Selenium Dynamics in Headwater Streams of the Central Appalachian Coalfields: An Investigation of Enrichment and Bioaccumulation

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ABSTRACT (Academic)

Selenium (Se) contamination in the Appalachian coalfields is a primary alteration to stream ecosystems caused by surface coal-mining. Selenium dynamics are complex and largely controlled by site-specific factors. We evaluated the degree and dynamics of Se enrichment and bioaccumulation in headwater streams influenced by coal-mining in central Appalachia. Based on Se concentrations in benthic macroinvertebrates collected from 23 headwater streams, nine sites were selected for further study: three reference streams with no history of coal-mining, and six streams influenced by coal mining. Mining-influenced streams were further separated into “high-Se” and “low-Se” categories based on macroinvertebrate tissue Se concentrations. Water-column, sediment, biofilm, leaf detritus, and prey and predator macroinvertebrates were collected and analyzed for Se concentration during two sample periods, Sept. - Oct. 2015 and Feb. - March 2016. Enrichment and trophic transfer factors describing Se dynamics were quantified using media Se concentrations. Selenium concentrations in all media were elevated in mining-influenced streams compared with reference streams and in high-Se streams over compared with low-Se streams. Selenium bioaccumulation processes did not exhibit major differences among streams of differing Se levels. Study results quantify Se dynamics in headwater streams, providing insight into the relationship between Se concentrations in the water column and in macroinvertebrate tissue samples. Findings from this study indicate headwater streams influenced by coal-mining play a significant role in the introduction of elevated Se concentrations into the aquatic food-chain.

ABSTRACT (Public)

Surface coal-mining is a source of selenium (Se) contamination in streams of the Appalachian coalfields. Selenium dynamics in aquatic systems are complex and largely controlled by site-specific factors, but have been understudied in Appalachian headwater streams. In this study, we evaluated the degree and dynamics of Se enrichment and bioaccumulation in headwater streams influenced by coal-mining. Based on Se concentrations in macroinvertebrates collected from 23 headwater streams, nine sites were selected for further study: three reference streams with no history of coal-mining, and six streams influenced by coal mining. Mining-influenced streams were further separated into “high-Se” and “low-Se” streams based on macroinvertebrate tissue Se concentrations. Water-column, sediment, biofilm, leaf detritus, and prey and predator macroinvertebrates were collected and analyzed for Se concentration during two sample periods, Sept. - Oct. 2015 and Feb.-March 2016. Selenium concentrations in all media were found to be elevated in mining-influenced over reference streams and in high-Se over low-Se streams. Selenium dynamics, enrichment in particulate media (sediment, biofilm and leaf detritus) and trophic transfer of Se to prey from particulate media and to predators from prey, did not exhibit major differences among streams of differing Se levels. Water column Se concentrations were predicative of Se concentrations in macroinvertebrate tissues. Findings from this study indicate headwater streams influenced by coal-mining are capable of a high degree of Se bioaccumulation in macroinvertebrate populations.

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CHAPTER 1. INTRODUCTION

Background

Surface coal-mining activities have altered the landscape of central Appalachia extensively and are the primary cause of land-conversion from forested woodland to reclaimed-mine sites (Sayler 2008). Within the central Appalachians' ecoregion, which is approximately 59,800 km^2 in size, 2,620 km^2 of forest land was converted to mined land from 1973 to 2000 (Sayler 2008). Within the coalfield region of Virginia, 8% of land cover was estimated to have been altered by mining practices (Li et al. 2015). Mining consequences are cumulative, and though the land-conversion rate of active coal-mining sites has declined in some areas, total land conversion continues to increase (Townsend et al. 2009).

Large-scale land conversion by mining has long-lasting effects on water resources (Pond et al. 2014; Negley & Eshleman 2006). Headwater streams, constituting 70-80% of surface-water stream length within the region, are severely impacted by mining activities (USEPA 2011). Ecosystem changes from coal mining alter headwater stream aquatic communities (Pond et al. 2008, 2014) and may alter the role of headwater streams as major contributors to nutrient cycling and carbon processing within larger river networks (Palmer et al. 2010; Meyer et al. 2007). However, mining effects on such ecosystem processes have not been well studied.

Surface coal-mining influences in headwater streams

Through the process of surface coal-mining, rock layers are removed to expose underlying coal-seams. Mining-disturbed rock, or overburden, is replaced on mined sites to restore the approximate original contour of the landscape, and excess overburden is often disposed of in adjacent valleys as valley fills (Palmer et al. 2010). Mining processes remove springs and both ephemeral and intermittent streams, and expose a surface of largely impermeable bedrock that is subsequently covered with overburden. Surplus overburden may cover additional ephemeral, intermittent, and permanent headwater streams. In central Appalachia, 1,944 km of headwater streams were estimated to be directly impacted by valley-fill burial from 1992 to 2002, a number that has increased with additional mining permits (USEPA 2011). Beyond direct stream loss, mining practices alter stream hydrology through watershed deforestation and soil compaction increasing downstream flow magnitudes and, in some cases, potential for flooding (Palmer et al. 2010; Negley and Eshleman 2006).

Stream chemistry is affected when water and oxygen come into contact with recently fractured and disturbed overburden material, causing geochemical reactions that convert major ions and trace elements into dissolved forms that can be transported into receiving streams. Effluent waters may contain higher concentrations of major ions and trace elements than would naturally occur in undisturbed systems. In streams, heightened concentrations of common ions such as Ca^{2+}, Mg^{2+}, SO_4^{2-}, and HCO_3^- which correspond with elevated total dissolved solids (TDS)

and specific conductance, are associated with shifts in benthic macroinvertebrate assemblages to more tolerant taxa (Timpano et al. 2015; USEPA 2011, Pond et al. 2008).

Selenium (Se), a trace element, has also been found at elevated levels in mine-affected streams of Appalachia (Lindberg et al. 2011; USEPA 2011). Elevated levels of Se have been identified as a primary source of change to stream ecosystems caused by surface-coal mining (USEPA 2011). By the same leaching process that cause elevated TDS concentrations in mining-influenced surface waters, Se enrichened in coal deposits and associated rock strata oxidizes to water-soluble selenite and selenate anions under increased weathering conditions (Young et al. 2010, Lussier et al. 2003). During and following mining, Se can be leached from disturbed rock and enter the aquatic environment at elevated concentrations (Pond et al. 2014).

Selenium in the aquatic ecosystem

Though Se is an essential nutrient for a wide diversity of biota from bacteria to humans, toxic effects of Se contamination can occur at only marginally elevated water-column concentrations. The most common toxic effects are reduced viability and juvenile deformities that are observed primarily in the most Se-sensitive organisms, oviparous vertebrates (Janz 2010; Arnold et al. 2014).

Complexity of Se dynamics within an aquatic ecosystem creates challenges for regulators who are tasked with setting an appropriate water-quality criterion. Enrichment or uptake of dissolved Se by bacteria, algae, and plants at the bottom of the food chain is the most-concentrating step of Se bioaccumulation within an ecosystem (USEPA 2016). Additional bioaccumulation occur through trophic transfer from food particulates to primary consumers, primary consumers to secondary consumers, and further up the food chain (Chapman et al. 2010). These transformations are controlled by site-specific factors, including both concentration and chemical form of dissolved Se entering the system, water residence time, and biotic community composition. Consequently, site-specific studies examining Se enrichment and trophic transfer are needed to inform appropriate resource management and protection practices (Presser & Luoma 2010).

Need for study

Surface coal-mining is widely acknowledged to be a potential source for Se in stream ecosystems (Arnold et al. 2014; Presser 2013; Lindberg et al. 2011; USEPA 2011). Despite the recognized threat, headwater streams receiving water exposed to mining-disturbed rock layers have been understudied as sites at risk for Se contamination, and integral parts of larger river networks. Because Se dynamics are complex and site-specific, the critical link between dissolved Se concentration and ecosystem-level impacts on headwater stream communities is not well understood. Field studies evaluating Se concentrations in media at low levels of the aquatic food chain are needed to quantify major processes of Se enrichment and trophic transfer that link dissolved Se to Se-sensitive taxa.

Research objectives and hypotheses

Objectives: (1) To evaluate Se concentrations in ecosystem media of central Appalachian headwater streams influenced by surface coal mining (2) To quantify major processes of Se enrichment and bioaccumulation in central Appalachian headwater streams influenced by surface coal mining.

Hypotheses: Based on previous studies (Arnold et al. 2014, Presser 2013), it is predicted that historical mining activities within a watershed will cause an increase in dissolved Se within the water column downstream, and a corresponding elevation in other ecosystem media through processes of enrichment and bioaccumulation. Furthermore, these measured ecosystem media are expected to exhibit highest Se concentrations in mining-influenced streams with relatively high water-column Se concentrations. Selenium dynamics (factors of enrichment and trophic transfer) are not predicted to differ among streams with different Se concentrations among measured media because, to the greatest extent possible, this study has been designed to minimize confounding site factors that may influence Se dynamics.

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CHAPTER 2. LITERATURE REVIEW

The trace element Se has garnered widespread concern from scientists and resource managers in the past few decades (Chapman et al. 2010). Rock formations enriched with Se are widespread and human activities that disturb these formations can release Se into the environment. Management of Se contamination is problematic because of its unusual and complex biogeochemical cycling. Selenium is an essential element, but potentially toxic at concentrations that are only marginally elevated above essential or reference levels. It bioaccumulates through the food chain with the most toxic effects reported in oviparous vertebrates (Janz 2010). To address these challenges, some researchers have suggested that water-quality criteria for Se should be more complex than water-based criteria that incorporate fixed thresholds, and other scientists who study Se advocate that Se contamination and potential toxicity should be assessed on a site-by-site basis (Luoma & Presser 2009, Lemly 2002).

Sources, speciation and biogeochemical cycling

Selenium is often enriched in ancient organic-rich marine deposits, such as coal and oil-shale, and in associated rock strata. Selenium may remobilize through human activities such as coal mining that disturb Se-rich rock strata (Young et al 2010). When Se-bearing minerals are exposed to oxygen (O_2) and water, Se oxidizes to water-soluble selenite and selenate anions that can be mobilized into adjacent streams (Lussier et al. 2003).

The tendency of Se to form oxyanions in aqueous solutions differs from many other trace elements that typically form cations (Young et al 2010). Additionally, unlike most other trace elements with chemistries that can be described using thermodynamics alone, biological mechanisms are a major driving factor in speciation transformations of Se (Luoma and Presser 2009). Four oxidative states can occur in natural environments: selenite (Se^{VI}), selenate (Se^{IV}), elemental Se (Se^{0}), and organic forms of Se (organo-selenide) (Se^{-II}). All of these states can be found naturally in sediments and soils, and all but elemental Se can be found in natural environments in dissolved forms.

Biogeochemical cycling of Se derives from its unusual chemical nature and occurs in a manner that is similar, in some respects, to cycling of mercury. Dissolved forms of Se enter an aquatic system, primarily in the form of inorganic selenite and selenite, though organic Se may also occur (William et al. 2010). Selenium may either remain in solution or become enriched in environmental media such as sediments and biofilms (Lemly 1999).

Enrichment, or environmental partitioning, is a process by which Se is transformed from a dissolved, primarily inorganic form of Se within the water column, to a particulate phase of Se at the base of the food-web. "Particulate" is a general term used to describe all living and non-living forms of Se that may serve as a pathway for Se bioaccumulation when eaten by primary consumers (Presser and Luoma 2010). The process of enrichment in particulate forms occurs by

ingestion or absorption by microbes, algae, or plants. Organisms that take up Se may transform inorganic forms of Se to organic forms. Once enriched in particulate phase, Se is bioavailable to higher trophic levels. Organic Se in living organisms may return to dissolved inorganic form through excretion or decomposition. Alternatively, Se-bearing mineral material and detritus may undergo sedimentation and subsequent transformation of Se to an elemental form through microbial activity (Luoma and Presser 2009; Lemly 1999).

Toxicity

Selenium is a micronutrient, incorporated into proteins as selenocysteine and selenomethionine. Selenium-containing proteins, called selenoproteins, are present in a wide range of taxa from bacteria to vertebrates, though the number of selenoproteins found among taxa varies (Janz 2011). As a micronutrient, Se plays a number of important roles including detoxification and DNA synthesis, and Se deficiencies can cause adverse effects such as reduced viability and juvenile deformities (Chapman et al. 2010).

Despite its role as an essential nutrient in most living organisms, toxic effects can occur when Se is ingested in quantities only marginally above healthy nutritional levels (Janz et al. 2010). At elevated concentrations in organisms, Se may be substituted for sulfur in amino acids, and Se may be involved in formation of reactive oxygen species, causing oxidative stress. Chronic toxicity of Se is the primary concern of ecological relevance. Acute toxicity can occur, but only at extreme concentrations not found even at the most contaminated sites (Janz 2011).

Detrimental effects caused by chronic Se exposure are sublethal, and primarily observed in organisms as reproductive impairment. The most pronounced effects are found in egg-laying (oviparous) vertebrates, the most sensitive and most well-studied taxonomic group (Young et al. 2010). Invertebrate taxa are generally less sensitive to Se contamination, and perhaps the most important role of invertebrates is bioaccumulation and as a dietary source of Se for vertebrate predators. However, studies have also shown that some invertebrates have a sensitivity to Se at levels considered safe for vertebrates (deBruyne and Chapman 2007, Conley et al. 2009).

Enrichment and bioaccumulation of Se in aquatic ecosystems

Unlike most trace elements, the dominant pathway of Se exposure in aquatic animals is dietary. Effects of direct exposure (i.e., dissolved Se in the water column) are largely insignificant as uptake of Se in any form through gills or skin is slow (Presser & Luoma 2010, Lemly 2002). Therefore, evaluating toxicity of Se in an aquatic ecosystem requires an understanding of food web dynamics as well as the thermodynamic and hydrological characteristics of the system. Major factors affecting enrichment and bioaccumulation of Se in a lotic system include 1) concentration and speciation of Se entering the system, 2) hydrodynamics of the stream, and 3) the aquatic community (Luoma & Presser 2009).

Selenium contamination generally enters an aquatic system in solution, primarily as selenite or selenate. Different sources of Se contamination may carry characteristic speciation signatures

(Cutter & Cutter 2004). Concentration and speciation of Se affect Se solubility, adsorption onto particles, and assimilation by biota (Maher et al. 2010). For example, the enrichment ratio of particulate Se to dissolved Se is magnitudes higher in selenite-dominant systems than in systems where selenate or organo-Se is abundant (Luoma& Presser 2009).

Retention time, or the time required by the "average" water molecule to move through a system, is a major hydrologic factor restricting the cycling of Se (Orr et al. 2006). Rapidly-moving streams may flush Se -enriched particles out of a stream system, preventing build-up of Se-rich sediments. Selenium is bioaccumulated most effectively in shallow, slow-moving streams where primary production and water residence times are high (Lemly 1999).

Perhaps the most important factor governing bioaccumulation of Se is the ecosystem community. The largest step in bioconcentration of Se is uptake of inorganic Se into autotrophic organisms (primary producers); but enrichment ratios differ among producers, and even among taxa within these broad groups (Stewart et al. 2010). Among biota capable of immobilizing Se (algae, bacteria and fungi), study of algae has been the most extensive, whereas study of bacteria and fungi is relatively limited. However, in a study conducted by Baines et al. (2004), the ratio of Selenite: Carbon was significantly higher in bacteria than in phytoplankton. This study suggests that bacteria may play an important role in environmental enrichment of Se, and further, that bacterivores may be exposed to higher concentrations of Se than herbivories. Studies of algae have shown that enrichment ratios vary among taxa with green algae accruing less Se than other forms such as diatoms (Janz et al. 2010).

Similar to the process of enrichment, assimilation efficiency of Se in consumers varies depending on the particle type being consumed as well as the consuming organism (Doblin et al. 2006, Presser and Luoma 2010). Trophic transfer concentrates Se to a far less extent than the process of enrichment. Nevertheless, Se continues to bio-accumulate and, in certain cases, bio-magnify at higher trophic levels (Janz 2011).

Early Se studies and regulatory history

The scientific community recognized Se as a contaminant before the metalloid was recognized as a nutrient. In the 1930's, studies conducted by the US Department of Agriculture identified Se as a toxic factor in diseases affecting cattle in the western U.S. (Chapman et al. 2010). Concern over Se grew rapidly through the 1970's and 1980's as major declines in the fisheries of Belews Lake, NC, Sweitzer Lake, CO, and Martin Lake, TX, and water bird mortalities in Kesterson Reservoir, CA were linked to Se contamination. These and other environmental crises motivated a flurry of field studies concerning Se in the environment (Hamilton 2004).

The U.S. Environmental Protection Agency (USEPA) first recommended aquatic-life criteria for Se in the 1980's. Total recoverable, inorganic Se in the water column was to not exceed an average of 35 $\mu g\ l^{-1}$ in a 24-hr period or 260 $\mu g\ l^{-1}$ at any given time (USEPA 1980). However, these initial criteria were developed considering chronic effects of direct water-column exposure only, and did not take bioaccumulation into account (USEPA 2016). Revised criteria

guidelines published in 1987 incorporated dietary pathways of Se bioaccumulation, lowering the freshwater Continuous Concentration Criteria to 5 µg l^{-1} and acute criteria of 20 µg l^{-1} based on field studies in Belews Lake, NC (USEPA 1987).

In 1998, the USEPA concluded that tissue-based criteria would be a more effective measure for assessing Se toxicity than water-column criteria. However, disagreement over how to implement nation-wide guidelines stalled the process of developing a new criterion (Hamilton 2004). To facilitate consensus among various stakeholders, the Society of Environmental Toxicology and Chemistry (SETAC) sponsored a workshop in 2009, "Ecological Assessment of Se in the Aquatic Environment," bringing together businesses, academia, government, and nongovernmental organizations (Chapman 2009).

Key findings from the workshop included: (1) Diet is the primary exposure pathway for consumers. (2) Using water-column dissolved concentrations of Se is not an effective method for predicting toxicity because site-specific factors control processes of enrichment and bioaccumulation. (3) Site-specific studies are needed to characterize risk of Se contamination in a given system (Chapman et al. 2009).

In 2016, the USEPA published its most recent aquatic-life criterion for Se (USEPA 2016). This criterion offers four elements for evaluating Se contamination - two water-based elements and two fish-tissue based elements. States and tribes may adopt concentration values developed by the USEPA as the four elements described above, or they may develop a site-specific water column criterion using a mechanistic modeling approach for relating dissolved Se concentrations to toxicity or lack of toxicity at a specific site based on Presser and Luoma (2010). The modeling method quantifies enrichment factors (EF), the ratio between concentration in a particulate organic material to concentration in the water, and trophic transfer factors (TFF), the ratio between concentrations in a consumer to concentration in the consumer's diet. Using this method, Se bioaccumulation can be described at a specific site in a way that allows comparison to other sites.

