benthic macroinvertebrates), we calculated eight biological metrics that represent a variety of ecological categories (from Barbour et al. 1999) and that were 1) observed to be responsive to salinity based on prior study, 2) related to ecosystem functions of interest in other components of this project (e.g., leaf litter breakdown), or 3) common indicators of perturbation. We then conducted Spearman correlations of each metric with conductivity measured concurrently with biological sampling. Next, we evaluated consistency of salinity-biota relationships by comparing correlation coefficient magnitude and direction through time. Finally, we noted whether salinity-biota relationships varied between Fall and Spring seasons.

Objective 5: Evaluate Trends in Conductivity, Ionic Composition, and Biology

<u>Conductivity Trends</u>
To detect temporal trends in conductivity, we calculated weekly mean SC values for each stream; and conducted seasonal Kendall analysis on the weekly means to investigate for trend over the full study period (Helsel and Hirsch 2002). Seasonal Kendall analysis is a non-parametric technique that is commonly used to analyze water quality data for temporal trends because of its ability to accommodate non-normal data distributions and missing data values while considering data seasonality.

We also calculated the Theil-Sen slopes (Helsel and Hirsch 2002) as indicators of trend magnitude. As with seasonal Kendall analysis, the Theil-Sen slope calculation is non-parametric and accommodates missing data values while considering seasonality.

We used the tau values generated by seasonal Kendall analysis as indicators of the strength for water-quality trends following techniques also used by Zipper et al. (2002). Tau is the non-parametric correlation coefficient generated by seasonal Kendall analysis. Tau magnitudes can range from +1 to -1. When statistically significant, positive tau values indicate increasing trends; and negative values indicate declining trends. Statistical significance of the temporal trends indicated by the tau values is determined by the absolute value of tau and numbers of observations. For analyses conducted with a given number of observations, a tau value with a larger absolute magnitude will correspond with a smaller p-value (i.e., a higher level of statistical significance) than a tau value with a smaller absolute magnitude.

Linking Mine-Site Characteristics to Conductivity Trends

As a means of aiding interpretation of findings concerning conductivity trends, we defined watersheds for each monitoring location; and determined the fraction of watershed area mined during the ~1980-2011, the 2011-2016, and the ~1980-2016 periods. The ~1980-2011 mining for Virginia study locations was determined using the geospatial database generated by Li et al. (2015) via analysis of Landsat satellite data. Using the same Landsat image stack as had been analyzed by Li et al. (2015), similar methods were applied to determine West Virginia mining, ~1980-2011. Mining during the 2011-2016 period was estimated by obtaining leaf-on clear-sky Landsat images for 2012, 2013, 2014, and 2016; and conducting manual analyses of those images. Mining during the ~1980-2016 period was determined by combining the ~1980-2011 and 2011-2016 data series. We also calculated a mean value of SC for each monitoring location as the mean of all daily mean values recorded by the continuous conductivity monitors.

Biological Metric and Ion Ratio Trend Analysis

We evaluated trends in biological metrics using the same metrics selected for assessment of salinity-biota relationships in Objective 4.

To facilitate evaluation of ionic composition trends, we selected two ion-ratio metrics as indicators of overall ionic composition. The ratio of sulfate:bicarbonate (SO_4:HCO_3 ratio) was selected as an anion matrix indicator. Analyses of leachates from central Appalachian mine spoils in laboratory columns demonstrates that sulfate is the dominant anion early in the leaching process, when TDS concentrations and SC are highest (Orndorff et al. 2015; Daniels et al. 2016). As leaching progresses and TDS/SC declines, bicarbonate concentrations tend to increase and sulfate concentrations tend to decline. We expect that results from column leachate studies are indicative of weathering processes in the field; hence, we expect that SO_4:HCO_3 ratios will decline with time in mined watersheds with no current mining. The calcium:magnesium (Ca:Mg) ratio was also selected as a cation matrix indicator because Ca and Mg occur at concentrations higher than all other cations in all of our study streams; and due to our observations that Ca:Mg ratios are often altered (occur at lower levels) at our mining-influenced test sites relative to reference sites.

We calculated trends for each site individually as mixed models with the biological metrics and ion ratios as the dependent variables; for each model, year (numeric) and season (categorical) were defined as independent variables. If the effect by year was significant ($p < 0.05$), we interpreted that effect as indicating that a temporal trend was present.

All analyses were conducted using R statistical software (R Core Team 2016) with test $\alpha = 0.05$.

Results and Discussion

Characterize Long-Term Salinity Patterns

The sinusoidal model identified a significant seasonal pattern of salinity, with minimum SC in Spring and maximum SC in Fall (Figure II-1). The model was centered on mean SC (i.e., SCRDM = 0; β_0 $p > 0.05$), with strong fit ($R^2 = 0.83$). The model predicted an annual minimum SC that was 21% below mean SC, occurring in late February, and an annual maximum SC that was 20% greater than mean SC, occurring in late August (Figure II-1). Mean SC (i.e., SCRDM = 0) occurs in late May and late November (Figure II-1).

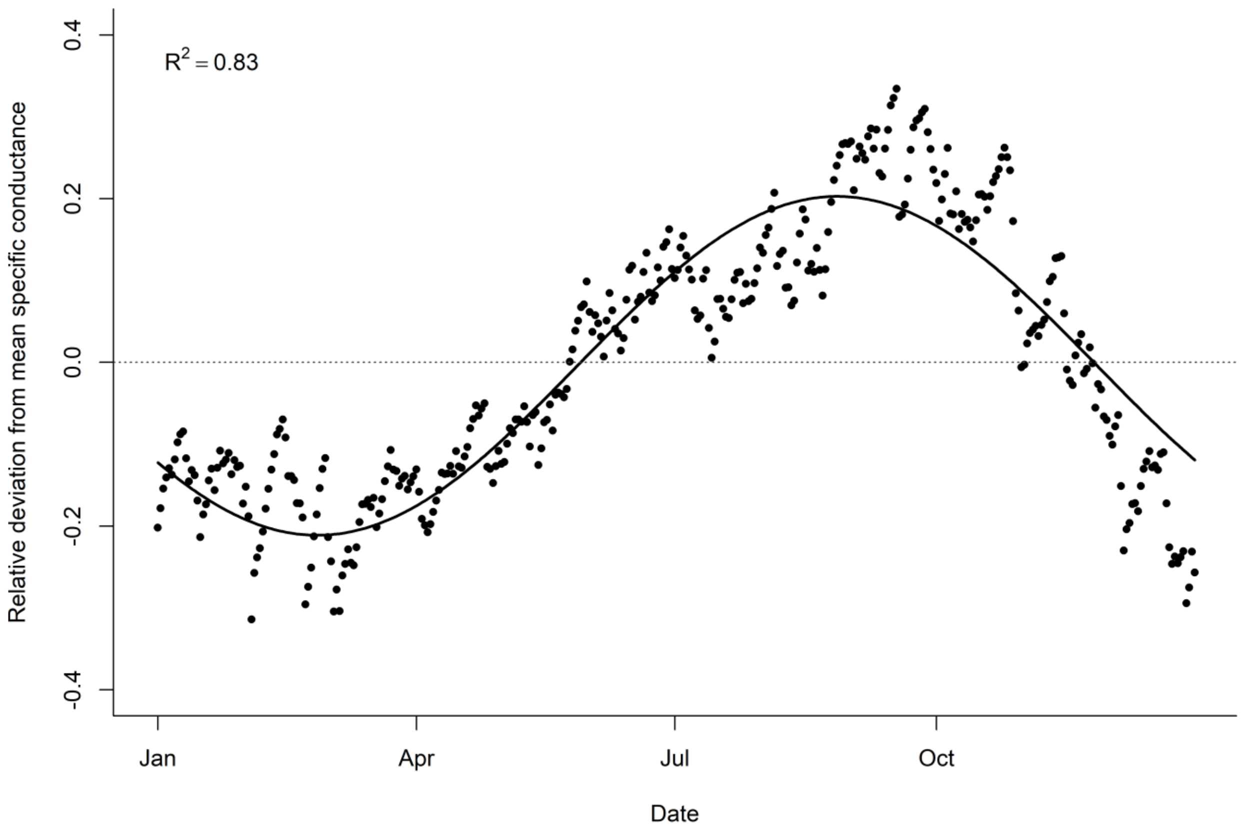

Figure II-1. Annual conductivity pattern: sinusoidal model of relative deviation from mean specific conductance (SCRDM) by date. Points are means of daily SCRDM for all streams over the study period. Solid line is fitted model, dotted line represents mean SC.

We found that both reference and test streams exhibited significant seasonal SC patterns, with Spring minima and Fall maxima. Reference and test models were comparable, with the reference model exhibiting a slightly greater amplitude and later occurrence of extrema as compared to the test site model (Figure II-2).

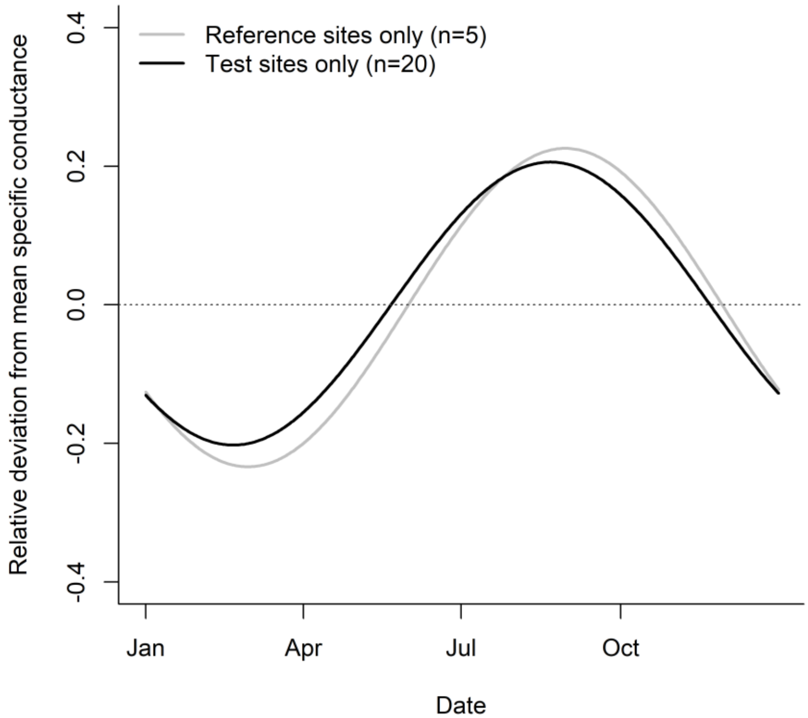

Figure II-2. Temporal pattern of specific conductance in test sites (black, n = 20) compared to reference sites (gray, n = 5). The dotted line represents mean SC.

<u>Seasonal and sub-seasonal variation</u>

Modeling of high-frequency data has revealed that in-stream concentration of major ions is non-constant and exhibits a non-random annual cycle. This overall pattern appears driven by hydrology, seasonal temperature cycles, and terrestrial vegetation. Specific conductance corresponds inversely to the general annual hydrologic pattern for forested catchments in the region, which is characterized by a drying trend through Summer (punctuated by localized thunderstorms), followed by groundwater recharge from Fall/Winter precipitation. Terrestrial vegetation also exerts a strong influence on hydrology by transpiring soil moisture to the atmosphere during the warm-weather seasons, when evaporation is also greater than during the Winter months. Evapotranspiration processes contribute to lower streamflows that are typically observed within the region during the Summer and early Fall. Those streamflow patterns correspond inversely with higher seasonal SC levels, and appear to contribute to the seasonal SC patterns represented by Figure II-1 and Figure II-2. In all of our study sites, non-mined areas are predominantly forested, and forest vegetation typically transpires more water than the herbaceous and shrub-like vegetation that is likely present on some or all of the mined areas.

Transition between seasonal extrema is not smooth, however; sub-seasonal variation was evident in the data and is not accounted for in our model. Such variation may be driven by intra-annual hydrologic patterns. Closer examination of daily salinity data while considering catchment hydrology yields several hypotheses to describe the salinity variation observed at the sub-seasonal temporal scale. Salinity begins to increase in early Spring, as evapotranspiration (ET) increases through initiation of the terrestrial growing season. Continued increase in ET gradually concentrates salts as stream discharge declines from March through June. The drying trend is punctuated in July and August with temporary declines in salinity, likely from intense isolated thunderstorms which appear to contribute little to groundwater recharge (SC values return quickly to near pre-storm levels). Salinity peaks during late Summer/early Fall (August – October), generally the driest period of the year for the region. The return of precipitation to the region in the Fall/early Winter appears to provide lasting dilution of salts, as rainfall and decline of ET appear to be sufficient for groundwater recharge (SC does not recover quickly from dilution, nor does it return as closely to pre-storm levels during this time of year). Decreasing ET through November and December further contributes to increased stream discharge and declining salinity during that period. January and February exhibit a gradual downward trend approaching annual minimum salinity, characterized by episodic SC dilution and recovery as Winter storm fronts deposit water on the region, after which the annual cycle repeats. Additional data incorporated into the sinusoidal model, such as precipitation and/or stream discharge measurements, may improve model performance.

The above hypothesis for deviation of measured water quality from the modeled seasonal pattern is consistent with our observations over the study period. The extent to which those patterns of deviation from the seasonal model are typical in most years, or are artifacts of specific weather patterns occurring during our study period, is not clear.

Salt source and hydrogeology influence salinity pattern

It is important to note that the specific patterns of salinity observed in our study streams are influenced by the nature of the salt source and catchment hydrogeology. We therefore caution that the sinusoidal model presented here may not be appropriate for other salinization sources, such as managed discharge from underground mines, pulsed inputs from road de-icing salts, agricultural runoff, or other industrial point-sources.

In reference streams free from mining influence, the bulk of in-stream dissolved major ions originate from natural mineral weathering and reach the stream through diffuse discharge of subsurface water. Runoff often causes abrupt dilution of in-stream salts, with salinity gradually returning to near pre-storm levels as discharge recedes. Over the course of a year, salinity increases as the relative proportion of base flow comprised of saline groundwater increases as a result of increased catchment ET and reduced precipitation.

A typical test stream in our study has a very similar SC pattern as reference streams, but with altered flow paths and an amplified weathering effect resultant from increased water contact with unweathered mine spoil placed in valley fills (Griffith et al. 2012). Such contact releases high concentrations of dissolved major ions upon infiltration by precipitation or groundwater, thereby elevating the salinity of baseflow[1] discharge. As in reference streams, test stream salinity responds inversely to precipitation.

[1] We use the term “baseflow” to refer to streamflow at any time of year when the stream is not influenced by runoff.

Validate Consistency of Salinity-Biota Relationships

Relationships of SC to the biological metrics selected for study were generally consistent over the study period (Table II-1), especially during the Spring season. Seven of the eight metrics (all but percent shredders) were correlated negatively with SC for all four Spring sampling events; and all but two of these relationships (Evenness in Spring 2012, 2013) were highly significant ($p < 0.01$). Correlation of shredder percent with SC was positive and highly significant ($p < 0.01$) for all four Spring seasons, except in Spring 2012 when it was only moderately significant ($p < 0.05$).

Across Fall sampling events, correlations by four of the eight metrics (Total Richness, EPT Richness, Ephemeroptera Richness, and Ephemeroptera Percent) with SC were also negative and highly significant ($p < 0.01$) for the four sampling events. Correlations of Evenness with SC were not statistically significant across three of the four Fall sampling events. Shredder percent during Fall sampling events was the only invertebrate metric that changed from a negative to a positive correlation; however, the strength of correlation between this metric and SC was never statistically significant in the Fall sampling events.

Table II-1. Coefficients of Spearman correlation between invertebrate metrics and specific conductance during Spring and Fall seasons during the study period (2011-2016).

	Fall				**Spring**			
Metric	**2011**	**2012**	**2013**	**2015**	**2012**	**2013**	**2014**	**2016**
Richness	-0.75**	-0.51**	-0.78**	-0.56**	-0.75**	-0.76**	-0.72**	-0.66**
Evenness	-0.46*	-0.26	-0.41	-0.38	-0.33	-0.42*	-0.75**	-0.63**
EPT richness	-0.80**	-0.62**	-0.71**	-0.59**	-0.81**	-0.81**	-0.81**	-0.82**
Ephemeroptera richness	-0.80**	-0.76**	-0.79**	-0.82**	-0.87**	-0.88**	-0.83**	-0.93**
Plecoptera richness	-0.63**	-0.43*	-0.41	-0.40*	-0.53**	-0.60**	-0.70**	-0.53**
Ephemeroptera percent	-0.73**	-0.79**	-0.76**	-0.84**	-0.69**	-0.87**	-0.86**	-0.83**
Predator percent	-0.67**	-0.41*	-0.48*	-0.25	-0.55**	-0.75**	-0.71**	-0.53**
Shredder percent	-0.06	0.11	0.25	0.27	0.41*	0.55**	0.70**	0.50**

* $p < 0.05$, ** $p < 0.01$

Evaluate Long-Term Trends in Water Chemistry and Biology

<u>Conductivity Trends</u>

We found both declining and increasing long-term trends in salinity at individual test and reference sites; however, 47% of study sites show no salinity trends (Figure II-3, Table II-2). When decreasing or increasing salinity trends did occur, the slopes of these trends were weak, ranging from maximum decreases of 31 µS/cm per year to maximum increases of 21.5 µS/cm (Table II-3). Seven test sites showed decreasing salinity trends, whereas three test sites showed increasing salinity trends (Table II-2). Two of the five reference sites had increasing salinity trends (Table II-2).

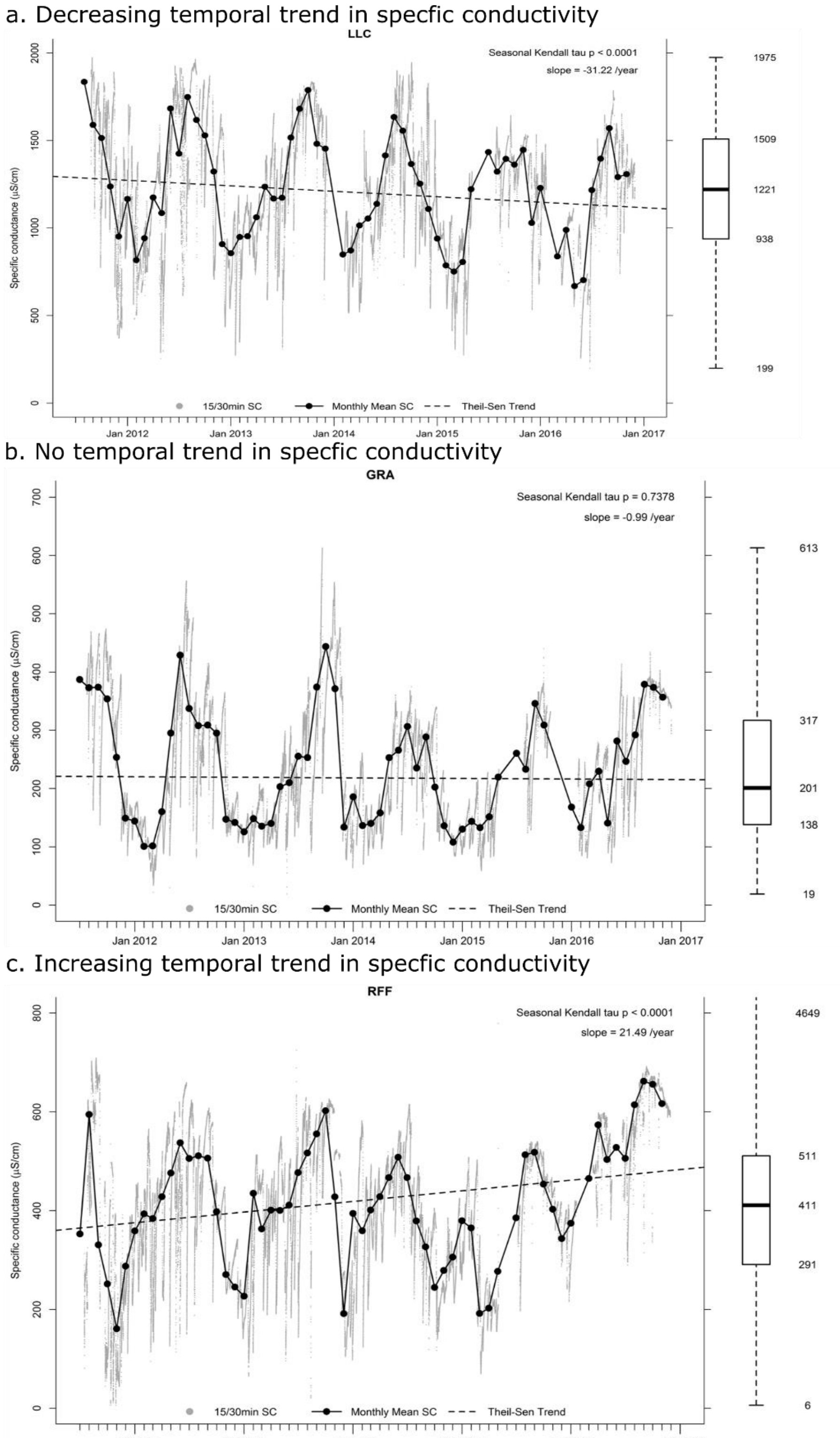

Figure II-3. Examples of long-term trends in specific conductance. Increasing trends (a.), no trends (b.), and decreasing trends (c.) were found across the 24 studies sites.

Mean tau values were nominally greater for reference sites than for test sites but did not differ significantly between the two site types (Figure II-4). Five sites exhibited increasing trends: two reference and three test sites (Table II-2 and Table II-3). All seven of the sites exhibiting declining trends were test sites.

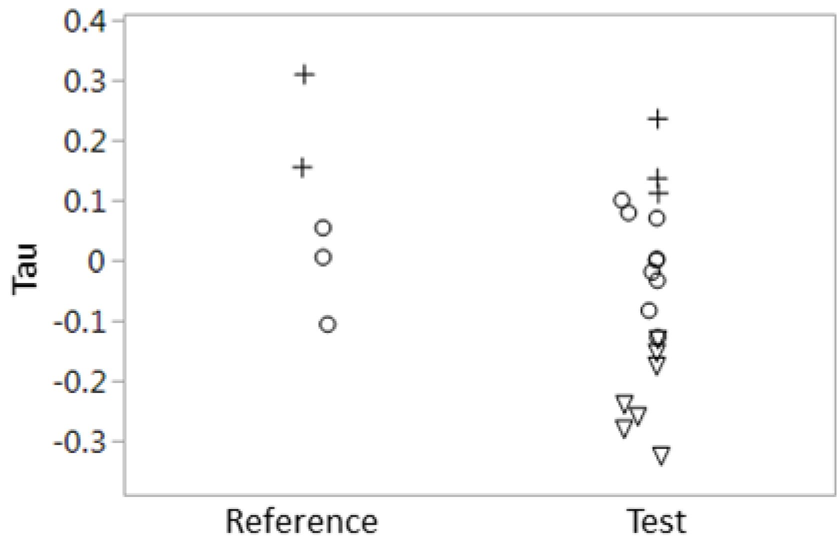

Figure II-4. Distribution of tau values for conductivity trend at reference (n = 5) and test (n = 19) sites. Significant trends ($p < 0.05$) are positive (+) or negative (triangles). Circles denote no significant trend ($p > 0.05$).

Table II-2. Mean tau values and numbers of increasing, declining, and non-significant temporal trends for specific conductance at reference and test sites.

			Number of Trends ‡		
Site Type	n	Mean Tau†	Increasing	N.S.	Declining
Reference	5	0.08 ± 0.07	2	3	0
Test	19	-0.06 ± 0.04	3	9	7

† Mean tau values are not significantly different ($p < 0.05$)
‡ N.S. = not significant at ($p > 0.05$)

<u>Linking Mine Site Characteristics to Conductivity Trends</u>
Percent mined area for the test sites, as determined by our methods, ranged from 0% to 68.4% of watershed area (Table II-3). One test site, CRA, had no mining recorded for the ~1980-2016 period, but inspection of the watershed defined by the monitoring point in Google Earth imagery revealed what appear to be pre-1977 highwall and bench areas. Percent of watershed area mined did not exhibit a significant correlation with any of the watershed-area mining indicators, when the analysis was performed with test sites only (Table II-4). When both reference and test sites were included in the analysis, tau values were negatively correlated with % mining ~1980-2011. We also conducted analyses intended to capture age-effects, such as conversion of %-mined area to a temporal-decay weighted indicator, which assigned differential weightings based on time passed since the mining disturbance (as illustrated by Zipper et al. 2016). However, these

exercises provided no improvement in capability to explain why certain sites exhibited declining temporal trends while others did not, so these results are not shown. Mean SC was negatively correlated with tau for the entire data set (n = 24; reference and test sites combined) and for the test sites only (n = 19).

Of the three test sites exhibiting increasing SC trends, two (FRY and RFF) had received additional mining over the 2011-2016 period. Two of the three sites exhibiting declining trends (KEL and KUT) drained watersheds that received additional mining over the 2011-2016 period, but this additional mining affected small fractions (< 2.5%) of overall watershed area and constituted a small increment of new mining relative to the area disturbed by mining over ~1980-2011.

Seven of the 19 test sites (37%) exhibited declining SC trends. These findings can be evaluated in light of the fact that two of the five reference sites EAS and HCN exhibited increasing SC trends over the study period. We are aware of no land-use effects within EAS or HCN that may have caused the increasing SC trends; hence, it is possible that weather/climate patterns over the study period were responsible for the observed reference site increases, and it is unlikely that weather/climate was responsible for the declining trends observed at seven test sites. Hence, we interpret the progressive nature of mine-spoil weathering processes as the cause for declining trends at seven test sites; and we interpret declining trends as evidence of water-quality recovery from the effects of mining disturbance. The observed tendency for SC to decline at some of the mining-influenced test sites is consistent with expectations based on understanding of mine spoil weathering processes that has been gained from prior study (e.g. Evans et al. 2014, Sena et al. 2014, Orndorff et al. 2015, Daniels et al. 2016).

However, the magnitudes of SC change (i.e., trend slope) were small when long-term trends were present. Associated Theil-Sen slopes, however, were of low magnitudes relative to mean SC levels. The mean rate of decline for SC at test sites with declining trends was 2.4% of mean SC/year; and the slope range was 0.8% to 4.2% per year.

Table II-3. Tau values, Theil-Sen slopes, mean SC values, and watershed characteristics (% of watershed mined during each of three time periods) for the 24 study sites.

Site code	Site Type	% Mined ~1980-2011	Additional % Mined 2011-2016	% Mined ~1980-2016	Mean SC	Tau	P-values	Trend ‡	Slope (μS/cm yr^{-1})	Slope (% of Mean SC yr^{-1})
BIR	Test	5.4	0.2	5.6	588	0.07	0.224	NS	0.4	0.1
COP	Ref	0	0	0	132	-0.11	0.074	NS	-1.1	-0.8
CRA	Test	0	0	0	425	-0.33	†	Neg	-18.0	-4.2
CRO	Ref	0	0	0	69	0.01	0.938	NS	-0.8	-1.2
EAS	Ref	0	0	0	26	0.31	<0.001	Pos	1.0	3.8
FRY	Test	4.5	0.8	5.4	382	0.14	0.015	Pos	6.1	1.6
GRA	Test	2.1	0	2.1	235	-0.02	0.738	NS	-1.0	-0.4
HCN	Ref	0	0	0	69	0.16	†	Pos	0.3	0.4
HUR	Test	20.7	0	20.7	383	0.00	0.972	NS	-1.2	-0.3
KEL	Test	56.3	2.4	58.7	758	-0.28	<0.001	Neg	-11.9	-1.6
KUT	Test	39.3	0.5	39.8	1093	-0.15	0.005	Neg	-8.2	-0.8
LAB	Test	7.6	0	7.6	616	-0.13	0.035	Neg	-11.1	-1.8
LLC	Test	19.2	5.7	24.9	1218	-0.26	<0.001	Neg	-31.2	-2.6
LLE	Test	10.9	0	10.9	574	-0.08	0.143	NS	-15.6	-2.7
LLW	Test	26.4	0	26.4	1079	-0.24	<0.001	Neg	-30.9	-2.9
MCB	Ref	0	0	0	52	0.05	0.369	NS	0.4	0.8
MIL	Test	51.6	0.8	52.4	634	-0.18	0.005	Neg	-20.3	-3.2
POW	Test	60.7	7.7	68.4	768	0.08	0.174	NS	11.8	1.5
RFF	Test	0.2	20.8	21	415	0.24	<0.001	Pos	21.5	5.2
RIC	Test	34.7	0	34.7	1444	-0.03	0.574	NS	-6.0	-0.4
ROC	Test	30.4	0.5	30.9	719	-0.13	0.051	NS	-3.3	-0.5
ROL	Test	29.9	0.1	30	632	0.00	1	NS	2.7	0.4
RUT	Test	10.2	0	10.2	566	0.10	0.089	NS	9.8	1.7
SPC	Test	3.8	0	3.8	366	0.11	0.044	Pos	7.4	2.0

† p not computed but assumed to be < 0.05 based on tau magnitude.
‡ Pos = increasing trend; Neg = decreasing trend; N.S. = not significant at p < 0.05

Table II-4. Spearman correlations of watershed mining indicators vs. Kendall's tau values.

	All sites (n = 24)		Test sites only (n = 19)	
Watershed Characteristic	rho	p-value	rho	p-value
Percent Mining ~1980-2011	-0.42	0.04	-0.33	0.17
Percent Mining 2011-2016	-0.07	0.73	0.07	0.78
Percent Mining ~1980-2016	-0.37	0.07	-0.26	0.28
Mean SC	-0.60	0.002	-0.54	0.02

Biological Metric and Ion Ratio Trend Analysis

Is biological recovery or degradation evident in monitored streams over the study period?
We found few significant trends of biological metrics at individual sites (Table II-5). Moreover, trends in biological metrics were not found consistently at those sites with increasing or decreasing trends in conductivity. For test sites with decreasing conductivity trends (CRA, KEL, KUT, LAB, LLE, LLC, LLW, MIL), we found that a significant increase in taxonomic richness occurred at only one site (LAB). Test sites with increasing conductivity trends (FRY, RFF, SPC), did not appear to have deteriorating biological conditions, as no biological metrics, except % Predator abundance at FRY, showed decreasing trends (Table II-5). At reference sites with increasing conductivity trends (EAS, HCN), we similarly found no indication of deteriorating biological condition because neither of these sites showed decreasing trends in biological metrics (Table II-5). We found no strong or consistent relationships between trends indicating improving or deteriorating biological conditions and SC trends or mean SC levels (Table II-6).

Are changes of ion matrix composition evident in monitored streams over the study period?
Seven of the 19 test sites exhibited significant declines of SO_4:HCO_3 ratios over the study period. Based on column leaching studies (Orndorff et al. 2015; Daniels et al. 2016) and comparisons of water chemistry between reference and test sites (Table I-4), we interpret declining SO_4:HCO_3 ratios as indicating a process of return to “natural background” conditions such as those occurring at reference sites. However, the declining SO_4:HCO_3 ratios do not exhibit any relationship or association with either declining SC trends or test-site SC levels that are low relative to test sites where declining SO_4:HCO_3 trends were not noted (Table II-6). Hence, the significance of this finding is not clear.

Data analyses conducted with all prior and current quarterly samples (2011-2016) confirmed the utility of SO_4:HCO_3 and Ca:Mg ratios as indicators of ion-matrix composition for our test streams influenced by surface mine drainage (Figure II-5). Both ratios, calculated on a millimolar (mmol) basis were analyzed for site-type effect with study site defined as a random variable. Ca:Mg ratios were higher at reference sites (1.65 ± 0.07 SE) than at test sites (1.11 ± 0.02 SE) ($p = 0.006$); and SO_4:HCO_3 ratios were higher at test sites (1.80 ± 0.08 SE) than at reference sites (0.37 ± 0.03 SE) ($p = 0.016$) (Figure II-5). Both ion ratios exhibited distinctive patterns relative to SC as measured at the time of water sampling, with SO_4:HCO_3 tending to increase and Ca:Mg tending to decline with increasing SC.

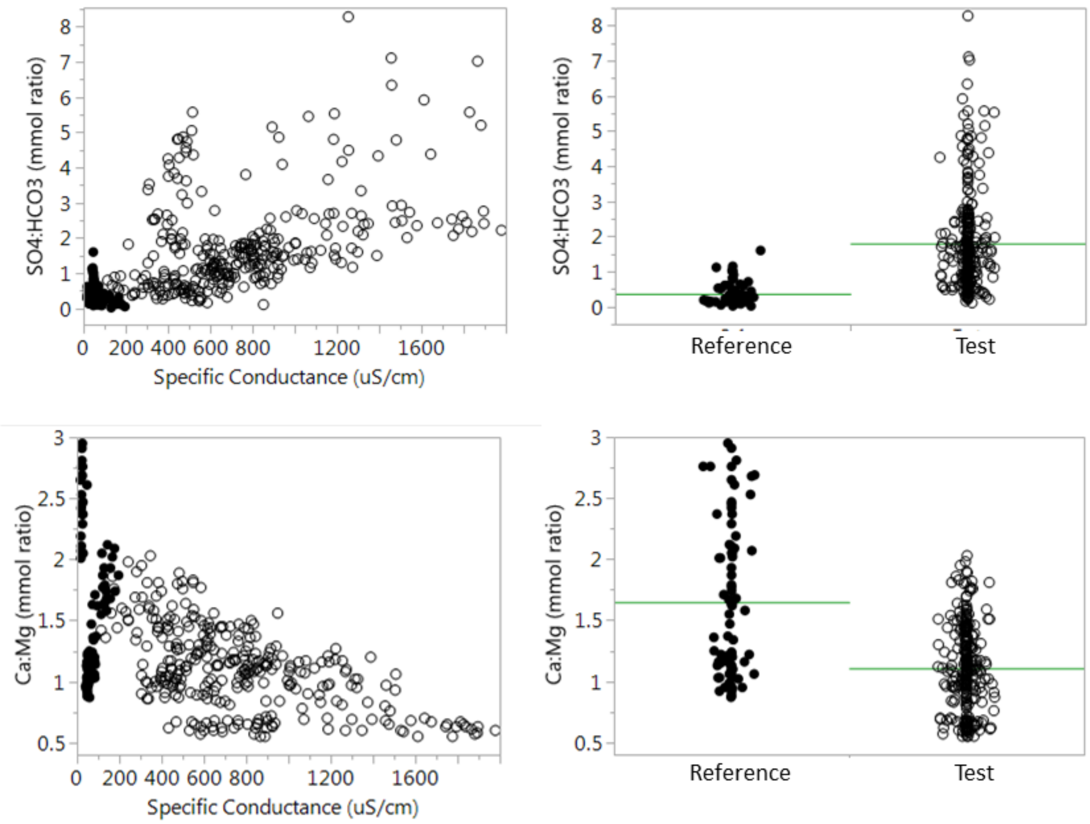

Figure II-5. Relationships of SO_4:HCO_3 and Ca:Mg ion ratios to SC (left); and ratio distributions for reference and test sites (right). Solid symbols are reference sites, and open symbols are test sites. Data include all prior and current quarterly samples (Q2, 2011 – Q4, 2016).

Similarly, declining Ca:Mg ratios were detected at three of the 19 test sites but no increasing trends were found (Table II-5 and Table II-6). However, we expected Ca:Mg ratios to increase with time and with continued geologic weathering at the mining-influenced test sites. Therefore, the significance of this finding is not clear.

Given basic geochemical concepts and the fact that central Appalachian landscapes have been formed from rocks similar to those disturbed by mining, it is reasonable to expect that water quality emanating from mined landscapes will return to a natural-background-like condition eventually. However, the time required to reach that level may be extensive, as the natural water chemistry observed currently is the result of thousands of years of weathering of the sedimentary rocks forming the central Appalachian landscape.

Several studies have evaluated changes in water chemistry produced by central Appalachian mine spoils over extended periods. All have found declining major-ion concentrations (measured directly as ion concentrations or TDS, or by proxy as SC) with continued weathering and ion leaching (Evans et al. 2014; Sena et al. 2014; Orndorff et al. 2015; Ross 2015; Daniels et al. 2016; Clark et al. 2017; and unpublished data resulting from continuation of the mine-spoil leaching study documented by Ross 2015), but none have found TDS/SC of mine-spoil leachates to have declined to levels characteristic of natural background. Therefore, the extent of time that will be required for water quality in central Appalachian mined landscapes to reach natural-background TDS/SC levels remains as a major unknown.

Table II-5. Temporal trends† of biological metrics and ion ratios at individual sites.

Site	Site Type	Rich ness	Evenness	EPT Richness	E Richness	P Richness	% Predators	% Shredders	% Ephemeroptera	SO_4: HCO_3	Ca: Mg	Tau	SC Trend	Mean SC (µS/cm)
BIR	Test	-	-	-	Pos	-	-	-	-	-	-	0.07	-	588
COP	Ref	-	-	-	-	-	-	-	-	-	-	-0.11	-	132
CRA	Test	-	-	-	-	-	-	-	-	-	-	-0.33	Neg	425
CRO	Ref	Pos	-	-	Pos	-	-	-	-	-	-	0.01	-	69
EAS	Ref	-	-	-	-	-	Neg	-	Pos	-	-	0.31	Pos	26
FRY	Test	-	-	-	-	-	Neg	-	-	-	-	0.14	Pos	382
GRA	Test	-	-	-	-	-	-	-	Pos	-	-	-0.02	-	235
HCN	Ref	-	-	-	-	-	-	-	-	-	-	0.16	Pos	69
HUR	Test	-	Pos	-	-	Neg	-	-	-	-	-	0	-	383
KEL	Test	-	-	-	-	-	-	-	-	Neg	Neg	-0.28	Neg	758
KUT	Test	-	-	-	Neg	-	-	Neg	Neg	Neg	-	-0.15	Neg	1093
LAB	Test	Pos	-	-	-	-	-	-	-	Neg	-	-0.13	Neg	616
LLC	Test	-	Neg	-	-	-	-	-	-	-	-	-0.26	Neg	1218
LLE	Test	-	Pos	-	-	-	-	-	-	Neg	-	-0.08	-	574
LLW	Test	-	-	-	-	Pos	-	-	-	-	-	-0.24	Neg	1079
MCB	Ref	-	-	-	-	-	-	-	-	-	Neg	0.05	-	52
MIL	Test	-	-	-	-	-	-	-	-	Neg	Neg	-0.18	Neg	634
POW	Test	-	-	-	-	-	-	-	-	Neg	-	0.08	-	768
RFF	Test	-	-	-	-	-	-	-	-	-	-	0.24	Pos	415
RIC	Test	-	-	-	-	-	-	-	-	-	-	-0.03	-	1444
ROC	Test	-	-	-	-	-	-	-	-	-	Neg	-0.13	-	719
ROL	Test	-	-	-	-	-	-	-	-	-	-	0	-	632
RUT	Test	-	-	-	-	-	-	-	-	Neg	-	0.1	-	566
SPC	Test	-	-	-	-	-	-	-	-	-	-	0.11	Pos	366

† Pos =increasing temporal trend; Neg = declining temporal trend; all other trends are not significant at $p<0.05$

Table II-6. **Numbers of sites (N), mean Kendall's tau values and specific conductance mean values of sites with increasing (Pos) and declining (Neg) trends for biological metrics and ion ratios, in comparison to sites with no significant trends (NS) for those same biological metrics and ion ratios; by site type.**

Metric or Ratio	**Site Type**	**- - - - -N - - - - -**			**- - - - - Tau† - - - - -**			**- -Mean SC (µS/ cm) - -**		
		Neg	**NS**	**Pos**	**Neg**	**NS**	**Pos**	**Neg**	**NS**	**Pos**
Richness	Ref	0	4	1		0.10	0.01		70	69
E Richness	Ref	0	4	1		0.10	0.01		70	69
% Predators	Ref	1	4	0	0.31	0.03		26	81	
% E	Ref	0	4	1		0.03	0.31		81	26
Richness	Test	0	18	1		-0.05	-0.13		682	616
Evenness	Test	1	16	2	-0.26	-0.05	-0.04	1218	670	479
E Richness	Test	1	17	1	-0.15	-0.09	0.07	1093	713	588
P Richness	Test	1	17	1	0.00	-0.05	-0.24	383	673	1079
% Predators	Test	1	18	0	0.14	-0.06		382	673	
% Shredders	Test	1	18	0	-0.15	-0.05		1093	656	
% E	Test	1	17	1	-0.15	-0.05	-0.02	1093	680	235
Ca:Mg	Ref	1	4	0	0.05	0.09		52	74	
SO_4:HCO_3	Test	7	12	0	-0.09	-0.002		716	657	
Ca:Mg	Test	3	16	0	-0.20	-0.03		704	674	

† Kendall's tau is an indicator of specific conductance (SC) trend; tau values that are sufficiently positive to be statistically significant indicated increasing SC trends; tau values that are sufficiently negative to be statistically significant indicate declining SC trends.