Selenium in central Appalachian streams

Surface coal-mining practices are fully acknowledged to be a potential source of Se contamination (Palmer et al. 2010, Chapman et al. 2010, Lemly 2002). The USEPA named elevated levels of Se in the water column and subsequent toxicity in fish as one of the five primary alterations to stream ecosystems caused by surface coal-mining (USEPA 2011). Despite this recognition, few field-studies have been conducted in lotic habitats within the region.

A survey of water quality in 37 streams in West Virginian coalfields conducted between 1999 and 2001 established a link between mountaintop/valley fill coal-mining and elevated levels of Se in the water column (Bryant et al. 2002). Multiple studies conducted in the Mud River Basin, WV indicated a connection between increases in water column Se to deformities in fish species (Arnold et al. 2014; Lindberg et al. 2011; WVDEP 2009). In addition to fish and water column

media, Arnold et al. (2014) collected benthic macroinvertebrates, reporting that macroinvertebrate tissues were also elevated in Se in waters receiving mining effluent.

To our knowledge, Presser (2013) is currently the only study documenting Se concentrations in particulate matter forming the base of the aquatic food chain, and thus the only study examining Se enrichment in the Appalachian coalfields. In 2010-2011, Presser (2013) collected samples of suspended particulate matter, benthic macroinvertebrates, and fish at fifteen sites in West Virginia ranging from high-elevation streams to lentic reservoirs. Also reported in Presser (2013) are a three sediment samples previously collected by the U.S. Geological Survey. This study found enrichment factors to vary greatly among sites and called for further study to improve scientific knowledge of Se bioaccumulation dynamics in the Appalachian coalfields.

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CHAPTER 3. MATERIALS AND METHODS

Site history

Twenty-seven stream sites were selected for research examining effects of elevated total dissolved solids (TDS), induced by surface coal mining, on benthic macroinvertebrate communities (Timpano et al. 2015; Timpano 2011). Sites were located in the coal-fields of southwestern Virginia and southern West Virginia (Figure 3-1). Candidate sites were required to meet "reference-like" physical and chemical conditions (Table 3-1) with the intent of minimizing potential confounding factors influencing benthic macroinvertebrate community composition. In contrast with other physical and chemical water-quality parameters, TDS concentrations ranged greatly across sites. Since study establishment in 2008, water chemistry and benthic macroinvertebrate samples have been collected on a quarterly and semi-annual basis, respectively. Since installation of *in-situ* probes (Onset Hobo U-24, Bourne, MA) in 2011, continuous readings of specific conductance have been recorded at 24 of these study sites.

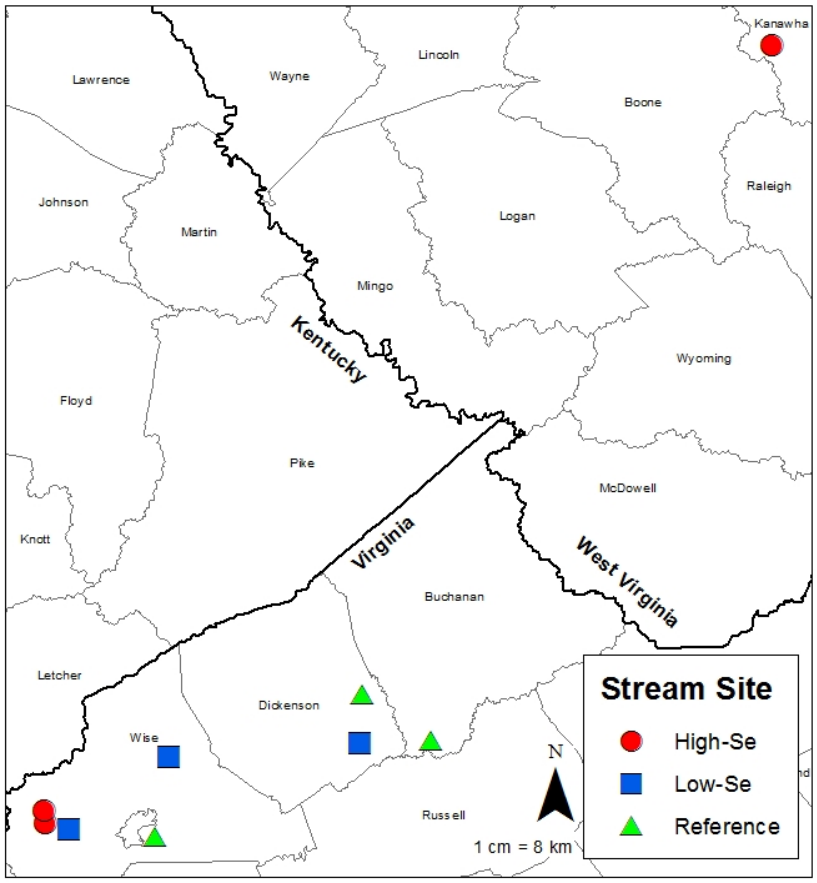

Figure 3-1. Nine stream sites selected for study in the coalfields of central Appalachia.

Table 3-1. Reference conditions applied for selection of study streams.

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

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

Dissolved trace element data were available at most of the study sites dating back to 2008. However, instrumental detection limits for Se were not lowered to ecologically relevant levels until 2013 (Appendix A). To meet study objectives, we conducted a water column and tissue-based assessment of Se in candidate stream sites, July 7th - August 8th 2015.

Selenium enrichment and bioaccumulation study – phase I

Selenium bioaccumulation values within headwater systems were established by collecting tissue samples of benthic macroinvertebrates from sites at 23 streams. This phase of the study enabled evaluation of a representative range of Se bioaccumulation values within headwater systems of central Appalachia. Results from phase I of the study informed the selection of sites for phase II.

Crayfish from family *Cambaridae,* and dragonfly nymphs from families *Gomphidae* and *Cordugastridae* were targeted for collection. These taxa were widely available at mining-influenced and reference streams, and relatively large body sizes allowed for lower sampling effort. Optimal habitat for these taxa was sampled using a D-frame net. Collected organisms were kept alive in site water throughout each sampling period. Sampling effort terminated in ≤ 4 hr or when sufficient mass had been collected for tissue analysis (Crawford and Luoma 1993). Samples were frozen on dry ice and transported to the lab where they were stored at -20 °C until analysis. Invertebrates were separated into taxa groups, and for each stream site, composite samples underwent an acid digestion (USEPA 1998). Samples were analyzed for Se concentrations using an inductively-coupled plasma mass spectrometer (ICP-MS) (Perkin-Elmer, Norwalk, CT) (Table 3-2). More detailed explanation of analytical procedures is provided below.

Table 3-2. Selenium (Se) concentrations in benthic macroinvertebrates and site-water collected from 23 central Appalachian headwater streams, July 7th – August 8th 2015.

	Tissue Samples (µg/g dry wt)[A]			Dissolved Se (mg/l)[B]
Site	***Gomphidae***	***Cordugastridae***	***Cambaridae***	**Water**
BIR [L]	4.02		2.75 [c]	0.6 [c]
COP [R]	2.88		1.24 [c]	< MDL
CRA			< MDL	< MDL
CRO [R]	2.28	1.41 [c]	< MDL	< MDL
EAS [R]			0.97 [c]	< MDL
FRY	5.17			< MDL
GRA	3.52			< MDL
HCN	2.11	< MDL	0.58 [c]	< MDL
HUR			3.71	0.5 [c]
KEL [H]	27.70		8.41	4.3
KUT [H]	23.62		7.02	8.8
LAB	14.65		5.48	4.7
LLC [H]	11.82	22.63	6.86	20.5
LLE		3.50	1.06 [c]	0.2 [c]
LLW			4.92	3.6
MCB	< MDL	1.80 [c]	0.45 [c]	< MDL
MIL [L]	5.05	4.57 [c]	1.87 [c]	0.7 [c]
RFF [L]	3.07 [A]		1.49 [c]	1
RIC	5.73			2
ROC			9.07	25.2
ROL			1.65 [c]	0.8 [c]
RUT	4.20			< MDL
SPC	4.68	3.22 [c]	0.71 [c]	< MDL

[A] < MDL designates tissue sample below minimum detection limit (.0005 µg Se l^{-1}). May be caused by low Se concentration and/or low sample wt.

[B] < MDL designates water samples below minimum detection limit of 0.0005 µg Se l^{-1}.

[C] sample > Method detection limit (.002 mg l^{-1}) < Minimum reporting level (.0005 mg l^{-1}).

[R] reference site selected for further sampling.

Selenium enrichment and bioaccumulation study – phase II

To examine Se dynamics of enrichment and trophic transfer at low levels within the aquatic food chain, we selected nine sites for further sampling based on Se concentrations in benthic macroinvertebrates collected during the first phase of this study (Table 3.2). Three sites with no history of mining activities within their watersheds were selected as reference streams. Six remaining sites had mining activities and were selected to be representative of Se accumulation levels found in headwater streams of central Appalachia. For the purpose of statistical analysis, these streams were separated into two groupings of three streams each: “high-Se” streams exhibiting high Se concentrations in macroinvertebrate tissue samples and “low-Se” streams exhibiting distinctly lower Se concentrations in tissue samples. 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 at some distance, in southern West Virginia, was selected for the purpose of maintaining a balanced study design despite its lack of geographic proximity (Figure 3-1).

Experimental design

The experimental design employed in this study was adapted from an ecosystem-scale methodology for studying Se developed by Presser and Luoma (2010). This methodology addresses the site-specific nature of Se bioaccumulation by quantifying major processes in Se bioaccumulation at each site. Enrichment factors (EFs) are quantified by relating Se concentrations dissolved in the water column to concentrations in living and nonliving particulates at the base of the food web. This transfer determines Se bioavailability to invertebrates (Presser & Luoma 2010). In this study, stream bed sediments, biofilm on rock or sand surfaces in the stream bed, and leaf detritus within the stream were selected as three distinct “particulate phases” of Se, having potential to become enriched in Se, and bioavailable to benthic macroinvertebrates. Enrichment factors were calculated as a ratio between Se concentrations in particulate phase to Se concentrations in the water column (Table 3-3).

Trophic transfer factors (TTF) were quantified by relating Se concentrations in particulate phase to macroinvertebrate population concentrations. A second trophic transfer to secondary consumer vertebrate populations (i.e. Se-sensitive fish species) was beyond the scope of this project. However, collected benthic macroinvertebrate populations were separated in the lab into primary consumer and secondary consumer groups, allowing a second trophic transfer factor to be quantified (Table 3-3). To develop EFs and TTFs in the selected stream sites, samples of the water column, stream bed sediments, biofilm, leaf detritus, and benthic macroinvertebrate samples were collected for Se and other trace element concentration analysis in September 2015 and March 2016 (Figure 3-2).

Table 3-3. Enrichment factors (EFs) and trophic transfer factors (TTFs) used to quantify Se dynamics within headwater streams in Central Appalachia.

Enrichment Factors	Trophic Transfer Factors	
EF [1]	TTF_1 [1]	TTF_2 [1]
$EF_{sediment} = \frac{\text{Sediment}}{\text{Water Column}}$	$TTF_{prey:sediment} = \frac{\text{Invertebrate Prey}}{\text{Sediment}}$	$TTF_{predator:prey} = \frac{\text{Invertebrate Predator}}{\text{Invertebrate Prey}}$
$EF_{biofim} = \frac{\text{Biofilm}}{\text{Water Column}}$	$TTF_{prey:biofilm} = \frac{\text{Invertebrate Prey}}{\text{Biofilm}}$	
$EF_{leaf\ detritus} = \frac{\text{Leaf Detritus}}{\text{Water Column}}$	$TTF_{prey:leaf\ detritus} = \frac{\text{Invertebrate Prey}}{\text{Leaf Detritus}}$	

[1] Factors are calculated as ratios of Se concentrations in media.

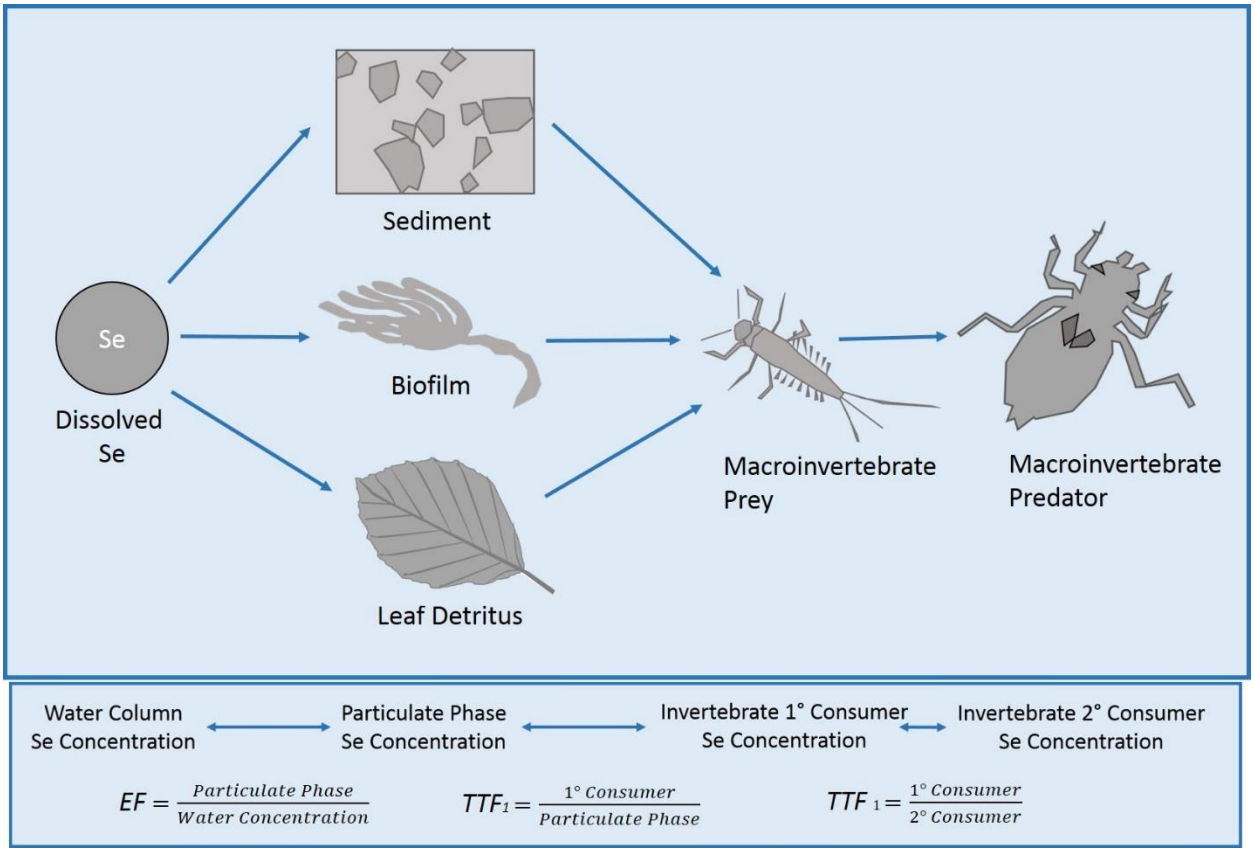

Figure 3-2. Conceptual diagram of experimental design, describing key transformations of Se that may occur in a headwater stream ecosystem.

Field methods & materials

At each stream, a study reach 100 m in length was delineated and marked with flagging tape. Reaches were centered on *in-situ* continuously-logging conductivity meters installed at sites in 2011. When necessary, study reaches were shifted upstream to avoid location below major tributaries draining expanded watersheds or to avoid roadways crossing the stream. To facilitate collection of all media evenly throughout the entire stream reach, each study reach was further divided into 10 sub-reaches, 10 m in length (Figure. 3-3).

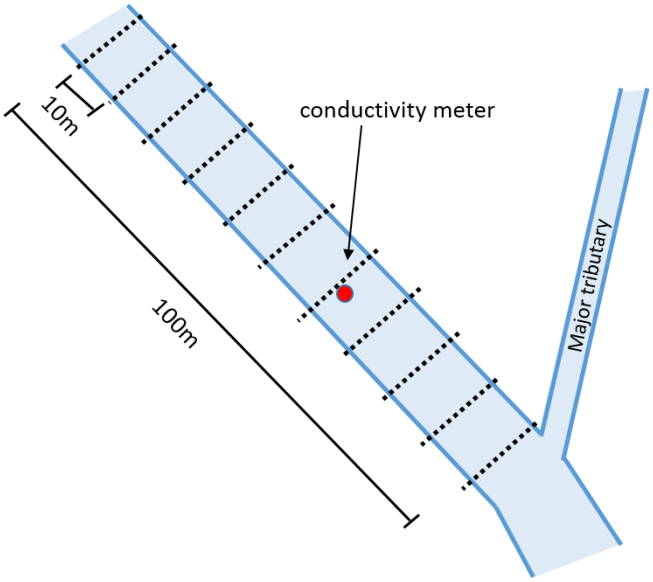

Figure 3-3. Stream delineation conceptual figure. The study reach is centered approximately on a conductivity meter.

Water sampling took place in the middle of the reach adjacent to conductivity meters. Sampling progressed so that collection for more easily disturbed media was completed before collection for other media began. Water sampling was completed at mid-study reach before upstream collection for stream bed sediment, leaf detritus within the stream and biofilm on rock or sand surfaces. Because benthic macroinvertebrate collection methods caused the most disruption to the study sites, macroinvertebrates were the last collected. Care was taken to progressively move upstream throughout the collection procedure.

Water column

Water samples for dissolved trace metals and total recoverable trace metals were collected at approximately mid-study reach, downstream of riffle habitat to ensure vertical mixing. For dissolved metals analysis, ~50 ml of water was filtered in the field using a 0.45 µm-pore filter

membrane. Water samples for total recoverable metal analysis were not filtered. Both sample types were preserved with trace-metal-grade nitric acid to <2 pH immediately following sampling, and stored in Nasco® whirl- pak sample bag on ice until transported back to the laboratory where they were stored at 4 °C.

Additional water samples were collected for analysis of total dissolved solids (TDS) and alkalinity at the same location sampled for metal analysis. For each analysis, ~100 ml were collected and filtered as above, but not acidified. All sampled were stored on ice until return to the lab where they were stored at 4 °C prior to analysis.

Stream bed sediment

Composite sediment samples were collected using a 237 ml plastic scoop. Sub-samples were taken at five evenly spaced intervals in three lateral transects per sub-reach and combined in light-excluding Fisherbrand® whirl-pak bags. Sample depth was restricted to 1- 3 cm, thus selecting for recently deposited, biologically active sediments (USEPA 2001). Stream-intervals where sediments could not be collected using this method (i.e., intervals falling on bed rock or with cobbles larger than the scoop volume) were not sampled. In this way, sampling methods were selected for areas of deposition within the stream. Samples were transported on dry ice back to the lab where they were stored at -20 °C until further processing.

Biofilm

Biofilm accumulations in riffle and run habitats were scraped from rock substrate using an acid-washed plastic knife. At each sub-reach, a maximum of three rocks carrying the largest accumulations of biofilm were pulled from the stream, and biofilm was scraped into light-excluding 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 surface of all sediments visibly carrying biofilm accumulation. The full visible extent of biofilm was collected within the reach. Composite biofilm samples were stored in light-excluding Fisherbrand® whirl-pak bags, and transported on dry ice back to the lab where they were stored at -20 °C until analysis (Orr et al. 2006, Casey 2005).