Conclusions

Modeling Salinity Patterns

As salinization increasingly threatens biodiversity of freshwaters globally (Cañedo et al 2016), tools for describing and estimating patterns of major ion concentration in streams and rivers will likely become increasingly critical for water resource managers seeking to mitigate salinization impacts. We have demonstrated that a sinusoidal model can be a useful tool for capturing overall seasonal salinity patterns in temperate forested headwater streams in the central Appalachian region. The model framework used here is also applicable to a single stream or single year to account for site-specific factors and inter-annual fluctuation in weather patterns influencing salinity. Further model development that incorporates precipitation and/or stream discharge should improve predictive power and utility of this modeling approach.

Validation of Salinity-Biota Relationships

We found strong consistency in the relationship of our eight chosen macroinvertebrate biotic metrics with SC across the study period. In general, seven of our chosen biotic metrics showed negative correlation with SC. In particular, total taxonomic richness, EPT richness, Ephemeroptera richness and percent Ephemeroptera had strong negative correlations with SC during all Spring and Fall sampling events. Therefore, these four metrics may be particularly useful when investigating potential negative effects of salinization on macroinvertebrate communities. However, Spring and Fall measures for these metrics should not be compared to one another given that levels for certain of these metrics (especially those that include Ephemeroptera) will vary among seasons (Boehme et al. 2016).

Interestingly, percent shredders was positively correlated positively with SC, especially during the Spring sampling season. Our data do not indicate a clear cause of this unexpected observation, but we hypothesize that an increasing percent shredder abundance may be related to reductions in predator abundance; we observed moderate to strong negative correlations between predator relative abundance and SC and percent Predator abundance. Considering the importance of these macroinvertebrate shredders to the organic matter breakdown process, these results underscore the relevance of our investigation of potential effects of salinity on leaf breakdown in the following section of this report.

Trends in Salinity, Ionic Composition, and Biology

Across our 19 test sites, we found no strong indication of rapid or significant declines of water quality with regard to salinity during the 2011-2016 study period, but gradual SC declines and apparent recovery from mining disturbance at some sites was evident. Long-term declining trends in salinity were found at seven test sites, while increasing trends were found at three sites, two of which had experienced additional mining over the 2011-2016 period. All other test sites showed no long-term salinity trends. In contrast, two of the five reference sites exhibited increasing SC trends and no declining trends were noted. Hence, it appears that gradual decline of SC is occurring at some test sites. However, the magnitudes of SC change (i.e., trend Theil-Sen slope) were small when long-term trends were present.

Furthermore, we found no indication of a consistent pattern of biological recovery at test sites over the five-year study period. Although long-term trends were found in biological metrics at some individual sites, those trends were not consistent across sites that had either decreasing or increasing trends in SC. The lack of strong, consistent trends in the biological metrics supports our finding that there appears to be no indication of improvement or deterioration of water quality or biological condition in these study streams over the period of study.

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III. INFLUENCE OF MINING-INDUCED SALINITY ON LEAF LITTER BREAKDOWN

Introduction

Biodiversity and ecosystem functions are major foci of global change research (Loreau et al. 2001, Cardinale et al. 2001, Hooper et al. 2012). The recent boom in empirical studies of these two facets of ecosystems is driven by the realization that biodiversity loss could result in drastic changes to ecosystem functions, resulting in impairment of ecosystem services that sustain human populations (Cardinale et al. 2012). Evidence of negative effects of biodiversity loss on the rates or efficiency of ecosystem function is widespread (Cardinale et al. 2000, Costantini and Rossi 2010, Fugère et al. 2012). However, much of the research generating these results is based on direct biodiversity manipulations in artificial systems (e.g. mesocosms), which may not capture realistic responses of communities to environmental change (De Laender et al. 2016). Field studies are needed to provide more realistic insights into potential losses of ecosystem functions as a result of biodiversity loss.

Organic matter breakdown is a primary ecosystem function occurring in central Appalachian headwater streams that could be altered by macroinvertebrate biodiversity loss and/or alteration of community structure (Webster et al. 1999, Fritz et al. 2010, Krenz III et al. 2016). Breakdown of organic matter such as leaves in headwater streams represents the major energy source and contributes greatly to downstream waters (Wallace et al. 1991, Gomi et al. 2002). Aquatic macroinvertebrates play a key role in the breakdown process by shredding coarse particulate plant material (Wallace and Webster 1996) therefore changes to their richness or composition, such as those associated with mining-induced salinization, could significantly alter breakdown rates (Gessner et al. 2010). However, not all invertebrate shredders are sensitive to salinity (Boehme et al. 2016, Voss et al. 2015). Therefore, decreases in rates of organic matter breakdown should not be assumed based on a general decline is species richness resulting from salinization.

Objectives

The objective of this portion of our study was to determine the influence of mining-induced salinity on leaf litter breakdown, a key carbon processing function, in central Appalachian headwater streams. To this end, we measured breakdown rates in 24 headwater streams across a gradient of mining-induced salinity from reference (no mining) to high levels (Timpano et al. 2015). Following the hypothesis that biodiversity has a positive influence on rates of ecosystem function, we first predicted that rates of organic matter breakdown would be reduced in streams with elevated levels of salinity.

Methods

Site Selection

The leaf litter breakdown study was conducted in the same 24 streams being studied as part of our larger investigation of mining impacts on water quality and aquatic life in headwater streams of the central Appalachian coalfield region (see Section I for site selection criteria and site attributes).

Leaf Litter Breakdown

During November 2015–October 2016, we measured leaf litter breakdown following standard protocol (Benfield 2006) and similarly to other studies investigating mining impacts in this region (Fritz et al. 2010, Krenz III et al. 2016). We collected freshly abscised leaf litter of *Quercus alba* (white oak) a common species throughout the region from a single location in Blacksburg, Virginia. Leaves were uniformly mixed, air-dried indoors, and weighed until constant mass was achieved. Six and one half g of dried leaves were then placed in ~28 cm x 30 cm nylon mesh (6mm) bags. Twenty-seven bags were anchored to the streambed at three separate locations within each study site in November 2015. Leaf bags were installed in glides (transitional zones between riffles and pools) to maximize likelihood of remaining inundated for the entirety of incubation and to minimize potential loss of bags caused by high streamflow. After 0 d (to estimate handling loss), ~30 d, ~60 d, ~90 d and ~150 d, ~ 180 d, ~210 d, ~270 d of inundation, leaf bags were collected in triplicate, stored on ice in polyethylene zip-top bags, and transported to the laboratory. In the lab, leaves were removed from the mesh bags, and rinsed over 250 μm sieves to separate leaf litter from mineral deposits and macroinvertebrates. Leaves were then dried to constant dry mass (DM) in an oven (60°C). Once dried, leaves were aggregated, milled, and ashed at 550°C. Percent organic matter was calculated and multiplied by DM to obtain ash-free dry mass (AFDM), and percent AFDM remaining was determined.

Benthic Macroinvertebrates and Water Quality

This analysis incorporated benthic macroinvertebrate and water quality data collected as part of Section II of this project. See Section II for detailed sample collection and analysis methods.

Data Analysis

Leaf breakdown rates (*k*) were determined based on a first-order decay model

$$M_t = M_0(e^{-kt})$$

where M_0 is the initial leaf pack mass (% AFDM) at the beginning of the experiment and M_t is leaf pack mass (% AFDM) at time t (days; Petersen and Cummins 1974, Webster and Benfield 1986), with adjustments for mass loss incurred from handling during transport and installation of the litter bags.

We calculated simple linear regressions using specific conductance (mean value during study period) as a predictor of leaf breakdown rate to test the effect of salinity on leaf breakdown rates. We then assessed the effect of salinity on biological variables known to strongly influence breakdown rates. For this, we focused our analysis on shredder richness (total number of shredder taxa) and shredder percent abundance (percent of total individuals that shred organic matter - such as leaves - for food), using the mean of Fall 2015 and Spring 2016 values for each macroinvertebrate metric, as determined in Section II. We tested the significance of salinity using simple linear regressions as described above. For each regression model, we visually assessed residual variances for homogeneity of variance and normality and log transformed response and explanatory variables to meet these assumptions when necessary. We performed all analyses using R statistical software (R Core Team 2016) and determined statistical significance at a test level of $\alpha = 0.05$.

Results and Discussion

Leaf litter breakdown rates over a 90-day inundation period (November-February) averaged 0.030 ± 0.005 day^{-1} (mean ± se; range: 0.008 – 0.053 day^{-1}) at test sites and 0.031 ± 0.007 day^{-1} (range: 0.019 – 0.040 day^{-1}) at reference sites (Table III-1). We found breakdown rates calculated for the 150-day period were 0.031 ± 0.003 day^{-1} (range: 0.012 – 0.053 day^{-1}) at test sites and 0.027 ± 0.002 day^{-1} (range: 0.025 – 0.033 day^{-1}) at reference sites. Rates calculated for the 270-day inundation period were 0.028 ± 0.002 day^{-1} (range: 0.020 – 0.039 day^{-1}) at test sites and 0.024 ± 0.002 day^{-1} (range: 0.019 – 0.028 day^{-1}) at reference sites. Mean leaf litter breakdown rates for test streams did not differ significantly ($p > 0.05$) from those for reference streams for 90-, 150-, or 270-day inundation periods. However, due to higher variance and some loss of within-site replication of litter bags, likely due to high flow events, in 150- and 270-day breakdown rates, we only present breakdown rates calculated on the 90-day inundation period.

Table III-1. Leaf litter breakdown rates (mean ± standard error) for each test and reference site. Rates based on a 90-day inundation period.

Site		Breakdown rate (day^{-1})		
		mean		se
Test				
	BIR	0.008	±	0.001
	CRA	0.031	±	0.003
	FRY	0.042	±	0.008
	GRA	0.048	±	0.002
	HUR	0.036	±	0.006
	KEL	0.030	±	0.001
	KUT	0.042	±	0.007
	LAB	0.053	±	0.003
	LLC	0.011	±	0.001
	LLE	0.022	±	0.005
	LLW	0.032	±	0.003
	MIL	0.023	±	0.005
	POW	0.015	±	0.018
	RFF	0.038	±	0.006
	RIC	0.015	±	0.001
	ROC	0.042	±	0.007
	ROL	0.024	±	0.003
	RUT	0.016	±	0.006
	SPC	0.036	±	0.003
	All test sites	*0.030*	±	*0.005*
Reference				
	COP	0.040	±	0.002
	CRO	0.019	±	0.008
	EAS	0.035	±	0.009
	HCN	0.027	±	0.008
	MCB	0.033	±	0.007
	All reference sites	*0.031*	±	*0.007*

Effect of Salinity on Leaf Litter Breakdown Rates

In this study, we aimed to determine the influence of mining-induced salinity on organic matter breakdown across 24 headwater streams variously impacted by mining. We found no measurable effect of specific conductance on the leaf breakdown rates calculated from a 90-day inundation period (ANOVA, $p > 0.05$; Figure III-1). When breakdown rates were calculated based on a 150- and 270-day inundation period, we also found no effect of specific conductance (ANOVA, $p > 0.05$; data not shown), a result that further justifies our focus on breakdown rates derived using 90-day inundation data. Previous studies investigating changes in leaf breakdown rates in central Appalachian headwater streams resulting from coal-mining water-quality impairments have generally found reduced rates associated with mining (Fritz et al. 2010, Petty et al. 2013, Krenz III et al. 2016). Our results do not agree with these previous studies; however, we conducted rigorous site selection so as to minimize potential effects of non-salinity stressors (e.g., sedimentation) on the stream ecosystem, and thus on breakdown rates.

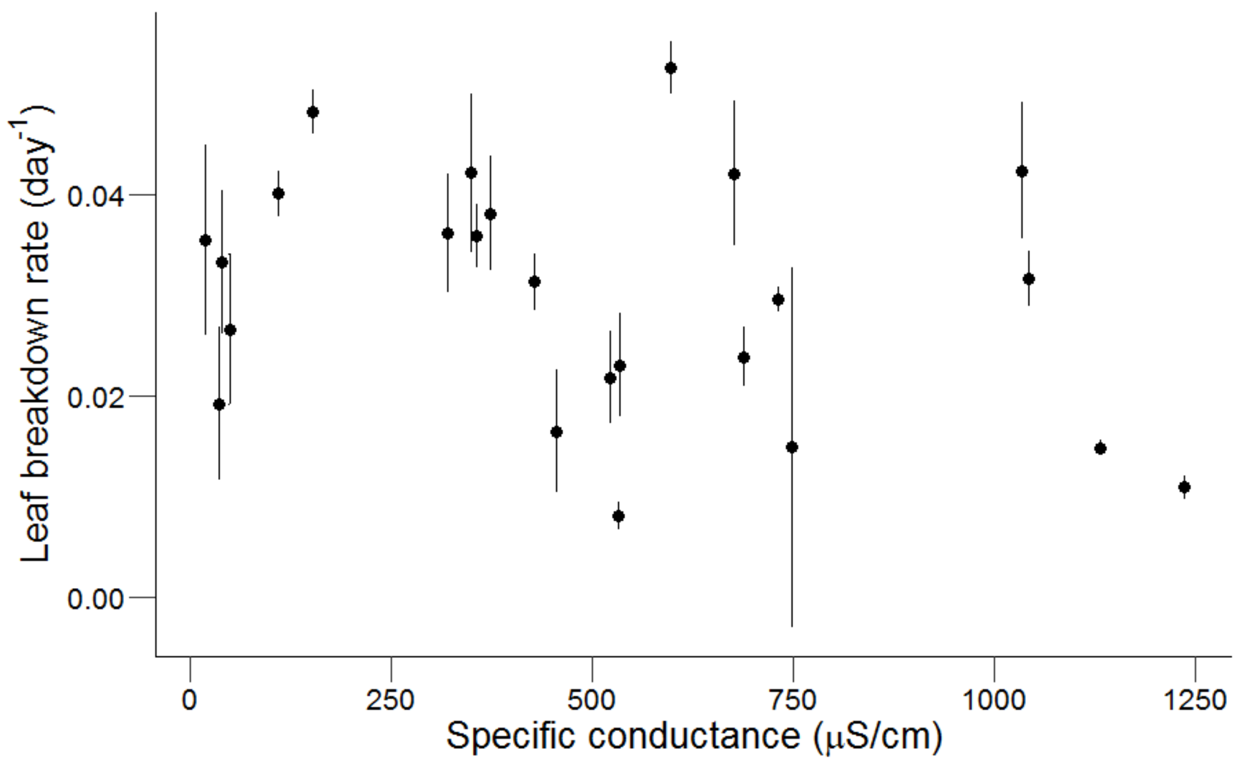

Figure III-1. Relationship between specific conductance and leaf breakdown rates (mean ± standard error). Rates based on a 90-day inundation period.

Linking Salinity, Benthic Macroinvertebrates, and Leaf Litter Breakdown Rates

Mean of Fall 2015 and Spring 2016 macroinvertebrate shredder richness was 3.2 ± 0.7 taxa (range: 1.5 – 5.5) at test sites and 4.3 ± 0.8 taxa (range: 3.0 – 7.0) at reference sites (Table III-2). Mean shredder percent abundance (Fall 2015 and Spring 2016) was 42 ± 8% (range: 21 – 72%) at test sites and 21 ± 10% (range: 12 – 33%) at reference sites. We found seven of the 19 test sites had mean shredder percent abundance > 50% (Table III-2).

Table III-2. Shredder richness and percent abundance for each test and reference site. Mean ± standard error of pooled (Fall 2015 and Spring 2016) values.

Site		Shredder richness			Shredder percent abundance		
		mean		s.e.	mean		s.e.
Test							
	BIR	1.5	±	0.5	72	±	3
	CRA	5.5	±	2.5	24	±	1
	FRY	4.0	±	1.0	37	±	5
	GRA	3.5	±	1.5	35	±	5
	HUR	3.0	±	1.0	49	±	4
	KEL	3.0	±	1.0	50	±	23
	KUT	2.0	±	0.0	25	±	15
	LAB	3.5	±	0.5	30	±	11
	LLC	3.5	±	0.5	37	±	18
	LLE	4.5	±	0.5	54	±	3
	LLW	5.0	±	0.0	29	±	6
	MIL	2.0	±	0.0	41	±	13
	POW	3.0	±	1.0	67	±	12
	RFF	2.5	±	0.5	23	±	6
	RIC	3.0	±	0.0	51	±	1
	ROC	2.5	±	0.5	21	±	10
	ROL	3.0	±	1.0	66	±	13
	RUT	2.0	±	0.0	52	±	7
	SPC	3.5	±	0.5	41	±	7
	All test sites	*3.2*	±	*0.7*	*42*	±	*8*
Reference							
	COP	4.0	±	1.0	19	±	4
	CRO	4.0	±	< 1	24	±	13
	EAS	7.0	±	1.0	17	±	< 1
	HCN	3.5	±	0.5	33	±	22
	MCB	3.0	±	< 1	12	±	1
	All reference sites	*4.3*	±	*0.8*	*21*	±	*10*

We found no measurable effect of specific conductance on shredder taxa richness (ANOVA, $p > 0.05$, Figure III-2a) or on percent shredder abundance (ANOVA, $p = 0.09$, Figure III-2b). This result provides a likely explanation for our finding that leaf breakdown rates were not reduced as salinity increased in our 24 study sites. There was a positive relationship between percent shredder abundance and mean SC at levels ≤ 750 µS/cm. Increasing salinity has been shown to reduce overall macroinvertebrate species richness in headwater streams salinized by mining in our study region (Timpano et al. 2015). However, these reductions in taxa richness do not occur randomly, affecting the entire macroinvertebrate community. Instead, our results show that shredding macroinvertebrates are not affected by increasing salinity over the range of SC observed at our sites. Perhaps, more importantly, we found that shredder percent abundance increased with salinity across our test sites. Boehme et al. (2016) also showed increased relative abundance of individuals from a dominant shredder family (Leuctridae) in headwater streams salinized by mining in our study region. Therefore, concomitant reduction in macroinvertebrate richness and leaf litter breakdown rates as a result of increasing salinity cannot be assumed in these headwater streams.

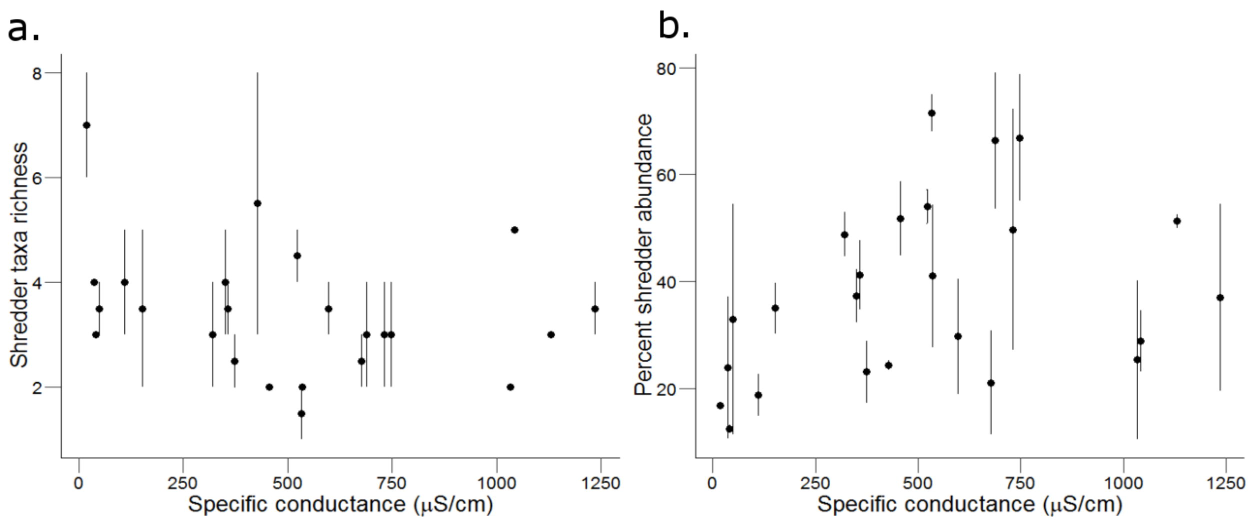

Figure III-2. Relationship between shredder taxa richness (a.), percent shredder abundance (b.) and mean specific conductance. Mean of pooled (Fall 2015 and Spring 2016) richness and percent abundance values.

Conclusions

Organic matter breakdown is a primary ecosystem function occurring in central Appalachian headwater streams that could be altered by macroinvertebrate biodiversity loss and/or alteration of community structure. Macroinvertebrates are particularly important contributors to leaf litter breakdown in headwater streams. Despite evidence that salinization negatively affects several important macroinvertebrate groups (e.g. EPT), we found no measurable effect of salinization on rates of leaf litter breakdown across our study sites. One likely explanation for this result is that a component of the shredder macroinvertebrate community (those that feed on leaf litter and associated microbial communities) is not particularly sensitive to salinization at the levels we observed in our study streams. We found no differences in shredder richness as salinity increased and some indication that shredder relative abundance increases with salinity, which is opposite of the expected general response of those taxa to perturbation.

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IV. TROPHIC TRANSFER AND BIOACCUMULATION OF TRACE ELEMENTS: SELENIUM

Introduction

Environmental contamination by the trace element selenium (Se) is a global concern for reasons that include the role of Se as a toxicant in aquatic environments (Lemly 2004). Many organisms require Se as an essential nutrient used in the formation of selenoproteins (Driscoll and Copeland 2003). However, because of its tendency to bioaccumulate, toxic effects in biota, particularly egg-laying vertebrates, may be observed at sites where Se concentrations in the water column are only marginally elevated above essential levels. Human activities including phosphate-, uranium-, and coal-mining, petroleum processing, and irrigation in regions with seleniferous soils, have increased Se inputs into the environment (Hamilton 2002). Environmental consequences of Se contamination have been documented, from fish kills in Belews Lake, NC (Lemly 2002, Lemly 1985) to embryonic deformities in water birds found at Kesterson Reservoir area, CA (Ohlendorf et al. 1986), to fish-tissue concentrations exceeding limits safe for human consumption in Lake Macquarie, Australia (Barwick and Maher 2003).

Ecosystem dynamics of Se enrichment and bioaccumulation are unique among trace elements. Selenium enrichment is the process of transformation from dissolved Se in the water column to biofilm and detritus (particulate matter). It is the most concentrating step of Se accumulation that can range from a 10^2 to 10^6 -fold increase in concentration (Stewart et al. 2010). Ecosystem enrichment of Se occurs through uptake of inorganic dissolved Se by bacteria, algae, or plants at the bottom of the food chain. The dominant pathway for Se bioaccumulation in consumer organisms is dietary. Smaller, but significant bio-concentrating steps occur through trophic transfer when biota consume particulate matter consisting of living or detrital particles containing Se. Additional trophic transfer may bio-concentrate Se when prey are consumed by predators (Presser and Luoma 2010).

Site-specific biogeochemical factors facilitate Se enrichment and bioaccumulation within an aquatic system. Source of Se contamination determines the dominant Se species, and thus the reactivity and efficiency of Se enrichment (Young et al. 2010). Water residency time influences enrichment and retention of Se; rapidly flowing streams limit reactivity time and may flush Se-enriched particles out of the stream system, restricting build-up of Se-enriched sediments and bioaccumulation through detrital pathways; hence lentic systems are thought to bioaccumulate Se more efficiently than lotic systems (Orr et al. 2006, Lemly 1999). Community composition at all levels of the aquatic food chain also controls enrichment and trophic transfer rates. Species differences in assimilation efficiencies, ingestion, and excretion rates may scale up to community-level differences (Presser and Luoma 2010). Because of the highly influential role that site-specific factors play in Se dynamics, linking dissolved water column concentrations to toxic effects within an ecosystem is a challenge for regulators seeking to create water-quality criteria, and for water resource managers. Site-specific studies examining Se enrichment and trophic transfer are needed to inform appropriate resource management and protection practices (Presser and Luoma 2010).

In central Appalachia, surface-coal mining is a source of Se contamination and is recognized to be a driver of change in water chemistry and aquatic communities in stream ecosystems (USEPA 2011). Surface-coal mining is widespread in central Appalachia, often dominating land-use change in mining-influenced watersheds and affecting stream ecosystems in numerous ways. Direct burial of headwater streams, watershed deforestation, and accelerated release of geologic-origin major ions and increases of total dissolved solids (TDS) concentrations in stream water are all possible consequences of mining-activities (USEPA 2011, Palmer et al. 2010). During the mining process, overburden rock layers are removed to uncover underlying coal-seams and overburden is often disposed in adjacent valleys (Palmer et al. 2010). Coal deposits and associated rock strata disturbed during the mining process often contain Se at concentrations greater than that of soil and near-surface, weathered rock. When exposed to rainfall, elemental Se oxidizes to water-soluble selenite and selenate anions and is transported in dissolved form into streams at elevated concentrations (Young et al. 2010, Lussier et al. 2003).

Despite the widely recognized source for Se contamination in central Appalachia, scientific knowledge of bioaccumulation processes in headwater streams within this ecoregion is limited, particularly in lower-levels of the aquatic food chain. In this portion of the study, we evaluated the degree and dynamics of Se enrichment and bioaccumulation in headwater streams. This was accomplished by determining Se tissue concentrations in benthic macroinvertebrates among 23 headwater streams, 18 of which were mining-influenced. For a subset of nine of those streams (six mining-influenced and three reference), Se concentrations in water, particulate forms, and benthic macroinvertebrate tissue, as well as Se enrichment and trophic transfer were measured through sampling of three reference streams and six mining-influenced streams.

Based on findings from previous studies (Arnold et al. 2014, Presser 2013), we predicted historical mining activities within a watershed would be a significant source of Se that would cause concentration increases of dissolved Se within the water column. By way of enrichment and bioaccumulation, we expected a corresponding Se concentration elevation in other ecosystem media relative to concentrations found in reference streams. Measured ecosystem media were expected to exhibit the highest Se concentrations in streams classified as high-Se and lower concentrations in streams classified as low-Se. Although individual ecosystem media were expected to differ among stream types, Se dynamics (factors of enrichment and trophic transfer) were not expected to differ, because differences in factors controlling Se dynamics among streams, such as selenium speciation and site hydrology (water residence time) were minimized. Prior research has demonstrated that Se bioaccumulation dynamics vary among ecosystem types, but all study streams were located within the same central Appalachian ecoregion had similar habitat quality that meets reference-like criteria (Timpano et al. 2015).

Methods

Site Selection

Candidate study sites were chosen from the 24 streams being studied as part of our larger investigation of mining impacts on water quality and aquatic life in headwater streams of the central Appalachian coalfield region (see Section I for site selection criteria and site attributes). Site selection proceeded in two phases (detailed below), ultimately resulting in nine streams (three reference, six test) retained for intensive trace element sampling.

Study Reach Delineation

Study reaches 100m in length were approximately centered on continuously-logging conductivity meters previously installed at the study sites. When necessary, study reaches were shifted upstream to avoid having downstream segments located below roadways or having tributaries draining expanded watersheds. Study reaches were subsequently divided into 10m sub-reaches to facilitate collection of all media evenly throughout the entire stream reach (Figure IV-1).

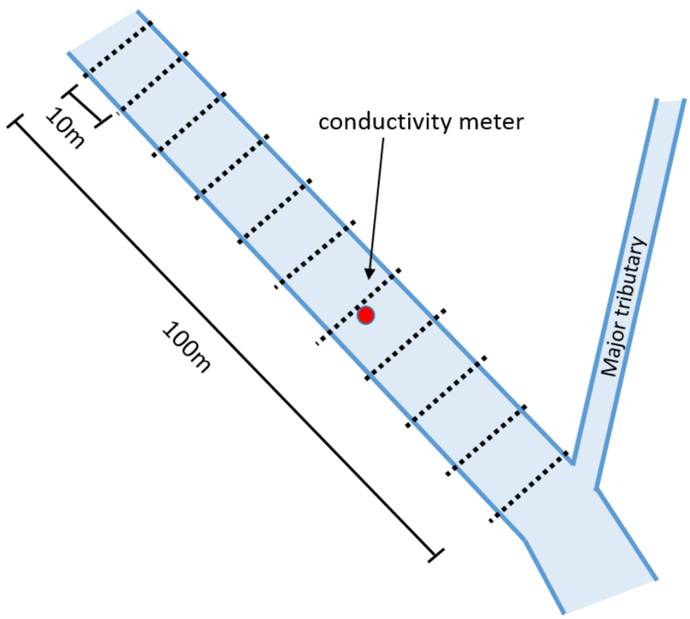

Figure IV-1. Conceptual figure of study reach delineation and sampling locations at study sites. The study reach, 100 m in length, is centered approximately on a conductivity datalogger.

Phase I

Selenium bioaccumulation was determined by collecting tissue samples of selected benthic macroinvertebrate taxa from 23 stream sites. Cambaridae and dragonfly nymphs from families Gomphidae and Cordulegastridae were targeted for collection at 23 of the 24 potential study streams between July 7^{th} and August 8^{th} 2015. These taxa were widely available at mining-influenced and reference streams, and their relatively large body sizes allowed for an efficient sampling effort. Optimal habitat for these taxa was sampled using a D-frame net. Samples were transported on dry ice to the laboratory where they were stored at -20 °C until analysis.

Macroinvertebrates from each stream site were separated into taxon groups. Composite samples by taxon underwent acid digestion and were analyzed for Se concentrations (
Figure IV-2) with an inductively-coupled mass spectrometer (ICP-MS) (Perkin-Elmer, Norwalk, CT).

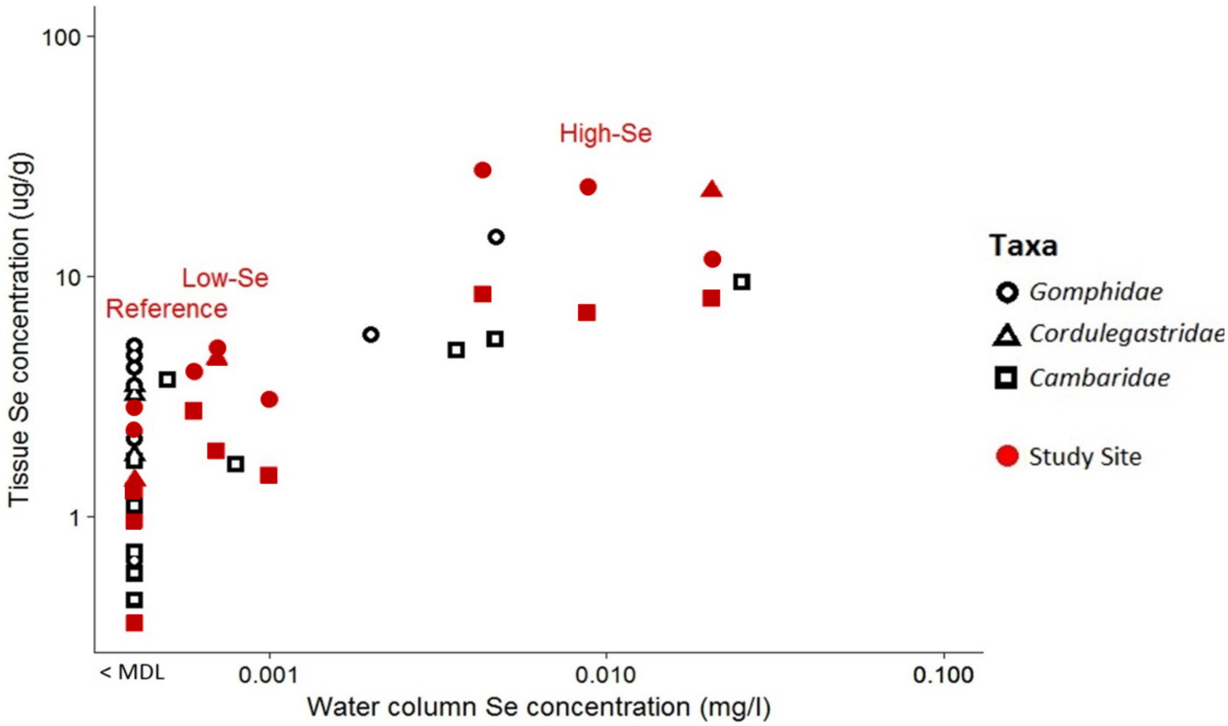

Figure IV-2. Dry mass Se concentrations in tissue samples of Cordulegastridae, Gomphidae, and Cambaridae in relation to water column Se concentration sampled in Phase I of study from streams in Central Appalachia used for selection of stream type. "< MDL" indicates water samples below minimum detection limit (< 0.0005 mg/L). Solid red symbols are sites selected for further sampling efforts in Fall 2015 and Spring 2016. "Reference," "Low-Se," and "High-Se," indicate stream type groupings.

Phase II

To examine Se dynamics of enrichment and trophic transfer, nine stream sites were selected for further study based on Se concentrations in benthic macroinvertebrates collected during Phase I (
Figure IV-2). Three stream sites with no history of mining activities within their watersheds were selected as reference streams. Six remaining stream sites had mining activities and were selected to represent the range of Se observed during Phase I. These mining-influenced streams were separated into two groupings of three streams each: "high-Se" streams exhibiting high Se concentration in macroinvertebrate tissue samples as measured in Phase I and "low-Se" streams exhibiting lower Se concentrations in tissue samples (Figure IV-2). Geographical proximity was also considered in selection of streams. Because only two high-Se sites were located in southwestern Virginia, a third high-Se site located in southern West Virginia was selected to include a broader range of sites representative of coalfield streams (Figure IV-3).

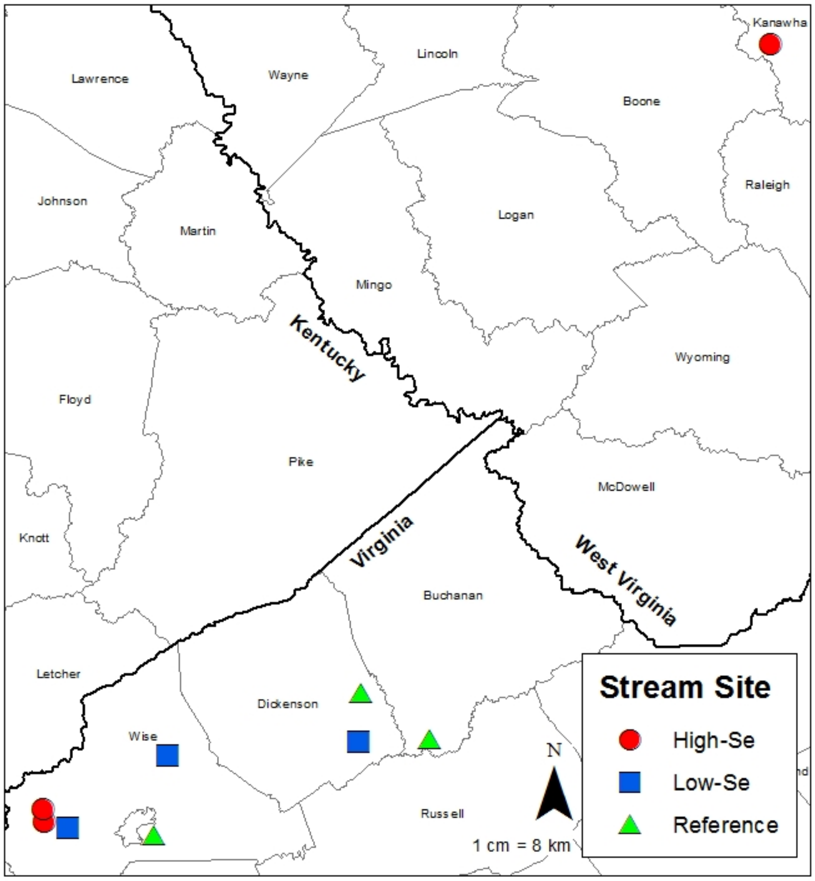

Figure IV-3. Location of stream sites selected for study in the coalfields of central Appalachia.

Sample Collection and Processing

Water Column

Water samples for dissolved trace elements were collected at approximately mid-study reach, downstream of riffle habitat to ensure vertical mixing. Approximately 50 ml of water was filtered in the field using a 0.45 µm-pore filter membrane, preserved with trace-metal-grade nitric acid to pH < 2 immediately following sampling, and stored in polyethylene bags on ice until transport back to the laboratory where they were stored at 4 °C. Acidified samples were analyzed on an ICP-MS without further processing (USEPA 1996).

Stream Bed Sediment

Composite stream bed sediment samples were collected using a 237 ml plastic scoop. Sample depth was restricted to 1- 3 cm, thus selecting for recently deposited, biologically active sediments (USEPA 2001). Sub-samples were taken at five evenly spaced intervals in three lateral transects per sub-reach, combined in light-excluding polyethylene bags, and stored at 4 °C. Samples were hand-pressed through a cleaned, 1mm stainless steel sieve into a stainless steel collection bowl. Extraneous materials such as leaves and twigs were removed during sieving. Sediments ≥ 1 mm were discarded. Sediments < 1 mm were homogenized and stored in 15 ml sterile glass vials. Samples were shaken vigorously to ensure thorough mixing and lyophilized for approximately 120 hr to ensure complete drying.