Leaf detritus

Leaf detritus originating from the tree canopy adjacent to each stream was collected from in-stream “leaf packs”, defined as an accumulation of more than three leaves within the stream. At each sub-reach, the largest leaf pack was identified, and up to ten leaves were collected. If the largest leaf pack contained fewer than ten leaves, the second largest leaf pack was identified. This process continued until a total of ten leaves was collected. Only completely brown and fully submerged leaves were collected from leaf packs. Leaf packs partially or completely covered in sediments were avoided. Each leaf was gently agitated in the water column of the stream in which it was collected to remove excess sediments before placement in a light-excluding Fisherbrand® whirl-pak bag. The composite samples were transported on dry ice back to the lab, where they were stored at -20 °C until analysis.

Benthic macroinvertebrates

Samples of benthic macroinvertebrate communities were collected using a D-frame dip net following field procedures for collecting aquatic insects for trace element analysis (Crawford and Luoma 1993; Barbour et al. 1999). Dip nets were emptied into plastic tubes filled with site water. Macroinvertebrates were removed from debris with stainless steel tweezers and placed into containers filled with site water. Macroinvertebrate predators were separated from non-predators ("prey") to reduce mortality during the collection and storage period.

Number of benthic macroinvertebrates collected from a stream site was intended to result in sufficient biomass for analysis (approximately 0.5 g dry wt), while optimizing sampling efficiency. To this end, the total number of macroinvertebrates collected from a stream site was dependent on the average size of taxa present within the stream. Number of individual prey taxa to be collected from each sub-reach was decided during sampling of the first sub-reach. Because of the large size disparity between macroinvertebrate prey and predator taxa, sufficient biomass of predator taxa was collected through the course of sampling to reach sufficient quantity of prey taxa. Total number of individuals collected at each site ranged from 482 to 1,199. Microhabitat quality within each sub-reach necessitated some variance in number of individuals collected among sub-reaches. Crayfish from family *Cambaridae* were also collected from streams by targeting optimal habitat and selecting up to five individuals measuring in length from 1.16 - 3.49 cm (mean 2.35 ± .54 cm) within and among stream sites.

Laboratory methods

Water column

An ICP-MS (Perkin-Elmer, Norwalk, CT) was used to analyze acidified water samples collected for dissolved and total recoverable metal analysis. Samples were analyzed for Se concentrations as well as Al, V, Cu, Zn, As, Sr, and Cd concentrations (Appendix B). Total dissolved solids (TDS) was measured by drying known sample volumes at 180°C (APHA 2005); total alkalinity was measured by titration with hydrochloric acid (APHA 2005).

Stream bed sediment

Composite sediment samples for each site were thawed and 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 the sieving process. Sediments larger than > 1mm were discarded. Sediments ≤ 1mm were homogenized manually using a stainless steel laboratory scoop and stored in Corning® brand 15ml sterile vials (EPA 2001). Excess site water was decanted after allowing sediments to settle out of the water column during a period of 20 min. Samples were shaken vigorously to insure thorough mixing and refrozen at -20 °C. Samples were freeze-dried (LABCONCO FreeZone, Kansas City, MO) for ~120 hr to ensure complete drying.

Biofilm

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 using a procedure adapted from Bell and Scudder (2007). 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 an addition of 50 ml deionized water, the new slurry was shaken once 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 Corning® brand 15ml sterile vials, refrozen, and lyophilized for ~ 120 hr. After complete drying, samples were ground using a mortar and pestle. Mortar and pestle were wiped clean with ethanol, followed by rinsing with deionized water between samples.

Leaf detritus

Leaves were thawed briefly, identified to tree species when possible, and agitated lightly in deionized water to remove sediments (Appendix C). Rhododendron leaves were removed from composite samples because of their low rates of decomposition and poor food quality for benthic macroinvertebrates (Eggert et al. 2003). Leaves were stored at 65 °C for ≥5 d to ensure thorough drying. After drying, leaf mid-veins were removed, and remaining leaf matter was ground using a cleaned, stainless steel ball-mill for ≥2 min at a vibration frequency of 25 sec^{-1}. Samples from each site were mixed thoroughly, stored in Corning® brand 15 ml sterile vials, and refrozen at -20 °C. Samples were freeze-dried for ~120 hr to ensure complete drying.

Benthic macroinvertebrates

Benthic macroinvertebrates were thawed briefly and identified to family. In cases where the family taxon group contained both predacious and non-predacious genera as specified in Poff et al. (2006) and Merritt & Cummins (1996), individuals were further identified to genus. Number of individuals belonging to each taxon group was recorded (Appendix B). Because of their disproportionally large body sizes, genera *Pteronarcys* and *Tipula* were separated from other prey taxa. *Pteranarchys* were collected in five streams in both the fall and spring. *Tipula* were collected at disproportionally large sizes only in the spring, and therefore were separated in spring samples from all nine streams. Samples were refrozen and freeze-dried for ~ 120 hr to ensure complete drying. The dried prey, predator, and *Pteronarchy* composite samples were weighed before they were ground with a mortar and pestle. Mortar and pestle were wiped clean with ethanol followed rinsing with deionized water between samples.

Digestion and analysis for Se and other trace elements

When sufficient material was available, composite media samples were subsampled for lab analysis three times to create three laboratory replications for each composite sample. In accord with laboratory equipment capacities, subsamples were analyzed in batches of 40 subsamples at a time. To estimate background trace element levels, three blanks exposed to the same reagents and laboratory equipment, were run concurrently with subsamples. After

analysis, the average Se concentration in the three blanks was subtracted from each batch test sample. To address low recovery rates of certified reference materials during Phase I, digestion procedures were adjusted between Phase I and Phase II. Digestions were completed using a MARS Xpress microwave system (MarsExpress, CEM Corp., Matthews, NC) with non-pressurized, teflon vessels. Digestion procedure was adapted from USEPA method 3015a for microwave assisted digestion (USEPA 1999).

Freeze-dried samples of leaf detritus, biofilm, and benthic macroinvertebrates weighing ≤0.5 g, and sediment samples weighing ≤2.0 g were placed in digestion vessels followed by addition of 5 ml of trace metal grade nitric acid (70% HNO_3). Vessels were allowed to vent for 18-24 hr before adding 0.5 ml of hydrogen peroxide, an oxidizing agent, to each vessel. Effervescence caused by the addition of hydrogen peroxide subsided in 2 - 4 hr whereupon an additional 1 ml of hydrogen peroxide was added. Further effervescence was allowed to subside once again.

Vessels were sealed and placed in the microwave digestion unit. The digestion unit was 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 quantitatively poured into a 50 ml volumetric flask and brought up to volume with deionized water. The volumetric flasks were inverted to thoroughly mix the sample and left to settle overnight. The following day, the solution underwent a further 1:5 dilution. The final solution was analyzed using an ICP-MS for Se (Appendix D), as well as V, Cu, Zn, As, Sr, and Cd (Appendix E).

Quality Control/Quality Assurance

Certified reference material (TORT-2 and TORT-3, National Research Council of Canada, Ottawa, Canada) was run in replicates of 3 in all sample batches. In phase I, recovery of Se (9.0 ± 0.49 μg g^{-1} Se) was less than the certified range (9.9 – 11.9 μg g^{-1} Se). Blanks were less than detection limits for Se. In all runs of Phase II material, recovery of Se (11.2 ± 0.39 μg g^{-1} Se) 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 ug l^{-1} Se. Differences between lab duplicates averaged 7.3%.

Digestion efficiency assessment

Digestions of all media consistently yielded some quantity of undigested particulate matter. To assess whether there was significant difference in mass of undigested material across streams, a protocol for assessing digestion efficiency was developed.

Following digestion, ~ 35 ml digesate was removed from the 50 ml volumetric flask for further dilution and analysis. Deionized water was added to the flask and mixed with remaining digestate to further dilute the solution. After allowing the particulates to settle out of solution, an additional ~ 35 ml was removed from the flask. The remaining digestate and particulates were poured into 50 ml beakers and placed onto a hot plate at a temperature that would allow the acid solution to evaporate without boiling. Once evaporation was completed, beakers were placed in a drying oven at 65 °C for 24 hrs. They were subsequently removed from the oven,

cooled to room temperature in a desiccator, and weighed. Beakers were ashed in a muffle furnace at 550 °C for 45 – 60 min to volatize organic material. Beakers were removed from the muffle furnace, wetted to rehydrate clay minerals, and dried at 65 °C for 24 hrs.

Data processing

Minimum detection limit (MDL) and minimum reporting level (MRL) for ICP-MS analyses were calculated using standard QAQC methods. The MRL, determined to be 0.002 mg l^{-1} Se, was adapted from methodology developed by the USEPA (Yang et al. 2013). The MDL, determined to be 0.0005 mg l^{-1} Se, was derived from the most conservative estimate of two different methods: three times the standard deviation of seven 'blank' readings and three times the standard deviation of seven MRL readings.

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, a value of 0.00025 mg l^{-1} Se. Prey concentrations were constituted mathematically by considering measured concentrations in the *Tipula* and *Pteranarchys* genera that had been separated from other prey taxa groups for analysis and weights of these taxonomic groups relative to the residual sample. When applicable, all sub-samples were averaged to calculate a value used for analysis. Dissolved Se concentrations in the water column that produced < MDL results were statistically analyzed as 0.00025 mg l^{-1} Se.

Calculating enrichment and trophic transfer factors

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.

Statistical analysis

R Studio software (RStudio, Boston, MA) was used to preform statistical analyses. 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 the significance of stream type (reference, low-Se, and high-Se), and season (fall and spring), and the interaction of stream type and season 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 with a Tukey HSD. An alpha level of 0.05 was used to determine significance for all statistical analyses.

Dissolved Se in the water column and leaf detritus, the only data sets that 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 analyses 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.

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CHAPTER 4. SELENIUM ENRICHMENT AND BIOACCUMULATION IN HEADWATER STREAMS OF THE CENTRAL APPALACHIAN COALFIELDS

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 water column concentration are only marginally elevated above essential levels. Human activities including phosphate, uranium, and coal mining, petroleum processing, and irrigation in regions with selenifrous 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 waterbird embryonic deformities 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. 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 aquatic community and water chemistry change 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 increased 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, 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 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 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 nine of those streams, six of which were mining influenced, Se concentrations in water, particulate forms, and benthic macroinvertebrate tissue, and on enrichment and trophic transfer were evaluated 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, 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 are located within the same Appalachian ecoregion, have similar gradient and catchment size, and have similar habitat quality that meets reference-like criteria (Timpano et al. 2015).

METHODS

Site Selection

Study sites were selected from a group of 27 streams in the coal fields of southwestern Virginia and southern West Virginia. Because these sites were originally selected to isolate effects of elevated TDS induced by surface coal mining on benthic macroinvertebrate communities with the intent of minimizing potential by non-TDS stressors on macroinvertebrate community composition (Timpano 2011; Timpano et al. 2015), they meet "reference-like" physical and chemical conditions (Table 4-1). In contrast with other water quality parameters and site characteristics, TDS concentrations ranged widely across the sites. Dissolved trace element concentration data, including Se, were available from previous research at most of the study sites.

Table 4-1. Reference conditions applied for selection of study streams in the coalfields of central Appalachia.

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

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

Selenium enrichment and bioaccumulation study – 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 *Cordugastridae* were targeted for collection at 23 of the 27 potential study streams between July 7th and August 8th 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 4-1) with an inductively-coupled mass spectrometer (ICP-MS) (Perkin-Elmer, Norwalk, CT) using the analytical procedure described in greater detail below.

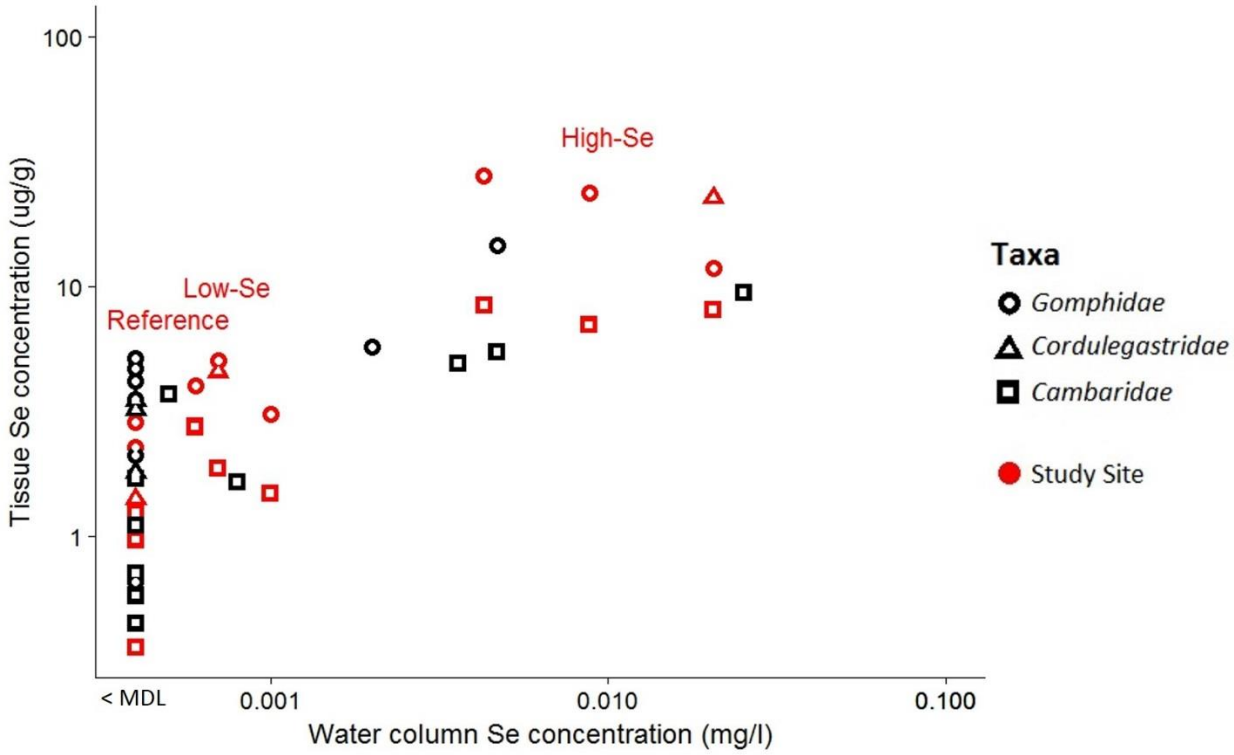

Figure 4-1. Dry mass Se concentrations in tissue samples of *Cordugastridatae, Gomphidae*, and *Cambaridae* sampled in Phase I of study from streams in Central Appalachia used for selection of stream type. "< MDL" indicates water samples below minimum detection limit (< 0.0005 mg l^{-1}). Red symbols are taxa groups collected from sites selected for further sampling efforts in fall 2015 and spring 2016. "Reference," "Low-Se," and "High-Se," indicate stream type groupings.

Selenium enrichment and bioaccumulation study – Phase II

To examine Se dynamics of enrichment and trophic transfer, nine sites were selected for further study based on Se concentrations in benthic macroinvertebrates collected during Phase I (Figure 4-1). 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 4-1). 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 maintain a balanced study design despite its lack of geographic proximity (Figure 4-2).

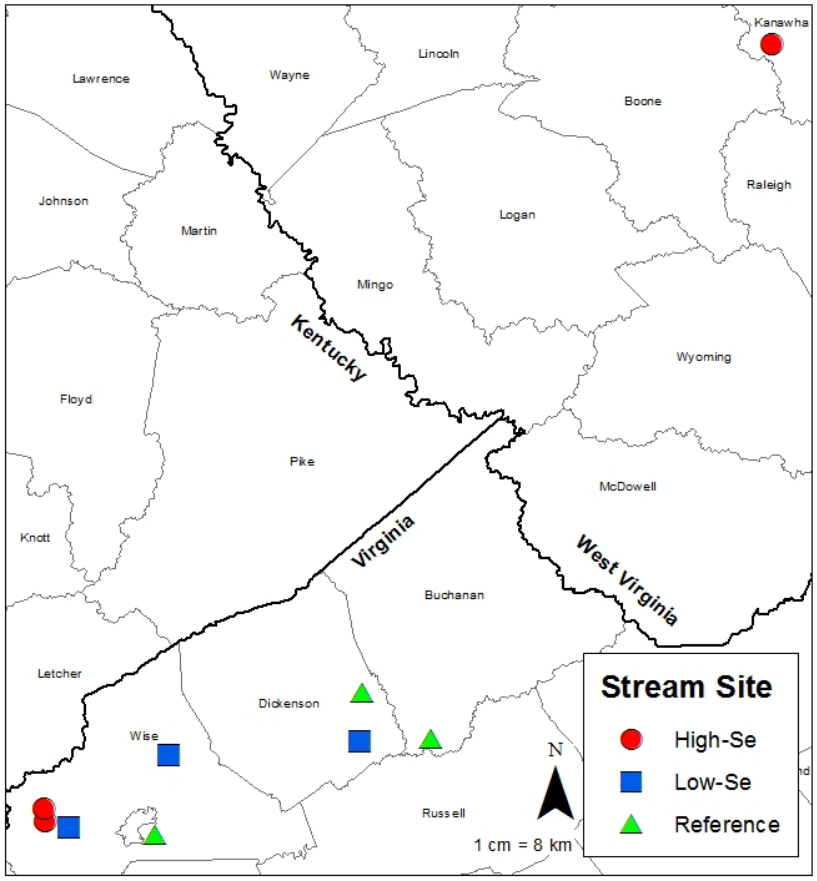

Figure 4-2. Location of stream sites selected for study in the coalfields of central Appalachia.

Study Site 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 4-3).

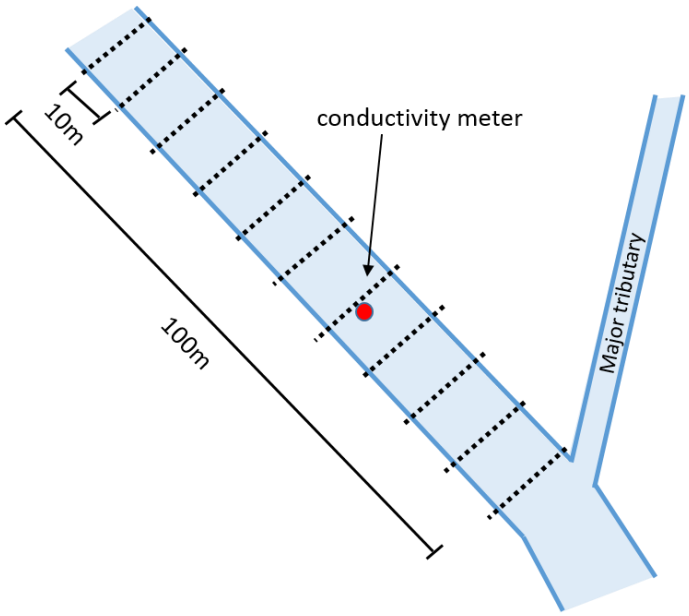

Figure 4-3. Conceptual figure of stream delineation and sampling locations at study sites. The study reach, 100 m in length, is centered approximately on a conductivity meter

Field Collection and Laboratory Sample Processing

Water column

Water samples for dissolved trace elements and total recoverable trace elements were collected at approximately mid-study reach, downstream of riffle habitat to ensure vertical mixing. For dissolved trace element analysis, ~50 ml of water was filtered in the field using a 0.45µm-pore filter membrane. Sample were preserved with trace-metal-grade nitric acid to <2 pH immediately following sampling and stored in Nasco® whirl-pak sample bags on ice until transported 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. Sub-samples were taken at five evenly spaced intervals in three lateral transects per sub-reach and combined in light-excluding Fisherbrand® whirl-pak bags. Sample depth was restricted to 1- 3 cm, thus selecting for recently deposited, biologically active sediments (USEPA 2001). Samples were thawed and 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 Corning® brand 15 ml sterile vials. Samples were shaken vigorously to insure thorough mixing, refrozen, 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 carrying the largest accumulations of biofilm were pulled from

the stream, and biofilm was scraped into light-excluding 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 Fisherbrand® whirl-pak bag, and transported on dry ice back to the laboratory (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 Corning® brand 15ml sterile vials, refrozen, and 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 in-stream 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 collected. Collected leaves were completely brown, fully submerged in water, and not covered by sediment. Composite leaf samples for each site were combined into a light-excluding Fisherbrand® whirl-pak bag, and transported on dry ice back to the laboratory. In the laboratory, leaves were briefly thawed, identified to tree species when possible, and agitated lightly in deionized water to remove excess sediments. Rhododendron leaves were removed from composite samples because of their low rates of decomposition and poor food quality for macroinvertebrates (Eggert et al. 2003). Leaves were dried at 65°C for ≥ 5 d to ensure thorough drying. 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 sec^{-1}.

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 for each stream that were comparable in size within and among streams (Appendix C). Macroinvertebrates were transported on dry ice back to the laboratory. In the laboratory, benthic macroinvertebrates 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* and *Tipula* were separated from other prey taxa. *Pteranarchys* 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 *Pteronarchy* composite samples collected in the fall were weighed. Spring samples were weighed by family taxa groups before compositing prey, predator, *Pteronarchy, and Tipula* groups. All samples were ground with a mortar and pestle.

Acid-digestion and analysis for Se

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 were subtracted from each batch's test samples.

Digestions were completed using a microwave digestion system (MarsExpress, CEM Corp., Matthews, NC) with non-pressurized, teflon 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 ml of trace metal grade nitric acid (70% HNO_3) were added to samples collected in phase I of this study. Five ml 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.

QA/QC

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 Se g^{-1} dry wt) was less than the certified range (9.9 – 11.9 µg Se g^{-1} 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 Se g^{-1} 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 Se g^{-1} 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 Se l^{-1} and 0.002 mg Se l^{-1}, 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 0.00025 mg Se l^{-1}. Prey concentrations were constituted mathematically by considering measured concentrations in the *Tipula* and *Pteranarchys* 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. Dissolved Se concentrations in the water column that produced < MDL results were statistically analyzed as 0.00025 mg Se l^{-1}.

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.

Statistical analysis

R Studio software (RStudio, Boston, MA) was used to perform statistical analyses. 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 with a Tukey HSD.

Dissolved Se in the water column and leaf detritus, which failed to meet assumptions of normality after log transformation, were 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 concentrations in media

Dissolved Se for water was < MDL in 4 of 9 streams in the fall and in 2 streams in the spring. Dissolved Se concentrations did not differ between seasons, but difference among stream type were detected. Mean water column concentrations in high-Se streams were 7.7 times greater than mean concentrations in low-Se streams (p-value = 0.000033), and 17 times greater than mean concentrations as estimated at half the method detection limit in reference streams (p-value = 0.00000078). Selenium-concentration differences between low-Se and reference streams were not significant (Figure 4-4. A).

Sediment and biofilm Se-concentrations did not vary by season, but were significantly different among all stream types with one exception of biofilm in the reference streams compared with low-Se streams. Highest concentrations of Se were found in high-Se streams and lowest concentrations in reference streams. Mean Se concentrations in sediments in high-Se streams were 2.2 times greater than the mean of low-Se streams (p-value = 0.0035), and mean concentrations in low-Se streams were 3.0 times greater than in reference streams (p-value = 0.0013). Mean Se concentrations in biofilm collected in high-Se streams were 3.4 times greater than in low-Se streams (p-value = 0.00053), and 6.0 times greater than in reference streams (p-value = 0.000015) (Figure 4-4. B & C).

Difference in leaf detritus Se concentrations between low-Se and high-Se streams was greater in the spring than 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. 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 greater than the mean in reference streams (p-value = 0.00052). In the spring, mean differences increased to 33.3 times greater concentrations of Se in leaf detritus in high-Se compared with reference streams (p-value = 0.00014). Mean leaf detritus Se concentration were 5.9 times greater in low-Se streams than in reference streams for fall samples (p-value = 0.0047) and, 16.4 times greater in high-Se streams than low-Se streams for spring samples (p-value = 0.00043) (Figure 4-4. D & E).

No significant difference was found in Se concentrations between prey composite samples with and without *Pteranarchys* and *Tipula* genera included; therefore, prey samples calculated to include all genera were used in further statistical analyses. Prey, predator, and *Cambaridae* (crayfish) 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 greater in prey samples (p-value = 0.000026), 4.3 times greater in predator samples (p-value = 0.0000009), and 4.0 times greater in *Cambaridae* samples (p-value = 0.0000013) than mean concentrations in low-Se streams. Mean Se concentration in low-Se streams were 4.3 times greater in prey samples (p-value = 0.000065), 2.3 times greater in predator samples (p-value = 0.00041), and 2.3 times greater in *Cambaridae* samples (p-value = 0.00018) than mean Se concentrations in reference streams (Figure 4. F, G, & H).

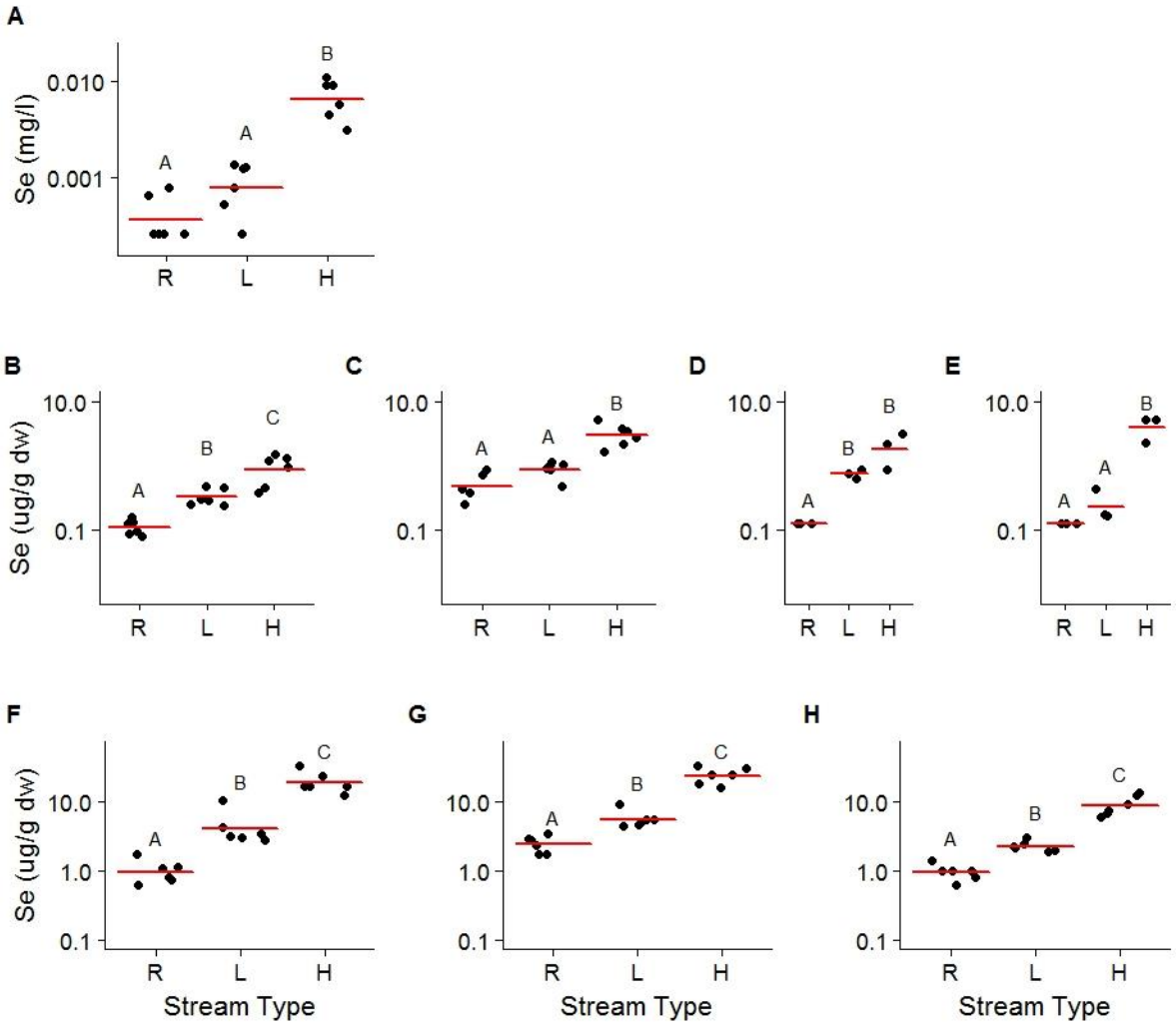

Figure 4-4. Selenium concentrations in (A) Water column (B) Sediment (C) Biofilm (D) Leaf Detritus, fall (E) Leaf Detritus, spring (F) Prey (G) Predator and (H) *Cambaridae* in reference (R), low-Se (L) and high-Se (H) headwater streams of the Appalachian coalfields. Horizontal lines indicate means and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

Enrichment and Trophic Transfer Factors

Enrichment factors describing the relationship between Se in the water column and Se in sediments ($EF_{sediment}$) did not differ by season or stream type (Figure 4-5A). Difference in mean $EF_{biofilm}$ values were not detected between seasons, but were detected between high-Se and reference streams. Mean $EF_{biofilm}$ values in reference streams were 4.1 times greater than in high-Se streams (p-value = 0.028) (Figure 4-5B). A significant interaction effect between season and stream type was detected in $EF_{leaf\ detritus}$ values (Figure 4-5C); however, further analysis detected no differences between seasons or among stream types.

Trophic transfer factors also did not differ significantly by season, but differences among stream types were detected for several of the TTF calculations. Mean $TTF_{prey:sediment}$ values were 2.7 times higher in high-Se streams than reference streams (p-value = 0.0068) (Figure 4-5E), and mean $TTF_{prey:biofilm}$ values were 3.0 times higher in high-Se streams than in reference streams (p-value = 0.012) (Figure 4-5 F&G). No differences among stream type were detected in $TTF_{prey:leaf\ detritus}$ (Figure 4-5H). Mean $TTF_{predator:prey}$ values were 2.0 times greater in reference streams than in both low-Se streams (p-value = 0.00022) and high-Se streams (p-value = 0.00079). Similar patterns of Se enrichment and trophic transfer were observed among stream types (Figure 4-5H).

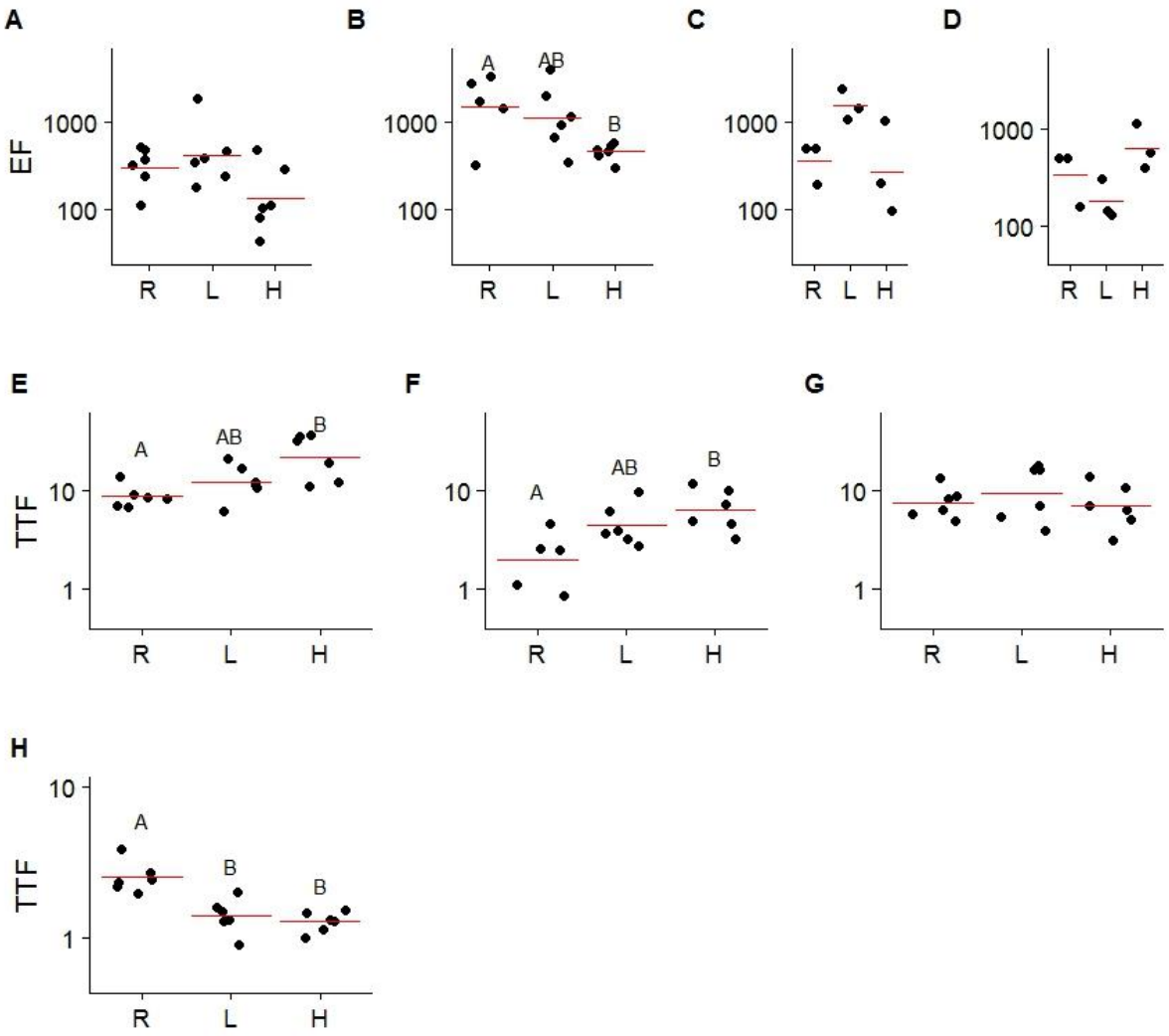

Figure 4-5. Selenium enrichment and trophic transfer factors in (A) $EF_{sediment}$ (B) $EF_{biofilm}$ (C) fall $EF_{leaf\ detritus.}$ (D) spring $EF_{leaf\ detritus}$ (E) $TTF_{prey:sediment}$ (F) $TTF_{prey\ :\ biofilm}$ (G) $TTF_{prey\ :\ leaf\ detritus}$ (H) $TTF_{predator:prey.}$in reference (R), low-Se (L) and high-Se (H) headwater streams in Appalachian coalfields. Horizontal lines indicate means by stream type and letters indicate significant differences among stream types for each media.

Though differences in EFs and TTFs were detected among stream types, overall differences were minimal. Viewing the data with a wider lens, Se dynamics do not appear to differ dramatically among stream types (Fig. 4-6). Minimal differences detected did not sum up to large differences in enrichment, trophic transfer, and overall link between water column and macroinvertebrate tissue concentrations.

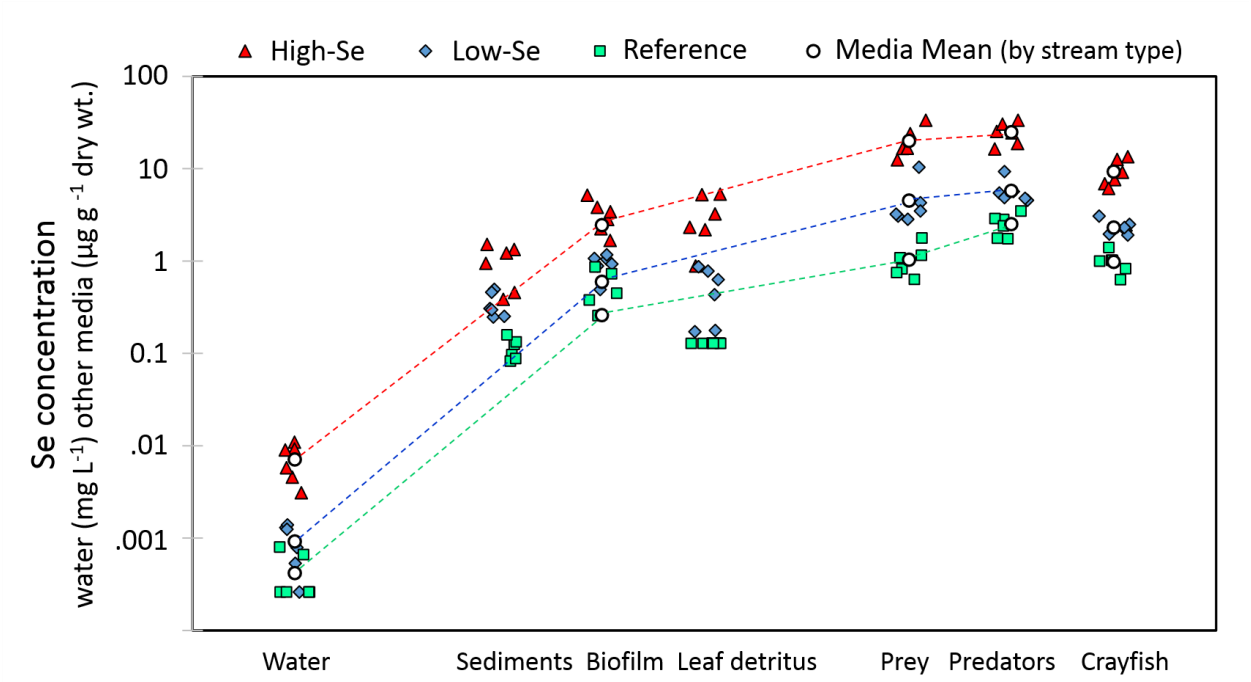

Figure 4-6. Selenium concentrations in all media by stream type. Media are ordered to illustrate Se pathways of enrichment from water column to particulate matter, and bioaccumulation pathways from particulate-phase media to prey taxa and from prey taxa to predator taxa. Media-mean concentrations for particulate-phase media are averaged. Dotted lines connect mean concentrations of media by stream type. *Cambaridae* Se concentration data are shown, but not included in illustration of Se pathways.