Biofilm

Biofilm accumulations in streams were collected from rock substrate using a plastic knife. At each sub-reach, ≤ three rocks with the largest accumulations of biofilm were removed from the stream, and biofilm was scraped into light-excluding polyethylene bags. During the Fall sample period, epilithic biofilm was not available for collection at all streams. When available, sandy substrate was sampled for biofilm by scooping the full visible extent of sediment surfaces visibly carrying biofilm accumulation within the reach. Composite biofilm samples were stored in light-excluding polyethylene bag, and transported on dry ice back to the laboratory for storage at 4 °C (Orr et al. 2006, Casey 2005). Biofilm samples, particularly samples collected from sandy deposits, contained a large quantity of sand and silt. Sand and silt portions of the sample were reduced by thawing the biofilm composite samples in an acid-washed, 500 ml beaker. The slurry was shaken for 30 sec, and decanted into another 500 ml beaker. Following addition of 50 ml deionized water, the new slurry was shaken again for 30 sec, and decanted. This process was repeated a third time to reach a final slurry of significantly reduced amount of sediment material (Bell and Scudder 2007). Samples were stored in 15ml sterile glass vials, and stored at 4 °C until they were lyophilized for approximately 120 hr. After complete drying, samples were ground using a mortar and pestle.

Leaf Detritus

Leaf detritus originating from the tree canopy bordering each stream was collected from leaf-packs defined as an accumulation ≥ three leaves within the stream. Within each sub-reach, the largest leaf-pack was identified and ≤ ten leaves were collected. If the largest leaf-pack contained < ten leaves, additional leaf-packs were identified for collection until a total of ten leaves per sub-reach was attained. Criteria for collected leaves included completely brown coloration, fully submerged in water, and not covered by sediment. Composite leaf samples for each site were combined into a light-excluding polyethylene bag, and transported on dry ice back to the laboratory where leaves were briefly thawed, identified to tree species when possible, and agitated lightly in deionized water to remove excess sediments. Leaves were dried at 65°C for ≥ 5 d. After drying, leaf mid-veins were removed, and remaining leaf matter was ground using a ball-mill for ≥ 2 min at a vibration frequency of 25 Hz.

Benthic Macroinvertebrates

Benthic macroinvertebrates were collected using a D-frame dip net following multi-habitat sampling procedures (Barbour et al. 1999). Dip net contents were emptied into plastic tubes filled with site water, and macroinvertebrates were removed from debris with stainless steel tweezers and placed into plastic containers filled with site water. Similar numbers of macroinvertebrates were collected from each sub-reach. Collection effort continued until sufficient biomass for analysis had been sampled. Because of difference in average macroinvertebrate taxa sizes among streams, total number of individuals collected ranged from 482 to 1,199 per sample. Crayfish from family Cambaridae were also collected from streams by targeting optimal habitat and selecting ≤ 5 individuals from each stream that were comparable in size within and among streams. Macroinvertebrates were transported on dry ice back to the laboratory where they were thawed briefly and identified to family. In cases where the family taxon group contained both predacious and non-predacious genera as specified in Merritt & Cummins (1996), individuals were further identified to genus. Numbers of individuals belonging to each taxon group were recorded. Because of their disproportionally large body sizes, genera *Pteronarcys* (Plecoptera: Pteronarcyidae) and *Tipula* (Diptera: Tipulidae) were separated from other prey taxa. *Pteronarcys* were found in five streams in both the Fall and Spring. *Tipula* were found at disproportionally large sizes only in the Spring, and therefore were separated in Spring samples from all nine streams. Samples were refrozen and lyophilized for approximately 120 hr to ensure complete drying. The dried prey, predator, and *Pteronarcys* composite samples collected in the Fall were weighed. Spring samples were weighed by family taxa groups before compositing prey, predator, *Pteronarcys, and Tipula* groups. All samples were ground with a mortar and pestle.

Sample Digestion and Analysis of Selenium

When sufficient material was available, composite media samples were subsampled for lab analysis three times to create three laboratory replicates for each composite sample. In accord with laboratory equipment capacities, analyses were run in batches of 40 subsamples. To estimate background trace element levels, three blanks exposed to the same reagents and laboratory equipment, were run concurrently with subsamples. After analysis, average Se concentration in the three blanks was subtracted from test sample concentrations from each batch.

Digestions were completed using a microwave digestion system (MarsExpress, CEM Corp., Matthews, NC) with non-pressurized, PTFE vessels (USEPA 1999). Leaf detritus, biofilm, and macroinvertebrates weighing ≤ 0.5 g, and sediment samples weighing ≤ 2.0 g were placed in digestion vessels. Ten milliliters of trace metal grade nitric acid (70% HNO_3) were added to samples collected in Phase I of this study. Five milliliters of trace metal grade nitric acid (70% HNO_3) and 1.5 ml of hydrogen peroxide (30% H_2O_2) were added to samples in Phase II of this study. Vessels were sealed and placed in the microwave digestion unit where they were brought to 200°C within a ramp time of 20 min, and held at 200°C for an additional 15 min. After vessels cooled to room temperature, the digestate was poured quantitatively into a 50 ml volumetric flask and brought up to volume with deionized water. After allowing time to settle, solutions were diluted with deionized water to a final solution of ~ 3% acid. Final solutions were analyzed for Se using an ICP-MS.

Certified reference material (TORT-2 and TORT-3, National Research Council of Canada, Ottawa, Canada) was run in replicates of three in all sample batches. In Phase I, recovery of Se (9.0 ± 0.49 µg/g Se dry wt) was less than the certified range (9.9 – 11.9 µg/g Se dry wt). Blanks were all less than detection limits for Se. In all runs of Phase II material, recovery of Se (11.2 ± 0.39 µg/g Se dry wt) was within the range of certified values. Average concentration in blanks run in parallel with samples was less than instrumental detection limits and ranged from -0.17 – 1.27 µg/g Se dry wt. Percent difference between lab duplicates averaged 7.3%.

Data Processing

Minimum detection limit (MDL) and minimum reporting level (MRL) for analyses were 0.0005 mg/L Se and 0.002 mg/L Se, respectively. Average blank concentrations calculated for each batch were subtracted from corresponding subsamples. Subsamples that were < MDL after blank subtraction were set at half the detection limit resulting in values of 0.0025 mg/L Se. Prey concentrations were constituted mathematically by considering measured concentrations in the *Tipula* and *Pteronarcys* genera that had been separated from other prey taxa groups for analysis and the weights of these taxonomic groups relative to the residual sample. When applicable, all sub-samples were averaged to calculate a value used for analysis.

Enrichment and Trophic Transfer Factors

The experimental design employed in this study was adapted from an ecosystem-scale methodology developed by Presser and Luoma (2001). This methodology addresses the site-specific nature of Se bioaccumulation by quantifying major processes in bioaccumulation at each site. The enrichment factor (EF) quantifies transformation of dissolved Se within the water column to Se in particulate phases (sediment, biofilm, and leaf detritus) forming the base of the food web. Enrichment factors are calculated as ratios of Se concentrations in particulate phases to concentrations in water. This step of enrichment determines Se bioavailability to primary consumers. The trophic transfer factors (TTF) quantify Se transfer to consumers from their food source and are calculated as ratios of concentration in consumers to concentrations in particulate phases. Additional TTFs can be calculated between predator species and their prey.

Enrichment factors were calculated for each stream by dividing particulate-phase Se concentrations by water-column Se concentrations. Trophic transfer factors were calculated for each stream by dividing prey Se concentrations by particulate-phase Se concentrations. A second-level TTF was calculated by dividing predator Se concentrations by prey Se concentrations. In this study, Cambaridae were excluded from calculations of Se dynamics.

Data Analysis

Statistical analyses were performed using R statistical software (R Core Team 2016). To meet ANOVA assumptions of normality and homoscedasticity, a log transformation was performed on all data sets. A two-way ANOVA was applied to all data sets meeting the assumption of normality after transformations. Two-way ANOVA was used to determine significance of stream type (reference, low-Se, and high-Se), and season (Fall and Spring), and the interaction of stream type and season as effects on media Se concentrations, EF, and TTF. Data sets that did not show a significant interaction effect were reanalyzed using ANOVA without the interaction effect as a factor. Data sets that showed an interaction effect between stream type and season were analyzed by Fall and Spring seasons separately. Data sets that showed a significant treatment effect were further analyzed for multiple comparisons by Tukey's HSD.

Dissolved Se in the water column and leaf detritus, which failed to meet assumptions of normality after log transformation, was analyzed using non-parametric methods. A modified Friedman's test for replicated block design was used to determine significance of season and stream type on water column concentrations, and non-parametric analysis were also used to determine significance of season and stream type interaction. Pairwise comparisons using the Bonferroni correction was used to detect differences among individual stream types.

Results

Selenium Concentration in Media

Dissolved Se in stream water was < MDL in four of nine streams in the Fall and in two of nine streams in the Spring. Dissolved Se concentrations in stream water did not differ between seasons, but differences among stream type were detected. Mean water column concentrations in high-Se streams were 7.7 times higher than mean concentrations in low-Se streams ($p < 0.0001$), and 17 times higher than mean concentrations as estimated at half the method detection limit in reference streams ($p < 0.0001$). Selenium-concentration differences between low-Se and reference streams were not significant (Figure IV-4 A).

Sediment and biofilm Se-concentrations did not vary by season. Sediment and biofilm Se-concentrations were significantly different among all stream types with the exception of biofilm Se in the reference streams, which did not differ from concentrations in low-Se streams. For sediments, mean Se concentrations in high-Se streams were 2.2 times higher than the mean of low-Se streams ($p = 0.0035$), and mean concentrations in low-Se streams were 3.0 times higher than in reference streams ($p = 0.0013$). For biofilm, mean Se concentrations were 3.4 times higher in high-Se streams than in low-Se streams ($p = 0.0005$), and 6.0 times higher than in reference streams ($p < 0.0001$) (Figure IV-4 B & C).

Difference in leaf detritus Se concentrations between low-Se and high-Se streams was significant in the Spring but not in the Fall, resulting in a significant interaction between season and stream type. Seasonal leaf detritus concentrations followed patterns among stream type observed in water and other particulate media. However, overall differences between seasons were not significant. In the Fall, mean concentrations of Se in leaf detritus of high-Se streams were 16.2 times higher than the mean in reference streams ($p = 0.0005$). In the Spring, mean differences increased to 33.3 times higher concentrations of Se in leaf detritus in high-Se compared with reference streams ($p = 0.0001$). Mean leaf detritus Se concentration were 5.9 times higher in low-Se streams than in reference streams for Fall samples ($p = 0.0047$) and, 16.4 times higher in high-Se streams than low-Se streams for Spring samples ($p = 0.0004$) (Figure IV-4 D & E).

No significant difference was found in Se concentrations between prey composite samples with and without *Pteronarcys* and *Tipula* genera included; therefore, prey samples calculated to include all genera were used in further statistical analyses. Concentrations of Se in benthic macroinvertebrate prey, predator, and Cambaridae samples did not differ by season. Macroinvertebrate media were significantly different in Se concentration among the three stream types. Mean macroinvertebrate Se concentrations in high-Se streams, were 4.4 times higher in prey samples ($p < 0.0001$), 4.3 times higher in predator samples ($p < 0.0001$), and 4.0 times higher in Cambaridae samples ($p < 0.0001$) than mean concentrations in low-Se streams. Mean Se concentration in low-Se streams were 4.3 times higher in prey samples ($p < 0.0001$), 2.3 times higher in predator samples (p-value = 0.0004), and 2.3 times higher in Cambaridae samples ($p = 0.0002$) than mean Se concentrations in reference streams Figure IV-4 F, G, & H).

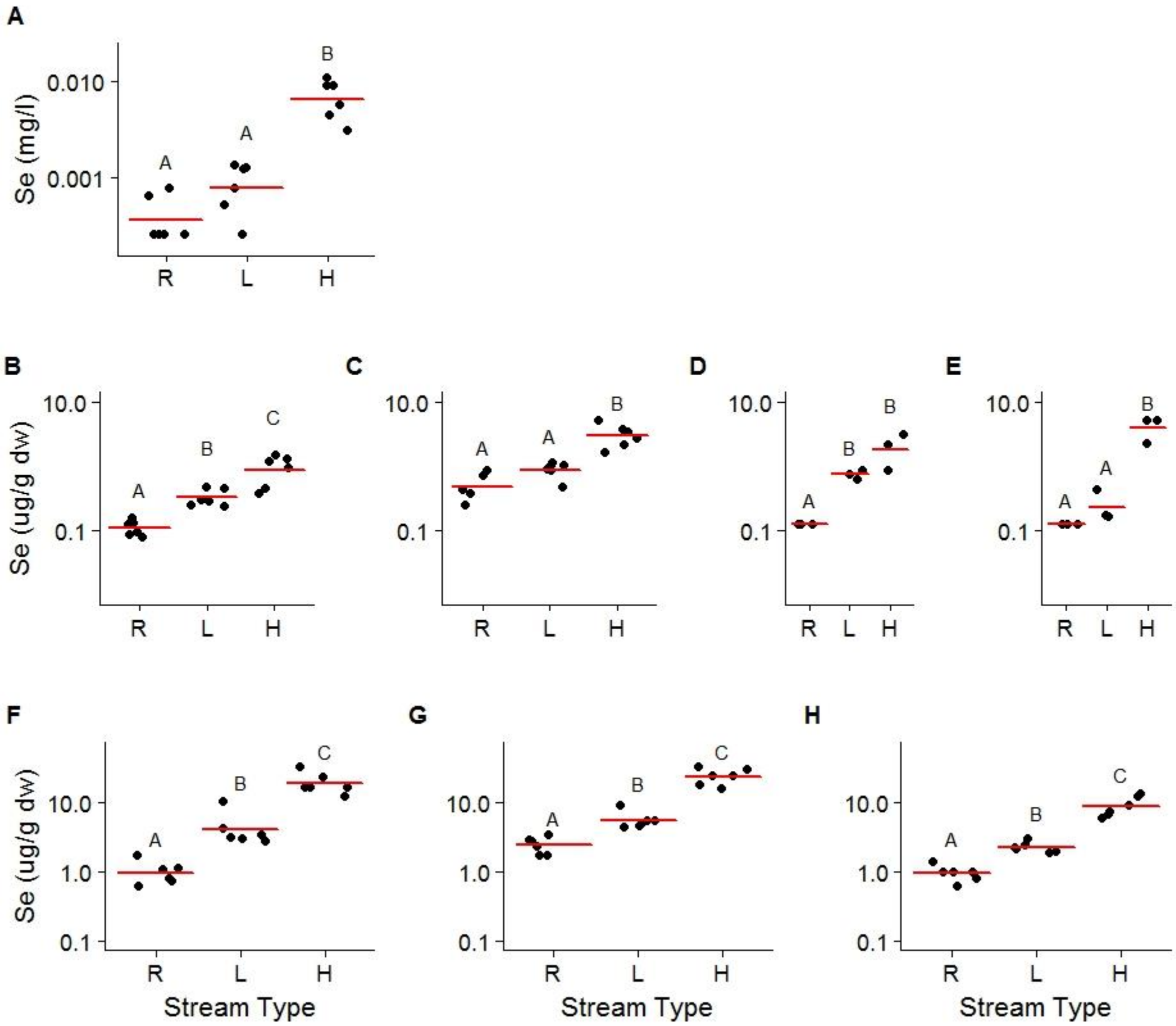

Figure IV-4. Selenium concentrations in (A) Water column, (B) Sediment, (C) Biofilm, (D) Fall Leaf Detritus, (E) Spring Leaf Detritus, (F) Prey, (G) Predator, and (H) Cambaridae in reference (R), low-Se (L) and high-Se (H) headwater streams of the central Appalachian coalfields. Horizontal lines indicate means and letters indicate significant differences among stream types for each medium.

Selenium Enrichment and Trophic Transfer Factors

Enrichment factors describe relationships between Se in the water column and Se in particulate media. Enrichment factors for sediments ($EF_{sediment}$) did not differ by season or stream type (Table IV-1). For $EF_{biofilm}$, differences of mean values were not detected between seasons, but high-Se and reference streams did differ significantly. Mean $EF_{biofilm}$ values in reference streams were 4.1 times higher than in high-Se streams ($p = 0.0280$). For $EF_{leaf\ detritus}$, a significant interaction between season and stream type was detected, but no differences between seasons or among stream types were revealed by further analysis.

Trophic transfer factors also did not differ significantly by season; but, for several TTFs, differences among stream types were detected. Mean $TTF_{prey:sediment}$ values in high-Se streams were 2.7 times higher than reference streams ($p = 0.0068$) (Table IV-2), and mean $TTF_{prey:biofilm}$ values in high-Se streams were 3.0 times higher than in reference streams ($p = 0.012$). For $TTF_{prey:leaf\ detritus}$, no differences among stream type were detected. Mean reference-stream $TTF_{predator:prey}$ values were 2.0 times higher than in both low-Se streams ($p = 0.0002$) and high-Se streams ($p = 0.0008$). Similar patterns of Se enrichment and trophic transfer were observed among stream types.

Table IV-1. Mean enrichment factors in particulate media of central Appalachian headwater streams, by stream type.

Se Site Type	Sediment/Water	Biofilm/Water		Leaf Detritus/ Water, Fall ‡	Leaf Detritus/ Water, Spring ‡
Reference	343 ± 63	1952 ± 534	a*	399 ±103	386 ± 103
Low Se	591 ± 269	1542 ± 559	ab	1674 ± 404	194 ± 58
High Se	186 ± 70	470 ± 41	b	447 ± 300	707 ± 227

* For each medium, site-type means followed by different letters are significantly different from one another ($p < 0.05$).
‡ Because statistical analyses revealed an interaction between season and stream type for leaf detritus, mean concentrations for the Fall and Spring seasons are listed separately.

Table IV-2. Mean trophic transfer factors of central Appalachian headwater streams, by stream type.

Se Site Type	Prey/Sediment		Prey/Biofilm		Prey/ Leaf Detritus	Predator/Prey	
Reference	9 ± 1	a*	2 ± 1	a	8 ± 1	2.6 ± 0.3	a
Low Se	13 ± 2	ab	5 ± 1	ab	11 ± 3	1.4 ± 0.1	b
High Se	25 ± 5	b	7 ± 1	b	8 ± 2	1.3 ± 0.1	b

* For each medium, site-type means followed by different letters are significantly different from one another ($p < 0.05$).

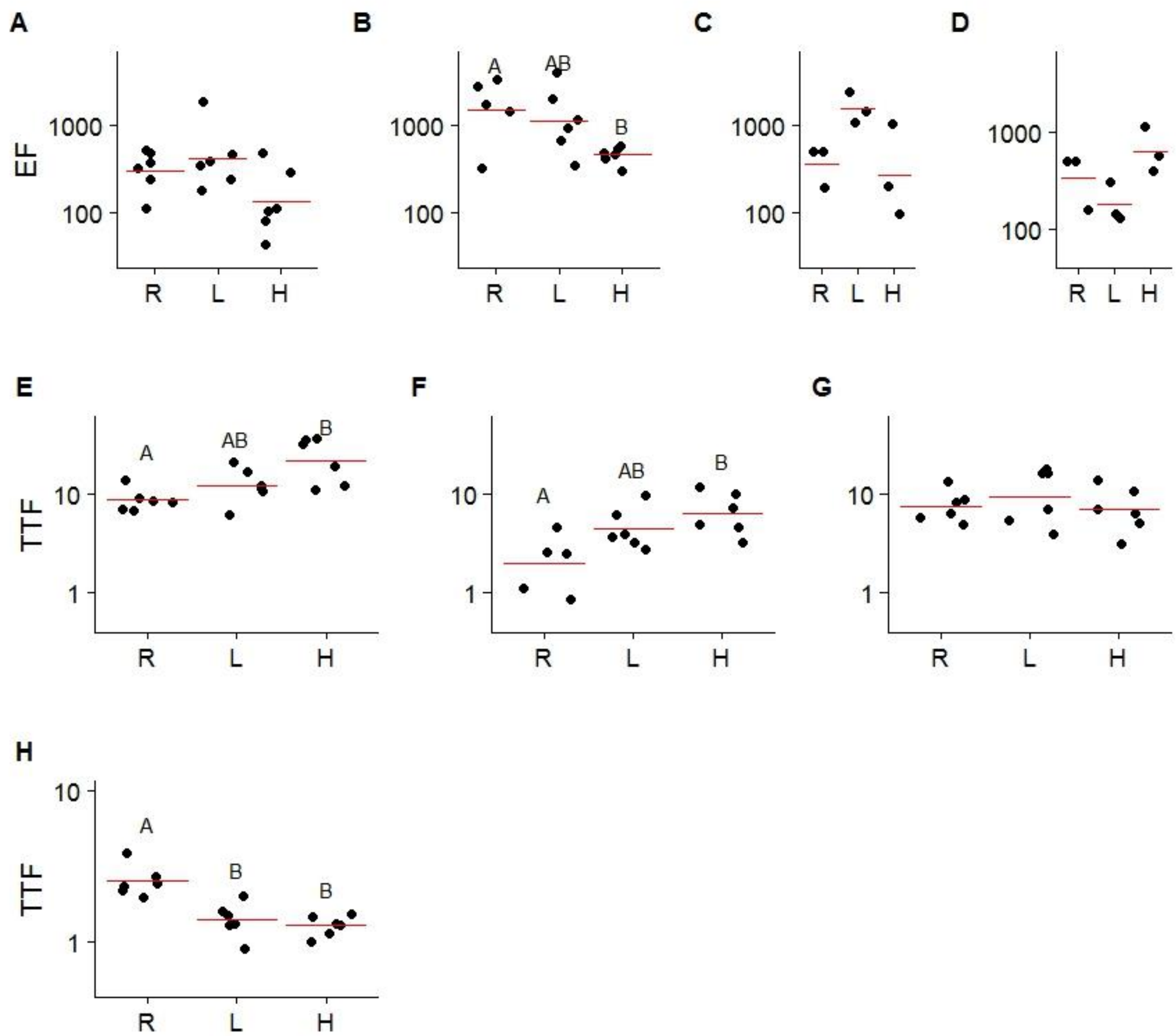

Figure IV-5. Selenium enrichment factors in (A) sediment, (B) biofilm, (C) Fall leaf detritus, and (D) Spring leaf detritus. Selenium trophic transfer factors for (E) prey:sediment, (F) prey:biofilm, (G) prey:leaf detritus, and (H) predator:prey. Stream types are Reference (R), low-Se (L) and high-Se (H). Horizontal lines indicate means by stream type and letters indicate significant differences among stream types for each medium.

Though differences in EFs and TTFs were detected among stream types, overall differences were minimal. Viewing the data with a wider lens, Se dynamics involving enrichment and trophic transfer do not appear to differ dramatically among stream types (Figure IV-6). Minimal differences detected did not sum up to large differences in enrichment, trophic transfer, and overall link between water column and macroinvertebrate tissue concentrations.

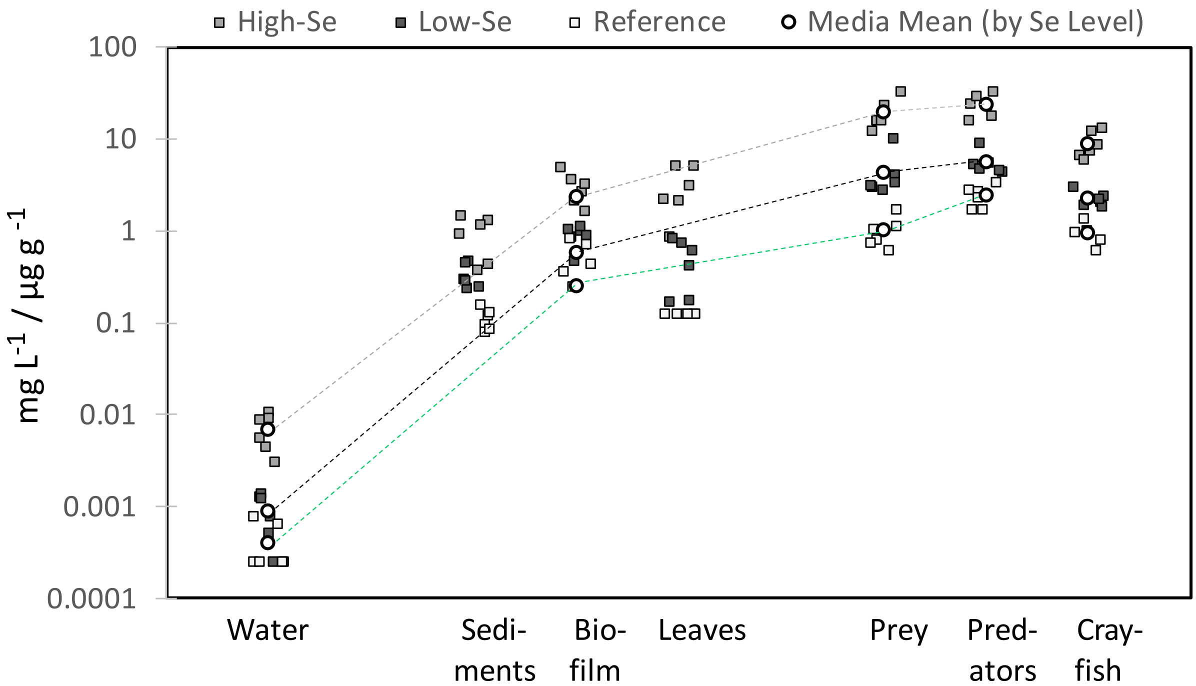

Figure IV-6. Selenium concentrations in all media by stream type. Dotted lines connect mean concentrations of media by stream type. Media are ordered to illustrate selenium enrichment and trophic-transfer pathways. Media-mean concentrations for particulate-phase media are averaged by stream type.

Discussion

Our results demonstrate that Se readily bioaccumulates in headwater streams affected by coal-mining, supporting our hypothesis that mining disturbances can act as a source of water-borne Se, and can result in Se enrichment in headwater stream ecosystems. Our hypothesis that Se dynamics (EFs and TTFs) would not vary significantly among sites was not fully supported by the results of this study. In some cases, differences in calculated factors were detected among stream types. However, those differences that were detected did not show consistent patterns, and general trends of enrichment and bioaccumulation were consistent among stream types (Figure IV-6).

Few studies have examined Se in lotic ecosystems of central Appalachia. Arnold et al. (2014) sampled benthic macroinvertebrates and fish within the Mud River Basin, WV, and concluded that the stream branch receiving mining-effluent was significantly affected based upon elevated Se concentrations in organisms and a higher incidence of juvenile fish deformities compared to the reference stream branch. Presser (2013) quantified EF and TTF values in West Virginia coal-field streams to develop a site-specific model of Se bioaccumulation. The 15 study sites ranged from high-elevation streams to lentic reservoirs. Presser (2013) found Se concentration in suspended particulate matter (the study’s chosen form of particulate medium) to correlate closely with Se concentrations within macroinvertebrate taxa, signifying low variability in TTF (mean: 2.5; range 1.5–3.6). Enrichment factors were much more variable, ranging from 108 to 1,811 (Presser 2013).

Selenium concentrations in ecosystem media

All media (water, sediment, biofilm, leaf detritus, and benthic macroinvertebrates) collected from streams in mined watersheds were elevated in Se concentration compared with media collected from reference streams. Further, media from mining-influenced streams chosen to represent the most severe conditions of ecosystem bioaccumulation among our study streams were elevated above media in streams representative of lower levels of bioaccumulation. Selenium concentrations in media were consistent with concentration values documented in current literature.

To our knowledge, only two studies (Arnold et al. 2014, Presser 2013) reported Se concentrations from lotic systems in central Appalachia in media other than water column and fish tissue. One study reported Se concentrations in two sediment samples (Presser 2013), and neither study reported concentrations of leaf detritus or biofilm. We expect that enrichment of leaf detritus has occurred due to uptake of water-column Se by microbial communities that become established on the exterior surfaces of the submerged leaves, but we did not conduct measurements to test that expectation. However, Se concentrations in these media may be compared to media in other lotic systems exposed to Se contamination. In reference streams, we found Se concentrations in water, sediment, biofilm, and benthic macroinvertebrates (mean: < 0.0005 mg/L Se; 0.11 μg/g Se dry wt, 0.53 μg/g Se dry wt, and 1.0 μg/g Se dry wt, respectively) were within or close to accepted background levels of freshwater environments: water (0.0001 - 0.0004 mg/L Se), sediment (0.2 – 2.0 μg/g Se dry wt), algae (0.1 – 1.5/g Se dry wt), and aquatic macroinvertebrates (0.4 – 4.5 Se μg/g Se dry wt) (USDOI 1998).

Particulate media collected from low-Se and high-Se streams were within ranges documented in the literature. Presser (2013) reported Se concentrations in suspended particulate matter (1.7 – 7.1 μg/g Se dry wt Se), similar to our range of 0.247 to 5.26 μg/g Se dry wt Se in three forms of particulate matter. Two sediment-Se concentrations from the Mud River Reservoir, WV were 0.9 and 2.8 μg/g Se dry wt (USGS 2008) and, were slightly elevated above our range of 0.25 to 1.5 μg/g Se in high-Se streams. Ranges of particulate concentration reported in Presser (2013) include samples collected from the Mud River Reservoir, a hydraulically lentic site likely capable of more efficient Se enrichment than the high-gradient streams sampled in our study (Orr et al. 2006). Differences in site hydrology may explain the higher range of Se concentrations.

A study of reference streams and streams influenced by coal-mines in west-central Alberta reported mean Se concentration in surface water, sediment, and biofilm in reference streams as 0.0002 mg/L Se, 0.2 μg/g Se dry wt and 1.0 μg/g Se dry wt, respectively and in mining-impacted streams as 0.0107 mg/L Se, 2.4 μg/g Se dry wt, and 3.2 μg/g Se dry wt, respectively (Casey 2005). Another study of reference streams and mining-impacted streams in the Canadian Rockies, reported Se concentrations in water (0.0008 mg/L Se, 0.010 mg/L Se, respectively) and biofilm (1.81 μg/g Se dry wt, 3.57 μg/g Se dry wt, respectively) (Kuchapski & Rasmussen 2015). Selenium concentrations in reference streams of our study were similar to those reported by Casey (2005) and by Kuchapski and Rasmussen (2015). Water and biofilm concentrations collected from mining-impacted streams in Casey (2005) and by Kuchapski and Rasmussen (2015) were similar to Se concentrations documented in high-Se streams of our study (0.0071 mg/L Se, and 3.2 μg/g Se dry wt, respectively). Sediment Se concentration in high-Se streams in our study was lower than literature values, possibly because of short residence times in our study's high-gradient streams.

Macroinvertebrate samples collected from a branch of the Mud River, WV receiving mining effluent had Se concentrations of 10.1 ± 0.2 μg/g Se dry wt Se (Arnold et al. 2010), occurring within the range of Se concentrations for high-Se and low-Se streams in our study (20 μg/g Se dry wt and 4.5 μg/g Se dry wt, respectively). Presser (2013) reported benthic macroinvertebrate Se concentrations (6.3–12 μg/g Se dry wt) within the Mud River Watershed, WV also within the range in low-Se and high-Se streams of our study. In lotic systems of west-central Alberta, Casey (2005) reported Se concentrations in benthic macroinvertebrates collected from reference streams (4.5 μg/g Se dry wt) that were higher than mean values for reference streams in our study (< 1.0 μg/g Se dry wt), and in streams impacted by coal mining (10.0 μg/g Se dry wt) that were within the range of our mining-impacted stream values.

Macroinvertebrate Se concentrations in high-Se streams from this study were two times higher on average than those of Arnold et al. (2014) and above maximum Se concentrations in aquatic insects reported in Presser (2013). Though macroinvertebrates have been collected at concentrations exceeding 100 μg/g Se dry wt (Ohlendorf et al. 1986, Lemly 1985), these extremely high concentrations were typically found in lentic lake and reservoir systems, capable of accumulating Se more readily than in lotic systems (Orr et al. 2006). Macroinvertebrate Se concentrations in the high-Se streams of our study were relatively high compared with Se concentrations reported in a number of other studies in lotic habitats (Presser and Luoma 2010). In previous studies, Se concentrations in media were found at the highest levels closest to the source of Se, with concentration decreasing as sampling effort continued downstream (Casey 2005). Therefore, high Se concentrations in this study may be due to the location of the headwater-stream study sites which, were relatively close to sources of mining effluent.

Enrichment and trophic transfer factors

Enrichment factors in biofilm samples differed among streams types with higher $EF_{biofilm}$ in reference streams than high-Se streams. Multiple factors may have influenced these results. Low concentrations in dissolved Se samples used to calculate EF increased uncertainty in EF values, particularly at reference streams where concentrations were < MDL in four of nine samples. Concentration-dependent mechanisms of enrichment may also explain EF differences. In a review of published studies, DeForest et al. (2007) found that enrichment ratios for Se and other metallic trace elements tend to decrease as exposure increases (i.e. enrichment can be "concentration dependent"), an observation that is consistent with our finding higher EFs at low-Se streams relative to high-Se streams. Lower Se EF values in streams with higher Se exposure was also reported in biofilm collected from streams in the Rocky Mountains (Kuchapski and Rasmussen 2015).

Enrichment factors are not only the most bio-concentrating step in Se accumulation, but also contribute the most uncertainty to Se-bioaccumulation models (Presser and Luoma 2010). Mean EF by stream type in our study ranged from 186 in high-Se stream sediment to 1,916 for biofilm in reference streams. Presser (2013) reported a similar range in values of 180 – 1,800 for enrichment in suspended particulate matter collected in West Virginia. Additional EF values from two studies of Se in the Canadian Rocky Mountains included 2,230 for Se enrichment in biofilm (Kuchapski and Rasmussen 2015) and a range of 224 – 5,000 for Se enrichment in sediment and biofilm (Casey 2005). A compilation of studies conducted in multiple freshwater systems was used to arrive at a range of 107 to > 3,000 for Se EFs (Presser and Luoma 2010).

Trophic transfer factors also differed among stream types. $TTF_{prey:sediment}$ and $TTF_{prey:biofilm}$ in reference streams were lower than in high-Se streams, and $TTF_{predator:prey}$ was higher in reference streams than in low-Se and high-Se streams. Differences in TTF may be attributed to differences in benthic macroinvertebrate communities collected at different sites. Taxa groups may differ in rate at which they ingest Se-enriched food and efficiency with which they assimilate Se into their tissues, as denoted by our Phase 1 data, which demonstrate tissue concentration differences at the same site between Cordulegastridae and Gomphidae, two families of dragonflies (Odonata) (Figure IV-2). Because of these differences, shifts in community assemblages may scale up to produce differences in Se bioaccumulation ratios for the whole community (Presser and Luoma 2010). In this study, the macroinvertebrate taxa differed among streams type. For example, on average, reference stream macroinvertebrate samples were comprised of 27% (Fall) and 33% (Spring) mayflies from the family Heptageniidae. In contrast, low-Se stream samples contained 12% and 2% Heptageniidae, respectively, and in high-Se streams no Heptageniidae were collected during either season. Differences among stream types for other taxa groups are also illustrative of this point.

Shifts in macroinvertebrate community assemblage may be driven by additional changes to stream ecosystems caused by coal-mining and associated with mining-related Se levels. Conductivity is a co-variant with Se in central Appalachian headwater streams, as observed by Pond et al. (2014) and as found in this study (data not shown). Changes in conductivity have been shown to correspond with shifts in macroinvertebrate community composition (Timpano et al. 2011, USEPA 2011, Pond et al. 2008).

First-level TTFs from particulate media to primary consumers were higher and more variable in this study than values reported in similar studies. Mean values of first-level TTF in this study range from 2.4 – 24.5, whereas first-level TTF was reported to range from 1.6 to 4.0 (Presser 2013, Kuchapski and Rasmussen 2015, Casey 2005, Presser and Luoma 2010). Presser (2013) found TTF to chironomid taxa (4.2) to be well above composite invertebrate samples collected at the same sites, suggesting difference in invertebrate community composition may contribute to uncertainty found in TTF values in this study. Mean second-level trophic transfer from prey macroinvertebrates to predator taxa (1.3 – 2.6) were less variable and within range of values reported for primary to secondary trophic transfer of Se (Presser and Luoma 2010).

Assessment of Potential Toxicity

Though toxic effects of Se in consumers were beyond the scope of this study, Se concentrations in other studies that evaluated toxicity may be useful for comparison. Conley et al. (2009) reported reduced fecundity when a laboratory mayfly was fed with a food source containing ≥ 4.2 μg/g Se dry wt, which is within the range of particulate media concentrations that we observed in high-Se streams. Reduced survival, however, was not observed unless food-source Se concentrations were ≥ 11.9 μg/g Se dry wt, approximately 2x the highest particulate concentrations observed in our study. Arnold et al (2014) reported increased occurrence of Se-related fish deformities in impacted streams that contained macroinvertebrate concentrations below those of macroinvertebrates collected at high-Se sites in our study.

Conclusions

This portion of the study shows that surface coal mining results in enrichment of Se in headwater streams. Selenium concentrations in stream water, sediment, biofilm, leaf detritus, and benthic macroinvertebrates were consistently elevated at mining-influenced sites and may serve as possible pathways for Se enrichment and bioaccumulation. Dynamics of Se enrichment and trophic transfer were observed to differ slightly among stream types. However, overall patterns of Se enrichment and bioaccumulation were similar among stream types. Enrichment and trophic-transfer factor values developed in this study may serve as a model for establishing preliminary linkages of water column Se concentrations to potential tissue concentrations in other headwater streams impacted by coal mining within the ecoregion of our study.

Enrichment and trophic transfer ratios were higher than values reported by similar studies of lotic systems in the central Appalachian coalfields. Furthermore, Se concentrations in media, particularly macroinvertebrate taxa sampled in high-Se streams, were elevated to concentrations that other studies suggest as toxic to consumers within the stream reach. Further studies are needed to fully investigate taxa at risk for Se toxicity, including mayfly species and insectivores, such as salamanders and fishes.

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V. TROPHIC TRANSFER AND BIOACCUMULATION OF TRACE ELEMENTS: OTHER TRACE ELEMENTS

Introduction

Selenium is well known for its bioaccumulation tendencies in freshwater aquatic ecosystems generally, including those influenced by central Appalachian coal mining. However, multiple major and minor ions are discharged by central Appalachian mines and mined lands at concentrations that exceed those present in unmined reference streams (Pond et al. 2008, 2014). Some of those elements may also be subject to ecosystem enrichment or bioconcentration in various compartments of the ecosystem. For example, Wayland and Crosley (2006) found As, Cd, Pb, and Zn, as well as Se, to be elevated in tissue of aquatic insects below coal mining sites in Alberta, Canada. Considering this background, we assessed the occurrence of other trace elements in environmental media collected during the study of Se bioaccumulation that was reviewed in the prior section of this report.

As with Se, the potential for bioconcentration of other elements in particulate and macroinvertebrate media is of environmental significance for several reasons, including potential toxicity to higher trophic level organisms. In this section, we evaluated bioconcentration of eight additional trace elements in environmental media of mining-influenced headwater streams. Because of the well-known and problematic bioaccumulation tendencies of Se, we included Se in this study to enable evaluation of the eight additional elements in comparison to Se.