DISCUSSION

Our data 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, difference in calculated factors were detected among stream types. However, those differences which were detected did not show consistent patterns, and general trends of enrichment and bioaccumulation were consistant among stream types (Figure 4-6).

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 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 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) report Se concentrations from lotic systems in central Appalachia in media other than water column and fish tissue. One study reports Se concentrations in two sediment samples (Presser 2013), and neither study reports 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 Se l^{-1}; 0.11 µg Se g^{-1} dry wt, 0.53 µg Se g^{-1} dry wt, and 1.0 µg Se g^{-1} dry wt, respectively) were within or close to accepted background levels of freshwater environments: water (0.0001 - 0.0004 mg Se l^{-1}), sediment (0.2 – 2.0 µg Se g^{-1} dry wt), algae (0.1 – 1.5 Se g^{-1} dry wt), and aquatic macroinvertebrates (0.4 – 4.5 Se µg Se g^{-1} 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 Se g^{-1} dry wt Se), similar to our range of 0.247 to 5.26 µg Se g^{-1} 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 Se g^{-1} dry wt) (USGS 2008) and, were slightly elevated above our range of 0.25 to 1.5 µg Se g^{-1} 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 Se l^{-1}, 0.2 µg Se g^{-1} dry wt and 1.0 µg Se g^{-1} dry wt, respectively and in mining-impacted streams as 0.0107 mg Se l^{-1}, 2.4 µg Se g^{-1} dry wt, and 3.2 µg Se g^{-1} 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 Se l^{-1}, 0.010 mg Se l^{-1}, respectively) and biofilm (1.81 µg Se g^{-1} dry wt, 3.57 µg Se g^{-1} 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 Se l^{-1}, and 3.2 µg Se g^{-1} 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 Se g^{-1} 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 Se g^{-1} dry wt and 4.5 µg Se g^{-1} dry wt, respectively). Presser (2013) reported benthic macroinvertebrate Se concentrations (6.3–12 µg Se g^{-1} 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 Se g^{-1} dry wt) that were higher than mean values for reference streams in our study (< 1.0 µg Se g^{-1} dry wt), and in streams impacted by coal mining (10.0 µg Se g^{-1} 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 greater 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 Se g^{-1} 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 this study's high-Se streams 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 have been found at the highest levels closest to the source of Se, with concentration decreasing as sampling effort continues 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 mining effluent sources.

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 be influencing these results. Low concentrations in dissolved Se samples used to calculate EF increase uncertainty in EF values, particularly at reference streams where concentrations were < MDL in 4 out of 9 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. that 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 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 include 2230 for Se enrichment in biofilm (Kuchapski and Rasmussen 2015) and a range 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 $TTF_{prey:sediment}$ 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 differ in rate at which they ingest Se-enriched food and the efficiency in which they assimilate Se into their tissues, as denoted by our Phase 1 data which demonstrate tissue concentration differences among *Cordugastridata*e that were *Gomphidae* at the same sites (Figure 4-3). 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 made up of 27% (fall) and 33% (spring) mayflies from the family *Heptigeniidae*. In contrast, low-Se stream samples contained 12% and 2% *Heptigeniidae*, respectively, and in high-Se streams no *Heptigeniidae* 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 Appalachian headwater streams, as observed by Pond et al. (2014) and as found in this study (Appendix F). 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 the literature. Mean values of first-level TTF in this study range from 2.4 – 24.5, whereas first-level TTF has been reported to be 1.6 – 4.0 (Presser 2013),

1.82 – 3.20 (Kuchapski and Rasmussen 2015), 2.6 – 4.5 (Casey 2005), and 2.8 (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 evaluate 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 Se g^{-1} 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 ≥ µg Se g^{-1} Se dry wt, approximately 2x the highest particulate concentrations observed in our study. Arnold et al (2014), however, reported increased occurrence of Se-related fish deformities in impacted streams that contained macroinvertebrate concentrations below those of macroinvertebrates collected at this studies high-Se sites.

CONCLUSIONS

This 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-influence sites and may serve as possible pathways for Se enrichment and bioaccumulation. Dynamics of Se enrichment and trophic transfer were observed to differ among stream types. However, differences were minor and overall patterns of Se enrichment and bioaccumulation among 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 available literature of lotic systems in the Appalachian coalfields. Furthermore, Se concentrations in media, particularly macroinvertebrate taxa sampled in high-Se streams, is 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 fish.

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CHAPTER 5. SUMMARY & CONCLUSIONS

Summary

Headwater streams in the coal-mining ecoregion of southern West Virginia and southwestern Virginia are negatively impacted by surface coal-mining activities (Palmer et al. 2010). Mining practices alter stream hydrology through watershed deforestation and soil compaction, altering downstream flow magnitude and, in some cases, potential for flooding (Palmer et al. 2010). Stream water chemistry is also altered when rock disturbed during the mining process is exposed to oxygen leaching, which dissolves and transports ions from un-weathered rock surfaces into streams at elevated concentrations. These changes to headwater streams may alter their role as major contributors to biodiversity and ecosystem processes within larger river networks (Meyer et al. 2007, USEPA 2016).

Elevated levels of Selenium (Se) have been identified as one of the primary sources of change to stream ecosystems caused by surface-coal mining (USEPA 2011). An essential element, but toxic at concentrations that are only marginally elevated above natural background, Se bio-concentrates through each step along the aquatic food chain. Toxic effects, including reduced viability and juvenile deformities, are primarily seen in oviparous vertebrates (Janz 2010). Selenium-enriched rock disturbed by coal mining can become a source of Se in the environment as those rocks are exposed by the mining process and subjected to accelerated weathering which enables Se to oxidize and leach out of the fractured rock materials (Young et al. 2010, Lussier et al. 2003). Dissolved Se entering a stream reach may be transformed to particulate form through uptake by bacteria and algae; this process is termed enrichment and is the most bio-concentrating step in Se accumulation (Young et al. 2010). Pathways of bioaccumulation to consumer organisms occurs through trophic transfer to consumers from their food source (Hamilton 2002).

Transformation of dissolved Se to tissues in Se-sensitive organisms is governed by site-specific factors within an aquatic ecosystem. To establish the relationship between dissolved Se concentrations and concentrations in tissues of Se-sensitive species, site-specific studies are needed to quantify the transformation processes of enrichment and trophic transfer (Ohlendorf et al. 2011, Stewart et al. 2010, Luoma and Presser 2009). Although Presser (2009) studied Se bioaccumulation in multiple Appalachian aquatic ecosystems that include headwaters, no published study to date has provided detailed examination of these processes in headwater streams of central Appalachia.

This study evaluated the dynamics of Se bioaccumulation that is occurring in headwater streams of central Appalachia, including streams influenced by coal mining. Ecosystem media were sampled to evaluate possible pathways of Se enrichment, trophic transfer, and bioaccumulation. Water column Se concentrations constituted the source of Se. Three media (stream-bed sediment, biofilm, and in-stream leaf detritus) termed “particulate phases” or “particulate matter”, were sampled as potential pathways of enrichment and bioaccumulation;

macroinvertebrates separated into prey and predator taxa groups to evaluate two levels of trophic transfer.

In phase I of this research, we assessed Se bioaccumulation in crayfish from family *Cambaridae* and dragonfly nymphs from families *Gomphidae* and *Cordugastridae* in 23 streams. Benthic macroinvertebrates collected during this sample period were analyzed for Se concentration, establishing a gradient representative of tissue-based Se concentrations within headwater ecosystems of the region influenced by coal mining activities. Reference streams, not influenced by mining and with minimal recent disturbance, consistently had some of the lowest concentrations of Se in all taxa collected.

In phase II of this research, we selected nine sites for further study to investigate Se dynamics at the base of the aquatic food chain in the fall of 2015 and spring of 2016. Sites were selected based upon the established range of Se tissue concentrations from Phase I. Streams were classified into three "stream types." Reference streams drained watersheds with no history of coal mining and minimal anthropogenic disturbance. Selenium concentration in media samples from reference streams were expected to represent background levels. Streams draining watersheds with a history of coal-mining activity were divided into two groups, "high-Se" and "low-Se" streams. High-Se streams were streams that contained benthic macroinvertebrates with Se concentrations at the high end of the range reported in phase I. Three streams that exhibited macroinvertebrate Se concentrations between reference and high-Se concentrations were selected as low-Se streams.

We expected media collected from mining-influenced streams to contain Se concentrations elevated above the reference streams. Tissue samples used to classify streams into categories of high-Se and low-Se were also expected to be indicative of concentrations in other media including stream bed sediment, substrate biofilm, and in-stream leaf detritus.

Selenium dynamics at stream sites were quantified for comparison by calculating enrichment factors (EF) and trophic transfer factors (TTF) at each site. Site-specific characteristics within an aquatic system primarily control these transformations. Speciation of the Se source, water residency time, and biological community composition are all known to influence enrichment and trophic transfer factors (Lemly 1985). We did not expect EF and TTF values to differ significantly among stream types because, by study design, site factors influencing Se dynamics had been minimized and all streams were similar in characteristics such as watershed position and watershed size.

Clear patterns of Se enrichment among stream types were found, in agreement with study hypotheses. Across all media, reference streams exhibited the lowest Se concentrations, low-Se streams were elevated above reference, and high-Se streams contained the highest Se concentrations in media. Most comparisons among sites were statistically significant (p-value <

0.05). No significant differences in Se concentrations were found between seasons in any media collected.

Our hypothesis that Se dynamics would not vary among streams was not fully supported by results of this study. Enrichment factors did not differ by season, but differences in $EF_{sediment}$ and $EF_{leaf\ detritus}$ were detected among streams. Trophic transfer factors also did not differ significantly by season, but differences among stream types were detected in $TTF_{prey:\ sediment}$ and $TTF_{predator:\ prey.}$ $TTF_{prey:\ sediment}$ were lower in reference and low-Se streams than in high-Se streams. $TTF_{predator:\ prey}$ was higher in reference streams than in high-Se streams. Selenium dynamics may differ at varying levels of Se exposure because of changes in biotic communities caused by Se or other stressors correlating with Se concentration increases. Concentration-dependent mechanisms of bioaccumulation may also be a factor contributing to these findings.

Conclusions

From phase I of this study, we were able to conclude that headwater streams in central Appalachia are capable of Se enrichment and bioaccumulation in benthic macroinvertebrate organisms. Results from phase II of this study indicated that elevated water-column Se concentrations in mining-influenced streams caused stream bed sediment, biofilms, and in-stream leaf detritus to become enriched in Se. Further, Se concentrations in benthic macroinvertebrate tissue samples correlate with Se levels in sediment, algae, and leaf detritus, suggesting that these media are viable pathways for enrichment within these stream ecosystems, and may act as pathways of bioaccumulation to consumers in the food web. These findings were consistent with findings by many other studies within a wide diversity of aquatic systems (Casey 2005).

Interpretation of Se dynamics is more complex. Values for EF and TTF differed significantly among stream types for some media ratios, but not others. The direction of differences detected among stream types also do not offer an easy and consistent pattern for interpretation. As a result, a number of possible explanations were put forth to explain variation in Se dynamics among stream types.

Enrichment Factor values were likely affected by methods used to address Se concentration data falling below detection limits (< MDL). Water concentration values that were < MDL were uniformly set at half the detection limit. Alteration of nominal values for < MDL Se concentrations relative to actual levels, would affect EF values that were calculated with water column Se concentration in the denominator of the calculated ratios.

Concentration-dependent mechanisms of enrichment may also explain EF differences. Selenium enrichment has been found to decrease as Se exposure increases (DeForest et al 2007), explaining higher EFs at low-Se streams compared with high-Se streams. Lower EF values in streams with higher Se exposure were also observed in biofilm collected from streams in the Rocky Mountains (Kuchapski and Rasmussen 2015).

Differences in TTF among the three stream types may be attributed to differences in benthic macroinvertebrate communities at study sites. Conductivity is a co-variant with Se and has been shown to be related to shifts in macroinvertebrate community composition (Timpano 2011; USEPA 2011, Pond 2008). Biological communities are driving factors in Se bioaccumulation, and shifts in community composition may produce differences in Se bioaccumulation ratios (Presser and Luoma 2010).

Presser and Luoma (2010) suggested that modeling of Se dynamics would be an appropriate method for developing water-quality criteria for Se, and that appropriate criteria for specific stream systems would be dependent on estimates of EF and TTF within a given stream system. The US Environmental Protection Agency adopted this approach in 2016 (USEPA 2016). Based on this study, one might conclude that Se dynamics are, in part, dependent on the level of Se contamination in water flowing through a given headwater stream ecosystem. However, overall, no major differences in Se enrichment and bioaccumulation pathways were detected among stream types, suggesting that EF and TTF values quantified in this study may be applicable to other headwater streams within the region and used to link dissolved water column concentrations to tissue concentrations in wildlife. However, further study of these issues is warranted.

It is also important to note the degree of uncertainty associated with all factors calculated. Previous studies have acknowledged EF to be the most uncertain Se transformation (Presser and Luoma 2010, Luoma and Rainbow 2005). Additional field studies evaluating Se concentrations in water column, particulate matter, and consumers, are needed to expand inference scale and to form more accurate estimates of EF and TTF ranges within headwater streams of the Appalachian coalfield. Lower instrumental detection limits would help to reduce the degree of uncertainty in Se concentrations in streams with low levels of Se exposure. A higher frequency of water-column measurements, so as to achieve greater precision in the estimated water-based Se exposures at a given site, would increase the accuracy of calculated enrichment and trophic-transfer factors.

High-Se streams in this study were chosen to be representative of sites subjected to maximum degree of Se accumulation in mining-influenced headwater streams of central Appalachia. By study design, sites were selected based on Se concentrations found in benthic macroinvertebrate tissue samples. Three streams, termed “high-Se,” containing the highest levels of Se concentrations were chosen for further study, thus selecting streams with potential for high levels of Se bioaccumulation relative to other streams in our study. This study found capacity for Se bioaccumulation in high-Se streams to be relatively high compared to other lotic systems. This finding was unexpected, as multiple environmental factors were expected to minimize Se bioaccumulation in headwater streams, including low residence times and Se speciation (Lemly 1986). However, this study observed benthic macroinvertebrate Se concentrations above previously reported values in streams within the same ecoregion (Arnold et al. 2014, Presser 2013). Most of the sites studied by those authors were in higher-order

streams; hence, our results were consistent with Casey (2005), who found the highest ecosystem-media concentrations of Se in streams closest to the source of Se and with concentrations decreasing as sampling effort continued downstream.

Headwater streams are an essential component of central Appalachian stream networks and are estimated to constitute 70-80% of total stream length within the region (USEPA 2011). Prior to this study, Se dynamics in headwater streams were largely unstudied, and their capacity for enrichment and bioaccumulation unquantified. All streams influenced by coal-mining sampled in Phase II of this study had Se concentrations in media measurably elevated above media sampled from reference streams. These findings suggest that mining-influenced headwater streams in central Appalachia likely play a significant role in Se loading within mining-influenced stream networks.

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APPENDIX A. HISTORICAL STREAM SITE DATA

Table A-1. Dissolved Se (µg/l) concentrations collected from 25 stream sites in central Appalachia between May 19, 2013 and Nov. 25, 2014

Site	5/19/13 - 5/23/13	7/17/13 - 7/20/13	9/20/16 - 9/23/16	11/9/13 - 11/17/13	1/17/14 - 1/21/14	3/9/14 - 3/12/14	4/11/14 - 4/23/14	7/20/14 - 7/24/14	10/17/14 - 10/19/14	11/21/14 - 11/25/14
BIR*							1.5		1.2	
COP*	0.9	0.5	0.7	0.5	0.3	0.7	0.8	0.7	0.5	0.6
CRA				0.5			1.3			
CRO*	0.5	0.6	0.9	1.6	1.1	0.4	0.6	0.9	(BDL)	0.7
DAV				4.4			3.4			
EAS*	0.8	0.5	0.8	0.9	0.6	0.5	0.8	0.8		0.5
FRY	1.4	1.0	0.7	0.7	2.2	1.3	1.4	1.1	0.9	1.0
GRA	0.7	0.5	0.6	0.5	0.6	0.8	0.5	0.8	(BDL)	1.6
HCN				0.5		0.3	0.6	0.7	0.5	(BDL)
HUR				0.8			1.7			
KEL*	3.4	3.8	3.2	3.5	4.6	4.2	3.7	3.7	4.0	3.5
KUT*	9.1	8.5	7.0	9.7	9.8	8.6	8.0	9.3	9.1	10.3
LAB				3.7			4.4			
LLC*				6.3	6.4	6.6	8.4	5.3		6.2
LLE				2.6			0.9			
LLW				0.3			3.9			
MCB							0.5			
MIL*	1.6	0.9	1.3	0.7	1.6	1.3	1.1	1.2		0.8
POW	2.2	1.8	1.7	0.8	2.8	5.0	2.0	3.4	3.1	1.7
RFF*	1.0	0.9	0.8		0.8	1.0	0.6	1.1	0.7	0.5
RIC	5.4	2.7	1.9	1.7	2.7	2.2	4.2	1.9	2.7	
ROC				8.0	12.4	11.9	15.7	10.4	11.8	24.3
ROL				0.7			1.4			
RUT		0.5		0.9			1.0			
SPC	1.2	1.5	1.1	0.5	0.7	0.7	0.6	0.8	0.6	0.6

(BDL) values are below detection limits (< .5 ug/l)

* sites selected for study investigating Se dynamics in headwater streams

APPENDIX B: RESULTS: TRACE ELEMENT WATER COLUMN DATA, PHASE II

Table B-1: Trace element concentrations (µg l^{-1}) in dissolved water column data.