Methods

The elements As, Cd, Cu, Ni, Se, and Zn were selected for study because they are known to be released in elevated concentrations from freshly fractured central Appalachian mine spoils and/or have been found to occur in elevated concentrations below mining operations (Clark 2017, Cravotta 2008, Pond et al. 2008, 2014). These elements have also been found to exhibit bioaccumulation tendencies (Eisler 1998, Rainbow 2002, Wayland and Crosley 2006). Aluminum was also selected because it occurs in elevated concentrations in freshly fractured central Appalachian mine spoil leachates and in mine drainage (Clark 2017, Cravotta 2008). Vanadium was selected because it occurs in carbonaceous rocks and in association with fossil fuels (McKelvey et al. 1996, Hope 1997) and as a groundwater pollutant in some areas (Wright and Belitz 2010), but has received limited study in the central Appalachian coalfield. Strontium was selected for study because it often occurs at elevated concentrations in coal mine discharges and has been reputed in scientific literature to have bioaccumulation tendencies (Chowdhury and Blust 2012).

Field and Laboratory Methods

Field and laboratory methods were identical to those described in Section IV (Selenium) for the nine sites that were studied intensively. The environmental media samples analyzed for Se were also analyzed for the additional elements that are the focus of this section.

Data Processing

Mean concentrations of each element in media collected from mining-influenced streams were compared to concentrations in the same media obtained from reference streams. After finding that particulate media concentrations (sediment, biofilm, and leaf tissue) were highly correlated with one another for most elements (Table V-1), mean particulate concentrations were calculated for each sample and used in selected analyses. Similarly, macroinvertebrate tissue concentrations (prey, predator, and Cambaridae) were also found to be highly correlated with one another (Table V-1), so mean macroinvertebrate tissue values were also calculated for each sample and used in selected analyses.

Similar to the Selenium-only analysis (Section IV), concentration ratios for selected media pairs were calculated for each element, and mining-influenced stream levels were compared to reference-stream levels. Those media pairs were selected to correspond with the enrichment, trophic transfer, and bioconcentration factors selected for study in the Section IV but are not described using those terms in this chapter because the processes responsible for differing concentrations at different trophic levels are not clearly defined for some of the elements under study. Selected concentration ratios were also compared among elements. For calculation of ratios, water concentrations were converted to mg/L (reported as µg/L in Table V-2) so that both water and other media concentrations were in parts per million. Ratios were calculated individually for each site/season, and the mean ratios were calculated for mined- and reference sites.

Statistical comparisons were prepared using JMP v13 (SAS Institute, Cary NC) as ANOVA mixed models with site defined as a random variable for the purpose of accommodating the repeated measures conducted at individual sites. For elemental discriminations (Table V-4), both site and season were defined as random variables.

Residuals were inspected for normality using the Shapiro-Wilk test ($\alpha = 0.05$) and for homogeneity of variance using Levene's test ($\alpha = 0.05$). Slight departures from variance homogeneity, as designated by Levene's test, were accepted if other equality-of-variance tests employed by the JMP software package (Brown-Forsythe and Bartlett tests) failed to confirm the departure from homogeneity at $\alpha = 0.05$. If visual inspection revealed complete separation of the quantities being compared (i.e., all mining-influenced observations exceeded all reference observations, or vice versa), such result was considered as a significant difference. In other cases where ANOVA residuals were not normal and/or heteroscedastic, a non-parametric procedure was employed by performing the same mixed-model ANOVA on the ranks.

As a final step of assessing bioconcentration potentials of the elements under study, correlations of particulate matter and macroinvertebrate tissue concentrations with water concentrations were performed as if all observations were independent. All statistical analyses were interpreted at a significance level of $\alpha = 0.05$.

During measurement of trace elements by ICP-MS, to evaluate our sample digestion and extraction procedures, we analyzed blanks and, where available, certified reference materials that contained a known quantity of the trace element of interest. Measurement of trace element concentrations in reference material that fall within the certified range indicate efficacy of our sample digestion and extraction procedures.

Aluminum (Al): Reference material was not certified for Al concentrations. The range of average concentrations of three blanks run in parallel with samples was 1.35 to 18.5 μg/L (MDL = 0.24 μg/L Al).

Arsenic (As): Average recovery of As was above the range of certified values (59.5 +/- 3.8 μg/g As). Average concentrations of three certified reference material run in parallel with samples was consistently above certified values and ranged from 66.5 to 73.4 μg/g As. The range of average concentration of three blanks run in parallel with samples was -0.042 to 0.57 μg/L (MDL = 0.11 μg/L As).

Cadmium (Cd): Recovery of Cd was within the range of certified values (42.3 +/- 1.8 μg/g Cd). Average concentrations of three certified reference material run in parallel with samples was within the range of certified values ranging from 38.7 to 41.7 μg/g Cd. The average concentration of three blanks run in parallel with samples was below instrumental detection limits (IDL = 0.11 μg/L Cd).

Copper (Cu): Average recovery of Cu was within the range of certified values (497 +/- 22 μg/g Cu). Average concentrations of three certified reference material run in parallel with samples was occasionally below certified concentration values and ranged from 452 to 497 μg/g Cu. The range of average concentrations in three blanks run in parallel with samples was -0.99 to 6.51 μg/L Cu (MDL = 0.094 μg/L Cu).

Nickel (Ni): Average recovery of Ni was within the range of certified values (5.30 +/- 0.24 μg/g Ni). Average concentrations of three certified reference material run in parallel with samples was occasionally below certified concentration values and ranged from 4.88 to 5.42 μg/g Ni. The range of average concentrations in three blanks run in parallel with samples was -0.10 to 0.34 μg/L Ni (MDL = 0.17 μg/L Ni)

Strontium (Sr): Average recovery of Sr was below the range of certified values (36.5 +/- 1.6 μg/g Sr). Average concentrations of three certified reference material run in parallel with samples was consistently below certified values and ranged from 32.6 to 34.7 μg/g Sr. The range of average concentration of three blanks run in parallel with samples was -0.15 to 0.10 μg/L Sr (MDL = 0.073 μg/L Sr).

Vanadium (V): Average recovery of V was within the range of certified values (9.1 +/- 0.4 μg/g V). Average concentrations of three certified reference material run in parallel with samples was occasionally below certified concentration values and ranged from 8.38 to 9.39 μg/g V. The range of average concentrations of three blanks run in parallel with samples was -0.10 to 0.34 μg/L V (MDL = 0.093 μg/L V).

Zinc (Zn): Average recovery of Zn was within the range of certified values (136 +/- 6 μg/g Zn). Average concentrations of three certified reference material run in parallel with samples ranged from 129 to 152 μg/g Zn. The range of average concentrations of three blanks run in parallel with samples was 0.46 to 17.7 μg/L Zn (MDL = 0.35 μg/L Zn).

Results

Trace Element Concentrations in Media

Water concentration differences were observed for four of the nine elements, as mean mining-influenced stream (mine) concentrations were 3.5x, 7x, 10x, and 7x greater than mean reference-stream concentrations for Cu, Ni, Se, and Sr, respectively (Table V-2). Water concentration differences for As, Cd, and V could not be assessed because most or all values for these elements were measured as < MDL and therefore were recorded as ½ the MDL value.

For particulate media, mean mine concentrations of Se were 6x, 4x, and 14x higher than reference concentrations for sediment, biofilm and leaf detritus, respectively, but few other significant differences were noted. Mean sediment concentrations in mine streams were 3x higher than reference concentrations for Sr, and 2x higher than reference concentrations for Zn. The mean value of mean-particulate Zn concentrations in mine streams exceeded reference concentrations by nearly 2x.

Both Ni and Se concentrations in mine streams exceeded reference concentrations for all three macroinvertebrate media (prey, predators, Cambaridae), with differences ranging from 2x (Ni, Cambaridae) to 12x (Se, prey). Mean Cd concentrations of Cambaridae in mine streams were approximately 3x those in reference streams, but mean Zn concentrations of prey in reference streams were approximately 2x higher than the mean concentrations of mine streams.

Concentration Ratios: Mining-Influenced vs. Reference Streams

Mean values for particulate media/water concentration ratios tended to be greater in reference streams than in mine streams; all significant differences followed this pattern (Table V-3). All three measured particulate/water (sediment/water, biofilm/water, and leaf detritus/water) concentration ratios were significantly different for Sr, and two of the three (biofilm/water, and leaf detritus/water) were significantly different for Cu and for Ni. Predator/prey concentration ratios in reference streams were approximately 2x higher than levels calculated in mine streams for Cd and Se.

A number of macroinvertebrate/water concentration-ratio differences were noted. Mean reference ratios for all three measures (prey/water, predator/water, and Cambaridae/water) were greater than mean values of mine-stream ratios for Cu, Ni, and Sr. Relative differences were greater for Sr (range 19x – 25x) than for Cu and Ni (range 3x – 7x). Mean Cambaridae/water ratios for Cd were approximately 2x greater in mine streams than in reference streams.

Concentration Ratios: Elemental Comparisons

Environmental media/Water concentration ratios in both mine and reference streams tended to be greatest for Al and least for Se and Sr (Table V-4), with Cu, Ni, and Zn ratios at intermediate levels. Mean Cu and Ni ratios for mean-particulate/water exceeded Zn ratios, and mean Cu ratios for mean-macroinvertebrate/water exceeded mean Zn ratios.

Relative to other elements, Se exhibited high prey/particulate and predator/prey ratios. Mean prey/particulate concentration ratio (7.7) for Se in mine streams exceeded those of all other elements, with the second- and third- largest ratios occurring for Cd (4.7) and Zn (2.7) respectively. In reference streams, Zn (8.2), Cd (6.9) and Se (4.4) exhibited the largest mean-values for prey/particulate concentration ratios. Selenium exhibited the highest predator/prey concentration ratio in reference streams (2.5), with Cu (1.3) as the only other element to exhibit and reference-stream predator/prey ratio with a value > 1.0. In mining-influenced streams Se (1.3), Cu (1.1), and Zn (1.0) all exhibited predator/prey concentration ratios with values > 1, and predator/prey concentration ratios for these elements exceeded those of all other elements.

Plots of mean media concentrations for Cu, Ni, Se, Sr, and Zn revealed similar bioconcentration profiles at mine and reference sites for individual elements but differences among elements (Figure V-1).

Correlations

Correlations of water with particulate-media concentrations (sediment, biofilm, and leaf detritus) were highly and consistently significant for Ni, Se, and Sr (Table V-1). Correlations of water with macroinvertebrate concentrations were highly and consistently significant for Ni and Se (Table V-1).

Discussion

Selenium exhibited different bioaccumulation patterns than any of the other elements studied. Alone among studied elements, concentrations of Se from mine streams exceeded concentrations in reference streams for all of the collected media. Particulate/water elemental ratios (which we interpret as enrichment factors for Se) were generally low relative to those calculated for other elements, but prey/particulate and predator/prey ratios for Se were generally high relative to other elements. Unlike all other elements except Cu, Se exhibited prey/particulate and predator/prey elemental ratios > 1.

Whereas both water-column and sediment concentrations for Ni, like Se, were greater in mine streams than in reference streams, other Ni particulate concentrations did not differ among mine and reference streams. Mine-stream Ni concentrations, however, were elevated above reference for all macroinvertebrate media. In addition, like Se, Ni concentrations in all particulate and macroinvertebrate media were correlated with water concentrations. These observations suggest that Ni is also bioaccumulating in benthic macroinvertebrate media in these streams. Unlike Se, however, macroinvertebrate:particulate ratios for Ni were < 1, suggesting that macroinvertebrate bioaccumulation processes for Ni differ fundamentally from those characteristic of Se.

Mine-stream As concentrations were elevated relative to reference levels in Cambaridae, also suggesting bioaccumulation of this element. However, mine-stream concentrations for prey and predator macroinvertebrates, although nominally higher than reference-stream concentrations, were not confirmed statistically. We were unable to determine if water concentrations differed for As because of the high frequency of < MDL values for this element. Although these data suggest the possibility for As bioaccumulation in stream macroinvertebrates, the evidence for that occurrence is not strong relative to Se and Ni.

Water concentrations for both Cu and Sr were higher in mine streams than in reference streams, but those differences did not appear to influence other media for Cu. Even nominal macroinvertebrate-media differences between mine and reference streams were minimal for Cu. Hence, these data provide no evidence for Cu bioaccumulation in these systems. Strontium, however, is enriched in mine-stream sediment (relative to reference), but no macroinvertebrate media differences among stream type were apparent. Also, particulate-media Sr concentrations were highly correlated with water concentrations, but those correlations for macroinvertebrate media were either not significant or not as strong. Hence, it appears that Sr is bioaccumulating in particulate media, but evidence for Sr bioaccumulation in macroinvertebrate media is weak.

These data provide no evidence for bioaccumulation of Cd and Zn in macroinvertebrates in these streams. Our data provide no evidence for elevated releases of either element from the mine sites that are influencing these streams. Yet, sediment and mean-particulate media in mine streams were elevated statistically for Zn, and all other particulate media measures for both elements were nominally elevated in mine streams, relative to reference. At the macroinvertebrate level, however, the only statistical difference demonstrated higher prey Zn concentrations in reference streams, relative to mine streams, and all nominal differences were for higher reference concentrations. Hence, it is possible that these elements are bioaccumulating in particulate media, but these data are not sufficient to demonstrate that such is (or is not) occurring. The data, however, provide no evidence for macroinvertebrate bioaccumulation of Cd or Zn.

The data gathered for Al or V do not demonstrate bioaccumulation tendencies for macroinvertebrates. Although particulate media were highly enriched in both elements relative to water, it is possible that some of this effect occurred due to mineral contamination. Although efforts were made to separate organic from mineral components when sampling these media, 100% separation was not possible. Low correlations of particulate-media concentrations for both elements also suggest that potential for mineral contamination of these samples to have influenced these results, especially for biofilm. Of the three particulate media, we expect that the cleanest materials were the leaves, and these exhibited the lowest particulate-media concentrations for both Al and V. Prey/particulate and predator/prey ratios for both elements, however, were lower for Al and V than most of the other elements studied, and were < 1.

All elements exhibited substantial enrichment in the particulate phase relative to water concentrations. In one sense, this is not surprising because most are utilized by biota for physiological functions. Aquatic organisms have the capacity to regulate uptake of metals and metalloids from the water column (Chapman 2010). We interpret the observation that reference stream particulate/water ratios exceeded those of mine streams for Cu, Ni, and Sr, all of which were elevated in mine streams, as indicating that the lower-trophic level organisms responsible for producing particulate-phase were exerting some level of uptake regulation of these elements based on physiological requirements and needs.

One limitation of this dataset is that results are based on only two water samples. Those sampling events occurred under baseflow (i.e., no runoff) conditions, and we presume those conditions to be representative of longer time frames. Of the elements that are subject to US EPA water quality criteria, no measured values approached or exceeded those criteria for any element other than Se (Table V-5).

Conclusions

Of the elements studied, Se demonstrated the most consistent and strongest bioaccumulation tendencies. Nickel also demonstrated a tendency to bioaccumulate in macroinvertebrates. Consequently, Se and Ni merit further study to help inform improved assessment of headwater streams influenced by mining in the central Appalachian coalfield, as well as to improve information available for regulatory considerations for water resource protection in this region.

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Figures and Tables

Table V-1. Pearson coefficients (r) and associated p-values (p) for correlations of elemental concentrations in particulate (left) and macroinvertebrate (right) media with other media of similar types, and with water concentrations.

Element	Variable	by Variable	r	p	Variable	by Variable	r	p
Al	Biofilm	Sediment	0.43	0.0866	Predator	Prey	***0.81***	***< 0.0001***
Al	Leaf detritus	Sediment	***0.78***	***0.0001***	Cambaridae	Prey	***0.73***	***0.0008***
Al	Leaf detritus	Biofilm	0.47	0.0543	Cambaridae	Predator	***0.79***	***< 0.0001***
Al	Water	Sediment	-0.02	0.9321	Water	Prey	0.09	0.7444
Al	Water	Biofilm	0.06	0.8227	Water	Predator	0.02	0.9313
Al	Water	Leaf detritus	0.05	0.8481	Water	Cambaridae	0.16	0.5271
As	Biofilm	Sediment	***0.67***	***0.0032***	Predator	Prey	***0.87***	***< 0.0001***
As	Leaf detritus	Sediment	***0.58***	***0.0109***	Cambaridae	Prey	***0.73***	***0.0005***
As	Leaf detritus	Biofilm	***0.67***	***0.0034***	Cambaridae	Predator	***0.68***	***0.0020***
Cd	Biofilm	Sediment	***0.81***	***< 0.0001***	Predator	Prey	***0.76***	***0.0003***
Cd	Leaf detritus	Sediment	***0.62***	***0.0063***	Cambaridae	Prey	***0.58***	***0.0116***
Cd	Leaf detritus	Biofilm	***0.66***	***0.0041***	Cambaridae	Predator	***0.66***	***0.0027***
Cu	Biofilm	Sediment	***0.62***	***0.0074***	Predator	Prey	0.44	0.0679
Cu	Leaf detritus	Sediment	***0.49***	***0.0369***	Cambaridae	Prey	***0.59***	***0.0101***
Cu	Leaf detritus	Biofilm	***0.52***	***0.0326***	Cambaridae	Predator	0.41	0.0880
Cu	Water	Sediment	***0.52***	***0.0264***	Water	Prey	0.01	0.9545
Cu	Water	Biofilm	0.36	0.1615	Water	Predator	-0.22	0.3752
Cu	Water	Leaf detritus	0.17	0.5075	Water	Cambaridae	0.30	0.2340
Ni	Biofilm	Sediment	***0.83***	***< 0.0001***	Predator	Prey	***0.90***	***0.0008***
Ni	Leaf detritus	Sediment	***0.81***	***< 0.0001***	Cambaridae	Prey	***0.95***	***< 0.0001***
Ni	Leaf detritus	Biofilm	***0.83***	***< 0.0001***	Cambaridae	Predator	***0.95***	***< 0.0001***
Ni	Water	Sediment	***0.83***	***< 0.0001***	Water	Prey	***0.94***	***0.0002***
Ni	Water	Biofilm	***0.67***	***0.0035***	Water	Predator	***0.83***	***0.0008***
Ni	Water	Leaf detritus	***0.70***	***0.0013***	Water	Cambaridae	***0.79***	***0.0035***

Correlations were performed on natural log-transformed pooled values for all media. Correlations with statistical significance ($p < 0.05$) are in bold italics. Correlations of particulate and macroinvertebrate media with water for As, Cd, and V are not represented because most or all water concentrations for those elements were < MDL.

Table V-1 (cont'd). Pearson coefficients (r) and associated p-values (p) for correlations of elemental concentrations in particulate (left) and macroinvertebrate (right) media with other media of similar types, and with water concentrations.

Element	Variable	by Variable	r	p	Variable	by Variable	r	p
Se	Biofilm	Sediment	***0.76***	***0.0004***	Predator	Prey	***0.98***	***< 0.0001***
Se	Leaf detritus	Sediment	***0.88***	***< 0.0001***	Cambaridae	Prey	***0.93***	***< 0.0001***
Se	Leaf detritus	Biofilm	***0.78***	***0.0002***	Cambaridae	Predator	***0.96***	***< 0.0001***
Se	Water	Sediment	***0.77***	***0.0002***	Water	Prey	***0.80***	***< 0.0001***
Se	Water	Biofilm	***0.81***	***< 0.0001***	Water	Predator	***0.86***	***< 0.0001***
Se	Water	Leaf detritus	***0.78***	***0.0001***	Water	Cambaridae	***0.92***	***< 0.0001***
Sr	Biofilm	Sediment	***0.62***	***0.0084***	Predator	Prey	***0.79***	***0.0001***
Sr	Leaf detritus	Sediment	***0.52***	***0.0282***	Cambaridae	Prey	***0.73***	***0.0050***
Sr	Leaf detritus	Biofilm	***0.60***	***0.0117***	Cambaridae	Predator	***0.60***	***0.0298***
Sr	Water	Sediment	***0.78***	***0.0002***	Water	Prey	***0.50***	***0.0359***
Sr	Water	Biofilm	***0.71***	***0.0013***	Water	Predator	0.19	0.4556
Sr	Water	Leaf detritus	***0.70***	***0.0011***	Water	Cambaridae	***0.69***	***0.0093***
V	Biofilm	Sediment	0.37	0.1465	Predator	Prey	***0.80***	***< 0.0001***
V	Leaf detritus	Sediment	***0.72***	***0.0008***	Cambaridae	Prey	***0.68***	***0.0020***
V	Leaf detritus	Biofilm	0.43	0.0849	Cambaridae	Predator	***0.74***	***0.0005***
Zn	Biofilm	Sediment	***0.69***	***0.0021***	Predator	Prey	***0.56***	***0.0164***
Zn	Leaf detritus	Sediment	***0.78***	***0.0001***	Cambaridae	Prey	-0.07	0.7975
Zn	Leaf detritus	Biofilm	***0.76***	***0.0003***	Cambaridae	Predator	-0.02	0.9401
Zn	Water	Sediment	0.12	0.6493	Water	Prey	0.15	0.5657
Zn	Water	Biofilm	0.47	0.0554	Water	Predator	0.24	0.3284
Zn	Water	Leaf detritus	0.11	0.6684	Water	Cambaridae	-0.30	0.2205

Correlations were performed on natural log-transformed pooled values for all media. Correlations with statistical significance ($p < 0.05$) are in bold italics. Correlations of particulate and macroinvertebrate media with water for As, Cd, and V are not represented because most or all water concentrations for those elements were < MDL.

Table V-2. Mean trace-element concentrations (µg/L for water, mg/kg dry wt for other media) in environmental media collected from mining-influenced (Mine) and reference (Ref) headwater streams in the central Appalachian coalfield.

Medium/ Site Type	Al	As	Cd	Cu	Ni §	Se	Sr	V	Zn
Water (µg/L)									
Mine	12 ± 3	0.09 ± 0.02 *	0.06 ± 0.0 *	0.7 ± 0.1 a ‡	1.5 ± 0.3 a ‡	4.0 ± 1.1 a ‡	784 ± 132 a †	0.06 ± 0.01 *	18 ± 3
Ref	10 ± 3	0.09 ± 0.02 *	0.06 ± 0.0 *	0.2 ± 0.1 b	0.2 ± 0.1 b	0.4 ± 0.1 b	109 ± 61 b	0.05 ± 0.00 *	18 ± 4
Sediment									
Mine	*7157 ± 907*	3.93 ± 0.49	0.13 ± 0.02	9.7 ± 1.2 †	25.3 ± 4.5 a ‡	0.7 ± 0.1 a ‡	23 ± 3 a	11.0 ± 1.2	61 ± 8 a
Ref	5074 ± 494	2.89 ± 0.44	0.06 ± 0.01	4.2 ± 0.4	7.6 ± 0.5 b	0.1 ± 0.0 b	7 ± 2 b	7.6 ± 0.7	25 ± 2 b
Biofilm									
Mine	16719 ± 2187	6.04 ± 0.48	0.34 ± 0.06	19.4 ± 2.1	49.9 ± 9.6	2.1 ± 0.4 a	62 ± 8	22.1 ± 2.3	124 ± 14
Ref	17000 ± 3407	6.51 ± 1.51	0.18 ± 0.05	14.8 ± 3.5	22.1 ± 4.0	0.5 ± 0.1 b	36 ± 2	21.9 ± 3.9	74 ± 16
Leaf detritus									
Mine	4009 ± 653	0.92 ± 0.13	0.14 ± 0.03	9.0 ± 0.8	15.5 ± 3.8 †	1.8 ± 0.5 a	180 ± 21	5.2 ± 0.8	40 ± 4
Ref	2443 ± 517	0.57 ± 0.14	0.10 ± 0.01	8.0 ± 1.2	6.7 ± 1.1	0.1 ± 0.0 b	158 ± 55	3.2 ± 0.6	26 ± 3
Mean Particulate									
Mine	9295 ± 1083	3.63 ± 0.34	0.20 ± 0.03	12.7 ± 1.1	30.2 ± 5.5	1.5 ± 0.3 a ‡	88 ± 9	12.8 ± 1.2	75 ± 8 a
Ref	8103 ± 1356	3.24 ± 0.60	0.11 ± 0.02	8.7 ± 1.5	12.1 ± 1.9	0.3 ± 0.0 b	73 ± 23	10.8 ± 1.5	42 ± 7 b
Prey									
Mine	3180 ± 357	2.48 ± 0.37	0.93 ± 0.24	22.2 ± 1.7	26.1 ± 9.1 a ‡	12.2 ± 2.8 a ‡	42 ± 4	4.9 ± 0.5	187 ± 17 b
Ref	1865 ± 508	1.51 ± 0.20	1.01 ± 0.36	23.4 ± 2.2	4.4 ± 0.4 b	1.0 ± 0.2 b	39 ± 8	3.4 ± 0.4	328 ± 54 a
Predator									
Mine	1175 ± 125	0.92 ± 0.11	0.40 ± 0.08 †	24.0 ± 1.0	7.9 ± 2.3 a ‡	15.2 ± 3.2 a ‡	23 ± 4	1.9 ± 0.2	180 ± 16 †
Ref	738 ± 145	0.57 ± 0.12	0.91 ± 0.28	28.4 ± 2.2	2.3 ± 0.3 b	2.5 ± 0.3 b	31 ± 9	1.1 ± 0.2	261 ± 17
Cambaridae									
Mine	484 ± 57	0.69 ± 0.09 a†	0.46 ± 0.07	91.4 ± 5.0	4.2 ± 0.4 a ‡	5.8 ± 1.2 a ‡	647 ± 88	0.8 ± 0.1	81 ± 3
Ref	360 ± 55	0.32 ± 0.02 b	0.84 ± 0.33	101 ± 17.6	2.1 ± 0.2 b	1.0 ± 0.1 b	486 ± 67	0.6 ± 0.1	95 ± 17

Mine and ref concentrations followed by different letters are significantly different ($p < 0.05$).
† Non-parametric analysis (ANOVA on ranks).
‡ Clear distinction between mine and ref: all mine observations are greater than all reference observations.
§ Macroinvertebrate tissue concentrations of nickel were available for Fall samples only.
* Most water samples for V and As, and all water samples for Cd, were < MDL.

Table V-3. Mean elemental concentration ratios for environmental media collected from mining-influenced (Mine) and reference (Ref) headwater streams in the central Appalachian coalfield, by element, for comparison by stream type.

	Al	As*	Cd*	Cu	Ni §	Se	Sr	V*	Zn
Sediment/Water									
Mine	1,098,702	53,162	2,436	16,842	18,881 †	389	41 b	223,472	4,911
Ref	870,163	44,216	1,153	36,167	52,617	343	193 a	163,597	1,900
Biofilm/Water									
Mine	2,565,700	81,893	6,101	34,176 b †	34,201 b †	1,006	95 b †	447,923	9,224
Ref	3,035,003	83,873	3,350	125,368 a	184,949 a	1,952	1,171 a	469,199	5,713
Leaf Detritus/Water									
Mine	593,011	12,442	2,552	16,596 b	10,499 b	756	271 b ‡	105,675	3,318
Ref	425,125	7,035	1,784	69,779 a	46,905 a	393	3,612 a	68,361	1,956
Mean Particulate / Water									
Mine	1,419,472	51,395	3,402	22,482 b	21,171 b	712	136 b ‡	242,053	5,817
Ref	1,502,148	44,376	1,834	76,511 a	94,043 a	903	1,518 a	215,587	3,351
Prey/Mean Particulate									
Mine	0.36	0.66	4.69	1.82	0.81 a	7.76 a	0.49	0.40	2.7 b
Ref	0.31	0.56	6.92	2.84	0.38 b	4.38 b	0.69	0.32	8.2 a
Predator/Prey									
Mine	0.38	0.39	0.50 b	1.12	0.40	1.35 b	0.53	0.39	1.03
Ref	0.31	0.37	0.98 a	1.27	0.46	2.56 a	0.73	0.32	0.88
Prey/Water									
Mine	459,381	32,890	16,793	38,645 b ‡	15,629 b ‡	6,574	65 b ‡	101,686	16,208
Ref	500,634	21,107	18,333	211,321 a	51,194 a	3,323	1,287 a	71,905	21,325
Predator/Water									
Mine	171,431	12,421	7,310 †	42,592 b ‡	6,058 b ‡	7,389	35 b ‡	37,534	14,209
Ref	154,602	7,417	16,503	249,573 a	19,369 a	7,588	879 a	23,144	18,583
Cambaridae/Water									
Mine	64,966	9,688	8,358 b	159,554 b ‡	2,019 b ‡	2,493	1,068 b ‡	15,460	6,942
Ref	73,068	4,530	15,281 a	768,440 a	14,274 a	2,812	20,402 a	12,098	8,382

Mine and ref concentrations followed by different letters are significantly different ($p < 0.05$).
† Non-parametric analysis (ANOVA on ranks).
‡ Clear distinction between mine and ref: all mine observations are greater than all reference observations.
§ Macroinvertebrate tissue concentrations of nickel were available for Fall samples only.
* Most water samples for V and As, and all water samples for Cd, were < MDL.

Table V-4. Mean elemental concentration ratios for environmental media collected from mining-influenced (Mine) and reference (Ref) headwater streams in the central Appalachian coalfield, by element, for comparison among elements.

	Al		As *	Cd *		Cu		Ni §		Se		Sr		V *		Zn	
Mean Particulate / Water																	
Mine	1,419,472	A†	51,395	3,402		22,482	B	21,171	B	712	D	136	E	242,053		5,817	C
Ref	1,502,148	A	44,376	1,834		76,511	B	94,043	B	903	C	1,518	C	215,587		3,351	C
Prey / Mean Particulate																	
Mine	0.36	E	0.66 D	4.69	B	1.82	C	0.81	D	7.76	A	0.49	DE	0.40	E	2.74	C
Ref	0.31	C	0.56 C	6.92	A	2.84	B	0.38	C	4.38	AB	0.69	C	0.32	C	8.16	A
Predator / Prey																	
Mine	0.38	B	0.39 B	0.50	B	1.12	A	0.40	B	1.35	A	0.53	B	0.39	B	1.03	A
Ref	0.31	D	0.37 CD	0.98	B	1.27	B	0.46	BCD	2.56	A	0.73	BC	0.32	D	0.88	B
Mean Macroinvertebrate / Water																	
Mine	231,970	A	19,184	9,959		80,032	B	8,559	CD	5,443	D	387	E	48,167		12,452	C
Ref	252,641	A	11,514	15,375		391,796	A	31,977	AB	4,516	B	7,624	B	33,341		16,096	B

† Elemental concentration ratios of each type, for each stream type, followed by different letters are significantly different ($p < 0.05$).
§ Macroinvertebrate tissue concentrations of nickel were available for Fall samples only.
* Most water samples for V and As, and all water samples for Cd, were < MDL. Therefore, these elements were excluded from comparisons of elemental concentration ratios that include water concentration as the denominator.

Table V-5. Chronic (CCC) and acute (CMC) water quality criteria for selected elements, compared to mean and highest observed values in mining-influenced streams.

Element	CCC †	CMC †	Mean	Maximum
	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Al	87	750	12.2	45.3
As	150	340	0.09	0.23
Cd	0.72	1.8	0.06	0.06
Cu	9	13	0.7	2.1
Ni	52	470	1.5	4.1
Se	3.1		4.0	10.9
Zn	120	120	17.5	44.3

† Freshwater CCC and CMC values for Al, As, Cd, Ni, and Zn are from US EPA (2017). The Cd and Ni values are hardness-dependent, with listed values calculated by EPA for waters with 100 mg/L hardness. The Cu values were calculated by Price et al. (2011) based on US EPA (2007), also for 100 mg/L hardness. The Se CCC is the default lotic monthly average criterion but can be adjusted by states that carry out studies to justify alternative values (US EPA 2016).

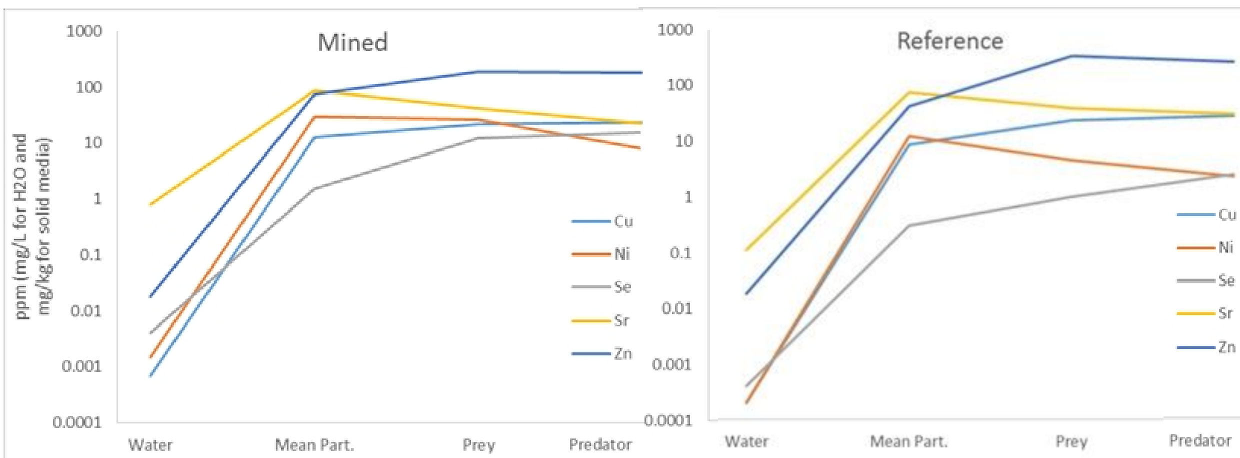

Figure V-1. Concentrations of Cu, Ni, Se, Sr, and Zn in all media by stream type. Dotted lines connect mean concentrations of each element for the different media by stream type. Media are ordered to illustrate enrichment and trophic-transfer pathways in a manner similar to that illustrated for Se in Figure IV-6. Media-mean concentrations for particulate-phase media (sediments, biofilm, leaf detritus) are averaged.

VI. SUMMARY AND CONCLUSIONS

SUMMARY

Objective 1: Assess long-term chemical and biological patterns in central Appalachian headwater streams salinized by coal mining

Both reference and test streams exhibited a significant seasonal pattern of SC, which was modeled using a sinusoidal function. The seasonal pattern appears to be driven by hydrology and evapotranspiration, as modeled mean-SC minima occurred in the late February – early March period of low evapotranspiration, maximum groundwater recharge, and generally high streamflows; whereas SC maxima tended to occur in the late August – early September period that is generally characterized by high evapotranspiration, minimum groundwater recharge, and low flows.

Collectively, the eight benthic macroinvertebrate structural metrics selected for focus were more sensitive to SC during the Spring than during the Fall seasons. Six of the eight metrics exhibited significant negative relationships with SC for all four Spring sampling events, whereas Evenness exhibited significant negative relationships for three of the four Spring sampling events and Shredder percent exhibited a significant positive relationship with SC for all four Spring sampling events. Within the Fall season, metric relationships with SC were also generally consistent over the study period, but not as consistent as in Spring. Metrics were likely more sensitive in Spring because samples from that season contain more salt-sensitive mayfly individuals, which in turn causes metrics that include mayflies to be more sensitive to salinity. In contrast, few mayfly specimens are found in Fall samples, resulting in less change in mayfly-influenced metrics across the salinity gradient during that season.

Across our 19 test sites, rapid or significant declines of water salinity during the 2011-2016 study period were not apparent but gradual SC declines and apparent recovery from mining disturbance at some sites were evident. Long-term decreasing trends in salinity were observed at seven test sites; whereas increasing trends were observed at three sites, two of which had additional mining during the 2011-2016 period. In contrast, two of the five reference sites exhibited increasing SC trends and no declining trends were noted. Hence, it appears that gradual decline of SC is occurring at some the test sites. However, the magnitude of SC change (i.e., trend slope) was small when long-term trends were present.

Furthermore, we found no indication of a consistent pattern of biological recovery at test sites over the five-year study period. Although long-term trends were found in biological metrics at some individual sites, those trends were not consistent across sites that had either decreasing or increasing trends in SC. The lack of strong, consistent trends in the biological metrics supports our finding that there appears to be no indication of recovering biological condition in these study streams over the period of study.

Objective 2: Determine influence of mining-induced salinity on leaf litter breakdown

Macroinvertebrates are important contributors to ecosystem functions in headwater streams, particularly leaf litter breakdown. Despite evidence that salinization negatively affects several important macroinvertebrate groups (e.g., EPT), we found no measurable effect of salinization on rates of leaf litter breakdown across our study sites. One likely explanation for this result is that many shredder macroinvertebrates (those that feed on leaf litter and associated microbial communities) are not particularly sensitive to salinization at the levels we observed in our study streams. We found no differences in shredder richness as salinity increased and some indication that shredder relative abundance increased with salinity, which is inconsistent with the expected response of those taxa to perturbation. However, we emphasize that our data cannot illuminate any effects salinity may have on other ecosystem functions, including those that are influenced by macroinvertebrates that are sensitive to salinization. Future investigations should test effects of salinization on other important ecosystem functions, such as primary productivity, biomass production, and carbon export, especially considering salt-sensitive macroinvertebrates (e.g., Ephemeroptera) may be more important to these ecosystem functions than they are to leaf litter breakdown. Investigating potential effects of mining-induced salinity on other ecosystem functions using our test- and reference-site approach could provide a more complete understanding of how mining influences central Appalachian headwater streams. We also note that the leaf breakdown rates appear as depressed at the two sites with the highest SC levels; and that scientific literature demonstrates the presence of mining-influenced central Appalachian streams with still-higher SC levels than were present at any of our study sites. Hence, our results should not be interpreted to suggest lack of salinity effects on leaf breakdown at higher SC levels than we were able to test.

Objective 3: Investigate trophic transfer and bioaccumulation of selenium and other trace elements

Selenium concentrations in all media were elevated in mining-influenced streams compared with reference streams and in high-Se streams compared with low-Se streams. Selenium bioaccumulation processes (enrichment, trophic transfer) did not exhibit major differences among stream types or seasons. Particulate-media Se concentrations in high-Se streams exceeded those found to cause fecundity impairments of benthic macroinvertebrates in laboratory studies described by scientific literature, and Se tissue concentrations in benthic macroinvertebrates of high-Se streams exceeded those reported in mining-influenced central Appalachian streams with Se-related fish deformities by other studies. However, fish were present in few of the headwater streams selected for our study, and we draw no conclusions concerning potentials for toxicity by the Se levels observed in our study streams.

Selenium was also included in the general trace element study to provide a basis for comparison for the other trace elements. Of the studied elements, only Se exhibited water concentrations approaching or exceeding US EPA recommended water quality criteria. All studied elements exhibited substantial enrichment in the particulate phase relative to water concentrations. However, concentrations in mining-influenced streams exceeded concentrations in reference streams for all of the collected media only for Se. Particulate/water concentration ratios (which we interpret as enrichment factors for Se) were generally higher for the other trace elements relative to those calculated for Se, but prey/particulate and predator/prey concentration ratios for Se were generally high relative to those calculated for other elements. Of the elements studied, only Se and Ni exhibited elevated concentrations in mining-influenced streams, relative to reference streams, for all three of the studied benthic macroinvertebrate media (predators, prey, and Cambaridae).