Site	Season	Al	V	Ni	Cu	Zn	As	Se	Sr	Cd
		µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}
Minimum Reporting Le		**5**	**1**	**1**	**1**	**5**	**0.5**	**2**	**1**	**1**
Method Detection Limi		**0.24**	**0.09**	**0.17**	**0.09**	**0.35**	**0.11**	**0.51**	**0.07**	**0.11**
KEL	Fall	6.4	< MDL	1.1	0.5 [A]	3.2	< MDL	3.1	802.9	< MDL
KEL	Spring	45.3	< MDL	1.1	0.5 [A]	14.7	< MDL	4.5	735.1	< MDL
KUT	Fall	4.2 [A]	< MDL	1.5	0.8 [A]	27.2	0.1 [A]	10.9	1658.0	< MDL
KUT	Spring	3.9 [A]	< MDL	1.1	0.7 [A]	19.7	< MDL	9.2	1380.0	< MDL
LLC	Fall	11.2	< MDL	1.9	0.7 [A]	21.1	< MDL	9.0	1359.0	< MDL
LLC	Spring	14.9	< MDL	4.0	0.3 [A]	17.6	< MDL	5.8	671.5	< MDL
COP	Fall	3.2 [A]	< MDL	0.3	< MDL	6.6	0.2 [A]	< MDL	378.7	< MDL
COP	Spring	2.6 [A]	< MDL	< MDL	< MDL	15.3	< MDL	< MDL	190.8	< MDL
CRO	Fall	15.9	< MDL	0.4	0.3 [A]	10.3	0.2 [A]	< MDL	41.8	< MDL
CRO	Spring	7.3	< MDL	< MDL	0.2 [A]	21.8	< MDL	< MDL	19.7	< MDL
EAS	Fall	21.2	< MDL	0.3	0.3 [A]	34.9	< MDL	0.7 [A]	12.9	< MDL
EAS	Spring	6.7	< MDL	< MDL	0.3 [A]	16.1	< MDL	0.8 [A]	10.0	< MDL
BIR	Fall	14.2	< MDL	2.0	0.6 [A]	44.3	0.2 [A]	< MDL	249.0	< MDL
BIR	Spring	5.7	< MDL	1.9	0.6 [A]	14.9	< MDL	1.3 [A]	195.6	< MDL
MIL	Fall	2.8 [A]	< MDL	1.0	0.5 [A]	9.7	0.1 [A]	0.5 [A]	721.5	< MDL
MIL	Spring	7.6	< MDL	0.9 [A]	2.1	15.2	< MDL	1.4 [A]	404.6	< MDL
RFF	Fall	6.5	0.1 [A]	0.6	0.3 [A]	8.8	0.2 [A]	0.8 [A]	610.4	< MDL
RFF	Spring	23.2	< MDL	0.5 [A]	0.8 [A]	14.0	< MDL	1.2 [A]	622.7	< MDL

Table B-2: Trace element concentrations (µg l^{-1}) in total recoverable water column data.

Season	Site	Al µg l^{-1}	V µg l^{-1}	Ni µg l^{-1}	Cu µg l^{-1}	Zn µg l^{-1}	As µg l^{-1}	Se µg l^{-1}	Sr µg l^{-1}	Cd µg l^{-1}
Minimum Reporting Level		5	1	1	1	5	0.5	2	1	1
Method Detection Limit		0.24	0.09	0.17	0.09	0.35	0.11	0.51	0.07	0.11
Fall	KEL	28.8	< MDL	1.1	0.4 [A]	6.3	< MDL	3.5	800.8	< MDL
Spring	KEL	2.4 [A]	< MDL	1.0	0.4 [A]	30.1	< MDL	5.2	741.3	< MDL
Fall	KUT	19.9	< MDL	1.4	0.6 [A]	12.5	0.1 [A]	10.5	1651.0	< MDL
Spring	KUT	13.1	< MDL	1.2	0.6 [A]	15.7	< MDL	10.1	1390.0	< MDL
Fall	LLC	57.7	< MDL	1.7	0.7 [A]	17.3	< MDL	8.6	1367.0	< MDL
Spring	LLC	44.3	< MDL	4.5	0.4 [A]	21.7	< MDL	6.3	669.5	< MDL
Fall	COP	61.6	0.1 [A]	0.4	< MDL	7.2	0.2 [A]	< MDL	378.9	< MDL
Spring	COP	37.6	< MDL	< MDL	0.2 [A]	13.4	< MDL	< MDL	189.5	< MDL
Fall	CRO	52.4	0.1 [A]	0.7	0.6 [A]	10.9	0.2 [A]	< MDL	42.3	< MDL
Spring	CRO	40.3	< MDL	< MDL	0.2 [A]	13.1	< MDL	< MDL	19.6	< MDL
Fall	EAS	64.1	0.1 [A]	0.4	< MDL	7.4	< MDL	< MDL	13.3	< MDL
Spring	EAS	32.1	< MDL	< MDL	0.1 [A]	12.4	< MDL	< MDL	9.7	< MDL
Fall	BIR	13.2	< MDL	1.7	0.1 [A]	11.8	0.2 [A]	0.6 [A]	247.2	< MDL
Spring	BIR	39.4	< MDL	2.3	0.6 [A]	20.1	< MDL	1.4 [A]	194.1	< MDL
Fall	MIL	14.3	< MDL	1.3	0.6 [A]	61.3	0.1 [A]	0.9 [A]	727.9	< MDL
Spring	MIL	27.3	< MDL	0.7 [A]	0.5 [A]	14.8	< MDL	1.2 [A]	404.9	< MDL
Fall	RFF	17.6	0.1 [A]	0.6	< MDL	15.5	0.2 [A]	0.8 [A]	613.1	< MDL
Spring	RFF	134.0	0.2 [A]	0.9 [A]	1.0 [A]	16.4	0.2 [A]	2.0	616.2	< MDL

APPENDIX C. SUPPLEMENTARY DATA OF MEDIA PROCESSED, PHASE II

Table C-1. Leaves, identified to tree genus collected as composite leaf detritus sample from 9 streams during 2 sample period, fall 2015 and spring 2016.

	Site	Date of Collection	*Acer Saccharam*	*Betula Lenta*	*Ericaceae rododendron*	*Fagus grandifolia*	*Hamamelis virginiana*	*Liriodendron tulipifera*	*Magnolia macrophylla*	*Platanus occidentalis*	*Prunus serotina*	*Quercus alba*	*Quercus montana*	*Quercus rubra*	*Tilia americana*	*Vitis spp.*	unidentifiable
Fall 2015	**KEL**	9/11/15	12							14		2					27
	KUT	9/6/15	43		1					21		3		1		3	28
	LLC	10/10/15	26					9		33		1					25
	MIL	9/20/15	5		17			8									7
	BIR	9/19/15	41		2			5	1			1		6			6
	RFF	9/27/15	12	5			1	17	3		4	18					
	COP	9/26/15	34		5												10
	CRO	9/21/15	5		6			13		3							4
	EAS	9/12/15	41		38			1						3	6		9
Spring 2016	**KEL**	3/6/16	5		2	47						12		5			9
	KUT	3/7/16			11	26				1		6		25			16
	LLC	3/5/16	2			33				31		6		1			25
	MIL	3/1/16	1		4	65						1					4
	BIR	3/1/16			7	26								19			23
	RFF	3/4/16	2		4	69				6		6		1			8
	COP	3/8/16	6		3	15		0		9		1	0	16			25
	CRO	2/1/16			1	35								2			33
	EAS	3/11/16	3		3	3		3				4	12	34			10

Table C-2. Count of macroinvertebrate "prey" taxa collected from 9 streams during 2 sample periods, fall 2015 and spring 2016.

Sample period	Family (*Genus*) [1]	Ameletidae	Baetidae	Chironomidae [5]	Chloroperlidae (*Alloperla*)	Dixidae	Dryopidae	Elmidae	Ephemerellidae	Ephemeridae	Gammaridae	Heptageniidae	Hydropsychidae	Lectradae	Leptophlebia	Limnephilidae	Nemouridae	Oligochaeta	Peltoperlidae	Philopotamidae	Psephenidae	Pteronarcyoidea	Simuliidae	Stratiomydae	Taeniopterygidae	Tipulidae (*Antocha*)	Tipulidae (*Tipula*)	Uenoidae
	FFG [2]	CG	CG; H	CG	CG	CF [3]	H	CG	CG; H	CG	CG [3]	CG; H	CF	SD	CF; H	CG; H; SD	5	CG [3]	SD [3]	CF	H	SD	CF [3]	CG [3]	H; SD	CG [3]	SD	H
Fall 2015	KEL		1										141	631			1	3		6				2			3	41	
	KUT		5	3		2							186	793										8				3	
	LLC		2	1			10	6					464	19					1	195	1	4						65	
	MIL		4	12		1	1						260	422				2		12	3		28					42	
	BIR		1	4				7					180	5				11		137			5					28	
	RFF		14	9	5		10	15	1	76		292	337					5	2	3	22	10						35	
	COP		37	1		6	4		3	27		107	128	3	8			5	27	5	8	8						47	
	CRO		6	11			1	4	7	79		117	10	4	18			1		3	19	3						2	
	EAS		9	14	4	2		2	21	33		76	229	21	28			7	22	11	13	16							
Spring 2016	KEL	36	91	3		1			12				435	42		2	263	1	1		8		5					30	
	KUT	2	101	13		1			1				733	21			92	1	1	1	3		20	1				35	
	LLC	1		2			7		6		4		262	44		2	245	2		21	4	4	4		40			81	34
	MIL	193	126	6					10			1	146	2		4	9	1	1	2	4		1		35			49	3
	BIR	113	103	26									70	1		3	79	12		18	3		49		4			50	
	RFF	66	105	25	11			3	23	10		33	325	3		1	13	4	1		4	10	1		20			20	5
	COP	21	1	4					161	2		185	171	4	11	15			73	3	3	30	6				27	
	CRO	111	1	1				1	39	13		278	95	9	95		1	2	2	8	8	13	48				8	38
	EAS	28	4						82	4		232	91	10	81	1			52	1	3	28	2				4	4

[1] Macroinvertebrates were identified to family level. In some cases, identification to genus level was needed for functional feeding group classification. When applicable, genus level identification is in parentheses.

[2] Functional feeding groups: (CG) collector-gatherers (CF) collector-filterers (H) herbivores (PR) predators (SD) shredder-derivers. Primary source for classification: Poff, N. LeRoy, et al. J. of the North Am. Benthological Soc. 25.4 **2006**: 730-755.

[3] Alternative source used to classify FFG: Merritt, R. W., & Cummins, K. W. (1996). An introduction to the aquatic insects of North America. Kendall Hunt

Table C-3. Count of macroinvertebrate "predator" taxa collected from 9 streams during 2 sample periods, fall 2015 and spring 2016.

Sample period	Family (*Genus*) [1]	Aeshnidae	Calopterygidae	Ceratopogonidae	Chloroperlidae *(Haploperla)*	Chloroperlidae *(Sweltza)*	Cordulegastridae	Corydalidae	Empididae	Gerridae	Gomphidae	Hydrophilidae (larvae)	Perlidae	Perlodidae	Polycentropodidae *(Polycentropus)*	Rhagionidae	Rhyacophilidae	Sialidae	Tipulidae *(Dicronata)*	Tipulidae *(Hexatoma)*	Veliidae
	FFG [2]	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR [3]	PR [3]	PR
Fall 2015	KEL		1				7		1		118							5		1	13
	KUT								1		30						2				3
	LLC	2	5					7			5	2	103			7	1	1	2	15	8
	MIL	2	1					3			22		1	1			3				20
	BIR	1	2				2	52			50					1	2	3			26
	RFF						4	56		1	9		32			1		4		6	9
	COP						1				17		8	17					1	9	9
	CRO	1			1		12	1			20		28		3		1			11	28
	EAS			1				2			11		72				11		2	20	51
Spring 2016	KEL						4				145		6		5		72	6		1	
	KUT						1				95				1		76				
	LLC	2						8	1		9		116	23		9	94	1		6	
	MIL	7	1				1	8	4		17		5	5			30			1	
	BIR						1	26			8						13	1		3	
	RFF						15	15			3		17	17		1	23	1		1	
	COP							1			9		20	40			5			6	
	CRO					5		1	4		7		67	14			6			9	
	EAS					5		1					39	47			10			9	

[1] Macroinvertebrates were identified to family level. In some cases, identification to genus level was needed for functional feeding group classification. When applicable, genus level identification is in parentheses.

[2] Functional feeding groups: (CG) collector-gatherers (CF) collector-filterers (H) herbivores (PR) predators (SD) shredder-derivers. Primary source for classification: Poff, N. LeRoy, et al. J. of the North Am. Benthological Soc. 25.4 **2006**: 730-755.

[3] Alternative source used to classify FFG: Merritt, R. W., & Cummins, K. W. (1996). An introduction to the aquatic insects of North America. Kendall Hunt

Table C-4. Cambaridae length (cm) collected from 9 headwater streams in central Appalachia during 2 sample period, fall 2015 and spring 2016.

		ave	n	stdev	min	max
Fall 2015	KEL	3.3	5	0.19	3.1	3.6
	KUT	4.5	2	1.20	3.6	5.3
	LLC	4.0	4	0.36	3.5	4.3
	MIL	3.5	5	0.83	2.1	4.2
	BIR	2.7	2	1.13	1.9	3.5
	RFF	3.4	4	0.35	3	3.8
	COP	3.2	7	0.20	3.1	3.6
	CRO	3.1	5	0.69	2.3	3.9
	EAS	3.1	4	0.30	2.8	3.4
Spring 2016	KEL	3.0	5	0.19	2.70	3.2
	KUT	3.1	5	0.27	2.70	3.4
	LLC	3.4	3	0.32	3.00	3.6
	MIL	3.2	5	0.26	2.80	3.5
	BIR	3.4	4	0.18	3.20	3.6
	RFF	3.3	5	0.30	2.90	3.6
	COP	3.1	5	0.17	2.80	3.3
	CRO	2.2	12	0.06	2.20	2.4
	EAS	3.3	5	0.23	3.05	3.6

APPENDIX D: PHASE II RESULTS: SE

Table D-1. Reported selenium concentrations (µg/g dry wt) in media collected from 9 streams in the fall 2015 and spring 2016.

	sediment					biofilm					leaf detritus				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se															
KEL															
Fall	1.51	0.51	3	0	0	1.67	0.11	3	0	0	3.22	0.28	3	0	0
Spring	1.34	0.01	3	0	0	2.23	0.28	3	0	0	5.22	0.40	3	0	0
KUT															
Fall	1.22	0.31	3	0	0	5.15	0.21	3	0	0	2.18	0.15	3	0	0
Spring	0.94	0.16	3	0	0	2.81	0.53	3	0	0	5.26	0.38	3	0	0
LLC															
Fall	0.38	0.02	3	0	0	3.79	0.32	3	0	0	0.88	0.04	3	0	3
Spring	0.46	0.05	3	0	0	3.40	0.17	3	0	0	2.31	0.21	3	0	0
Low-Se															
BIR															
Fall	0.49	0.07	3	0	0	1.05	0.10	3	0	1	0.63	0.21	3	0	3
Spring	0.46	0.05	3	0	0	0.88	0.10	3	0	3	0.17	0.08	3	2	3
MIL															
Fall	0.25	0.07	3	0	2	1.07	0.32	3	0	1	0.78	0.22	3	0	2
Spring	0.25	0.02	3	0	2	0.49	0.05	3	0	3	0.43	0.04	3	0	3
RFF															
Fall	0.30	0.01	3	0	0	0.92	0.03	3	0	3	0.87	0.11	3	0	3
Spring	0.30	0.03	3	0	0	1.16	0.10	3	0	0	0.18	0.08	3	2	3
Reference															
COP															
Fall	0.13	0.01	3	0	3	0.38 [1]	0.11	3	0	3	0.13	0.00	3	3	3
Spring	0.13	0.04	3	0	3	0.45	0.07	3	0	3	0.13	0.00	3	3	3
CRO															
Fall	0.10	0.03	3	0	3	0.73 [1]	0	3	0	3	0.13	0.00	3	3	3
Spring	0.08	0.01	3	0	0	0.86	0.17	3	0	3	0.13	0.00	3	3	3
EAS															
Fall	0.16	0.01	3	1	3						0.13	0.00	3	3	3
Spring	0.09	0.01	3	0	3	0.26	0.22	3	2	3	0.13	0.00	3	3	3

[1] Biofilm samples collected from sandy substrates

Table D-1. (*Continued*) Reported selenium (µg/g dry wt) concentrations in media collected from 9 streams in the fall 2015 and spring 2016.

	Pteronarcys					***Tipula***					**prey - *Tipula* - *Pteronarcys***					**prey [2]**	**predator**					***Cambaridae***				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se																										
KEL																										
Fall											16.65		1	0	0	16.65	24.34	0.31	2	0	0	7.60		1	0	0
Spring						17.04	0.41	3	0	0	15.90	0.63	3	0	0	16.47	25.17	0.70	3	0	0	12.58	0.58	3	0	0
KUT																										
Fall											23.86		1	0	0	23.86	30.34		1	0	0	9.09	0.08	2	0	0
Spring						41.27	0.61	3	0	0	20.58	1.11	3	0	0	33.42	33.46	0.98	3	0	0	13.43	0.98	3	0	0
LLC																										
Fall	10.61		1	0	0						12.72	0.6	2	0	0	12.43	16.35		1	0	0	6.86		1	0	0
Spring	9.33		1	0	0	17.36	0.57	4	0	0	13.27		1	0	0	16.62	18.61	0.18	3	0	0	6.09	0.22	3	0	0
Low-Se																										
BIR																										
Fall											10.32		1	0	0	10.32	9.28		1	0	0	2.12		1	0	0
Spring						2.14	0.14	3	0	0	4.24	0.37	2	0	0	2.82	5.62	0.01	2	0	0	1.95	0.18	4	0	0
MIL																										
Fall											4.26		0	0	0	4.26	5.43		1	0	0	1.91		1	0	0
Spring						2.71	0.19	5	0	0	3.55	0.20	4	0	0	3.05	4.56	0.19	2	0	0	2.49		1	0	0
RFF																										
Fall	1.40	0.17	2	0	0						5.43	0.09	2	0	0	3.47	4.52	0.21	3	0	0	2.29	0.10	2	0	0
Spring	2.19	0.12	4	0	0	4.48	0.47	2	0	0	3.30	0.22	3	0	0	3.20	4.76	0.28	3	0	0	3.06	0.15	2	0	0
Reference																										
COP																										
Fall	0.86		1	0	1						2.34		1	0	0	1.77	3.46		1	0	0	0.81	0.24	2	0	2
Spring	0.80	0.01	3	0	3	0.45	0.29	3	1	3	2.21	0.20	4	0	0	1.15	2.80	0.27	3	0	0	1.00		1	0	1
CRO																										
Fall	0.37		1	0	1						1.00		1	0	1	0.82	1.76	0.47	2	0	0	1.02		1	0	0
Spring	0.33	0.06	3	0	2	0.40		1	0	1	1.24	0.25	3	0	1	0.75	1.74	0.16	3	0	0	0.63	0.31	3	0	3
EAS																										
Fall	0.57		1	0	1						1.80		1	0	0	1.08	2.88		1	0	0	1.39		1	0	0
Spring	0.30	0.20	4	2	4	0.48		1	0	1	1.06	0.10	4	0	3	0.63	2.40	0.38	2	0	0	1.02	0.27	2	0	1

[2] Calculated value

APPENDIX E. PHASE II RESULTS: OTHER TRACE ELEMENTS

Table E-1. Mean aluminum concentrations (µg g^{-1} dry wt.) in particulate matter subsamples by stream and season.

	sediment					biofilm					leaf detritus				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**6927**	**4002**				**17623**	**8697**				**3637**	**2588**			
KEL	**8817**	**2232**				**15192**	**2263**				**6311**	**2581**			
Fall	9439	3074	3			17150	621	3			8524	860	3		
Spring	8195	1357	3			13234	954	3			4098	1106	3		
KUT	**9518**	**3566**				**22665**	**13019**				**3168**	**1101**			
Fall	12450	2383	3			33782	6364	3			4051	603	3		
Spring	6586	567	3			11548	3533	3			2285	571	3		
LLC	**2446**	**567**				**15012**	**6061**				**1432**	**468**			
Fall	2556	858	3			20270	2288	3			1068	216	3		
Spring	2335	180	3			9754	1915	3			1795	324	3		
Low-Se	**7387**	**3130**				**15815**	**7532**				**4380**	**1883**			
BIR	**9282**	**3609**				**15721**	**8501**				**5263**	**2681**			
Fall	11145	4681	3			22402	6469	3			7687	471	3		
Spring	7420	499	3			9039	2207	3			2840	363	3		
MIL	**5234**	**2240**				**11822**	**7754**				**3474**	**1434**			
Fall	6303	3007	3			17560	7053	3			4680	872	3		
Spring	4164	267	3			6084	1329	3			2268	140	3		
RFF	**7646**	**2305**				**19903**	**4613**				**4403**	**945**			
Fall	8967	2786	3			22633	4664	3			5004	286	3		
Spring	6325	535	3			17173	3016	3			3802	1032	3		
Reference	**5074**	**1572**				**17000**	**7362**				**2443**	**1236**			
COP	**5018**	**1621**				**12982**	**2093**				**2580**	**1198**			
Fall	6108	1724	3			13836	2863	3			3590	709	3		
Spring	3929	196	3			12128	755	3			1570	151	3		
CRO	**4865**	**1007**				**25091**	**3079**				**3391**	**998**			
Fall	5474	1032	3			25850	4577	3			4226	558	3		
Spring	4256	599	3			24331	1008	3			2556	299	3		
EAS	**5338**	**2148**				**8857**	**711**				**1359**	**503**			
Fall	6746	2358	3								1811	74	3		
Spring	3929	156	3			8857	711	3			906	104	3		

Table E-2. Mean aluminum concentrations (µg g^{-1} dry wt.) in macroinvertebrate subsamples by stream and season.

	Pteronarcys					*Tipula*					prey - *Tipula* - *Pteronarcys*					predator					*Cambaridae*				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**1228**	**910**				**2666**	**1109**				**3158**	**1168**				**1289**	**532**				**461**	**265**			
KEL						**4180**	**588**				**4188**	**1201**				**1844**	**236**	**5**			**813**	**116**			
Fall											5966	-	1			1608	186	2			708	-	1		
Spring						4180	588	3			3595	238	3			2001	34	3			848	114	3		
KUT						**2305**	**139**				**2701**	**843**				**1202**	**196**	**4**			**383**	**64**			
Fall											3947	-	1			929	-	1			340	93	2		
Spring						2305	139	3			2285	175	3			1293	89	3			412	29	3		
LLC	**1228**	**910**				**1801**	**157**				**2395**	**429**				**681**	**41**	**4**			**208**	**21**			
Fall	1871	-	1								2381	605	2			731	-	1			220	-	1		
Spring	584	-	1			1801	157	4			2424	-	1			665	30	3			204	24	3		
Low-Se	**2237**	**755**				**2868**	**825**				**2473**	**1097**				**1117**	**380**	**#**			**523**	**124**			
BIR						**2307**	**288**				**3165**	**1350**				**1166**	**404**	**3**			**526**	**93**			
Fall											4723	-	1			1467	-	1			588	-	1		
Spring						2307	288	3			2386	43	2			1015	435	2			510	100	4		
MIL						**2650**	**314**				**1642**	**823**				**813**	**468**	**3**			**413**	**2**			
Fall											3072	-	1			1354	-	1			411	-	1		
Spring						2650	314	5			1284	225	4			543	3	2			414	-	1		
RFF	**2237**	**755**				**4256**	**727**				**2992**	**552**				**1245**	**296**	**6**			**575**	**168**			
Fall	1426	58	2								3795	-	1			1466	259	3			459	111	2		
Spring	2642	540	4			4256	727	2			2725	168	3			1024	76	3			692	136	2		
Reference	**1040**	**876**				**1760**	**307**				**2778**	**1168**				**684**	**322**				**361**	**238**			
COP	**2465**	**94**				**1931**	**162**				**2969**	**1205**				**991**	**203**				**536**	**338**			
Fall											5076	-	1			1280	-	1			579	400	3		
Spring	2465	94	3			1931	162	3			2442	293	4			894	79	3			408	-	1		
CRO	**574**	**145**				**1732**					**3303**	**468**				**579**	**303**				**245**	**70**			
Fall	788	-	1								3917	-	1			885	235	2			345	-	1		
Spring	502	30	3			1732	-	1			3098	277	3			376	23	3			212	25	3		
EAS	**557**	**243**				**1274**	**-**				**2167**	**1424**				**449**	**166**				**283**	**87**			
Fall	983	-	1								4704	-	1			639	-	1			383	-	1		
Spring	451	57	4			1274	-	1			1533	156	4			354	29	2			233	15	2		

Table E-3. Mean vanadium concentrations (µg g^{-1} dry wt.) in particulate matter subsamples by stream and season.

	sediment					biofilm					leaf detritus				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**10.15**	**4.76**				**22.82**	**9.13**				**4.59**	**3.08**			
KEL	**12.53**	**2.34**				**20.87**	**1.79**	**6**			**7.95**	**2.65**			
Fall	12.77	3.44	3			22.20	1.09	3			10.01	1.53	3		
Spring	12.30	1.30	3			19.54	1.25	3			5.88	1.54	3		
KUT	**13.24**	**4.18**				**28.31**	**13.63**	**6**			**4.12**	**1.03**			
Fall	16.40	3.61	3			39.99	6.25	3			4.92	0.61	3		
Spring	10.08	0.78	3			16.63	3.99	3			3.31	0.57	3		
LLC	**4.69**	**0.40**				**19.27**	**6.22**				**1.70**	**0.57**			
Fall	4.71	0.59	3			24.64	1.90	3			1.24	0.24	3		
Spring	4.67	0.21	3			13.89	2.51	3	0	1	2.15	0.37	3		
Low-Se	**11.90**	**3.79**				**21.44**	**7.97**				**5.88**	**2.22**			
BIR	**15.73**	**3.07**				**22.04**	**9.18**				**6.93**	**3.19**			
Fall	17.46	3.79	3			29.20	7.03	3			9.82	0.47	3		
Spring	14.01	0.53	3			14.88	2.66	3			4.04	0.41	3		
MIL	**8.26**	**1.74**				**17.60**	**9.23**	**6**			**4.77**	**1.62**			
Fall	9.27	2.10	3			25.18	6.22	3			6.08	1.16	3		
Spring	7.25	0.27	3			10.03	1.52	3			3.46	0.17	3		
RFF	**11.71**	**1.67**				**24.69**	**3.98**	**6**			**5.94**	**1.10**			
Fall	12.87	1.68	3			26.44	4.38	3			6.53	0.26	3		
Spring	10.55	0.29	3			22.94	3.36	3			5.36	1.38	3		
Reference	**7.64**	**1.73**				**21.90**	**8.41**				**3.19**	**1.56**			
COP	**6.74**	**1.16**				**16.35**	**2.23**				**3.42**	**1.54**			
Fall	7.63	0.97	3			16.47	3.37	3			4.72	0.95	3		
Spring	5.86	0.17	3			16.24	1.00	3			2.13	0.15	3		
CRO	**7.10**	**0.99**				**31.42**	**2.99**				**4.39**	**1.17**			
Fall	7.93	0.30	3			31.66	4.52	3			5.16	1.20	3		
Spring	6.27	0.51	3			31.19	1.33	3			3.62	0.40	3		
EAS	**9.06**	**2.03**				**13.95**	**0.88**				**1.75**	**0.53**			
Fall	10.54	1.94	3								2.23	0.04	3		
Spring	7.59	0.24	3			13.95	0.88	3			1.28	0.13	3		

Table E-4. Mean vanadium concentrations (µg g^{-1} dry wt.) in macroinvertebrate taxa subsamples by stream and season.

	Pteronarcys					***Tipula***					**prey - *Tipula* - *Pteronarcys***					**predator**					***Cambaridae***				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**1.87**	**1.56**				**4.32**	**2.10**				**4.96**	**1.55**				**2.02**	**0.94**				**0.73**	**0.42**			
KEL						**7.19**	**0.22**				**6.54**	**1.24**				**2.92**	**0.61**				**1.28**	**0.22**			
Fall											8.37	-	1			2.34	0.17	2			0.97	-	1		
Spring						7.19	0.22	3			5.93	0.25	3			3.31	0.40	3			1.39	0.10	3		
KUT						**3.96**	**0.07**				**4.27**	**0.94**				**1.94**	**0.42**				**0.61**	**0.07**			
Fall											5.63	-	1			1.41	-	1			0.55	0.07	2	0	1
Spring						3.96	0.07	3			3.82	0.30	3			2.12	0.28	3			0.65	0.04	3		
LLC	**1.87**	**1.56**				**2.43**	**0.16**				**3.75**	**0.48**				**0.98**	**0.16**				**0.33**	**0.02**			
Fall	2.97	-	1								4.02	0.17	2			1.14	-	1			0.33	-	1	0	1
Spring	0.77	-	1			2.43	0.16	4			3.22	-	1			0.93	0.14	3			0.32	0.03	3	0	3
Low-Se	**3.49**	**1.17**				**5.15**	**1.01**				**3.83**	**1.44**				**1.76**	**0.49**				**0.85**	**0.18**			
BIR						**4.17**	**0.44**				**4.95**	**1.79**				**1.89**	**0.50**				**0.86**	**0.13**			
Fall											7.01	-	1			2.28	-	1			0.75	-	1		
Spring						4.17	0.44	3			3.92	0.21	2			1.70	0.52	2			0.88	0.13	4		
MIL						**5.17**	**0.61**				**2.76**	**1.12**				**1.41**	**0.74**				**0.70**	**0.10**			
Fall											4.74	-	1			2.26	-	1			0.63	-	1		
Spring						5.17	0.61	5			2.26	0.20	4			0.99	0.03	2			0.77	-	1		
RFF	**3.49**	**1.17**				**6.56**	**0.63**				**4.34**	**0.53**				**1.88**	**0.34**				**0.90**	**0.26**			
Fall	2.08	0.13	2								4.94	-	1			2.16	0.18	3			0.72	0.08	2		
Spring	4.20	0.52	4			6.56	0.63	2			4.14	0.42	3			1.59	0.10	3			1.09	0.24	2		
Reference	**1.64**	**1.23**				**2.85**	**0.57**				**4.03**	**1.36**				**1.03**	**0.40**				**0.56**	**0.30**			
COP	**3.62**	**0.26**				**3.20**	**0.26**				**4.01**	**1.11**				**1.43**	**0.11**				**0.78**	**0.43**			
Fall											5.98	-	1			1.59	-	1			0.84	0.51	3	0	1
Spring	3.62	0.26	3			3.20	0.26	3			3.52	0.21	4			1.38	0.03	3			0.61	-	1		
CRO	**1.05**	**0.35**				**2.71**	-				**4.79**	**0.44**				**0.90**	**0.39**				**0.41**	**0.09**			
Fall	1.55	-	1								5.36	-	1			1.31	0.21	2			0.55	-	1		
Spring	0.88	0.10	3			2.71	-	1			4.59	0.26	3			0.62	0.04	3			0.37	0.03	3	0	3
EAS	**0.93**	**0.32**				**1.96**	-				**3.44**	**1.90**				**0.72**	**0.24**				**0.48**	**0.12**			
Fall	1.49	-	1								6.83	-	1			1.00	-	1			0.62	-	1		
Spring	0.79	0.05	4			1.96	-	1			2.59	0.03	4			0.58	0.00	2			0.41	0.01	2	0	2

Table E-5. Mean copper concentration (µg g^{-1} dry wt.) of particulate matter subsamples by stream and season.

	sediment					biofilm					leaf detritus				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**9.08**	**4.65**				**21.35**	**8.99**				**8.05**	**1.85**	**18**		
KEL	**11.09**	**1.15**				**19.89**	**2.05**				**9.41**	**1.75**	**6**		
Fall	10.45	1.42	3			19.13	2.57	3			10.93	0.64	3		
Spring	11.73	0.28	3			20.64	1.49	3			7.89	0.54	3		
KUT	**13.07**	**2.28**				**30.81**	**9.11**				**8.74**	**0.85**	**6**		
Fall	13.38	3.15	3			39.01	0.88	3			9.39	0.59	3		
Spring	12.75	1.69	3			22.62	2.29	3			8.08	0.42	3		
LLC	**3.10**	**0.29**	**6**			**13.35**	**0.74**				**6.01**	**0.17**	**6**		
Fall	2.85	0.08	3			13.33	1.14	3			6.06	0.19	3		
Spring	3.34	0.16	3			13.37	0.23	3			5.95	0.16	3		
Low-Se	**10.24**	**3.49**				**17.50**	**4.37**				**9.95**	**2.99**	**18**		
BIR	**14.14**	**1.64**				**17.27**	**2.80**				**8.61**	**2.90**	**6**		
Fall	15.41	1.26	3			19.80	0.47	3			11.22	0.70	3		
Spring	12.86	0.46	3			14.75	0.41	3			6.00	0.06	3		
MIL	**6.19**	**0.40**				**14.28**	**5.23**				**10.15**	**2.53**	**6**		
Fall	6.36	0.51	3			19.01	0.94	3			12.39	0.98	3		
Spring	6.01	0.19	3			9.54	0.49	3			7.91	0.13	3		
RFF	**10.40**	**0.76**	**6**			**20.94**	**1.70**				**11.10**	**3.43**	**6**		
Fall	10.70	0.90	3			19.40	0.23	3			14.18	0.79	3		
Spring	10.11	0.59	3			22.47	0.31	3			8.01	0.51	3		
Reference	**4.23**	**0.90**				**14.77**	**7.25**				**7.95**	**2.89**	**18**		
COP	**3.48**	**0.38**				**9.49**	**1.69**				**6.84**	**1.99**	**6**		
Fall	3.80	0.22	3			8.00	0.18	3			8.64	0.31	3		
Spring	3.15	0.05	3			10.97	0.73	3			5.04	0.12	3		
CRO	**4.70**	**0.71**	**6**			**23.22**	**0.87**				**9.07**	**2.44**	**6**		
Fall	5.30	0.37	3			22.70	0.22	3			11.22	0.93	3		
Spring	4.11	0.25	3			23.75	1.00	3			6.92	0.33	3		
EAS	**4.52**	**1.02**	**6**			**8.43**	**0.15**				**7.95**	**3.95**	**6**		
Fall	5.37	0.61	3								11.55	0.45	3		
Spring	3.67	0.22	3			8.43	0.15	3			4.36	0.14	3		

Table E-6. Mean copper concentration (µg g^{-1} dry wt.) of particulate macroinvertebrate subsamples by stream and season.

	Pteronarcys					*Tipula*					prey - *Tipula* - *Pteronarcys*					predator					*Cambaridae*				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**17.6**	**11.7**				**21.7**	**7.9**				**25.8**	**5.4**				**25.7**	**3.3**				**99.3**	**25.5**			
KEL						**20.3**	**0.8**				**25.2**	**0.9**				**26.8**	**1.6**				**97.8**	**8.7**			
Fall											26.3	-	1			28.0	2.4	2			85.8	-	1		
Spring						20.3	0.8	3			24.9	0.8	3			26.0	0.0	3			101.8	4.0	3		
KUT						**32.6**	**1.5**				**31.4**	**1.7**				**28.6**	**0.8**				**121.0**	**25.0**			
Fall											33.8	-	1			28.4		1			94.3	0.2	2		
Spring						32.6	1.5	3			30.7	0.8	3			28.6	0.9	3			138.8	7.8	3		
LLC	**17.6**	**11.7**				**14.7**	**0.6**				**19.0**	**2.2**				**21.4**	**0.7**				**73.6**	**5.7**			
Fall	9.4	-	1								18.7	3.0	2			22.3	-	1			81.8	-	1		
Spring	25.9	-	1			14.7	0.6	4			19.6	-	1			21.1	0.3	3			70.9	2.0	3		
Low-Se	**26.8**	**2.0**				**18.9**	**1.3**				**17.5**	**2.9**				**21.6**	**2.0**				**86.4**	**9.0**			
BIR						**17.7**	**0.4**				**16.0**	**0.9**				**22.4**	**3.6**				**84.1**	**11.0**			
Fall											16.4	-	1			26.6	-	1			96.7	-	1		
Spring						17.7	0.4	3			15.7	1.1	2			20.3	0.4	2			80.9	9.8	4		
MIL						**20.0**	**0.5**				**19.6**	**3.0**				**21.4**	**2.1**				**80.8**	**4.5**			
Fall											24.7	-	1			23.8	-	1			77.6	-	1		
Spring						20.0	0.5	5			18.4	1.0	4			20.2	0.3	2			83.9	-	1		
RFF	**26.8**	**2.0**				**18.1**	**0.7**				**16.0**	**2.5**				**21.2**	**1.1**				**92.1**	**5.1**			
Fall	28.8	2.0	2								12.6	-	1			21.7	1.5	3			90.4	6.7	2		
Spring	25.8	1.3	4			18.1	0.7	2			17.2	1.1	3			20.7	0.5	3			93.8	4.5	2		
Reference	**31.4**	**3.1**				**13.0**	**1.9**				**17.0**	**2.8**				**28.8**	**5.3**				**91.3**	**37.0**			
COP	**29.3**	**1.4**				**11.9**	**0.2**				**15.6**	**1.4**				**25.0**	**1.7**				**63.1**	**22.3**			
Fall											13.3	-	1			22.9	-	1			58.4	24.7	3		
Spring	29.3	1.4	3			11.9	0.2	3			16.2	0.6	4			25.7	1.1	3			77.3	-	1		
CRO	**31.3**	**3.1**				**16.1**	-				**18.0**	**3.0**				**33.9**	**2.9**				**88.1**	**4.7**			
Fall	26.7	-	1								13.6	-	1			36.6	3.2	2			93.0	-	1		
Spring	32.8	0.8	3			16.1	-	1			19.5	0.7	3			32.2	0.7	3			86.5	4.2	3		
EAS	**32.7**	**3.6**				**13.0**	-				**17.6**	**3.5**				**25.2**	**4.0**				**133.1**	**42.7**			
Fall	39.0	-	1								23.8	-	1			29.9	-	1			182.3	-	1		
Spring	31.1	0.9	4			13.0	-	1			16.1	0.7	4			22.9	0.6	2			108.5	2.7	2		

Table E-7. Mean zinc concentration (µg g-1 dry wt.) of particulate matter subsamples by stream and season.

	sediment					biofilm					leaf detritus				
	mean	sd	n	n < mdl	n < mdl	mean	sd	n	n < mdl	n < mdl	mean	sd	n	n < mdl	n < mdl
High-Se	**55.21**	**12.12**				**143.91**	**43.42**				**41.67**	**14.55**			
KEL	**65.56**	**4.14**				**104.17**	**10.45**				**38.03**	**4.80**			
Fall	63.66	5.53	3			96.30	8.81	3			41.93	2.83	3		
Spring	67.46	1.17	3			112.04	3.06	3			34.13	1.97	3		
KUT	**59.23**	**6.70**				**150.38**	**30.66**				**39.08**	**9.54**			
Fall	59.51	10.24	3			177.22	7.37	3			30.62	2.13	3		
Spring	58.95	2.63	3			123.53	11.58	3			47.55	2.83	3		
LLC	**40.83**	**6.46**				**177.17**	**45.71**				**47.90**	**23.14**			
Fall	35.02	0.33	3			135.89	10.61	3			26.80	1.30	3		
Spring	46.64	1.72	3			218.44	0.53	3			69.00	1.15	3		
Low-Se	**66.08**	**36.85**				**105.04**	**46.25**				**38.59**	**12.69**			
BIR	**115.87**	**3.75**				**163.66**	**12.92**				**55.71**	**2.48**			
Fall	117.75	3.96	3			173.83	2.23	3			54.98	3.48	3		
Spring	113.99	2.98	3			153.50	10.11	3			56.44	1.30	3		
MIL	**33.69**	**1.54**				**61.39**	**18.36**				**28.27**	**1.63**			
Fall	34.59	1.65	3			77.89	4.63	3			28.36	2.56	3		
Spring	32.79	0.89	3			44.88	1.97	3			28.17	0.31	3		
RFF	**48.67**	**1.93**				**90.06**	**9.46**				**31.79**	**1.87**			
Fall	49.70	2.16	3			81.84	3.09	3			33.05	0.62	3		
Spring	47.64	1.23	3			98.29	3.32	3			30.53	1.90	3		
Reference	**25.23**	**4.76**				**74.46**	**32.95**				**26.44**	**6.98**			
COP	**21.83**	**1.83**				**49.58**	**10.12**				**21.17**	**3.23**			
Fall	23.19	1.64	3			40.55	2.17	3			24.07	0.78	3		
Spring	20.47	0.37	3			58.60	2.72	3			18.27	0.53	3		
CRO	**30.88**	**3.03**				**112.07**	**10.46**				**35.08**	**3.54**			
Fall	33.05	0.47	3			103.02	2.12	3			32.43	2.55	3		
Spring	28.71	2.93	3			121.11	4.86	3			37.72	1.96	3		
EAS	**22.99**	**2.52**				**49.03**	**0.89**				**23.07**	**2.51**			
Fall	24.84	2.30	3								25.34	0.33	3		
Spring	21.13	0.53	3			49.03	0.89	3			20.80	0.48	3		

Table E-8. Mean zinc concentration (µg g^{-1} dry wt.) of macroinvertebrate subsamples by stream and season.