CONCLUSIONS

Seasonality should be considered when monitoring mining-influenced streams for water quality and for benthic macroinvertebrate community structural measures. Specific conductance varies seasonally, whereas certain benthic macroinvertebrate structural measures tend to respond more directly and consistently to elevated SC in the Spring season than in the Fall season. However, richness of total taxa, EPT taxa, and Ephemeroptera taxa, and relative abundance (percent) of Ephemeroptera appear as robust measures of community response to elevated SC that exhibited consistent negative relationships with SC across all study years and in both Spring and Fall seasons.

We found no measurable effect of salinization on rates of leaf litter breakdown. We interpret this result as occurring because the benthic macroinvertebrates that perform leaf-breakdown functions (i.e., shredders) appeared tolerant of salinity at the levels we observed. Taxa of the group most affected by salinity (mayflies) do not shred leaves but do perform other roles in the processing of carbon in headwater streams. Therefore, although leaf-breakdown – an important component of the carbon cycle in these streams – appears unaffected by salinity at levels we observed, effects of mayfly loss on other ecosystem functions in our study streams remain unknown.

Findings indicate that headwater streams influenced by coal-mining play a significant role in the introduction of elevated Se concentrations into the aquatic food-chain. Bioaccumulation tendencies for Se appear as unique among the other trace elements studied because Se concentrations in all studied particulate and macroinvertebrate media were elevated in mining-influenced streams relative to reference streams. Of the other trace elements studied, results for Ni provided some concern because macroinvertebrate tissue concentrations were consistently elevated in mining-influenced streams relative to reference streams.

The results we observed regarding salinity, leaf litter breakdown, and selenium are specific to the region and systems studied, but our approach is broadly transferrable. As all of our study sites were in small first-order forested headwater streams, we would expect to find similar results in other streams with comparable conditions. Our approach can be adapted to a variety of riverine systems, allowing region-specific assessment of stream ecosystem response to mining influence.

The information conveyed in this report contributes to our knowledge of water quality, stream function, and benthic macroinvertebrate conditions in headwater streams influenced by coal mining in the central Appalachians. Consequently, these methods and findings can be used to inform monitoring and management of these vital aquatic ecosystems.

ACKNOWLEDGEMENTS

This research was supported by OSMRE through the Applied Science Program (Cooperative Agreement S15AC20028). Our prior work contributing to this study was supported by OSMRE Applied Science (Cooperative Agreement S11AC20004), and by a follow-on study funded by OSMRE (Cooperative Agreement S12AC20023) through Virginia Department of Mines, Minerals, and Energy (DMME). Complementary work was supported by Virginia Department of Environmental Quality, Virginia DMME, and Powell River Project. The authors thank the many landowners and others for providing study site access and orientation. We appreciate the generous assistance with site selection provided by past and current personnel from Virginia DMME, West Virginia Department of Environmental Protection, U.S. EPA Region 3, and University of Kentucky – Robinson Forest. Thanks to Patricia Donovan for generating watershed areas, and for determining the fraction of each watershed that had been disturbed by mining over the study period. We thank also the many Virginia Tech students and staff who assisted with preparation of leaf litter bags, data collection, sample processing, and sample analysis, including Elizabeth Sharp, Megan Underwood, Tyler Weiglein, Jenny Hwang and Rachel Pence. We greatly appreciate feedback on our litter breakdown study design from Fred Benfield.

RELEASE FROM OBLIGATIONS

Virginia Polytechnic Institute and State University considers the cooperative agreement "Stream Ecosystem Response to Mining-Induced Salinization in Appalachia" S15AC20028 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 – CONTINUOUS CONDUCTIVITY BY SITE

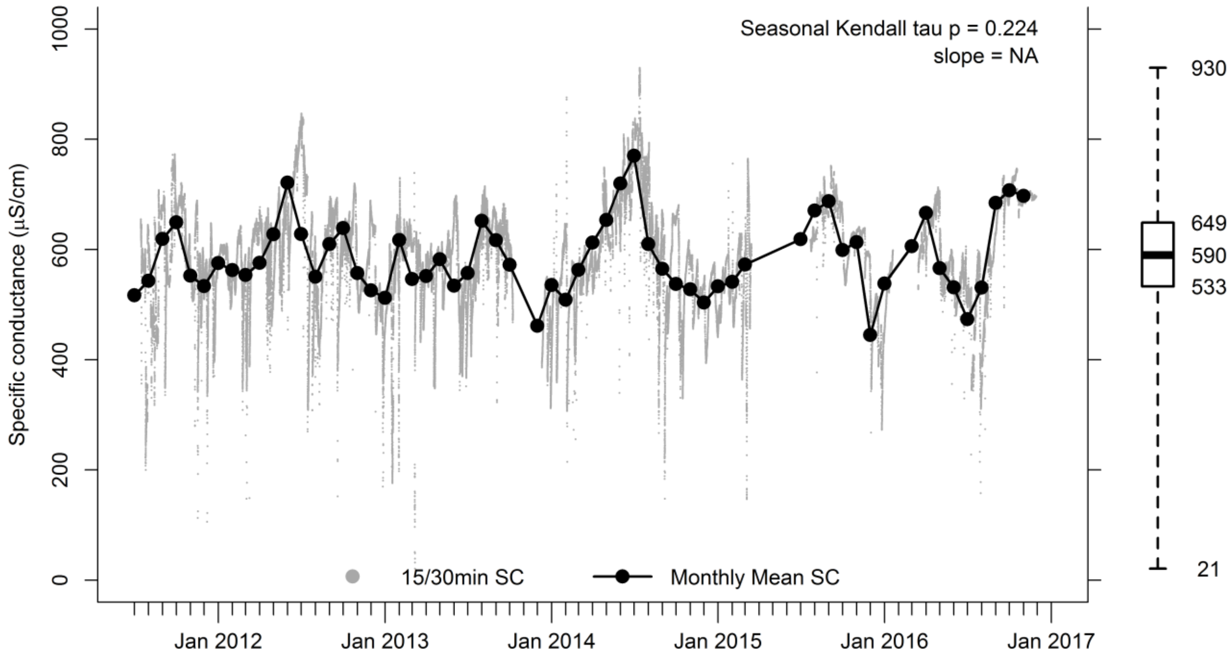

Figure A - 1. Birchfield Creek (BIR, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant (p < 0.05). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

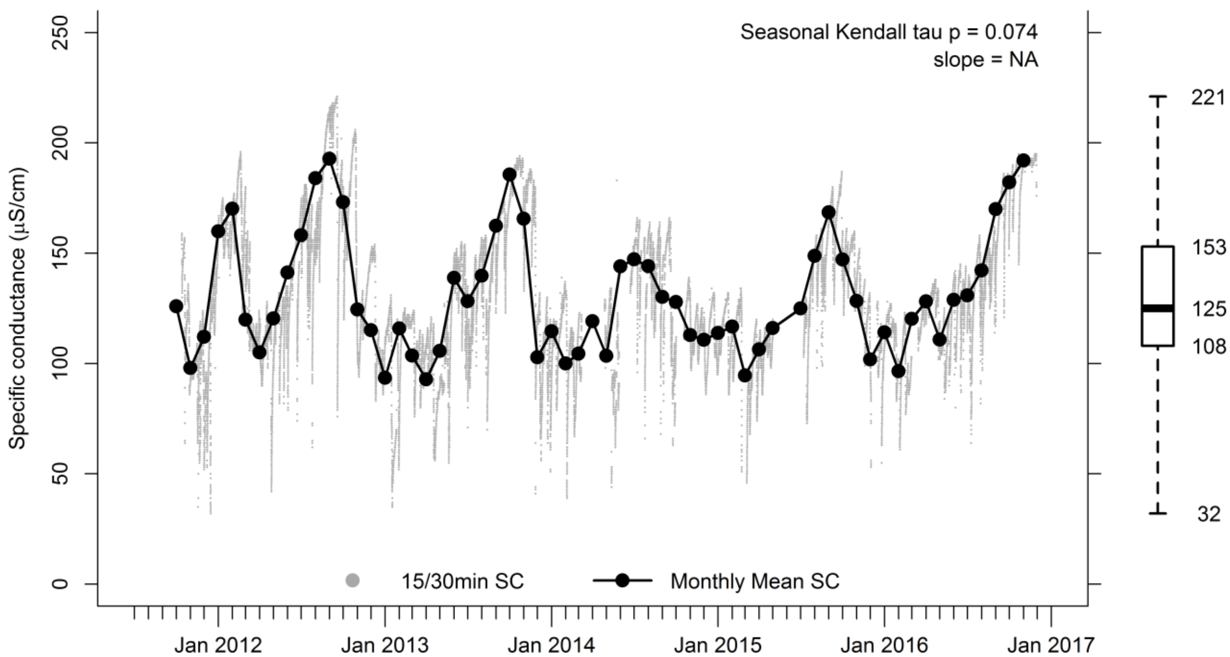

Figure A - 2. Copperhead Branch (COP, reference site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant (p < 0.05). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

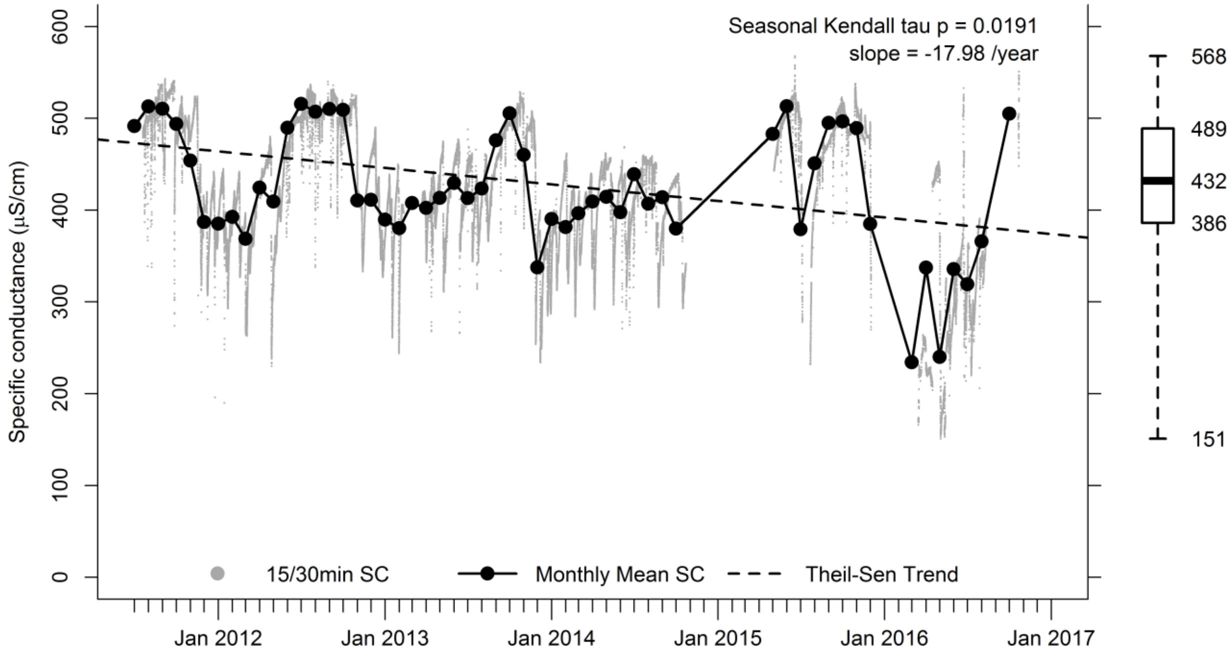

Figure A - 3. Crane Fork (CRA, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

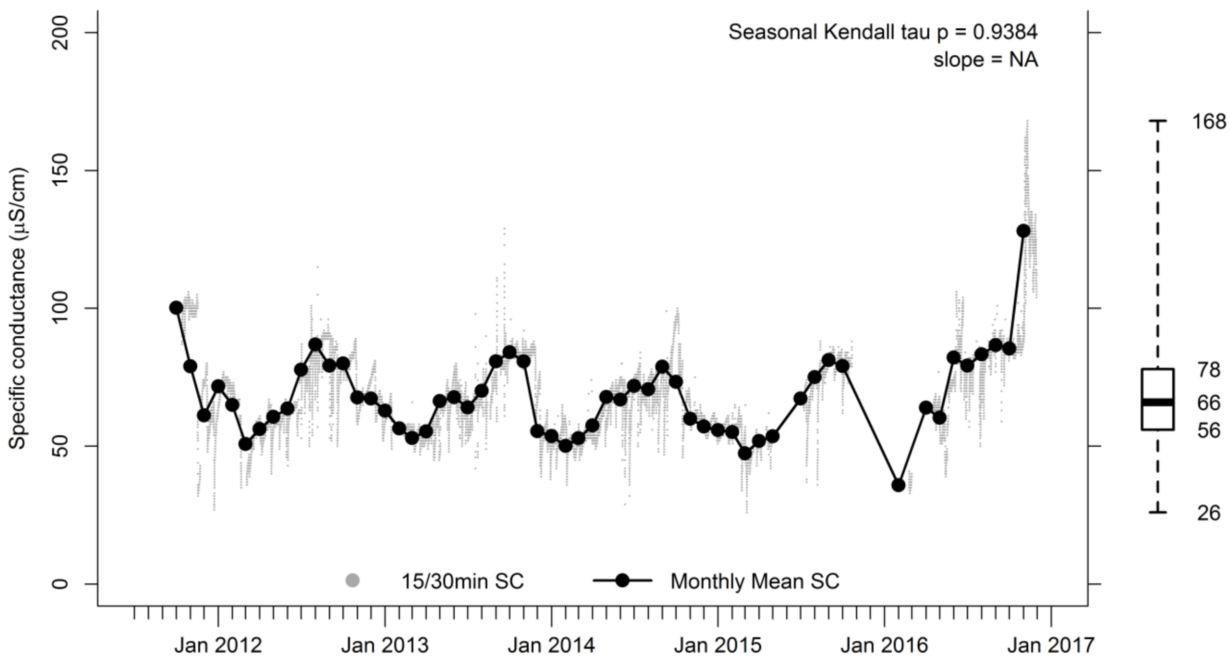

Figure A - 4. Crooked Branch (CRO, reference site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

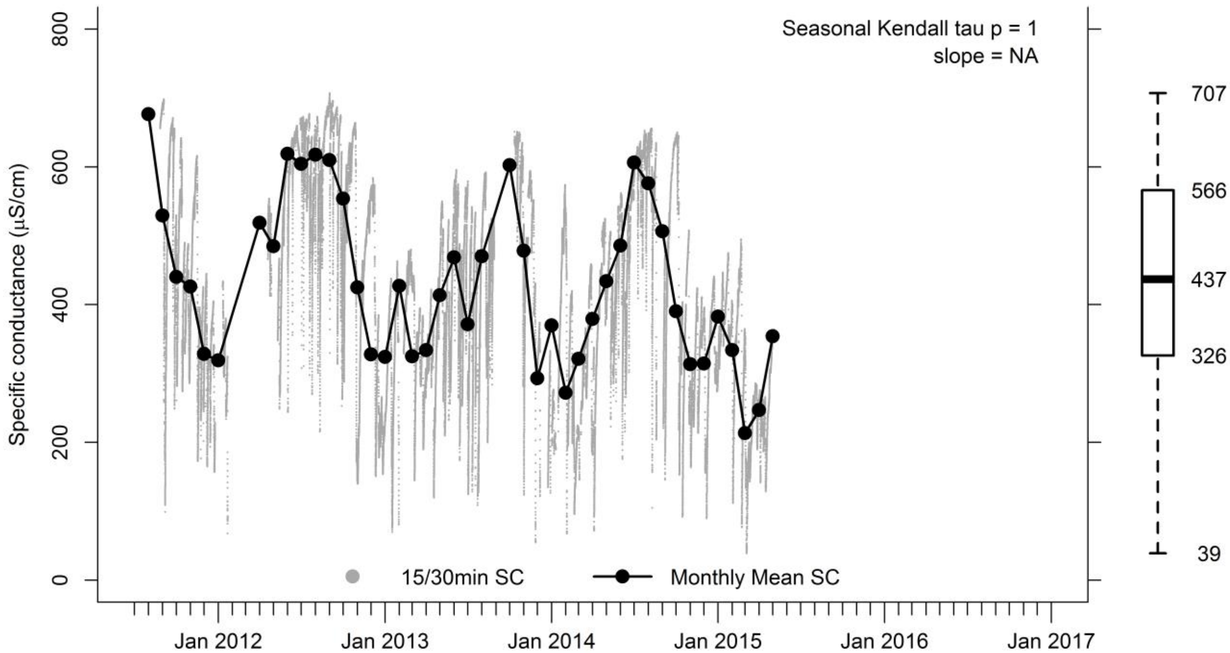

Figure A - 5. Dave Branch (DAV, test site). 15-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line). Box plot of 15 min SC showing median, inter-quartile range, and extreme values. Note: Data from 2011-2015 (shown) were used for SC pattern modeling, but Dave Br. was not sampled as part of the 2015-2016 study, so trend analyses were not conducted for this stream.

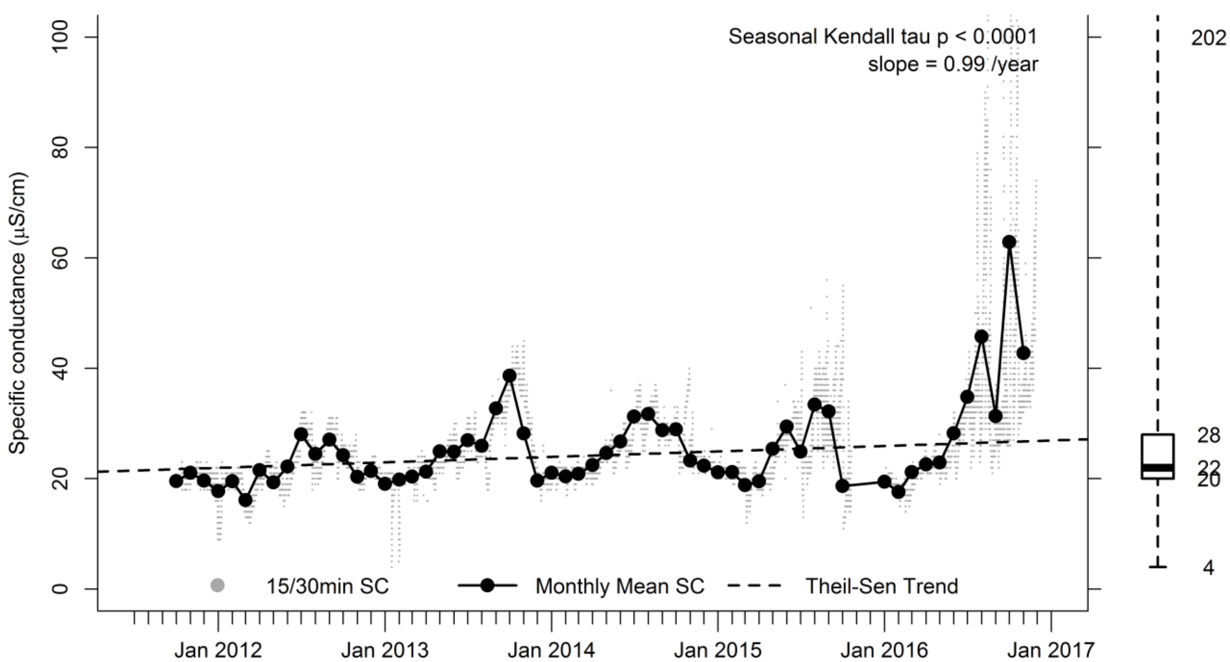

Figure A - 6. Eastland Creek (EAS, reference site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

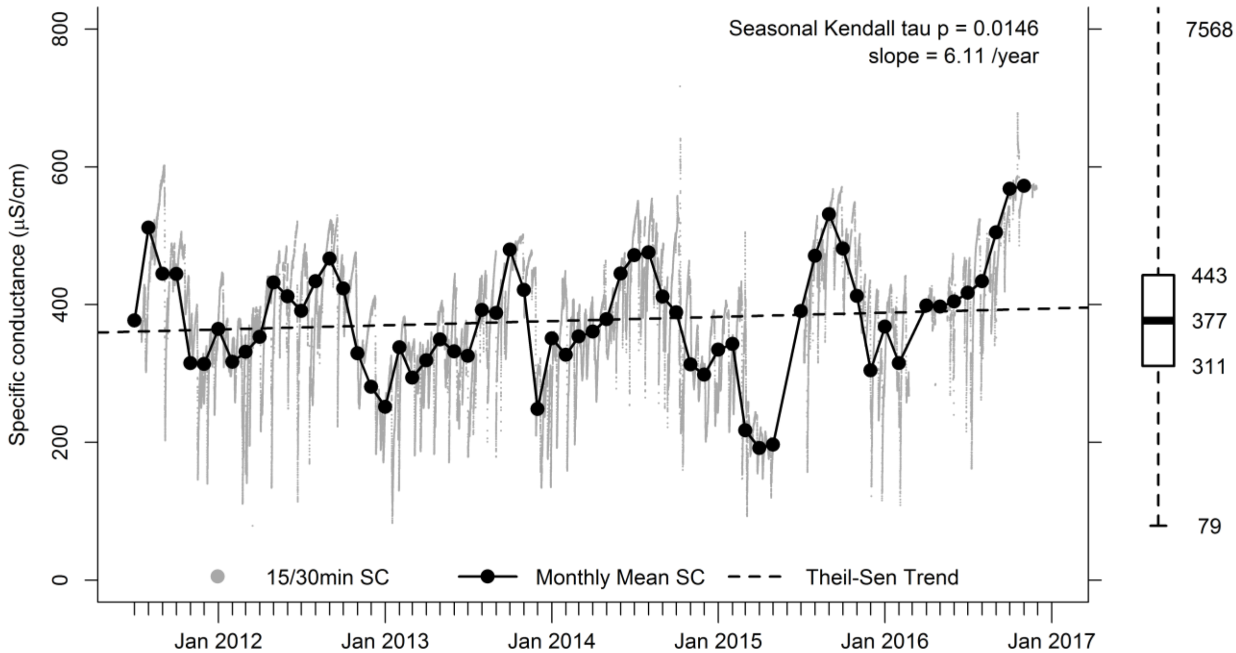

Figure A - 7. Fryingpan Creek (FRY, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant (p < 0.05). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

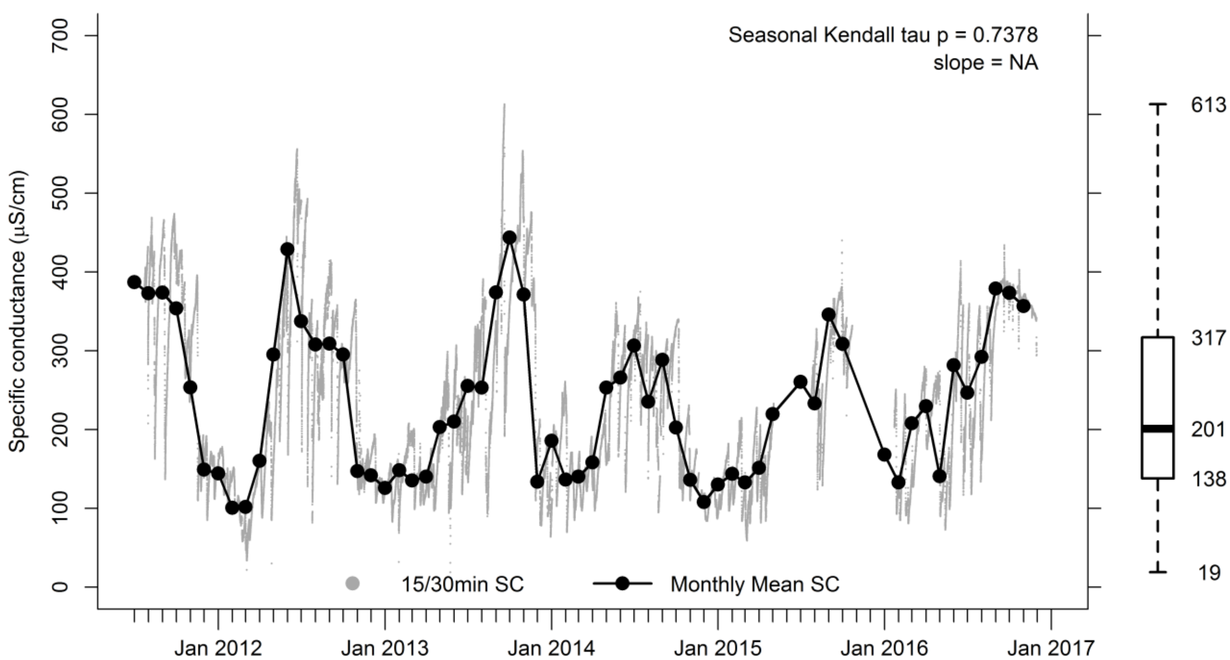

Figure A - 8. Grape Branch (GRA, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant (p < 0.05). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

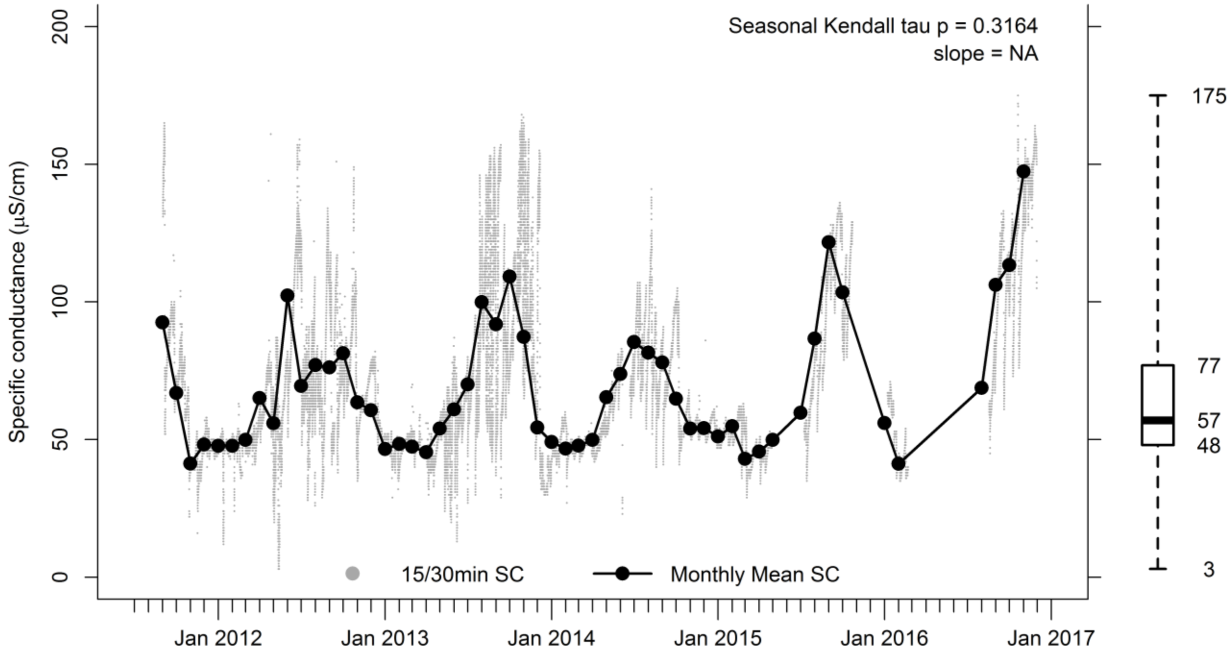

Figure A - 9. Hurricane Branch, WV (HCN, reference site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant (p < 0.05). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

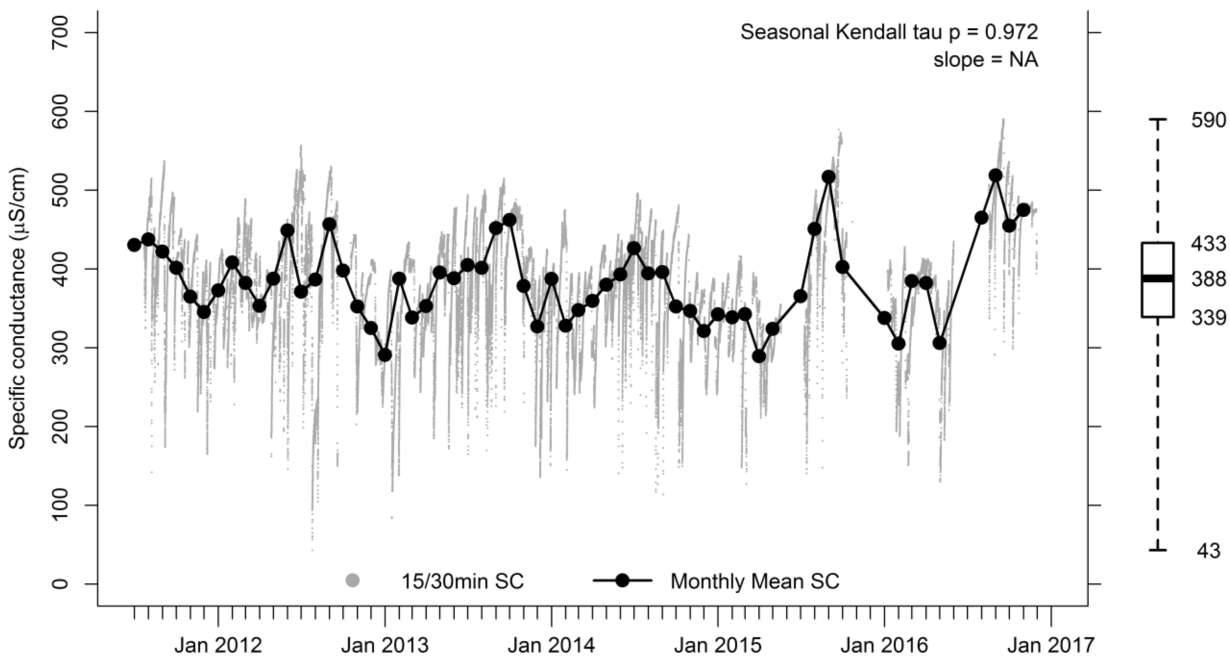

Figure A - 10. Hurricane Fork, VA (HUR, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant (p < 0.05). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

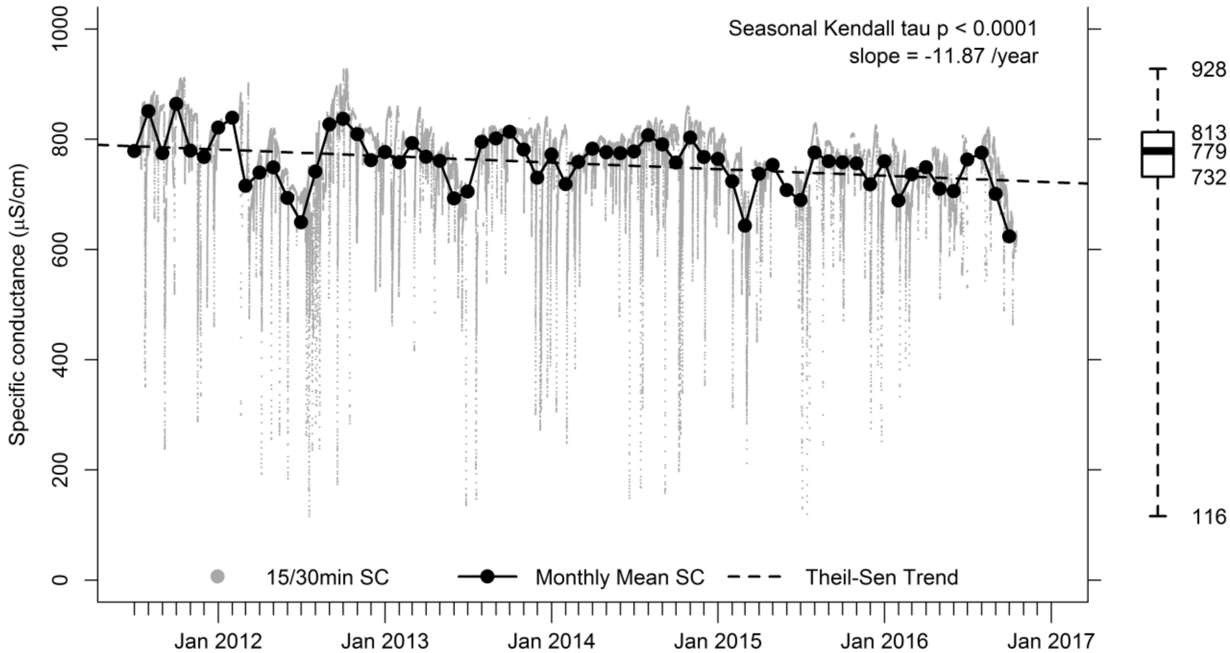

Figure A - 11. Kelly Branch (KEL, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

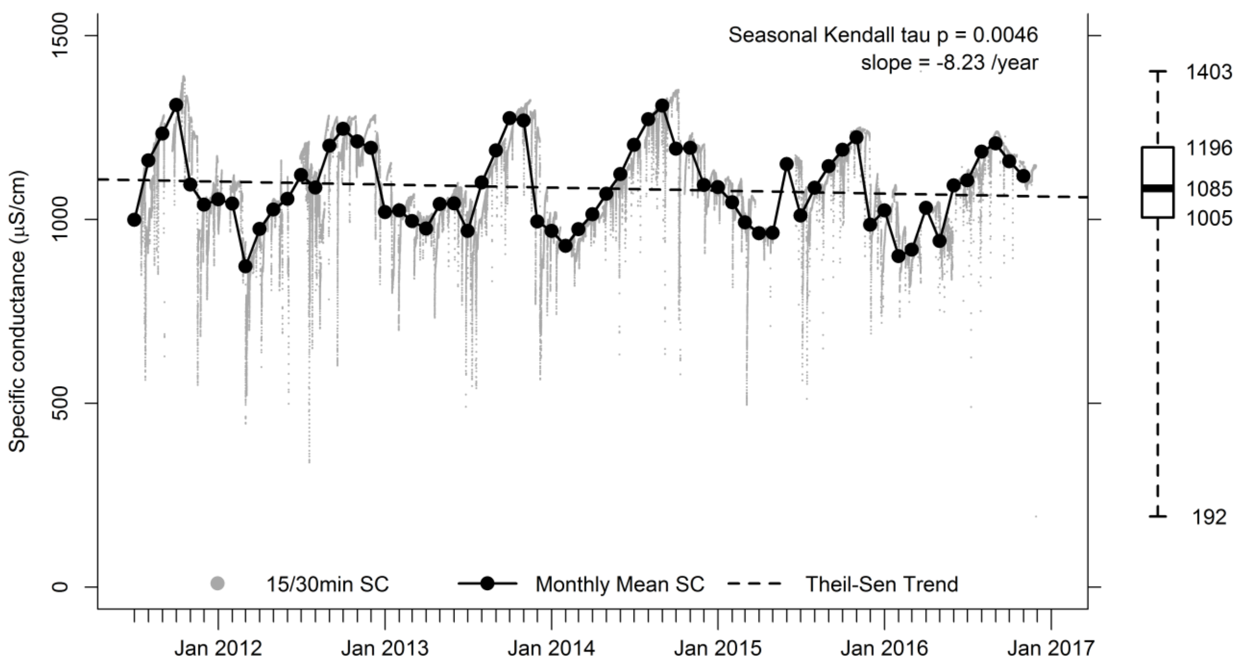

Figure A - 12. Kelly Branch Unnamed Tributary (KUT, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

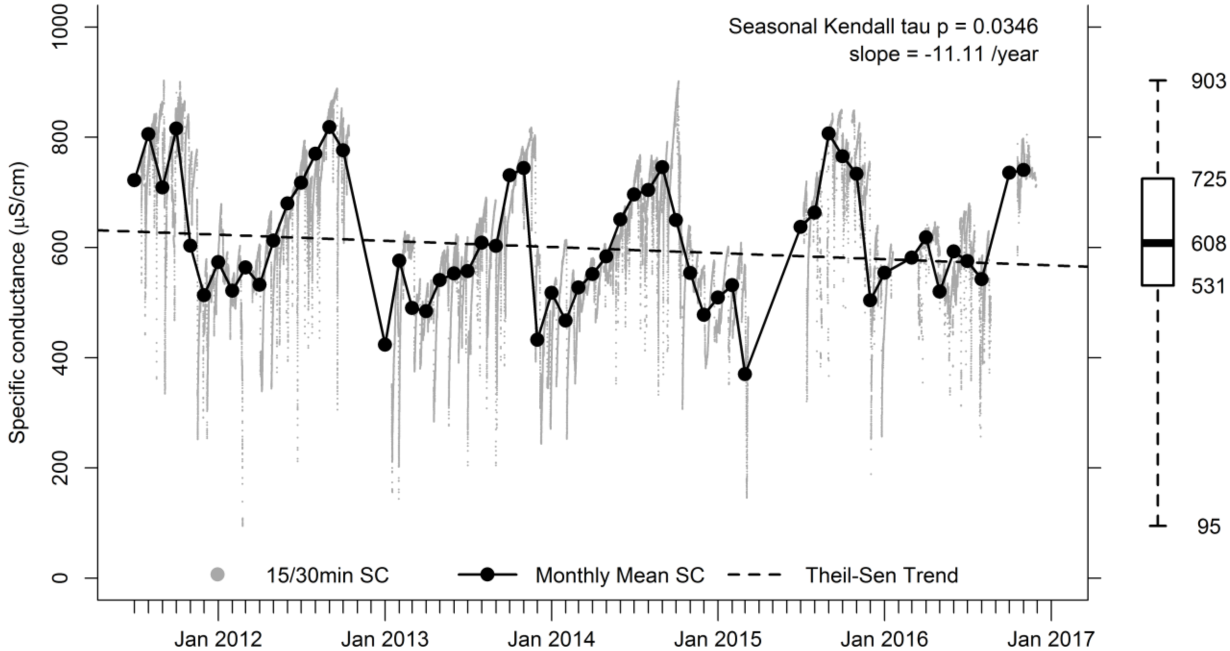

Figure A - 13. Laurel Branch (LAB, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

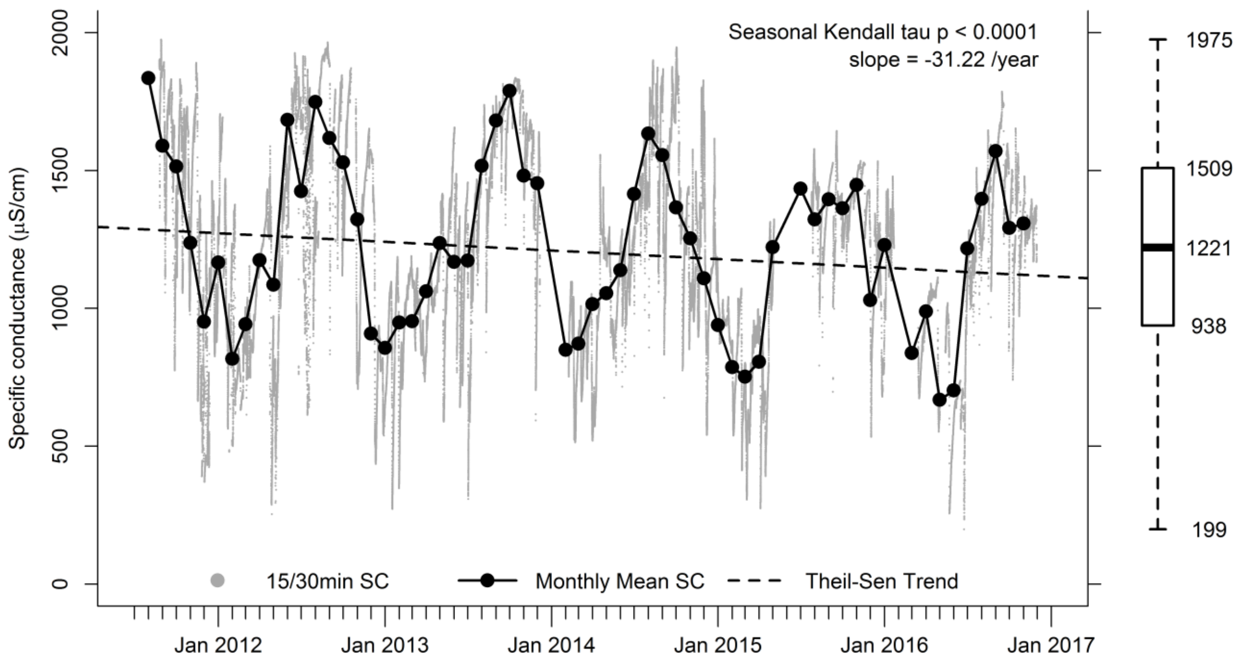

Figure A - 14. Left Fork of Long Fork of Coal Fork (LLC, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

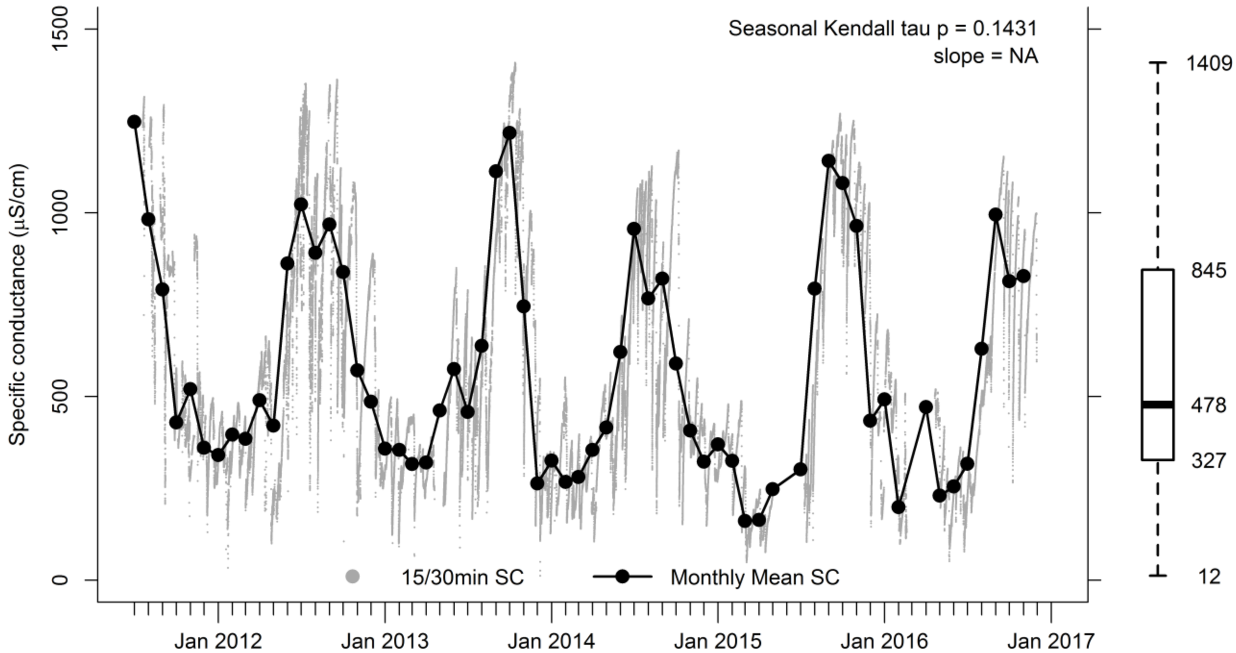

Figure A - 15. Longlick Branch East Fork (LLE, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

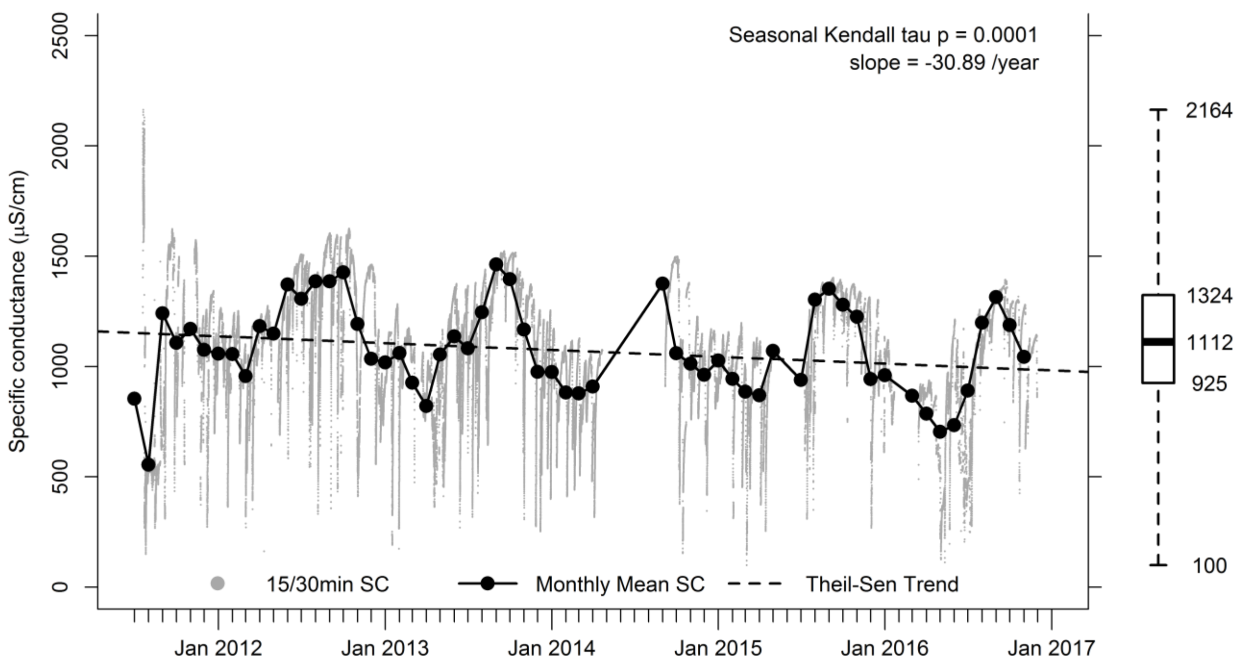

Figure A - 16. Longlick Branch West Fork (LLW, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

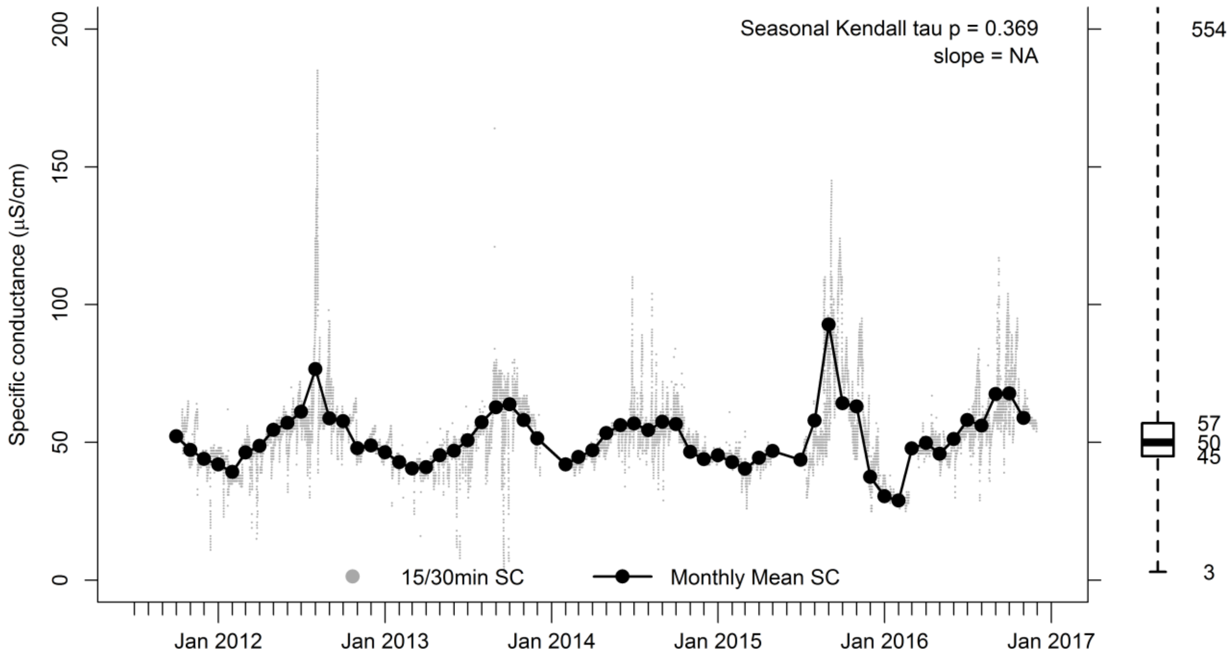

Figure A - 17. Middle Camp Branch (MCB, reference site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

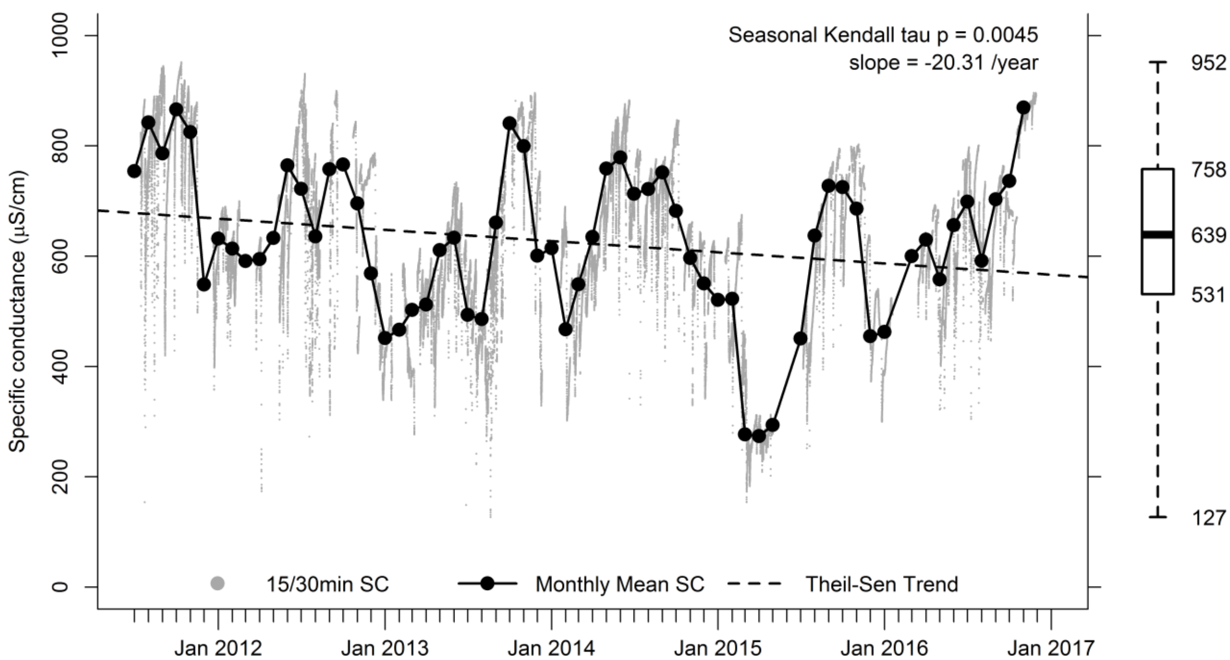

Figure A - 18. Mill Branch West Fork (MIL, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

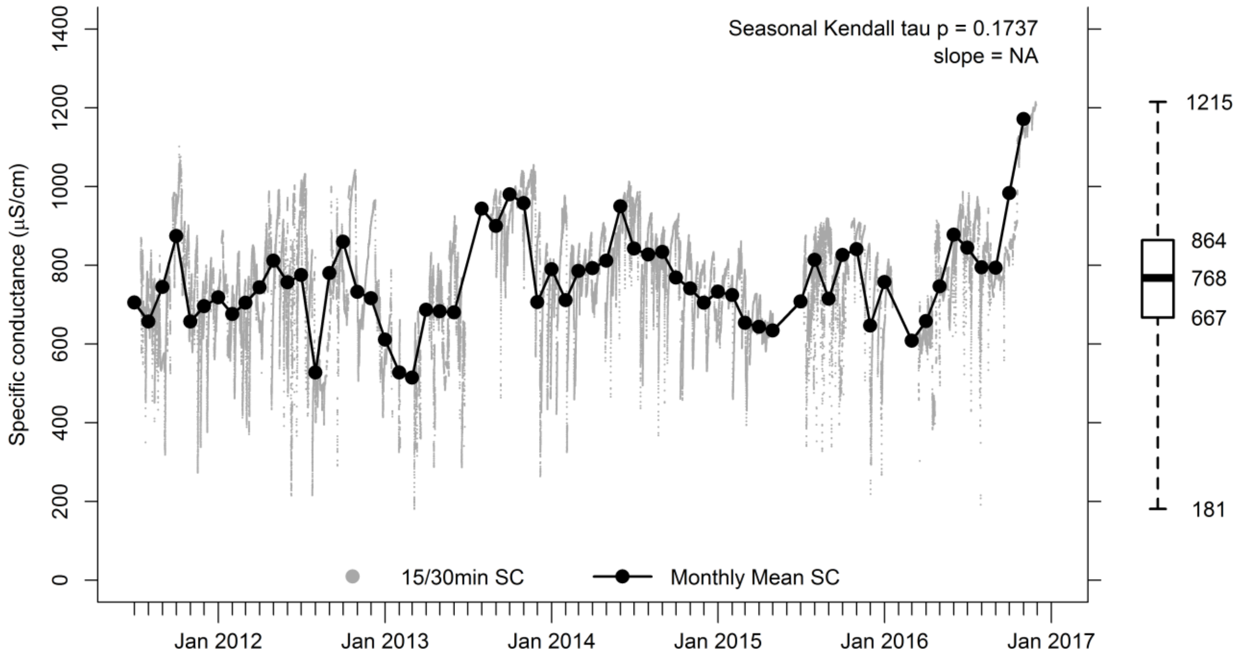

Figure A - 19. Powell River (POW, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

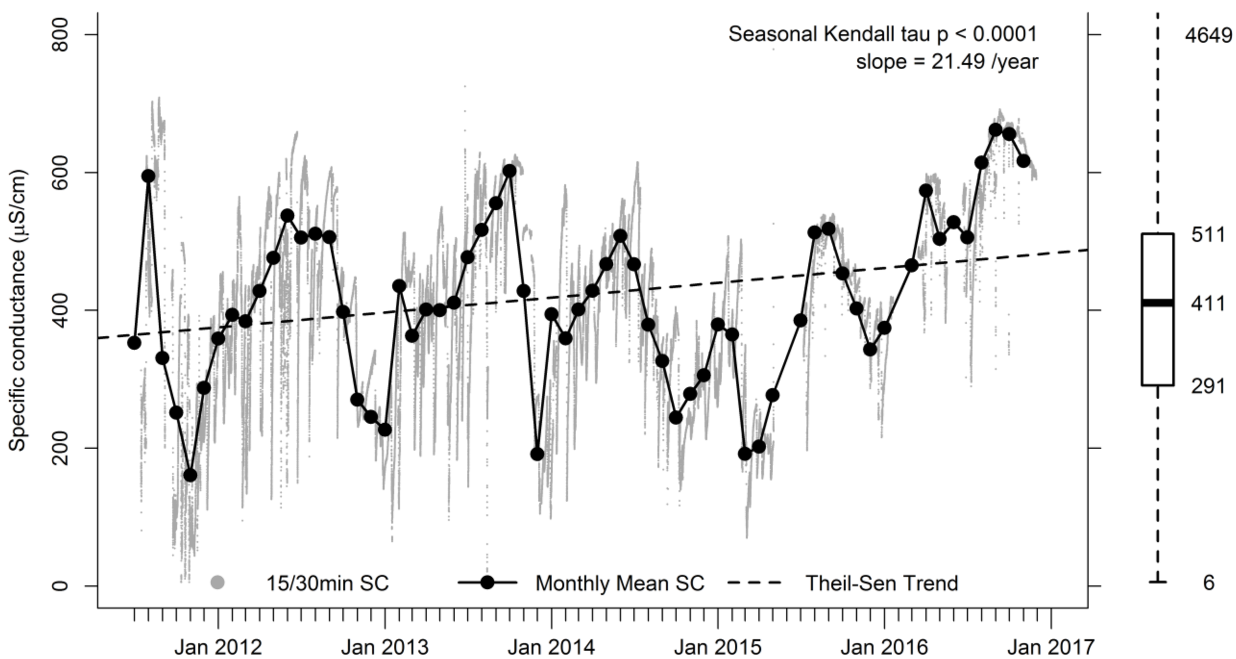

Figure A - 20. Right Fork Fryingpan Creek (RFF, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

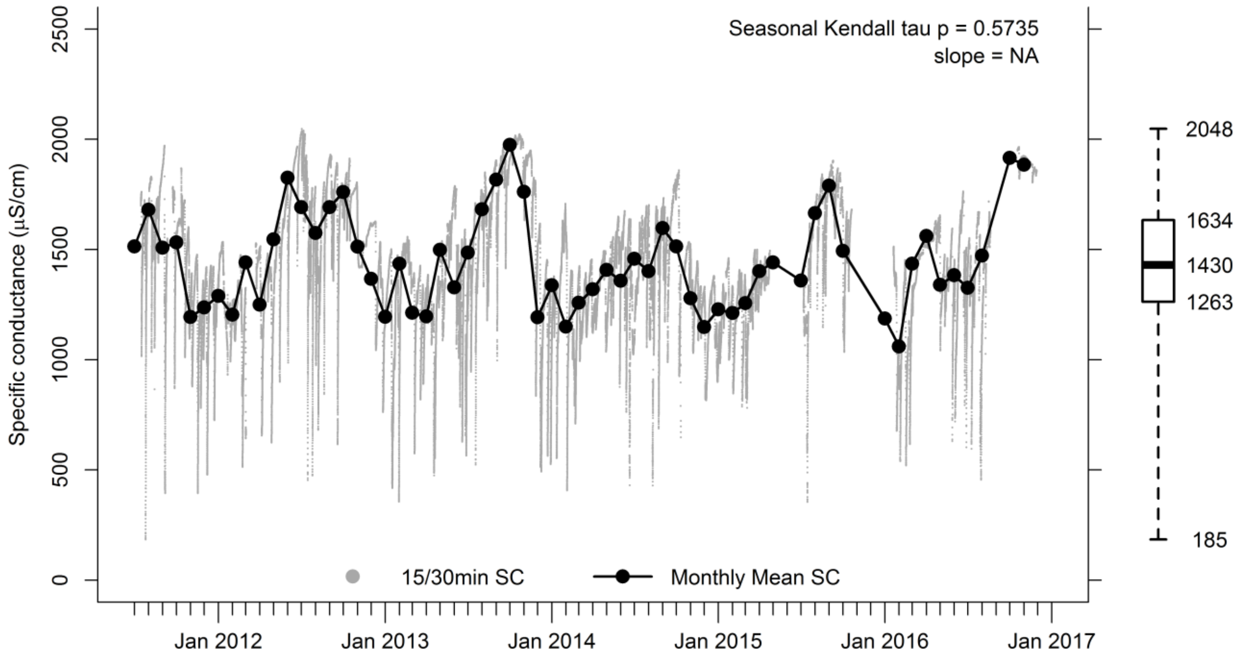

Figure A - 21. Rickey Branch (RIC, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

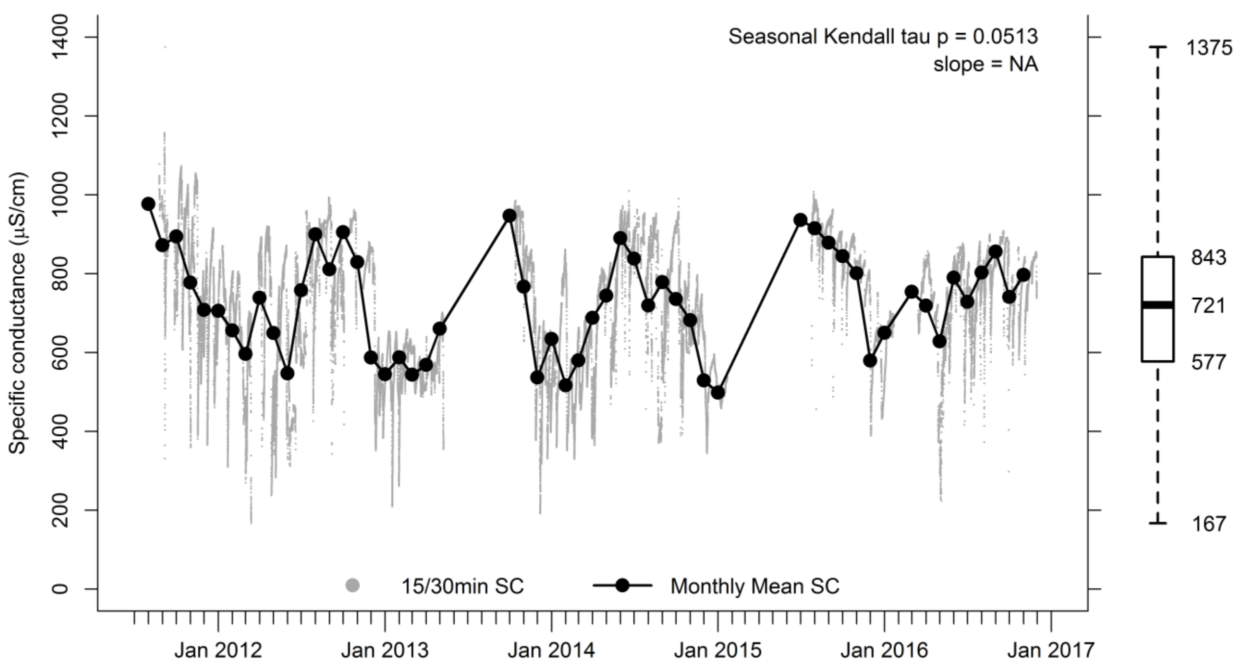

Figure A - 22. Rockhouse Fork (ROC, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

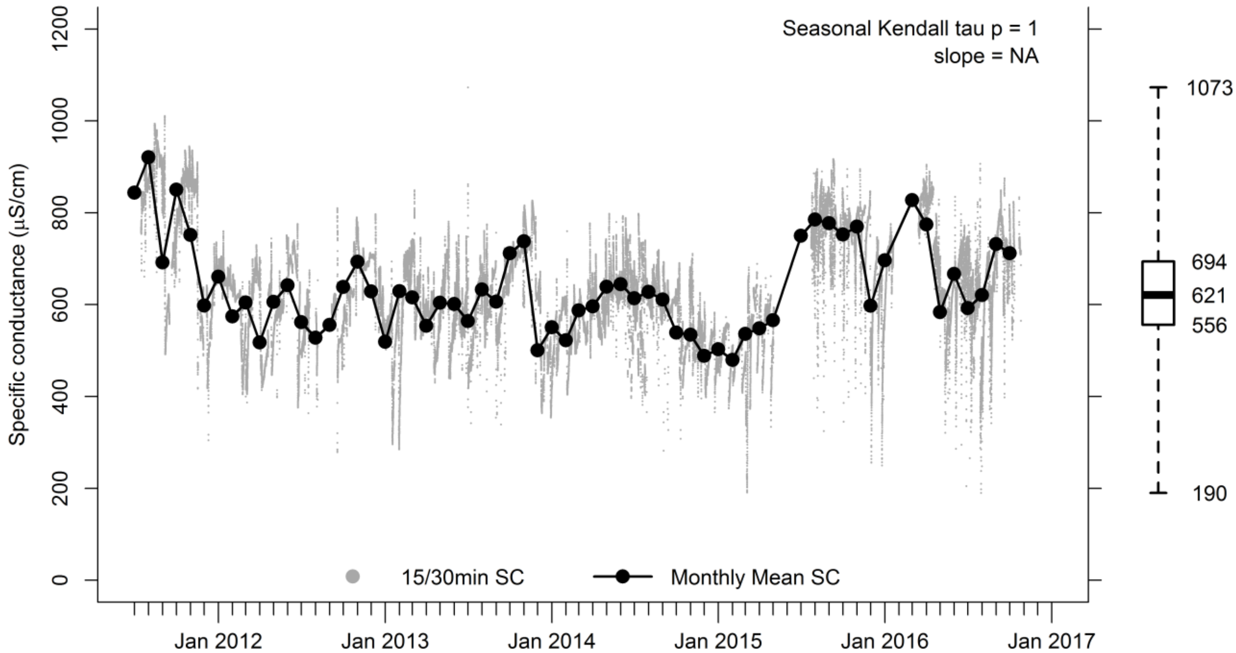

Figure A - 23. Roll Pone Branch (ROL, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

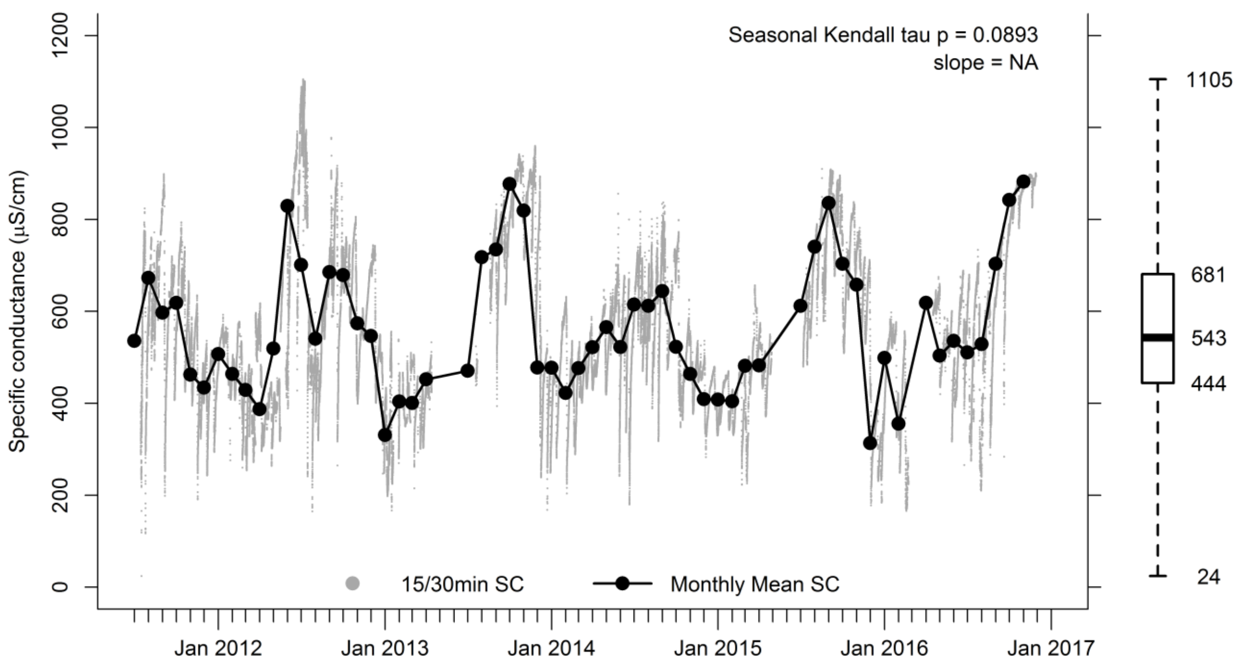

Figure A - 24. Rickey Branch Unnamed Tributary (RUT, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

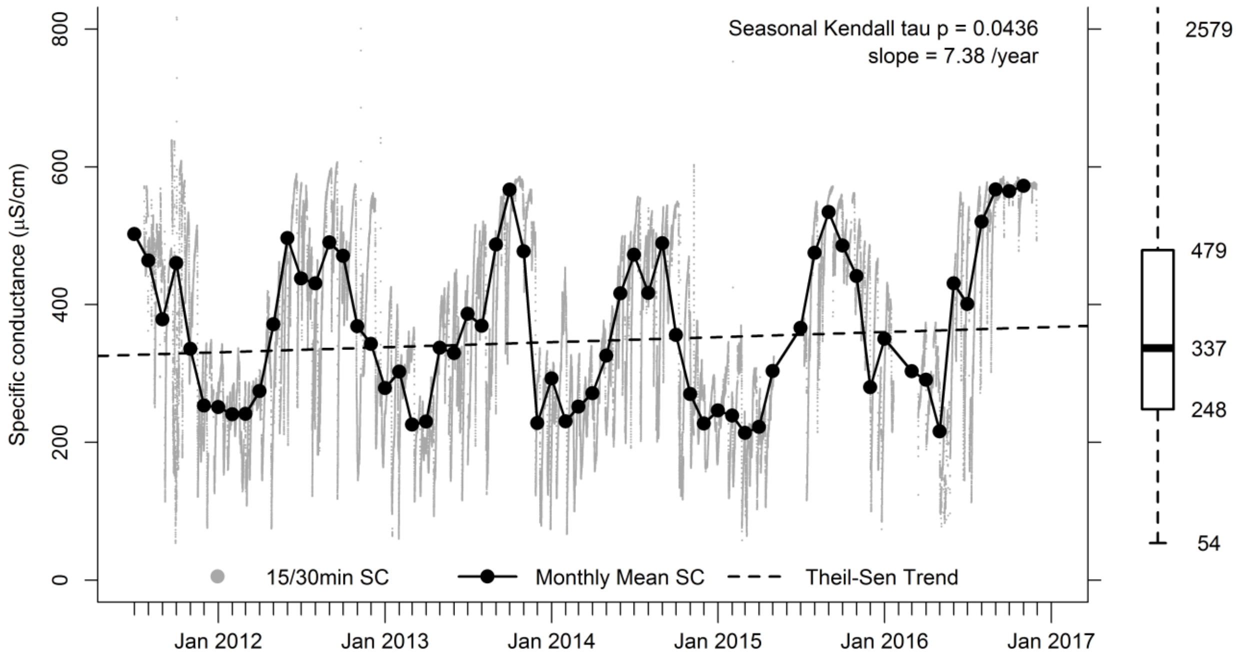

Figure A - 25. Spruce Pine Creek (SPC, test site). 15/30-minute specific conductance (SC; gray dots) with mean monthly values (black circles with black line), Seasonal Kendall tau p-value, and Thiel-Sen slope when tau is significant ($p < 0.05$). Theil-Sen trend line is shown for significant trends (dashed line). Box plot of 15/30 min SC showing median, inter-quartile range, and extreme values.

APPENDIX B – BIOLOGICAL METRICS & TAXA COUNTS

Table B - 1. Benthic macroinvertebrate metrics.

Year	Season	Date	Site ID	Site Type	No. Total Taxa	No. Ephemeroptera Taxa	No. EPT Taxa	No. Plecoptera Taxa	Pielou's Evenness	% Ephemeroptera	% Predators	% Shredders
2015	Fall	10/21/2015	COP	Ref	28	8	20	6	0.78	39.1	9.1	22.7
2015	Fall	10/21/2015	CRO	Ref	29	8	22	6	0.83	30.3	5.3	37.2
2015	Fall	10/19/2015	EAS	Ref	29	6	22	9	0.83	30.5	9.5	16.8
2015	Fall	10/20/2015	HCN	Ref	21	3	13	5	0.69	6.2	7.7	54.4
2015	Fall	10/21/2015	MCB	Ref	20	5	12	3	0.72	33.7	6.3	13.1
2015	Fall	10/20/2015	BIR	Test	14	1	7	1	0.42	0.5	3.1	75.0
2015	Fall	10/27/2015	CRA	Test	25	4	21	10	0.82	15.8	5.9	25.2
2015	Fall	10/20/2015	FRY	Test	18	3	13	5	0.64	2.1	0.5	42.3
2015	Fall	10/22/2015	GRA	Test	20	4	15	4	0.80	11.8	8.0	30.2
2015	Fall	10/22/2015	HUR	Test	26	6	17	6	0.70	15.3	6.9	52.9
2015	Fall	10/19/2015	KEL	Test	15	0	7	2	0.76	0.0	12.9	27.2
2015	Fall	10/19/2015	KUT	Test	11	0	6	3	0.58	0.0	8.2	10.4
2015	Fall	10/21/2015	LAB	Test	29	5	18	5	0.63	4.2	7.4	18.9
2015	Fall	10/27/2015	LLC	Test	19	0	12	4	0.59	0.0	3.2	54.5
2015	Fall	10/27/2015	LLE	Test	22	1	14	8	0.78	1.2	17.9	50.9
2015	Fall	10/27/2015	LLW	Test	17	0	10	4	0.79	0.0	3.1	34.5
2015	Fall	10/19/2015	MIL	Test	16	2	10	2	0.63	1.0	7.8	27.7
2015	Fall	10/20/2015	POW	Test	9	0	3	1	0.39	0.0	1.5	78.8
2015	Fall	10/20/2015	RFF	Test	18	2	13	3	0.74	3.7	5.2	28.8
2015	Fall	10/20/2015	RIC	Test	15	0	9	3	0.73	0.0	2.9	50.0
2015	Fall	10/27/2015	ROC	Test	15	1	9	2	0.76	3.0	4.5	30.8
2015	Fall	10/21/2015	ROL	Test	22	2	8	3	0.71	2.9	18.2	53.5
2015	Fall	10/20/2015	RUT	Test	14	0	8	2	0.70	0.0	7.3	44.8
2015	Fall	10/22/2015	SPC	Test	17	2	13	5	0.71	4.1	4.6	47.7
2016	Spring	4/16/2016	COP	Ref	28	6	18	6	0.77	36.3	8.2	14.8
2016	Spring	4/16/2016	CRO	Ref	33	11	21	7	0.79	34.9	8.5	10.6
2016	Spring	4/15/2016	EAS	Ref	29	9	21	8	0.76	49.3	6.5	16.7
2016	Spring	4/17/2016	HCN	Ref	18	6	12	4	0.68	13.9	4.1	11.3
2016	Spring	4/17/2016	MCB	Ref	26	9	18	7	0.85	34.8	14.4	11.6
2016	Spring	4/15/2016	BIR	Test	18	2	10	2	0.55	14.8	2.3	68.1
2016	Spring	4/19/2016	CRA	Test	20	3	14	5	0.76	32.0	11.2	23.4
2016	Spring	4/16/2016	FRY	Test	22	7	17	5	0.79	26.0	6.8	32.3
2016	Spring	4/17/2016	GRA	Test	24	6	17	6	0.76	27.3	7.2	39.7
2016	Spring	4/17/2016	HUR	Test	22	4	13	5	0.78	10.8	15.2	44.6
2016	Spring	4/15/2016	KEL	Test	14	2	7	2	0.59	1.0	2.6	72.2
2016	Spring	4/15/2016	KUT	Test	13	0	6	2	0.66	0.0	3.4	40.2
2016	Spring	4/16/2016	LAB	Test	23	3	11	4	0.73	5.1	8.7	40.5
2016	Spring	4/19/2016	LLC	Test	22	1	12	5	0.69	0.5	6.3	19.5
2016	Spring	4/20/2016	LLE	Test	23	4	15	8	0.71	6.7	6.7	57.2
2016	Spring	4/20/2016	LLW	Test	17	1	9	5	0.74	4.3	5.4	23.1
2016	Spring	4/15/2016	MIL	Test	15	1	8	2	0.64	1.4	3.8	54.3
2016	Spring	4/20/2016	POW	Test	19	2	10	6	0.70	10.4	3.8	55.0
2016	Spring	4/16/2016	RFF	Test	23	5	17	5	0.78	35.9	6.8	17.3
2016	Spring	4/16/2016	RIC	Test	19	1	10	4	0.65	0.5	4.9	52.5
2016	Spring	4/19/2016	ROC	Test	17	2	11	3	0.61	9.7	2.6	11.3
2016	Spring	4/16/2016	ROL	Test	11	1	6	4	0.57	0.5	2.3	79.1
2016	Spring	4/16/2016	RUT	Test	23	3	13	4	0.64	4.6	4.1	58.7
2016	Spring	4/17/2016	SPC	Test	18	5	14	4	0.76	24.9	3.1	34.7

Table B - 2. Benthic macroinvertebrate taxa count data, Fall 2015.

Year	Season	Date	Site	Site Type	Abundance	*Acentrella*	*Acerpenna*	*Acroneuria*	*Allocapnia*	*Ameletus*	*Amphinemura*	*Antocha*	*Asellus*	*Attenella*	*Baetis*	*Bezzia*	*Boyeria*	*Brachycentrus*	*Calopteryx*	*Ceratopsyche*	*Chelifera*	*Cheumatopsyche*	*Chimarra*	Chironomidae
2015	Fall	10/21/2015	COP	Ref	220	0	0	0	16	0	0	0	0	67	1	0	0	0	0	0	0	7	0	11
2015	Fall	10/21/2015	CRO	Ref	188	0	0	2	15	0	0	0	0	0	4	0	0	0	0	1	0	2	0	14
2015	Fall	10/19/2015	EAS	Ref	220	0	0	3	16	0	0	0	0	9	0	2	0	0	0	0	0	0	0	43
2015	Fall	10/20/2015	HCN	Ref	195	0	0	0	85	0	0	0	0	0	0	6	0	0	0	0	0	6	0	29
2015	Fall	10/21/2015	MCB	Ref	175	0	0	0	4	0	0	0	0	0	1	3	0	0	0	0	0	0	0	65
2015	Fall	10/20/2015	BIR	Test	192	0	0	0	144	1	0	0	1	0	0	0	0	0	0	0	0	13	7	5
2015	Fall	10/27/2015	CRA	Test	202	2	0	1	2	0	2	0	0	0	16	1	0	0	0	0	0	11	0	17
2015	Fall	10/20/2015	FRY	Test	194	0	0	1	76	0	0	0	0	0	1	0	0	0	0	0	0	10	0	6
2015	Fall	10/22/2015	GRA	Test	212	0	0	9	50	0	0	0	0	0	0	0	0	0	0	0	0	9	0	8
2015	Fall	10/22/2015	HUR	Test	189	0	0	1	80	2	0	0	0	0	12	3	0	0	0	0	0	0	0	17
2015	Fall	10/19/2015	KEL	Test	202	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	21	0	17
2015	Fall	10/19/2015	KUT	Test	183	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	20	0	8
2015	Fall	10/21/2015	LAB	Test	190	0	0	1	28	0	0	0	0	0	2	1	0	0	1	0	0	10	0	11
2015	Fall	10/27/2015	LLC	Test	220	0	0	1	118	0	0	0	0	0	0	0	0	0	0	0	0	20	0	21
2015	Fall	10/27/2015	LLE	Test	173	0	0	2	58	0	0	0	0	0	0	3	0	0	0	0	0	1	0	10
2015	Fall	10/27/2015	LLW	Test	194	0	0	0	43	0	0	0	0	0	0	0	0	0	0	0	0	10	0	12
2015	Fall	10/19/2015	MIL	Test	206	0	0	0	53	0	0	0	0	0	1	0	0	0	0	0	0	5	1	76
2015	Fall	10/20/2015	POW	Test	198	0	0	0	155	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
2015	Fall	10/20/2015	RFF	Test	191	0	0	0	51	0	0	0	0	0	2	0	0	0	0	6	0	13	0	4
2015	Fall	10/20/2015	RIC	Test	204	0	0	0	63	0	0	0	0	0	0	0	0	0	0	0	0	38	0	8
2015	Fall	10/27/2015	ROC	Test	198	0	0	1	59	0	0	0	0	0	6	0	0	0	0	15	0	33	0	25
2015	Fall	10/21/2015	ROL	Test	170	0	0	0	66	0	0	0	1	0	4	17	0	0	0	0	0	0	0	24
2015	Fall	10/20/2015	RUT	Test	192	0	0	2	81	0	0	0	0	0	0	0	0	0	0	1	0	10	0	5
2015	Fall	10/22/2015	SPC	Test	195	0	0	1	61	0	0	0	0	0	0	0	0	0	0	0	0	14	0	8

Table B - 2 (cont'd). Benthic macroinvertebrate taxa count data, Fall 2015.

Year	Season	Date	Site	Site Type	Abundance	*Chrysops*	*Cinygmula*	*Clinocera*	*Cordulegaster*	*Cyrnellus*	*Dibusa*	*Dicranota*	*Diphetor*	*Diplectrona*	*Diploperla*	*Dixa*	*Dolophilodes*	*Drunella*	*Eccoptura*	*Ectopria*	*Epeorus*	*Ephemera*	*Ephemerella*	*Eurylophella*
2015	Fall	10/21/2015	COP	Ref	220	0	0	0	0	0	0	0	1	16	0	2	3	0	0	5	5	1	4	0
2015	Fall	10/21/2015	CRO	Ref	188	0	1	0	0	2	0	0	0	7	0	0	0	0	0	7	0	0	0	7
2015	Fall	10/19/2015	EAS	Ref	220	0	0	0	0	3	0	2	0	21	0	0	1	0	0	4	28	0	10	3
2015	Fall	10/20/2015	HCN	Ref	195	0	0	0	0	1	0	0	0	2	0	0	0	0	0	6	0	0	0	2
2015	Fall	10/21/2015	MCB	Ref	175	0	0	0	1	0	0	0	0	4	0	1	0	0	0	8	0	1	0	28
2015	Fall	10/20/2015	BIR	Test	192	0	0	0	0	0	0	1	0	9	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/27/2015	CRA	Test	202	0	0	0	0	0	0	0	3	33	0	0	12	0	0	0	0	0	0	0
2015	Fall	10/20/2015	FRY	Test	194	0	0	0	0	2	0	0	0	44	0	0	1	0	0	1	0	0	0	2
2015	Fall	10/22/2015	GRA	Test	212	0	0	0	0	0	0	0	0	52	0	0	6	0	0	4	0	0	0	1
2015	Fall	10/22/2015	HUR	Test	189	0	0	0	0	0	0	0	10	16	0	5	2	0	0	1	3	0	0	1
2015	Fall	10/19/2015	KEL	Test	202	0	0	0	0	4	0	4	0	56	0	1	0	0	0	1	0	0	0	0
2015	Fall	10/19/2015	KUT	Test	183	0	0	0	0	0	0	0	0	112	0	1	0	0	0	2	0	0	0	0
2015	Fall	10/21/2015	LAB	Test	190	0	0	0	1	0	0	0	0	90	0	1	5	0	0	3	1	0	0	0
2015	Fall	10/27/2015	LLC	Test	220	0	0	0	0	6	0	0	0	16	1	0	10	0	0	0	0	0	0	0
2015	Fall	10/27/2015	LLE	Test	173	0	0	0	0	0	0	0	0	19	0	2	0	0	0	2	0	0	2	0
2015	Fall	10/27/2015	LLW	Test	194	0	0	0	0	0	0	0	0	52	0	1	0	0	0	9	0	0	0	0
2015	Fall	10/19/2015	MIL	Test	206	0	0	0	0	0	0	0	0	40	0	2	4	0	2	0	0	0	0	0
2015	Fall	10/20/2015	POW	Test	198	0	0	0	0	0	0	0	0	23	0	0	0	0	0	4	0	0	0	0
2015	Fall	10/20/2015	RFF	Test	191	0	0	0	0	2	0	0	0	35	0	0	1	0	0	0	0	0	0	0
2015	Fall	10/20/2015	RIC	Test	204	0	0	0	0	2	0	0	0	13	0	0	0	0	0	1	0	0	0	0
2015	Fall	10/27/2015	ROC	Test	198	0	0	0	0	1	0	0	0	30	0	0	12	0	0	1	0	0	0	0
2015	Fall	10/21/2015	ROL	Test	170	1	0	0	0	0	0	3	0	3	0	1	0	0	3	3	0	0	0	0
2015	Fall	10/20/2015	RUT	Test	192	0	0	1	0	0	0	0	0	44	0	0	0	0	0	7	0	0	0	0
2015	Fall	10/22/2015	SPC	Test	195	0	0	0	0	0	0	0	0	52	0	0	2	0	1	0	0	0	0	0

Table B - 2 (cont'd). Benthic macroinvertebrate taxa count data, Fall 2015.

Year	Season	Date	Site	Site Type	Abundance	*Forcipomyia*	*Glossosoma*	*Glutops*	*Habrophlebiodes*	*Haploperla*	*Hemerodromia*	*Heptagenia*	*Hexatoma*	*Hydropsyche*	*Hydroptila*	*Isonychia*	*Isoperla*	*Lanthus*	*Leuctra*	*Limnophila*	*Maccaffertium*	*Macronychus*	*Megaselia*	*Micrasema*
2015	Fall	10/21/2015	COP	Ref	220	0	0	0	0	1	0	0	2	7	0	0	2	1	0	0	3	0	0	1
2015	Fall	10/21/2015	CRO	Ref	188	0	0	0	8	1	0	0	2	3	0	0	2	1	0	0	7	0	0	0
2015	Fall	10/19/2015	EAS	Ref	220	0	0	0	0	0	0	2	1	2	0	0	0	0	7	0	0	0	0	0
2015	Fall	10/20/2015	HCN	Ref	195	0	0	0	0	0	0	0	0	3	0	0	2	0	1	1	6	0	0	0
2015	Fall	10/21/2015	MCB	Ref	175	0	0	0	0	0	0	0	1	0	1	0	4	0	5	0	11	0	0	0
2015	Fall	10/20/2015	BIR	Test	192	2	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0
2015	Fall	10/27/2015	CRA	Test	202	0	0	0	0	0	0	0	1	28	0	0	1	0	3	0	0	0	0	0
2015	Fall	10/20/2015	FRY	Test	194	0	0	0	0	0	0	0	0	26	0	0	0	0	1	0	1	0	0	0
2015	Fall	10/22/2015	GRA	Test	212	0	0	0	0	0	0	0	0	8	0	0	1	6	14	0	14	0	0	0
2015	Fall	10/22/2015	HUR	Test	189	1	0	0	0	0	0	0	0	0	0	0	3	2	6	0	1	0	0	0
2015	Fall	10/19/2015	KEL	Test	202	0	0	0	0	0	0	0	0	0	0	0	0	4	48	0	0	0	0	0
2015	Fall	10/19/2015	KUT	Test	183	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
2015	Fall	10/21/2015	LAB	Test	190	1	0	0	2	1	0	0	0	4	0	0	1	2	2	0	2	0	0	0
2015	Fall	10/27/2015	LLC	Test	220	0	1	0	0	0	1	0	0	13	1	0	0	0	1	0	0	0	1	0
2015	Fall	10/27/2015	LLE	Test	173	1	0	0	0	1	0	0	0	2	0	0	7	0	6	0	0	0	0	0
2015	Fall	10/27/2015	LLW	Test	194	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	3	0	0
2015	Fall	10/19/2015	MIL	Test	206	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
2015	Fall	10/20/2015	POW	Test	198	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0
2015	Fall	10/20/2015	RFF	Test	191	0	0	0	0	0	0	0	0	41	0	0	0	0	0	0	5	0	0	1
2015	Fall	10/20/2015	RIC	Test	204	0	0	0	0	0	1	0	1	21	0	0	2	0	0	0	0	0	0	0
2015	Fall	10/27/2015	ROC	Test	198	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/21/2015	ROL	Test	170	1	0	0	0	0	0	0	0	5	0	0	0	1	10	0	0	0	0	0
2015	Fall	10/20/2015	RUT	Test	192	0	0	0	0	0	0	0	0	7	0	0	0	2	0	0	0	0	0	0
2015	Fall	10/22/2015	SPC	Test	195	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	6	0	0	0

Table B - 2 (cont'd). Benthic macroinvertebrate taxa count data, Fall 2015.

Year	Season	Date	Site	Site Type	Abundance	*Molophilus*	*Nemoura*	*Neophylax*	*Neoplasta*	*Nigronia*	*Ochrotrichia*	Oligochaeta	*Optioservus*	*Oulimnius*	*Paracapnia*	*Paraleptophlebia*	*Pedicia*	*Peltoperla*	*Plauditus*	*Polycentropus*	*Psephenus*	*Pteronarcys*	*Pycnopsyche*	*Remenus*
2015	Fall	10/21/2015	COP	Ref	220	0	2	0	0	0	0	0	13	0	28	4	0	0	0	0	0	0	0	0
2015	Fall	10/21/2015	CRO	Ref	188	0	0	0	0	0	0	1	11	0	41	20	0	0	0	0	2	1	13	0
2015	Fall	10/19/2015	EAS	Ref	220	0	0	0	0	0	0	9	9	0	7	15	0	2	0	0	0	2	1	0
2015	Fall	10/20/2015	HCN	Ref	195	0	0	0	0	0	0	4	0	11	0	4	0	0	0	4	0	0	0	0
2015	Fall	10/21/2015	MCB	Ref	175	0	0	0	0	0	0	0	0	3	0	18	0	0	0	1	0	0	14	0
2015	Fall	10/20/2015	BIR	Test	192	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/27/2015	CRA	Test	202	0	0	0	0	0	0	0	0	0	32	0	0	2	11	0	0	0	1	0
2015	Fall	10/20/2015	FRY	Test	194	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	3	1	0	0
2015	Fall	10/22/2015	GRA	Test	212	0	0	2	0	0	0	0	8	0	0	1	0	0	0	0	6	0	0	0
2015	Fall	10/22/2015	HUR	Test	189	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
2015	Fall	10/19/2015	KEL	Test	202	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	2	0
2015	Fall	10/19/2015	KUT	Test	183	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/21/2015	LAB	Test	190	0	0	0	0	0	0	0	2	0	0	0	0	0	1	1	2	0	3	0
2015	Fall	10/27/2015	LLC	Test	220	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0	1	0	0	0
2015	Fall	10/27/2015	LLE	Test	173	0	0	0	0	0	0	0	7	0	0	0	0	16	0	0	0	0	0	0
2015	Fall	10/27/2015	LLW	Test	194	0	0	0	0	0	0	1	22	0	0	0	0	8	0	1	0	1	0	0
2015	Fall	10/19/2015	MIL	Test	206	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/20/2015	POW	Test	198	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/20/2015	RFF	Test	191	0	0	0	0	2	0	0	16	0	0	0	0	0	0	0	1	0	0	0
2015	Fall	10/20/2015	RIC	Test	204	0	0	1	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/27/2015	ROC	Test	198	1	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/21/2015	ROL	Test	170	1	0	0	0	0	0	3	1	0	0	1	0	0	0	0	0	0	14	0
2015	Fall	10/20/2015	RUT	Test	192	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/22/2015	SPC	Test	195	0	0	0	0	0	0	0	3	0	5	0	0	0	0	1	1	0	0	0

Table B - 2 (cont'd). Benthic macroinvertebrate taxa count data, Fall 2015.

Year	Season	Date	Site	Site Type	Abundance	*Rhamphomyia*	*Rhyacophila*	*Roederiodes*	*Sialis*	*Simulium*	Sisyridae	*Stenacron*	*Stenonema*	*Stylogomphus*	*Suwallia*	*Sweltsa*	*Taeniopteryx*	*Tallaperla*	*Tipula*	*Tipulidae*	*Wormaldia*	*Yugus*
2015	Fall	10/21/2015	COP	Ref	220	0	8	1	0	0	0	0	0	0	0	0	0	0	3	0	0	5
2015	Fall	10/21/2015	CRO	Ref	188	0	2	0	0	0	0	1	9	0	0	0	0	0	0	0	1	0
2015	Fall	10/19/2015	EAS	Ref	220	0	12	0	0	0	0	0	0	0	0	1	1	1	0	0	3	0
2015	Fall	10/20/2015	HCN	Ref	195	0	0	0	0	0	0	0	0	1	0	1	17	0	3	0	0	0
2015	Fall	10/21/2015	MCB	Ref	175	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
2015	Fall	10/20/2015	BIR	Test	192	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2015	Fall	10/27/2015	CRA	Test	202	0	3	0	0	0	0	0	0	0	0	4	7	0	2	0	6	1
2015	Fall	10/20/2015	FRY	Test	194	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0
2015	Fall	10/22/2015	GRA	Test	212	0	1	0	0	0	0	0	9	0	0	0	0	0	0	0	3	0
2015	Fall	10/22/2015	HUR	Test	189	0	1	0	0	0	0	0	0	0	0	3	9	0	5	0	1	0
2015	Fall	10/19/2015	KEL	Test	202	0	18	0	0	3	0	0	0	0	0	0	0	0	1	0	0	0
2015	Fall	10/19/2015	KUT	Test	183	0	13	0	0	2	0	0	0	0	2	0	0	0	0	0	0	0
2015	Fall	10/21/2015	LAB	Test	190	0	5	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0
2015	Fall	10/27/2015	LLC	Test	220	0	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
2015	Fall	10/27/2015	LLE	Test	173	0	7	1	0	0	0	0	0	0	0	10	2	0	6	0	8	0
2015	Fall	10/27/2015	LLW	Test	194	0	5	0	0	0	0	0	0	0	0	0	9	0	6	0	1	0
2015	Fall	10/19/2015	MIL	Test	206	0	10	2	0	2	0	0	0	0	0	0	0	0	4	0	0	0
2015	Fall	10/20/2015	POW	Test	198	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
2015	Fall	10/20/2015	RFF	Test	191	1	5	0	0	0	0	0	0	0	0	2	3	0	0	0	0	0
2015	Fall	10/20/2015	RIC	Test	204	0	2	0	0	0	0	0	0	0	0	0	37	0	2	0	0	0
2015	Fall	10/27/2015	ROC	Test	198	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
2015	Fall	10/21/2015	ROL	Test	170	0	0	0	1	0	0	0	0	6	0	0	0	0	0	0	0	0
2015	Fall	10/20/2015	RUT	Test	192	0	9	0	0	0	0	0	0	0	0	0	0	0	5	0	4	0
2015	Fall	10/22/2015	SPC	Test	195	0	6	0	0	0	0	2	0	0	0	0	25	0	2	0	0	0

Table B - 3. Benthic macroinvertebrate taxa count data, Spring 2016.

Year	Season	Date	Site	Site Type	Abundance	*Acentrella*	*Acerpenna*	*Acroneuria*	*Allocapnia*	*Ameletus*	*Amphinemura*	*Antocha*	*Asellus*	*Attenella*	*Baetis*	*Bezzia*	*Boyeria*	*Brachycentrus*	*Calopteryx*	*Ceratopsyche*	*Chelifera*	*Cheumatopsyche*	*Chimarra*	Chironomidae
2016	Spring	4/16/2016	COP	Ref	182	0	0	0	0	0	15	0	0	0	8	2	0	0	0	0	0	0	0	41
2016	Spring	4/16/2016	CRO	Ref	189	2	0	3	0	1	4	0	0	0	16	1	0	0	0	0	2	0	0	52
2016	Spring	4/15/2016	EAS	Ref	215	0	0	0	0	2	8	0	1	0	5	1	0	0	0	0	0	0	0	26
2016	Spring	4/17/2016	HCN	Ref	194	19	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	71
2016	Spring	4/17/2016	MCB	Ref	181	0	0	2	0	0	11	0	0	0	15	1	0	0	0	0	0	0	0	34
2016	Spring	4/15/2016	BIR	Test	216	0	0	0	0	0	117	0	0	0	30	0	0	0	0	0	0	2	0	16
2016	Spring	4/19/2016	CRA	Test	197	35	2	0	0	0	37	0	0	0	25	2	0	1	0	0	0	3	0	40
2016	Spring	4/16/2016	FRY	Test	192	25	0	3	0	0	40	0	0	0	10	0	0	0	0	0	0	0	0	40
2016	Spring	4/17/2016	GRA	Test	194	30	0	5	0	0	17	0	0	0	4	0	0	0	0	0	0	0	0	20
2016	Spring	4/17/2016	HUR	Test	204	0	0	0	0	0	39	0	0	0	0	2	0	0	0	0	0	0	0	21
2016	Spring	4/15/2016	KEL	Test	194	1	0	0	0	0	47	0	0	0	1	0	0	0	0	0	0	1	0	22
2016	Spring	4/15/2016	KUT	Test	204	0	0	0	0	0	10	1	0	0	0	0	1	0	0	0	0	1	0	66
2016	Spring	4/16/2016	LAB	Test	195	5	1	0	0	0	42	0	0	0	3	4	0	0	0	0	2	0	0	46
2016	Spring	4/19/2016	LLC	Test	190	0	0	1	0	0	25	0	0	0	0	1	0	0	0	0	0	3	0	83
2016	Spring	4/20/2016	LLE	Test	208	3	0	1	0	6	57	0	0	0	3	0	0	0	0	0	0	0	0	34
2016	Spring	4/20/2016	LLW	Test	186	8	0	0	0	0	21	0	0	0	0	0	0	0	0	0	6	0	0	66
2016	Spring	4/15/2016	MIL	Test	208	0	0	0	0	0	41	0	0	0	3	0	0	0	0	0	3	1	0	57
2016	Spring	4/20/2016	POW	Test	211	0	0	0	0	1	66	0	0	0	21	0	0	0	0	0	4	0	0	15
2016	Spring	4/16/2016	RFF	Test	220	58	0	1	0	0	31	0	0	0	11	1	0	0	0	0	0	1	0	23
2016	Spring	4/16/2016	RIC	Test	204	1	0	2	0	0	30	0	0	0	0	0	0	0	0	4	1	23	0	48
2016	Spring	4/19/2016	ROC	Test	195	3	0	0	0	0	14	0	0	0	16	0	0	0	0	0	0	1	0	108
2016	Spring	4/16/2016	ROL	Test	220	0	0	2	0	1	97	0	0	0	0	0	0	0	0	0	2	0	0	27
2016	Spring	4/16/2016	RUT	Test	196	7	0	2	0	0	52	0	0	0	1	0	0	0	0	3	1	1	0	29
2016	Spring	4/17/2016	SPC	Test	193	27	0	0	0	0	14	0	0	0	6	0	0	0	0	0	2	0	0	43

Table B - 3 (cont'd). Benthic macroinvertebrate taxa count data, Spring 2016.

Year	Season	Date	Site	Site Type	Abundance	*Chrysops*	*Cinygmula*	*Clinocera*	*Cordulegaster*	*Cyrnellus*	*Dibusa*	*Dicranota*	*Diphetor*	*Diplectrona*	*Diploperla*	*Dixa*	*Dolophilodes*	*Drunella*	*Eccoptura*	*Ectopria*	*Epeorus*	*Ephemera*	*Ephemerella*	*Eurylophella*
2016	Spring	4/16/2016	COP	Ref	182	0	0	1	0	0	0	0	3	8	0	1	2	4	0	5	13	0	36	0
2016	Spring	4/16/2016	CRO	Ref	189	0	0	0	0	0	0	0	3	8	0	1	0	1	0	3	5	0	18	3
2016	Spring	4/15/2016	EAS	Ref	215	0	14	0	0	0	0	0	0	5	0	0	0	3	0	1	65	0	11	0
2016	Spring	4/17/2016	HCN	Ref	194	0	0	5	0	0	0	0	0	0	0	0	3	1	0	0	0	0	4	0
2016	Spring	4/17/2016	MCB	Ref	181	0	2	0	0	0	0	0	0	7	0	0	12	2	0	2	3	0	20	0
2016	Spring	4/15/2016	BIR	Test	216	0	0	0	0	0	0	0	2	2	0	0	2	0	0	1	0	0	0	0
2016	Spring	4/19/2016	CRA	Test	197	0	0	1	0	0	0	3	0	16	0	0	0	0	0	0	0	0	3	0
2016	Spring	4/16/2016	FRY	Test	192	0	0	0	0	0	0	0	0	2	0	0	2	5	0	0	1	0	6	0
2016	Spring	4/17/2016	GRA	Test	194	0	2	0	0	0	0	0	0	12	0	0	7	6	0	0	1	0	10	0
2016	Spring	4/17/2016	HUR	Test	204	0	2	0	0	0	0	0	9	9	0	2	13	0	0	0	1	0	10	0
2016	Spring	4/15/2016	KEL	Test	194	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0
2016	Spring	4/15/2016	KUT	Test	204	0	0	0	0	0	0	0	0	25	0	0	0	0	0	4	0	0	0	0
2016	Spring	4/16/2016	LAB	Test	195	0	0	0	0	0	0	0	0	15	0	0	3	0	0	1	0	0	0	0
2016	Spring	4/19/2016	LLC	Test	190	0	0	0	0	0	0	0	0	3	0	0	10	1	0	1	0	0	0	0
2016	Spring	4/20/2016	LLE	Test	208	0	0	0	0	1	0	0	0	2	0	2	0	0	0	0	0	0	2	0
2016	Spring	4/20/2016	LLW	Test	186	0	0	1	0	1	0	0	0	12	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/15/2016	MIL	Test	208	0	0	0	0	0	0	0	0	4	0	0	2	0	0	1	0	0	0	0
2016	Spring	4/20/2016	POW	Test	211	0	0	0	0	0	0	0	0	13	0	1	0	0	0	1	0	0	0	0
2016	Spring	4/16/2016	RFF	Test	220	0	1	0	0	3	0	0	0	9	0	0	7	7	0	1	0	0	2	0
2016	Spring	4/16/2016	RIC	Test	204	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/19/2016	ROC	Test	195	0	0	1	0	0	0	0	0	3	0	0	12	0	0	0	0	0	0	0
2016	Spring	4/16/2016	ROL	Test	220	0	0	0	0	0	0	0	0	3	0	0	0	0	0	2	0	0	0	0
2016	Spring	4/16/2016	RUT	Test	196	0	0	1	0	0	1	0	0	6	0	0	0	0	0	2	0	0	1	0
2016	Spring	4/17/2016	SPC	Test	193	0	0	0	0	0	0	0	0	3	0	0	8	1	0	0	0	0	11	3

Table B - 3 (cont'd). Benthic macroinvertebrate taxa count data, Spring 2016.

Year	Season	Date	Site	Site Type	Abundance	*Forcipomyia*	*Glossosoma*	*Glutops*	*Habrophlebiodes*	*Haploperla*	*Hemerodromia*	*Heptagenia*	*Hexatoma*	*Hydropsyche*	*Hydroptila*	*Isonychia*	*Isoperla*	*Lanthus*	*Leuctra*	*Limnophila*	*Maccaffertium*	*Macronychus*	*Megaselia*	*Micrasema*
2016	Spring	4/16/2016	COP	Ref	182	0	0	0	0	1	1	0	0	0	0	0	2	0	9	0	0	0	0	0
2016	Spring	4/16/2016	CRO	Ref	189	0	0	0	11	1	1	1	1	0	0	5	2	0	12	0	0	0	0	3
2016	Spring	4/15/2016	EAS	Ref	215	0	0	0	2	0	0	3	2	1	0	0	1	0	13	0	0	0	0	0
2016	Spring	4/17/2016	HCN	Ref	194	0	0	0	1	0	0	0	0	0	0	0	1	0	17	0	1	0	0	0
2016	Spring	4/17/2016	MCB	Ref	181	0	0	0	16	2	3	0	3	0	0	0	5	2	9	0	1	0	0	0
2016	Spring	4/15/2016	BIR	Test	216	0	0	0	0	0	0	0	0	1	0	0	0	1	30	0	0	0	0	0
2016	Spring	4/19/2016	CRA	Test	197	0	0	0	0	9	0	0	0	6	0	0	1	0	7	0	0	0	0	0
2016	Spring	4/16/2016	FRY	Test	192	0	0	0	0	0	0	2	0	3	0	0	3	0	20	0	0	0	0	0
2016	Spring	4/17/2016	GRA	Test	194	0	0	0	0	1	0	0	0	4	0	0	0	0	57	0	0	0	0	0
2016	Spring	4/17/2016	HUR	Test	204	0	0	0	0	12	1	0	3	1	0	0	5	0	52	0	0	0	0	0
2016	Spring	4/15/2016	KEL	Test	194	0	0	0	0	0	1	0	0	0	0	0	0	1	93	0	0	0	0	0
2016	Spring	4/15/2016	KUT	Test	204	0	0	0	0	0	0	0	0	0	0	0	0	1	72	0	0	0	0	0
2016	Spring	4/16/2016	LAB	Test	195	0	0	0	0	9	0	0	0	0	0	0	1	1	36	0	0	0	0	0
2016	Spring	4/19/2016	LLC	Test	190	0	0	0	0	0	3	0	0	9	0	0	3	0	10	0	0	0	0	0
2016	Spring	4/20/2016	LLE	Test	208	0	0	0	0	2	0	0	0	0	0	0	5	0	44	0	0	0	0	0
2016	Spring	4/20/2016	LLW	Test	186	0	0	0	0	0	0	0	0	3	0	0	1	0	15	0	0	0	0	0
2016	Spring	4/15/2016	MIL	Test	208	0	0	0	0	0	3	0	0	0	0	0	0	0	72	0	0	0	0	0
2016	Spring	4/20/2016	POW	Test	211	0	0	0	0	1	0	0	0	0	0	0	0	2	48	0	0	0	0	0
2016	Spring	4/16/2016	RFF	Test	220	0	0	0	0	1	0	0	0	8	0	0	4	0	7	0	0	0	0	0
2016	Spring	4/16/2016	RIC	Test	204	0	0	1	0	0	1	0	0	3	0	0	0	3	72	0	0	0	0	0
2016	Spring	4/19/2016	ROC	Test	195	0	0	0	0	1	1	0	0	5	0	0	0	0	8	0	0	0	0	0
2016	Spring	4/16/2016	ROL	Test	220	0	0	0	0	0	0	0	0	0	0	0	3	0	77	0	0	0	0	0
2016	Spring	4/16/2016	RUT	Test	196	0	0	0	0	0	2	0	0	0	0	0	1	1	63	0	0	0	0	0
2016	Spring	4/17/2016	SPC	Test	193	0	0	0	0	0	0	0	0	0	0	0	3	0	52	0	0	0	0	0

Table B - 3 (cont'd). Benthic macroinvertebrate taxa count data, Spring 2016.

Year	Season	Date	Site	Site Type	Abundance	*Molophilus*	*Nemoura*	*Neophylax*	*Neoplasta*	*Nigronia*	*Ochrotrichia*	Oligochaeta	*Optioservus*	*Oulimnius*	*Paracapnia*	*Paraleptophlebia*	*Pedicia*	*Peltoperla*	*Plauditus*	*Polycentropus*	*Psephenus*	*Pteronarcys*	*Pycnopsyche*	*Remenus*
2016	Spring	4/16/2016	COP	Ref	182	0	0	1	1	0	0	0	16	0	0	0	0	0	0	1	0	3	0	0
2016	Spring	4/16/2016	CRO	Ref	189	0	0	0	5	0	0	0	14	0	0	0	0	0	0	0	2	1	0	0
2016	Spring	4/15/2016	EAS	Ref	215	0	0	0	1	0	0	0	23	0	0	1	0	2	0	0	0	7	0	2
2016	Spring	4/17/2016	HCN	Ref	194	0	0	0	0	0	0	6	5	0	0	0	1	0	0	0	0	2	0	0
2016	Spring	4/17/2016	MCB	Ref	181	0	0	0	3	0	0	0	18	0	0	3	0	1	0	0	0	0	0	0
2016	Spring	4/15/2016	BIR	Test	216	0	0	0	2	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/19/2016	CRA	Test	197	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
2016	Spring	4/16/2016	FRY	Test	192	0	0	0	0	0	0	7	12	0	0	0	1	0	0	0	3	2	0	0
2016	Spring	4/17/2016	GRA	Test	194	0	0	0	1	0	0	1	1	0	0	0	0	1	0	0	2	0	0	0
2016	Spring	4/17/2016	HUR	Test	204	0	0	0	3	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/15/2016	KEL	Test	194	0	0	0	2	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/15/2016	KUT	Test	204	0	0	0	1	0	11	7	0	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/16/2016	LAB	Test	195	0	0	0	0	1	0	5	11	0	0	2	0	0	0	0	1	0	0	0
2016	Spring	4/19/2016	LLC	Test	190	0	0	0	1	0	0	5	4	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/20/2016	LLE	Test	208	0	0	0	3	0	0	2	16	0	0	0	0	2	0	0	0	0	0	0
2016	Spring	4/20/2016	LLW	Test	186	0	0	0	8	0	0	4	31	0	0	0	0	5	0	0	0	0	0	0
2016	Spring	4/15/2016	MIL	Test	208	0	0	0	3	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0
2016	Spring	4/20/2016	POW	Test	211	0	0	0	4	0	1	0	22	0	0	0	0	0	0	0	0	1	0	0
2016	Spring	4/16/2016	RFF	Test	220	0	0	0	0	0	0	12	25	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/16/2016	RIC	Test	204	0	0	0	3	1	1	1	3	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/19/2016	ROC	Test	195	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/16/2016	ROL	Test	220	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/16/2016	RUT	Test	196	0	0	0	0	0	0	1	16	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/17/2016	SPC	Test	193	0	0	0	0	0	0	6	0	0	0	0	0	0	0	1	1	0	0	0

Table B - 3 (cont'd). Benthic macroinvertebrate taxa count data, Spring 2016.

Year	Season	Date	Site	Site Type	Abundance	*Rhamphomyia*	*Rhyacophila*	*Roederiodes*	*Sialis*	*Simulium*	Sisyridae	*Stenacron*	*Stenonema*	*Stylogomphus*	*Suwallia*	*Sweltsa*	*Taeniopteryx*	*Tallaperla*	*Tipula*	*Tipulidae*	*Wormaldia*	*Yugus*
2016	Spring	4/16/2016	COP	Ref	182	0	1	0	0	1	0	0	2	0	0	0	0	0	0	1	2	1
2016	Spring	4/16/2016	CRO	Ref	189	0	0	0	0	2	0	0	0	0	0	1	0	0	0	1	3	0
2016	Spring	4/15/2016	EAS	Ref	215	0	3	0	0	0	0	0	0	0	0	0	0	5	1	0	5	1
2016	Spring	4/17/2016	HCN	Ref	194	0	0	0	0	49	0	0	1	0	0	0	0	0	0	0	4	0
2016	Spring	4/17/2016	MCB	Ref	181	0	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0
2016	Spring	4/15/2016	BIR	Test	216	0	1	0	0	3	0	0	0	0	0	0	0	0	0	0	3	0
2016	Spring	4/19/2016	CRA	Test	197	0	2	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0
2016	Spring	4/16/2016	FRY	Test	192	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0
2016	Spring	4/17/2016	GRA	Test	194	0	1	0	0	2	0	0	0	0	0	0	0	1	1	0	7	0
2016	Spring	4/17/2016	HUR	Test	204	0	0	0	0	1	0	0	0	0	0	5	0	0	0	0	11	0
2016	Spring	4/15/2016	KEL	Test	194	0	1	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/15/2016	KUT	Test	204	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/16/2016	LAB	Test	195	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	3	0
2016	Spring	4/19/2016	LLC	Test	190	0	2	0	0	13	0	0	0	0	0	0	1	0	1	1	9	0
2016	Spring	4/20/2016	LLE	Test	208	0	1	1	0	3	0	0	0	0	0	0	0	16	0	1	0	1
2016	Spring	4/20/2016	LLW	Test	186	0	0	0	0	2	0	0	0	0	0	0	1	0	1	0	0	0
2016	Spring	4/15/2016	MIL	Test	208	0	1	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/20/2016	POW	Test	211	0	0	0	0	7	0	0	0	0	0	0	0	1	0	1	0	1
2016	Spring	4/16/2016	RFF	Test	220	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	5	0
2016	Spring	4/16/2016	RIC	Test	204	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	1	0
2016	Spring	4/19/2016	ROC	Test	195	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	13	0
2016	Spring	4/16/2016	ROL	Test	220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2016	Spring	4/16/2016	RUT	Test	196	0	1	0	0	2	0	0	0	0	0	0	0	0	0	1	1	0
2016	Spring	4/17/2016	SPC	Test	193	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	10	0

APPENDIX C – QUARTERLY WATER CHEMISTRY

Table C - 1. Quarterly water chemistry.

											Major Ions									Trace Elements‡					
				SC*	Temp	pH	D.O.	TDS†	Total Alkalinity	Total Hardness	Cl^-	SO_4^{2-}	CO_3^{2-}	HCO_3^-	Ca^{2+}	K^+	Mg^{2+}	Na^+	Sum of 8 Major Ions	Al	Cu	Fe	Mn	Se	Zn
Sampling Quarter	Date	Site	Site Type	μS/cm	°C	S.U.	mg/L	mg/L	mg/L as $CaCO_3$	mg/L as $CaCO_3$	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L
2015Q3	7/23/2015	COP	Ref	138	17.8	7.7	8.4	75.4	44.4	51.9	1.4	18.7	0	54.2	13.0	1.6	4.7	4.8	98.4	3.8	< 1	< 10	2.7	< 2.5	< 10
2015Q3	7/7/2015	CRO	Ref	66	19.5	7.6	8.2	< 42.8	19.7	22.5	1.6	6.8	0	24.0	4.7	1.6	2.6	2.5	43.9	6.4	< 1	39.4	3.4	< 2.5	< 10
2015Q3	7/23/2015	EAS	Ref	24	16.1	7.0	8.6	< 42.8	6.3	8.4	0.3	3.2	0	7.7	2.4	0.4	0.6	0.6	15.1	4.9	< 1	< 10	< 1	< 2.5	13.8
2015Q3	7/24/2015	HCN	Ref	62	18.2	7.2	8.3	45.6	18.1	17.8	0.9	8.1	0	22.1	3.8	1.6	2.0	3.5	42.1	4.0	< 1	61.4	7.8	< 2.5	< 10
2015Q3	7/8/2015	MCB	Ref	50	18.0	7.2	8.5	< 42.8	11.3	15.5	0.3	7.9	0	13.8	3.0	1.4	1.9	1.9	30.3	7.0	< 1	< 10	2.4	< 2.5	< 10
2015Q3	7/22/2015	BIR	Test	616	23.8	7.4	8.0	380.6	118.4	284.7	7.0	185.9	0	144.5	52.8	3.4	37.2	17.4	448.2	3.0	< 1	< 10	7.6	< 2.5	< 10
2015Q3	7/24/2015	CRA	Test	334	15.9	7.6	9.3	216.0	24.8	141.0	1.4	120.3	0	30.2	28.1	1.9	17.3	5.5	204.7	48.7	< 1	< 10	63.2	< 2.5	10.1
2015Q3	7/23/2015	FRY	Test	436	18.9	8.1	8.3	267.0	89.2	171.6	6.5	120.1	0	108.9	38.8	2.2	18.2	19.3	314.0	3.9	< 1	< 10	1.7	< 2.5	< 10
2015Q3	7/23/2015	GRA	Test	231	19.7	7.8	8.0	121.0	59.1	84.0	1.9	42.5	0	72.0	20.6	1.9	8.0	11.1	157.9	6.4	< 1	< 10	2.1	< 2.5	< 10
2015Q3	7/8/2015	HUR	Test	305	18.1	7.5	8.9	187.6	38.3	129.9	0.4	94.7	0	46.7	24.8	2.3	16.6	6.5	191.9	7.0	< 1	< 10	8.5	< 2.5	< 10
2015Q3	8/1/2015	KEL	Test					568.6	124.0	435.4	1.2	317.3	0	151.3	84.2	4.7	54.8	12.2	625.7	5.7	< 1	< 10	11.1	4.3	< 10
2015Q3	7/22/2015	KUT	Test	1014	15.4	8.1		721.4	150.9	487.9	0.9	406.4	0	184.1	97.4	6.0	59.6	31.1	785.5	5.0	< 1	< 10	4.8	8.8	< 10
2015Q3	7/7/2015	LAB	Test	648	15.6	7.8	8.9	409.4	112.2	271.7	1.4	205.4	0	136.9	60.7	3.5	29.3	28.0	465.1	4.9	< 1	< 10	7.5	4.7	< 10
2015Q3	7/24/2015	LLC	Test	1375	20.2	7.0	7.8	976.4		459.3	16.2	618.5			83.3	14.5	61.2	82.1		8.8	< 1	< 10	323.7	20.5	18.1
2015Q3	7/24/2015	LLE	Test	326	16.9	7.9	8.8	212.8	26.8	138.2	0.4	129.4	0	32.7	27.7	1.9	16.8	5.3	214.2	8.2	< 1	< 10	< 1	< 2.5	< 10
2015Q3	7/24/2015	LLW	Test	1152	16.6	8.2	9.2	880.2	135.6	666.1	1.3	541.2	0	165.5	121.5	5.6	88.4	10.2	933.6	7.8	< 1	< 10	2.1	3.6	< 10
2015Q3	8/9/2015	MIL	Test					443.6	138.9	351.8	0.9	224.5	0	169.5	75.3	4.6	39.9	12.6	527.2	3.4	< 1	< 10	17.2	< 2.5	< 10
2015Q3	8/1/2015	POW	Test					655.6	127.8	496.2	0.5	363.5	0	155.9	105.4	4.4	56.8	9.8	696.3	9.6	< 1	< 10	9.7	< 2.5	< 10
2015Q3	7/23/2015	RFF	Test	422	18.3	8.2	8.7	249.0	114.6	127.2	3.7	90.6	0	139.8	30.1	2.6	12.7	36.6	316.0	6.9	< 1	< 10	1.2	< 2.5	< 10
2015Q3	7/22/2015	RIC	Test	1532	20.2	8.2	8.3	1232.4	191.7	948.7	8.5	741.1	0	233.9	143.3	5.8	144.0	14.0	1290.6	7.3	< 1	< 10	11.9	< 2.5	< 10
2015Q3	7/24/2015	ROC	Test	838	18.9	8.3	8.8	580.2	194.0	456.3	1.4	260.2	0	236.6	66.2	6.1	70.9	5.5	647.0	12.9	< 1	< 10	14.8	25.2	< 10
2015Q3	7/23/2015	ROL	Test	852	18.1	7.8	8.1	591.0	100.4	413.5	2.1	21.1	0	122.4	89.1	3.5	46.5	18.4	303.1	5.6	< 1	< 10	4.4	< 2.5	< 10
2015Q3	7/22/2015	RUT	Test	532	20.6	8.1	7.9	343.0	79.9	255.5	4.5	169.3	0	97.5	38.6	3.0	38.8	5.6	357.3	7.1	< 1	< 10	4.8	< 2.5	48.8
2015Q3	7/8/2015	SPC	Test	375	17.9	7.9	8.6	207.2	120.8	112.4	5.0	61.7	0	147.4	28.4	1.8	10.1	34.9	289.3	5.4	< 1	56.8	4.8	< 2.5	< 10
2015Q4	10/21/2015	COP	Ref	158	10.1	7.9	8.2	82.4	61.2	59.7	1.5	13.8	0	74.7	15.7	1.6	5.0	6.7	119.0	3.3	< 1	19.5	1.8	< 2.5	19.4
2015Q4	10/21/2015	CRO	Ref	83	8.1	8.1	9.4	51.8	27.5	29.5	1.7	7.8	0	33.6	6.5	1.7	3.3	2.6	57.1	2.2	1.1	< 10	1.9	< 2.5	28.3
2015Q4	10/19/2015	EAS	Ref	24	9.4	8.2	9.7	< 42.8	6.8	8.8	0.3	3.3	0	8.3	2.6	0.4	0.6	0.6	16.1	5.4	< 1	26.9	1.5	< 2.5	29.4
2015Q4	10/22/2015	HCN	Ref	128	11.5	7.5	6.4		63.2	30.6	1.0	2.8	0	77.1	8.1	1.3	2.5	14.4	107.1	2.4	< 1	340.3	35.4	< 2.5	23.2
2015Q4	10/21/2015	MCB	Ref	56	9.5	7.6	8.2	< 42.8	18.9	19.3	0.3	6.7	0	23.0	4.0	1.6	2.3	2.1	40.0	3.7	1.0	11.3	1.4	< 2.5	22.4
2015Q4	10/20/2015	BIR	Test	685	9.1	8.0	9.1	442.0	146.3	311.6	8.3	191.5	0	178.5	62.8	4.0	37.7	18.5	501.4	3.6	< 1	30.6	8.4	< 2.5	19.9
2015Q4	10/27/2015	CRA	Test	520	11.9	8.0	8.9	356.8	26.6	237.5	1.3	222.3	0	32.4	50.5	2.6	27.1	5.6	341.8	7.8	< 1	< 10	10.2	< 2.5	15.9
2015Q4	10/20/2015	FRY	Test	519	9.6	8.2	9.0	320.4	110.4	211.4	7.3	131.8	0	134.7	50.7	2.4	20.7	22.9	370.4	2.1	< 1	< 10	< 1	< 2.5	14.3
2015Q4	10/22/2015	GRA	Test	332	8.8	7.9	9.0	187.4	75.5	114.4	2.5	91.3	0	92.1	29.5	1.9	9.9	19.4	246.7	2.3	< 1	< 10	5.1	< 2.5	14.4
2015Q4	10/22/2015	HUR	Test	460	10.8	7.9	8.6	294.2	45.0	201.6	0.6	170.4	0	54.9	40.5	2.6	24.5	7.9	301.4	1.7	< 1	< 10	2.8	< 2.5	12.9
2015Q4	10/19/2015	KEL	Test	807	7.5	8.1	10.6	569.4	118.7	408.4	1.5	308.8	0	144.8	86.9	4.4	46.6	11.4	604.3	4.1	< 1	58.1	11.3	< 2.5	30.2
2015Q4	10/19/2015	KUT	Test	1221	11.5	8.3	9.6	919.6	196.8	576.3	1.3	489.1	0.1	239.9	125.3	6.9	64.2	43.2	970.0	6.1	1.1	< 10	7.0	9.1	< 10
2015Q4	10/21/2015	LAB	Test	824	8.5	8.1	9.5	568.0	130.2	336.1	1.7	296.3	0	158.8	80.9	3.7	32.6	41.0	615.1	3.0	< 1	28.1	1.6	2.5	18.2
2015Q4	10/27/2015	LLC	Test	1457	10.9	8.0	9.4	1108.8	51.3	621.2	16.0	700.0	0	62.6	110.8	11.8	83.9	68.9	1054.0	3.4	< 1	< 10	5.8	5.9	14.1
2015Q4	10/27/2015	LLE	Test	1158	11.1	7.8	8.0	899.0	82.6	623.9	1.0	580.1	0	100.7	134.4	4.6	70.2	18.9	909.9	5.4	1.3	< 10	3.7	5.1	17.3
2015Q4	10/27/2015	LLW	Test	1315	11.4	7.9	8.1	1059.6	132.4	748.7	1.4	667.7	0	161.5	151.6	6.4	90.2	10.6	1089.3	4.2	< 1	< 10	6.6	< 2.5	16.4
2015Q4	10/19/2015	MIL	Test	819	8.6	8.3	10.1	563.2	163.9	411.6	1.1	279.7	0	200.0	95.0	4.4	42.5	14.9	637.4	3.5	< 1	64.4	10.6	< 2.5	22.4
2015Q4	10/20/2015	POW	Test	942	8.3	7.6	9.2	693.2	137.2	502.5	0.4	387.7	0	167.4	113.4	4.3	53.4	8.9	735.5	3.2	1.1	27.5	7.2	< 2.5	20.3
2015Q4	10/20/2015	RFF	Test	450	10.1	8.3	9.2	263.6	117.8	144.4	3.7	113.8	0	143.7	35.8	2.7	13.4	34.6	347.6	2.8	< 1	< 10	1.1	< 2.5	24.2
2015Q4	10/20/2015	RIC	Test	1814	8.0	8.2	10.0	1564.2	202.2	1065.5	10.1	948.7	0	246.6	172.0	5.7	155.0	14.2	1552.3	3.7	1.3	157.3	13.7	< 2.5	35.6
2015Q4	10/27/2015	ROC	Test	860	11.6	8.1	9.0	594.4	157.4	447.0	1.9	305.5	0	192.0	73.1	5.5	64.4	9.6	652.0	4.2	1.0	< 10	2.8	11.1	13.4
2015Q4	10/21/2015	ROL	Test	765	7.6	8.1	8.3	538.6	99.2	371.4	2.1	296.5	0	121.1	86.5	3.1	37.9	15.5	562.6	2.9	< 1	< 10	3.2	< 2.5	16.8
2015Q4	10/20/2015	RUT	Test	807	7.2	8.3	9.7	560.4	107.8	414.9	4.2	315.3	0	131.5	65.3	3.4	61.4	6.2	587.2	4.5	< 1	28.4	3.1	< 2.5	45.5
2015Q4	10/22/2015	SPC	Test	549	7.8	8.0	9.4	316.6	195.9	135.5	4.4	77.5	1.2	236.6	35.1	1.9	11.7	64.1	432.4	2.3	< 1	18.4	2.0	< 2.5	13.9

* specific conductance at 25 °C; † total dissolved solids; ‡ total dissolved concentrations

Table C - 1 (cont'd). Quarterly water chemistry.

											Major Ions									Trace Elements‡					
				SC*	Temp	pH	D.O.	TDS†	Total Alkalinity	Total Hardness	Cl^-	SO_4^{2-}	CO_3^{2-}	HCO_3^-	Ca^{2+}	K^+	Mg^{2+}	Na^+	Sum of 8 Major Ions	Al	Cu	Fe	Mn	Se	Zn
Sampling Quarter	Date	Site	Site Type	µS/cm	°C	S.U.	mg/L	mg/L	mg/L as $CaCO_3$	mg/L as $CaCO_3$	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
2016Q1	1/20/2016	COP	Ref	122		7.4		62.2	35.5	45.7	1.3	16.7	0	43.3	11.7	1.2	4.0	4.0	82.2	3.3	< 1	< 10	4.6	< 2.5	16.1
2016Q1	1/20/2016	CRO	Ref	62		7.2		< 42.8	13.1	20.6	2.7	10.2	0	16.0	4.5	1.2	2.3	2.4	39.4	8.8	< 1	< 10	1.2	< 2.5	12.1
2016Q1	1/18/2016	EAS	Ref	23	1.8	7.9		< 42.8	5.0	8.0	0.3	3.8	0	6.1	2.4	0.3	0.5	0.6	14.0	6.3	< 1	< 10	< 1	< 2.5	16.4
2016Q1	1/21/2016	HCN	Ref	56	0.3	7.9		< 42.8	10.9	15.9	1.1	9.5	0	13.2	3.5	1.1	1.7	2.6	32.8	7.5	< 1	54.7	4.3	< 2.5	14.2
2016Q1	1/20/2016	MCB	Ref	46		7.3		< 42.8	6.0	13.5	1.4	18.5	0	7.3	2.7	1.0	1.6	1.7	34.3	13.4	< 1	< 10	1.1	< 2.5	15.2
2016Q1	1/19/2016	BIR	Test	635		7.7		405.4	108.4	279.2	11.6	193.5	0	132.2	56.5	3.2	33.6	18.4	449.1	1.3	< 1	22.6	52.0	< 2.5	17.6
2016Q1	1/21/2016	CRA	Test	405	4.8	7.9		266.0	20.9	183.9	1.1	163.6	0	25.5	38.8	1.9	21.2	5.4	257.6	17.3	< 1	< 10	33.9	< 2.5	15.9
2016Q1	1/19/2016	FRY	Test	383		8.0		221.8	64.9	147.2	7.9	108.9	0	79.2	34.8	1.7	14.7	15.0	262.1	1.7	< 1	< 10	< 1	< 2.5	11.2
2016Q1	1/20/2016	GRA	Test	212	0.7	7.4		111.4	30.4	66.9	4.6	106.1	0	37.0	16.5	1.3	6.2	12.9	184.6	4.0	< 1	< 10	2.8	< 2.5	12.3
2016Q1	1/20/2016	HUR	Test	369		7.6		212.2	36.6	152.1	0.6	133.4	0	44.6	30.5	2.0	18.5	8.0	237.6	2.7	< 1	< 10	3.6	< 2.5	14.0
2016Q1	1/18/2016	KEL	Test	795	2.7	8.3		544.0	111.2	402.3	1.4	317.7	0	135.7	84.0	4.4	46.9	10.5	600.5	1.2	< 1	< 10	8.5	3.5	11.5
2016Q1	1/18/2016	KUT	Test	1049	7.7	8.2		766.0	159.1	518.8	1.3	424.3	0.4	193.3	111.8	6.1	58.4	26.6	822.1	2.7	< 1	< 10	6.6	10.1	77.6
2016Q1	1/19/2016	LAB	Test	601	1.5	8.0		384.6	100.4	237.6	1.4	184.3	0	122.5	56.5	3.0	23.5	25.4	416.6	2.5	< 1	< 10	9.8	3.4	15.4
2016Q1	1/21/2016	LLC	Test	1254		7.6		871.0	34.2	487.1	16.8	543.9	0	41.7	87.6	9.8	65.4	60.7	825.9	3.7	< 1	< 10	5.3	8.5	13.3
2016Q1	1/21/2016	LLE	Test	486	0.3	7.5		292.0	27.9	218.4	0.6	194.4	0	34.1	46.6	1.9	24.9	7.1	309.5	4.0	< 1	< 10	< 1	< 2.5	< 10
2016Q1	1/21/2016	LLW	Test	883	2.2	8.0		851.8	106.5	610.0	0.9	523.4	0	130.0	121.9	4.7	74.4	8.6	863.8	2.8	< 1	< 10	< 1	< 2.5	14.5
2016Q1	1/18/2016	MIL	Test	685		8.1		437.2	125.0	332.7	0.6	219.6	0	152.5	73.5	3.5	36.3	10.6	496.5	1.0	< 1	< 10	19.2	< 2.5	13.4
2016Q1	1/18/2016	POW	Test	910	0.3	8.0		668.0	129.9	485.2	1.0	374.4	0	158.4	107.6	3.8	52.7	9.1	707.0	1.6	< 1	< 10	11.2	< 2.5	11.4
2016Q1	1/19/2016	RFF	Test	514	0.2	8.3		296.8	153.6	137.4	2.1	64.5	1.1	185.1	34.2	2.5	12.6	50.1	352.1	3.5	< 1	< 10	18.7	< 2.5	14.6
2016Q1	1/19/2016	RIC	Test	1579		7.9		1254.2	173.7	903.7	8.0	783.4	0	211.9	147.3	5.0	130.6	12.3	1298.6	1.7	< 1	< 10	11.9	< 2.5	12.8
2016Q1	1/21/2016	ROC	Test	712	0.3	7.9		463.0	123.9	355.9	14.4	236.7	0	151.2	57.8	3.9	51.6	6.8	522.4	2.8	< 1	< 10	< 1	11.5	15.5
2016Q1	1/19/2016	ROL	Test	763		7.9		479.6	87.0	349.1	2.2	288.0	0	106.1	80.0	2.5	36.4	17.2	532.4	1.6	< 1	< 10	4.1	< 2.5	12.6
2016Q1	1/19/2016	RUT	Test	543		7.7		341.6	60.3	256.0	4.8	190.9	0	73.5	41.1	2.5	37.4	5.6	355.8	2.2	< 1	< 10	1.0	< 2.5	14.6
2016Q1	1/20/2016	SPC	Test	275	0.1	7.6		137.2	65.0	81.2	4.4	52.7	0	79.4	20.4	1.4	7.3	18.6	184.2	7.8	< 1	40.7	32.4	< 2.5	14.2
2016Q2	4/16/2016	COP	Ref	138	13.5	7.8	10.1	47.4	43.9	50.5	1.5	18.4	0	53.5	12.9	1.5	4.5	4.9	97.2	4.9	< 1	48.4	2.5	< 2.5	14.4
2016Q2	4/16/2016	CRO	Ref	66	14.6	7.9	9.6	< 42.8	16.8	20.8	2.8	9.1	0	20.5	4.5	1.7	2.4	2.8	43.8	6.0	< 1	25.3	2.2	< 2.5	13.8
2016Q2	4/15/2016	EAS	Ref	24	10.4	7.4	10.7	< 42.8	3.1	8.6	0.4	3.7	0	3.8	2.5	0.5	0.6	0.6	12.1	4.0	< 1	< 10	2.0	< 2.5	14.3
2016Q2	4/17/2016	HCN	Ref	59	15.6	7.6	10.3	< 42.8	12.1	15.8	1.3	10.2	0	14.8	3.4	1.4	1.7	3.2	36.0	3.6	< 1	65.8	4.4	< 2.5	11.9
2016Q2	4/17/2016	MCB	Ref	49	12.0	7.5	10.9	< 42.8	11.4	14.1	0.5	10.3	0	13.9	2.7	1.2	1.8	1.9	32.4	4.6	< 1	51.6	1.2	< 2.5	15.8
2016Q2	4/15/2016	BIR	Test	657	15.4	8.1	9.0	422.2		303.7	11.2	210.1			57.9	3.4	38.8	17.7		2.3	< 1	109.3	4.6	< 2.5	21.2
2016Q2	4/19/2016	CRA	Test	471	14.4	7.8	9.9	281.4	20.6	184.3	1.1	192.2	0	25.1	37.4	2.1	22.2	5.6	285.7	30.2	< 1	< 10	20.5	< 2.5	24.1
2016Q2	4/16/2016	FRY	Test	403	14.4	8.4	10.2	258.0	76.8	153.5	6.7	101.8	0	93.7	35.5	2.0	15.8	17.5	273.0	2.2	< 1	< 10	1.7	< 2.5	< 10
2016Q2	4/17/2016	GRA	Test	243	9.6	7.9	11.4	131.6	40.8	78.5	2.3	74.0	0	49.8	18.9	1.5	7.7	14.2	168.4	3.0	< 1	< 10	2.2	< 2.5	< 10
2016Q2	4/17/2016	HUR	Test	377	17.3	7.7	9.3	226.0	37.0	153.5	0.6	150.0	0	45.1	30.1	2.5	19.1	9.0	256.5	10.9	< 1	19.6	6.5	< 2.5	11.5
2016Q2	4/15/2016	KEL	Test	757	10.4	8.2	10.5	545.0	106.0	376.7	1.2	294.6	0	129.3	75.2	4.2	46.0	10.4	561.0	2.4	< 1	38.8	5.1	3.7	19.7
2016Q2	4/15/2016	KUT	Test	996	13.4	8.3	10.4	733.8	154.2	468.9	0.9	407.0	0	188.1	94.3	6.0	56.9	30.4	783.7	2.7	< 1	< 10	4.0	9.1	< 10
2016Q2	4/16/2016	LAB	Test	620	14.4	8.3	10.1	410.2	108.2	249.3	1.5	205.1	0	132.1	57.3	3.3	25.9	28.4	453.5	7.0	< 1	86.3	8.3	4.8	14.3
2016Q2	4/19/2016	LLC	Test	1065	16.0	7.9	10.1	803.8	48.3	441.8	7.7	505.6	0	59.0	77.8	8.2	60.3	39.0	757.6	44.4	2.6	4999	31.5	6.3	721.5
2016Q2	4/20/2016	LLE	Test	491	11.7	7.9	10.5	329.2	36.0	223.2	0.6	206.5	0	43.9	45.2	2.2	26.9	7.8	333.0	4.5	1.3	24.1	< 1	< 2.5	< 10
2016Q2	4/20/2016	LLW	Test	1092	11.3	8.1	10.8	849.0	110.8	598.4	0.8	542.1	0	135.2	113.4	4.9	76.8	9.3	882.5	3.5	< 1	18.0	2.7	2.9	19.9
2016Q2	4/15/2016	MIL	Test	669	15.6	8.3	9.7	443.2	129.6	325.2	0.9	241.9	0	158.1	69.1	3.8	37.2	12.0	523.0	2.2	< 1	39.5	7.5	< 2.5	189.1
2016Q2	4/20/2016	POW	Test	900	17.2	8.0	9.1	689.6	122.0	471.9	0.6	375.9	0	148.9	101.2	4.0	53.4	10.4	694.4	5.5	< 1	419.6	6.7	< 2.5	31.9
2016Q2	4/16/2016	RFF	Test	587	15.3	8.5	10.5	373.4	154.8	168.7	6.9	145.9	0	188.9	40.6	2.8	16.4	53.4	455.0	20.4	< 1	< 10	2.8	< 2.5	< 10
2016Q2	4/16/2016	RIC	Test	1544	8.1	8.0	11.3	1295.4	157.5	922.4	6.5	827.5	0	192.2	137.4	5.2	141.2	12.9	1322.9	4.4	1.4	< 10	9.8	< 2.5	12.1
2016Q2	4/19/2016	ROC	Test	684	17.3	8.5	10.1	502.8	121.1	346.8	1.4	234.7	0	147.7	53.5	4.6	52.0	7.9	501.8	8.1	< 1	< 10	< 1	11.8	12.1
2016Q2	4/16/2016	ROL	Test	740	10.6	8.1	11.2	498.6	87.8	344.7	1.8	295.0	0	107.1	76.4	2.8	37.5	18.6	539.1	4.2	< 1	< 10	2.2	< 2.5	11.9
2016Q2	4/16/2016	RUT	Test	600	7.9	8.0	11.6	397.6	74.0	293.8	0.5	220.1	0	90.3	44.3	2.7	44.7	6.0	408.6	5.6	< 1	120.3	2.8	< 2.5	14.7
2016Q2	4/17/2016	SPC	Test	341	8.4	8.0	11.6	177.8	95.7	100.9	4.6	71.5	0	116.7	25.0	1.6	9.4	27.4	256.2	5.4	< 1	62.9	8.5	< 2.5	10.8

* specific conductance at 25 °C; † total dissolved solids; ‡ total dissolved concentrations

Table C - 1 (cont'd). Quarterly water chemistry.

Sampling Quarter	Date	Site	Site Type	SC* (µS/cm)	Temp (°C)	pH (S.U.)	D.O. (mg/L)	TDS† (mg/L)	Total Alkalinity (mg/L as $CaCO_3$)	Total Hardness (mg/L as $CaCO_3$)	Major Ions: 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)	Sum of 8 Major Ions (mg/L)	Trace Elements‡: Al (µg/L)	Cu (µg/L)	Fe (µg/L)	Mn (µg/L)	Se (µg/L)	Zn (µg/L)
2016Q3	8/16/2016	COP	Ref	140	18.9	7.6	7.9	125.0	59.2	58.3	1.6	16.2	0	72.2	14.3	1.6	5.5	6.3	117.7	4.5	1.2	27.7	2.4	< 2.5	10.5
2016Q3	8/16/2016	CRO	Ref	84	20.4	7.6	7.0	< 42.8	29.8	29.9	2.6	5.5	0	36.3	6.1	1.9	3.6	3.1	59.0	5.4	2.5	30.3	3.3	< 2.5	17.2
2016Q3	8/15/2016	EAS	Ref	29	18.8	7.4	7.9		9.0	11.6	0.3	2.8	0	11.0	3.1	0.6	0.9	0.7	19.4	16.9	18.7	717.7	5.2	< 2.5	54.7
2016Q3	8/17/2016	HCN	Ref	61	20.1	7.5	8.1	149.0	18.6	18.3	0.7	7.8	0	22.6	3.8	1.7	2.1	3.2	42.0	6.3	< 1	59.2	8.3	< 2.5	12.6
2016Q3	8/16/2016	MCB	Ref	59	22.3	7.2	6.2	103.4	21.8	20.2	0.4	6.0	0	26.6	3.8	1.7	2.6	2.2	43.1	9.5	1.7	26.4	5.5	< 2.5	15.8
2016Q3	8/15/2016	BIR	Test	596	23.1	8.0	7.2	333.2	121.5	259.0	8.8	157.7	0	148.2	48.9	3.4	33.4	17.9	418.4	4.2	1.1	< 10	3.9	< 2.5	15.0
2016Q3	8/17/2016	CRA	Test	429	18.2	7.5	8.6	278.8	25.3	186.9	0.9	186.6	0	30.9	37.0	2.2	23.0	5.6	286.2	23.1	2.1	17.6	9.2	< 2.5	17.6
2016Q3	8/16/2016	FRY	Test	530	19.8	8.0	8.0	322.8	105.9	212.8	8.3	160.8	0	129.2	48.3	2.5	22.5	21.5	393.0	3.8	2.5	< 10	1.6	< 2.5	12.1
2016Q3	8/16/2016	GRA	Test	340	21.4	7.4	7.4	201.8	68.4	111.0	2.2	93.1	0	83.4	27.4	2.2	10.4	20.4	239.0	5.6	1.1	< 10	4.6	< 2.5	13.8
2016Q3	8/17/2016	HUR	Test	466	19.9	7.7	7.8	338.2	53.6	201.4	0.5	186.5	0	65.4	38.1	2.8	25.9	10.3	329.4	3.9	< 1	15.6	12.0	< 2.5	13.0
2016Q3	8/15/2016	KEL	Test	809	17.0	7.9	8.7		115.7	408.4	1.3	320.7	0	141.2	80.3	4.2	50.7	12.7	610.9	1.7	< 1	< 10	9.6	< 2.5	11.8
2016Q3	8/15/2016	KUT	Test	1194	15.0	7.8	9.5	924.6	193.3	563.0	1.2	514.1	1.0	233.9	111.5	6.4	69.3	47.5	984.8	4.2	1.8	34.6	7.0	4.6	13.4
2016Q3	8/16/2016	LAB	Test	633	16.0	7.7	8.7	419.8	118.7	252.4	1.1	202.0	0	144.8	56.9	3.3	26.9	28.2	463.1	6.6	5.7	31.5	7.4	< 2.5	23.1
2016Q3	8/17/2016	LLC	Test	1459	21.9	7.6	8.2	1155.8	58.2	608.7	21.0	709.0	0	71.0	99.3	10.8	87.9	83.5	1082.5	7.0	1.7	< 10	2.7	2.7	14.8
2016Q3	8/17/2016	LLE	Test	621	19.8	7.6	8.1	421.0	50.1	289.8	0.6	267.6	0	61.1	58.1	2.9	35.3	10.3	435.7	10.5	1.4	12.6	1.5	< 2.5	15.0
2016Q3	8/17/2016	LLW	Test	1228	18.1	7.8	8.8	1011.6	138.2	677.0	0.5	618.5	0	168.6	124.0	5.6	89.5	10.4	1017.1	14.6	3.9	42.5	2.4	< 2.5	25.1
2016Q3	8/15/2016	MIL	Test	568	20.9	8.1	8.1	431.2	122.5	271.6	0.6	167.5	0	149.5	60.1	3.9	29.6	8.5	419.8	3.5	< 1	< 10	16.1	< 2.5	12.3
2016Q3	8/15/2016	POW	Test	866	20.1	7.9	7.4	1168.8	139.7	505.0	0.5	405.9	0	170.4	105.3	4.4	59.0	9.0	754.6	6.6	1.4	< 10	7.6	< 2.5	12.3
2016Q3	8/16/2016	RFF	Test	631	19.5	8.1	8.4		163.3	198.8	5.9	152.9	0	199.2	47.0	3.2	19.8	56.2	484.3	8.1	3.3	32.8	1.8	< 2.5	18.6
2016Q3	8/15/2016	RIC	Test	1776	20.8	8.0	7.9	1449.2	220.9	1061.8	11.3	949.5	1.4	266.7	151.5	6.4	166.6	15.5	1568.9	15.9	22.7	201.3	14.6	< 2.5	40.0
2016Q3	8/17/2016	ROC	Test	889	21.8	7.8	8.0	666.6	190.5	477.1	19.4	255.1	0	232.4	68.1	5.9	74.9	8.4	664.1	10.5	1.7	< 10	1.2	9.5	14.1
2016Q3	8/16/2016	ROL	Test	738	18.2	7.8	8.1	497.2	100.7	344.9	2.1	294.5	0	122.8	75.0	3.1	38.4	18.1	554.0	6.8	4.4	25.6	4.1	< 2.5	31.7
2016Q3	8/15/2016	RUT	Test	646	21.0	8.0	7.8	445.6	89.6	324.1	4.3	233.1	0	109.3	48.8	3.4	49.3	6.2	454.3	6.8	3.9	58.8	6.8	< 2.5	25.0
2016Q3	8/16/2016	SPC	Test	552	21.2	7.8	7.6	319.6	175.8	140.0	6.1	106.7	0.5	213.5	34.4	2.1	13.2	60.0	436.4	7.7	1.9	41.0	4.3	< 2.5	16.9
2016Q4	10/18/2016	COP	Ref	196	14.3	7.8	7.0	85.0	89.3	72.9	1.7	7.8	0	109.0	19.1	1.8	6.2	11.8	157.3	5.0	< 1	< 10	4.5	< 2.5	23.5
2016Q4	10/18/2016	CRO	Ref	86	13.8	8.2	7.1	< 42.8	32.6	32.6	1.4	5.0	0	39.8	7.0	1.9	3.7	3.0	61.8	4.4	< 1	< 10	6.4	< 2.5	15.8
2016Q4	10/17/2016	EAS	Ref	47	14.6	8.3	5.0	< 42.8	13.0	12.9	0.6	2.1	0	15.9	3.8	0.5	0.9	0.8	24.6	3.3	< 1	< 10	3.0	< 2.5	16.4
2016Q4	10/19/2016	HCN	Ref	131	13.3	7.4	5.5	45.7	62.4	30.8	0.9	2.4	0	76.2	7.9	1.3	2.7	15.0	106.3	2.4	< 1	223.1	41.0	< 2.5	14.8
2016Q4	10/18/2016	MCB	Ref	66	15.6	7.1	5.7	< 42.8	22.8	21.8	0.3	4.4	0	27.8	4.4	2.0	2.6	2.4	43.9	9.9	3.1	21.4	3.0	< 2.5	19.3
2016Q4	10/17/2016	BIR	Test	759	15.2	7.8	7.9	487.8	161.0	379.1	1.4	190.2	0	196.5	74.0	4.5	47.3	19.8	533.8	5.8	1.0	< 10	6.2	< 2.5	22.2
2016Q4	10/19/2016	CRA	Test	485	16.2	7.4	8.5	398.6	21.9	226.7	0.8	186.4	0	26.7	47.5	2.4	26.3	6.0	296.2	21.7	< 1	19.8	12.1	< 2.5	17.5
2016Q4	10/18/2016	FRY	Test	689	13.4	8.0	9.0	422.6	122.2	299.1	8.6	178.8	0	149.1	71.0	3.0	29.7	30.5	470.7	4.7	< 1	< 10	2.3	< 2.5	15.1
2016Q4	10/18/2016	GRA	Test	385	15.3	7.7	7.7	192.8	79.4	129.4	2.3	94.9	0	96.9	33.4	2.4	11.2	27.0	268.1	4.2	1.1	< 10	8.8	< 2.5	20.0
2016Q4	10/19/2016	HUR	Test	455	14.6	7.5	8.1	352.2	51.5	210.6	0.6	154.8	0	62.8	41.8	2.9	25.9	8.3	297.1	6.0	1.6	15.1	7.7	< 2.5	15.8
2016Q4	10/17/2016	KEL	Test	836	12.2	8.2	9.5	611.2	119.2	443.1	1.6	288.0	0	145.5	92.2	4.4	51.8	13.3	596.8	5.8	1.4	< 10	8.6	< 2.5	15.7
2016Q4	10/17/2016	KUT	Test	1274	12.8	8.0	9.4	923.2	211.6	622.4	1.1	471.8	0.5	257.1	130.8	7.1	72.1	59.1	999.5	10.3	2.8	33.8	8.8	9.5	23.3
2016Q4	10/18/2016	LAB	Test	835	12.4	7.8	8.5	564.8	142.3	349.5	4.0	258.0	0	173.6	83.3	3.9	34.4	47.0	604.3	5.7	1.2	< 10	4.4	< 2.5	17.2
2016Q4	10/19/2016	LLC	Test	1644	17.6	7.6	8.0	1365.0	87.0	766.3	27.5	731.4	0	106.1	126.3	12.6	109.9	87.5	1201.3	9.4	1.6	< 10	4.9	13.6	15.2
2016Q4	10/19/2016	LLE	Test	1009	14.8	7.6	9.2	831.8	77.4	542.9	0.9	413.1	0	94.4	115.4	3.9	62.1	17.0	706.8	9.5	< 1	< 10	2.1	< 2.5	14.7
2016Q4	10/19/2016	LLW	Test	1323	14.7	7.6	9.5	1110.8	141.5	781.4	1.0	566.9	0	172.6	152.5	6.1	97.6	11.0	1007.8	6.8	1.1	85.2	2.8	< 2.5	13.6
2016Q4	10/17/2016	MIL	Test	810	13.9	7.9	9.0	521.0	162.7	427.4	0.8	267.8	0	198.5	96.5	4.6	45.4	16.5	630.0	5.0	1.1	19.0	10.8	< 2.5	16.1
2016Q4	10/18/2016	POW	Test	1082	14.0	7.9	8.2	815.8	161.2	631.3	0.5	399.4	0	196.7	136.6	4.8	70.7	11.9	820.5	4.3	< 1	< 10	4.2	< 2.5	14.8
2016Q4	10/18/2016	RFF	Test	647	13.6	7.9	9.3	382.0	165.1	220.8	5.7	138.2	0	201.5	53.9	3.3	21.0	52.8	476.4	4.5	< 1	< 10	1.9	< 2.5	16.1
2016Q4	10/17/2016	RIC	Test	1979	15.0	7.9	8.1	1729.0	221.8	1292.9	8.2	947.2	0	270.6	195.4	7.2	196.2	17.1	1641.9	4.8	1.5	< 10	13.2	< 2.5	20.8
2016Q4	10/19/2016	ROC	Test	856	17.1	7.6	8.6	652.8	170.4	467.1	1.8	249.8	0	207.9	73.6	5.6	69.1	9.0	616.8	8.8	1.2	< 10	1.8	11.1	16.0
2016Q4	10/17/2016	RUT	Test	897	14.3	7.9	8.5	672.2	109.6	499.3	4.1	309.3	0	133.8	76.9	4.0	74.9	6.8	609.7	7.6	1.1	< 10	11.2	< 2.5	16.2
2016Q4	10/18/2016	SPC	Test	586	15.5	7.9	8.4	349.2	217.1	129.6	4.0	66.9	1.9	260.9	33.2	2.2	11.4	81.0	461.5	4.5	< 1	13.3	2.9	< 2.5	16.4

* specific conductance at 25 °C; † total dissolved solids; ‡ total dissolved concentrations

Table C - 1 (cont'd). Quarterly water chemistry.

Sampling Quarter	Date	Site	Site Type	SC*	Temp	pH	D.O.	TDS†	Total Alkalinity	Total Hardness	Major Ions Cl^-	SO_4^{2-}	CO_3^{2-}	HCO_3^-	Ca^{2+}	K^+	Mg^{2+}	Na^+	Sum of 8 Major Ions	Trace Elements‡ Al	Cu	Fe	Mn	Se	Zn
				µS/cm	°C	S.U.	mg/L	mg/L	mg/L as $CaCO_3$	mg/L as $CaCO_3$	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Final	11/28/2016	COP	Ref	199	8.5	8.1	10.4	127.0	83.2	66.0	1.6	6.3	0	101.5	17.3	1.8	5.5	11.2	145.3	6.9	< 1	< 10	2.8	< 2.5	11.2
Final	11/28/2016	CRO	Ref	80	8.1	8.3	8.6	64.6	25.6	28.9			0	31.2	6.1	3.1	3.3	2.6		9.8	2.3	29.1	7.9	< 2.5	19.8
Final	11/28/2016	EAS	Ref	40	7.3	9.2	9.2	< 42.8	10.5	11.3	0.4	3.6	0	12.8	3.3	0.6	0.8	1.1	22.5	4.7	1.1	< 10	2.3	< 2.5	15.1
Final	11/28/2016	HCN	Ref	105	10.9	8.0	5.6	92.3	41.4	25.9	0.7	6.2	0	50.5	6.6	1.5	2.3	9.8	77.5	4.2	< 1	109.9	21.7	< 2.5	13.6
Final	11/28/2016	MCB	Ref	67	8.3	8.5	9.1	60.8	19.8	19.0	0.4	4.3	0	24.2	3.8	2.2	2.3	2.2	39.3	12.7	< 1	38.0	3.9	< 2.5	12.4
Final	11/28/2016	BIR	Test	785	6.9	9.8	9.7	516.8	153.7	374.7	7.4	195.4	0	187.5	70.7	4.5	48.3	21.2	534.9	9.7	2.5	20.7	12.9	< 2.5	17.6
Final	11/28/2016	CRA	Test	538	11.3	7.9	10.2	362.8	22.2	245.8	0.8	196.3	0	27.1	50.9	2.3	28.9	5.8	312.2	10.4	< 1	< 10	7.3	< 2.5	17.9
Final	11/28/2016	FRY	Test	613	5.8	8.3	9.3	403.4	123.2	252.0	7.2	144.2	0	150.3	58.8	2.3	25.6	33.0	421.4	3.2	1.0	< 10	1.1	< 2.5	13.1
Final	11/28/2016	GRA	Test	309	8.5	8.1	7.8	206.6	62.7	107.7	2.2	71.3	0	76.5	27.5	2.2	9.5	19.7	208.9	7.2	2.2	< 10	6.3	< 2.5	18.4
Final	11/28/2016	HUR	Test	389	10.9	8.1	10.8	292.6	40.1	172.5	0.7	131.2	0	49.0	33.7	3.3	21.6	7.9	247.3	7.1	1.6	< 10	10.1	< 2.5	16.6
Final	11/28/2016	KEL	Test	870	5.0			632.2	111.3	446.9	1.6	284.5	0	135.8	91.5	4.3	53.2	13.5	584.3	3.4	1.6	< 10	6.3	< 2.5	19.1
Final	11/28/2016	KUT	Test	1353	8.4			1025.6	201.4	621.4	1.1	471.8	0	245.7	129.2	6.8	72.8	60.6	987.9	3.2	1.1	33.3	8.8	9.0	13.4
Final	11/28/2016	LAB	Test	850	7.2	8.3	9.1	629.8	132.3	346.4	1.3	262.7	0	161.4	81.5	3.6	34.8	49.2	594.4	4.4	3.0	< 10	2.9	< 2.5	15.0
Final	11/28/2016	LLC	Test	1370	8.5	8.0	10.3	1067.8	58.8	638.1	15.7	456.1	0	71.7	111.5	10.0	87.7	67.6	820.2	4.4	< 1	< 10	2.0	6.3	14.7
Final	11/28/2016	LLE	Test	660	9.6	8.1	6.8	499.0	43.3	330.7		246.8	0	52.9	69.6	2.9	38.2	10.8		8.8	< 1	< 10	1.5	< 2.5	11.1
Final	11/28/2016	LLW	Test	998	10.2	8.1	10.1	770.2	104.9	563.7	0.9	386.8	0	128.0	109.5	5.1	70.7	8.4	709.5	8.9	< 1	< 10	2.5	< 2.5	15.8
Final	11/28/2016	MIL	Test	911	4.8			646.8	166.0	459.4	0.8	267.8	0	202.6	101.3	4.0	50.3	18.9	645.6	3.1	1.3	12.5	11.7	< 2.5	14.5
Final	11/28/2016	POW	Test	232	6.7			931.8	159.0	690.4	0.5	441.9	0	193.9	144.8	4.3	80.1	12.5	877.9	2.9	< 1	< 10	2.7	< 2.5	14.0
Final	11/28/2016	RFF	Test	570	6.1	8.4	10.3	370.6	128.0	207.0	4.6	139.9	0	156.2	49.1	2.8	20.5	41.5	414.5	3.1	< 1	< 10	1.7	< 2.5	12.1
Final	11/28/2016	RIC	Test	1857	5.1	8.8	12.0	1677.4	195.5	1236.8	7.7	934.5	0	238.5	190.5	6.0	185.5	16.4	1579.0	3.5	1.7	11.8	8.6	< 2.5	15.7
Final	11/28/2016	ROC	Test	780	9.8	8.1	6.8	539.2	138.9	419.9	0.6	230.7	0	169.5	66.6	4.8	61.8	8.9	542.9	5.7	< 1	< 10	2.1	9.9	13.3
Final	11/28/2016	RUT	Test	911	4.9	8.8	11.3		98.0	502.8	3.5	341.5	0	119.5	75.1	3.7	76.9	6.7	626.8	4.3	1.8	< 10	3.2	< 2.5	16.4
Final	11/28/2016	SPC	Test	502	8.8	8.2	6.7	326.0	180.8	108.0	4.2	64.3	0.3	220.0	27.5	2.0	9.5	69.6	397.5	8.8	3.2	32.3	4.6	< 2.5	15.6

* specific conductance at 25 °C; † total dissolved solids; ‡ total dissolved concentrations