	Pteronarcys					*Tipula*					prey - *Tipula* - *Pteronarcys*					predator					*Cambaridae*				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**289.9**	**20.9**				**136.7**	**32.4**				**205.7**	**34.3**				**191.4**	**65.3**				**90.7**	**14.3**			
KEL						**101.7**	**2.4**				**200.8**	**12.9**				**157.0**	**16.0**				**90.9**	**3.4**			
Fall											183.3	-	1			140.6	5.3	2			88.5	-	1		
Spring						101.7	2.4	3			206.6	6.8	3			167.8	7.5	3			91.7	3.7	3		
KUT						**124.3**	**5.0**				**237.6**	**8.3**				**143.0**	**6.0**				**95.4**	**22.2**			
Fall											247.6	-	1			134.1	-	1			71.6	4.9	2		
Spring						124.3	5.0	3			234.3	6.0	3			145.9	1.5	3			111.3	5.4	3		
LLC	**289.9**	**20.9**				**172.3**	**7.4**				**169.7**	**38.5**				**282.9**	**19.7**				**84.5**	**7.2**			
Fall	275.2	-	1								147.7	7.8	2			311.4	-	1			73.8	-	1		
Spring	304.7	-	1			172.3	7.4	4			213.8	-	1			273.5	6.6	3			88.0	1.5	3		
Low-Se	**437.3**	**96.1**				**102.7**	**28.2**				**167.5**	**71.4**				**168.6**	**18.3**				**76.3**	**5.9**			
BIR						**142.9**	**4.4**				**261.6**	**98.7**				**168.6**	**4.5**				**81.0**	**3.4**			
Fall											148.6	-	1			172.6	-	1			80.2	-	1		
Spring						142.9	4.4	3			318.1	18.7	2			166.6	4.0	2			81.2	3.9	4		
MIL						**82.4**	**1.4**				**137.7**	**8.6**				**140.6**	**2.7**				**71.8**	**3.2**			
Fall											148.3	-	1			143.6	-	1			74.1	-	1		
Spring						82.4	1.4	5			135.0	7.1	4			139.0	0.2	2			69.5	-	1		
RFF	**437.3**	**96.1**				**93.2**	**3.3**				**134.2**	**17.1**				**182.7**	**3.5**				**72.6**	**5.2**			
Fall	560.6	12.4	2								110.2	-	1			181.7	3.2	3			70.3	7.3	2		
Spring	375.6	11.0	4			93.2	3.3	2			142.2	7.2	3			183.7	4.3	3			75.0	2.5	2		
Reference	**551.4**	**161.3**				**78.4**	**10.7**				**203.7**	**71.0**				**267.2**	**39.9**				**104.8**	**92.7**			
COP	**388.0**	**17.5**				**75.8**	**1.8**				**141.8**	**13.8**				**235.7**	**14.7**				**152.6**	**154.4**			
Fall											120.3	-	1			214.5	-	1			179.0	177.6	3		
Spring	388.0	17.5	3			75.8	1.8	3			147.1	7.7	4			242.7	5.2	3			73.3	-	1		
CRO	**486.6**	**156.1**				**96.5**	-				**186.5**	**30.0**				**275.4**	**43.7**				**75.9**	**3.8**			
Fall	253.2	-	1								142.9	-	1			227.7	1.6	2			79.7	-	1		
Spring	564.4	15.0	3			96.5	-	1			201.1	8.7	3			307.1	5.5	3			74.6	3.4	3		
EAS	**701.3**	**27.8**				**68.0**	-				**279.4**	**57.3**				**295.8**	**35.3**				**79.6**	**3.2**			
Fall	668.2	-	1								177.7	-	1			255.2	-	1			82.8	-	1		
Spring	709.6	23.9	4			68.0	-	1			304.9	8.2	4			316.1	3.9	2			78.0	2.3	2		

Table E-9. Mean arsenic concentration (µg g^{-1} dry wt.) of particulate matter subsamples by stream and season.

	sediment					biofilm					leaf detritus				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**2.94**	**1.16**				**5.22**	**1.51**				**0.69**	**0.41**			
KEL	**3.86**	**0.38**				**5.64**	**0.41**				**1.18**	**0.13**			
Fall	3.79	0.45	3			5.35	0.23	3			1.29	0.10	3		
Spring	3.94	0.37	3			5.94	0.35	3			1.08	0.05	3		
KUT	**3.53**	**0.49**				**6.62**	**0.97**				**0.66**	**0.03**			
Fall	3.50	0.44	3			7.47	0.24	3			0.65	0.03	3		
Spring	3.56	0.63	3			5.77	0.39	3			0.67	0.04	3		
LLC	**1.42**	**0.15**				**3.40**	**0.24**				**0.24**	**0.11**			
Fall	1.42	0.24	3			3.45	0.35	3			0.14	0.02	3	0	3
Spring	1.43	0.01	3			3.35	0.11	3			0.34	0.01	3		
Low-Se	**4.92**	**1.60**				**6.85**	**1.36**				**1.15**	**0.39**			
BIR	**6.71**	**0.47**				**7.97**	**1.29**				**1.41**	**0.60**			
Fall	6.99	0.49	3			9.10	0.31	3			1.96	0.07	3		
Spring	6.43	0.26	3			6.84	0.48	3			0.86	0.04	3		
MIL	**3.13**	**0.56**				**6.01**	**1.33**				**1.04**	**0.10**			
Fall	3.41	0.71	3			7.16	0.46	3			1.00	0.14	3		
Spring	2.85	0.17	3			4.85	0.47	3			1.09	0.01	3		
RFF	**4.92**	**0.67**				**6.57**	**0.63**				**0.98**	**0.15**			
Fall	5.17	0.93	3			6.21	0.72	3			0.85	0.05	3		
Spring	4.67	0.29	3			6.93	0.30	3			1.11	0.07	3		
Reference	**2.89**	**1.09**				**6.51**	**3.13**				**0.57**	**0.37**			
COP	**1.87**	**0.20**				**3.75**	**0.30**				**0.49**	**0.10**			
Fall	1.99	0.22	3			3.78	0.46	3			0.58	0.03	3		
Spring	1.74	0.06	3			3.72	0.13	3			0.40	0.01	3		
CRO	**2.62**	**0.38**				**9.73**	**2.28**				**0.94**	**0.42**			
Fall	2.95	0.12	3			11.79	0.60	3			1.17	0.52	3		
Spring	2.28	0.04	3			7.68	0.17	3			0.70	0.04	3		
EAS	**4.18**	**0.71**				**5.57**	**0.33**				**0.27**	**0.05**			
Fall	4.36	1.03	3								0.31	0.03	3		
Spring	4.00	0.28	3			5.57	0.33	3			0.23	0.02	3		

Table E-10. Mean arsenic concentration (µg g-1 dry wt.) in macroinvertebrate subsamples by stream and season.

	Pteronarcys					***Tipula***					**prey - *Tipula* - *Pteronarcys***					**predator**					***Cambaridae***				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**1.27**	**0.12**				**1.59**	**0.82**				**2.03**	**0.58**				**0.76**	**0.33**				**0.55**	**0.20**			
KEL						**2.38**	**0.17**				**2.24**	**0.17**				**0.94**	**0.12**				**0.60**	**0.03**			
Fall											2.44	-	1			0.81	0.01	2			0.58	-	1		
Spring						2.38	0.17	3			2.17	0.12	3			1.03	0.04	3			0.60	0.04	3		
KUT						**2.03**	**0.09**				**2.47**	**0.25**				**0.99**	**0.09**				**0.62**	**0.29**			
Fall											2.10	-	1			0.87	-	1			0.30	0.03	2	0	1
Spring						2.03	0.09	3			2.59	0.05	3			1.03	0.04	3			0.83	0.07	3		
LLC	**1.27**	**0.12**				**0.66**	**0.06**				**1.19**	**0.04**				**0.29**	**0.04**				**0.41**	**0.04**			
Fall	1.35	-	1								1.20	0.05	2			0.33	-	1	0	1	0.37	-	1		
Spring	1.19	-	1			0.66	0.06	4			1.17	-	1			0.28	0.03	3	0	1	0.43	0.02	3		
Low-Se	**3.99**	**2.54**				**2.54**	**0.18**				**2.66**	**1.30**				**1.03**	**0.30**				**0.94**	**0.25**			
BIR						**2.29**	**0.05**				**3.89**	**1.70**				**1.50**	**0.12**				**1.11**	**0.08**			
Fall											5.85	-	1			1.64	-	1			1.24	-	1		
Spring						2.29	0.05	3			2.91	0.13	2			1.43	0.04	2			1.08	0.04	4		
MIL						**2.65**	**0.05**				**1.69**	**0.11**				**0.88**	**0.17**				**0.46**	**0.08**			
Fall											1.86	-	1			1.08	-	1			0.41	-	1		
Spring						2.65	0.05	5			1.64	0.07	4			0.79	0.03	2			0.52	-	1		
RFF	**3.99**	**2.54**				**2.67**	**0.01**				**2.95**	**1.02**				**0.87**	**0.08**				**0.96**	**0.05**			
Fall	0.73	0.01	2								4.47	-	1			0.94	0.01	3			1.00	0.03	2		
Spring	5.62	0.25	4			2.67	0.01	2			2.45	0.12	3			0.80	0.02	3			0.92	0.02	2		
Reference	**1.12**	**0.64**				**1.21**	**0.22**				**1.90**	**0.42**				**0.59**	**0.27**				**0.31**	**0.08**			
COP	**2.09**	**0.00**				**1.27**	**0.03**				**1.89**	**0.07**				**0.57**	**0.07**				**0.31**	**0.13**			
Fall											1.90	-	1			0.48	-	1			0.32	0.16	3	0	1
Spring	2.09	0.00	3			1.27	0.03	3			1.89	0.09	4			0.60	0.03	3			0.29	-	1		
CRO	**1.00**	**0.37**				**1.39**	-				**2.43**	**0.08**				**0.74**	**0.36**				**0.33**	**0.05**			
Fall	1.55	-	1								2.45	-	1			1.13	0.02	2			0.39	-	1		
Spring	0.81	0.07	3			1.39	-	1			2.43	0.09	3			0.48	0.05	3			0.30	0.04	3		
EAS	**0.64**	**0.11**				**0.83**	-				**1.48**	**0.25**				**0.35**	**0.03**				**0.29**	**0.04**			
Fall	0.46	-	1								1.91	-	1			0.39	-	1			0.34	-	1		
Spring	0.69	0.02	4			0.83	-	1			1.37	0.08	4			0.33	0.00	2			0.27	0.02	2		

Table E-11. Mean strontium concentration (µg g-1 dry wt.) in particulate matter subsamples by stream and season.

	sediment					biofilm					leaf detritus				
	mean	sd	n	mdl	mrl	mean	sd	n	mdl	mrl	mean	sd	n	n <	n <
High-Se	**22.47**	**13.68**				**66.94**	**29.30**				**196.24**	**29.99**			
KEL	**27.04**	**3.94**				**45.61**	**6.37**				**176.84**	**9.58**			
Fall	23.42	3.80	2			40.06	1.45	3			183.76	7.27	3		
Spring	29.45	1.43	3			51.15	2.63	3			169.92	5.75	3		
KUT	**36.61**	**6.63**				**105.10**	**15.49**				**235.37**	**6.81**			
Fall	40.02	5.95	2			118.40	4.28	3			235.51	5.90	3		
Spring	34.34	7.13	3			91.81	7.12	3			235.23	9.01	3		
LLC	**6.87**	**0.79**				**50.11**	**2.39**				**176.52**	**12.81**			
Fall	6.50	0.61	3			50.73	3.08	3			165.00	3.43	3		
Spring	7.24	0.88	3			49.49	1.90	3			188.05	0.19	3		
Low-Se	**22.22**	**4.46**				**57.17**	**22.07**				**163.37**	**95.43**			
BIR	**22.57**	**4.51**				**34.97**	**10.62**				**74.72**	**7.71**			
Fall	25.43	4.98	3			43.41	7.76	3			81.70	1.33	3		
Spring	19.72	1.23	3			26.53	2.84	3			67.74	0.97	3		
MIL	**19.15**	**2.17**				**68.96**	**25.36**				**135.82**	**25.62**			
Fall	20.47	2.42	3			91.61	4.12	3			158.57	9.21	3		
Spring	17.84	0.85	3			46.31	7.21	3			113.06	1.56	3		
RFF	**24.94**	**4.73**				**67.59**	**3.42**				**279.56**	**60.86**			
Fall	28.45	4.35	3			67.07	4.31	3			334.52	11.61	3		
Spring	21.43	0.37	3			68.11	3.14	3			224.59	7.82	3		
Reference	**7.46**	**4.73**				**36.16**	**5.56**				**157.85**	**127.21**			
COP	**13.05**	**3.90**				**41.17**	**2.68**				**311.15**	**111.34**			
Fall	16.51	1.40	3			40.41	2.59	3			412.68	2.26	3		
Spring	9.59	0.25	3			41.93	3.09	3			209.61	7.46	3		
CRO	**5.27**	**1.08**				**33.90**	**3.82**				**88.71**	**13.09**			
Fall	6.17	0.45	3			36.61	3.18	3			100.43	3.41	3		
Spring	4.36	0.52	3			31.18	2.06	3			76.99	2.15	3		
EAS	**4.07**	**1.57**				**30.66**	**5.07**				**73.69**	**3.79**			
Fall	5.29	1.28	3								70.71	0.25	3		
Spring	2.85	0.27	3			30.66	5.07	3			76.67	3.02	3		

Table E-12. Mean strontium concentration (µg g^{-1} dry wt.) in macroinvertebrate subsamples by stream and season

	Pteronarcys					***Tipula***					**prey - *Tipula* - *Pteronarcys***					**predator**					***Cambaridae***				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**55.9**	**19.4**				**65.8**	**7.8**				**33.8**	**8.5**				**24.8**	**10.1**				**-**	**290.3**			
KEL	**74.2**	**-**				**56.2**	**1.2**				**32.7**	**7.8**				**20.2**	**7.5**				**-**	**158.0**			
Fall											44.4	-	1			12.2	0.0	2			834.6	-	1		
Spring						56.2	1.2	3			28.7	0.3	3			25.6	1.8	3			-	50.6	3		
KUT						**74.0**	**2.2**				**39.3**	**10.2**				**18.1**	**1.4**				**-**	**399.7**			
Fall											54.6	-	1			16.1	-	1			821.7	20.9	2		
Spring						74.0	2.2	3			34.2	0.1	3			18.8	0.3	3			-	90.1	3		
LLC	**46.8**	**15.9**				**66.9**	**4.0**				**28.2**	**1.5**				**37.2**	**5.7**				**890.8**	**27.3**			
Fall	58.0	-	1								29.0	0.7	2			28.8	-	1			917.5	-	1		
Spring	35.6	-	1			66.9	4.0	4			26.5	-	1			40.1	0.7	3			881.9	25.3	3		
Low-Se	**67.7**	**11.7**	**6**			**44.8**	**17.5**				**19.2**	**7.2**				**26.6**	**18.4**				**658.3**	**401.8**			
BIR						**25.9**	**0.7**				**13.4**	**2.8**				**7.2**	**0.5**				**313.6**	**10.9**			
Fall											16.7	-	1			7.5	-	1			326.9	-	1		
Spring						25.9	0.7	3			11.8	0.7	2			7.1	0.6	2			310.2	9.2	4		
MIL						**44.6**	**0.7**				**18.6**	**8.9**				**15.1**	**0.5**				**542.9**	**32.0**			
Fall											34.5	-	1			15.7	-	1			520.3	-	1		
Spring						44.6	0.7	5			14.6	0.3	4			14.9	0.3	2			565.6	-	1		
RFF	**67.7**	**11.7**				**73.9**	**1.5**				**24.2**	**3.2**				**42.0**	**12.4**				**-**	**110.7**			
Fall	82.7	1.7	2								29.0	-	1			30.7	1.0	3			-	52.9	2		
Spring	60.3	1.4	4			73.9	1.5	2			22.6	0.2	3			53.3	1.6	3			-	69.5	2		
Reference	**44.9**	**19.8**				**50.5**	**26.9**				**14.9**	**8.9**				**35.1**	**21.6**				**579.1**	**345.5**			
COP	**76.5**	**2.0**				**70.2**	**1.1**				**25.6**	**5.5**				**58.7**	**10.0**				**832.2**	**501.9**			
Fall											35.3	-	1			44.0	-	1			712.2	539.9	3		
Spring	76.5	2.0	3			70.2	1.1	3			23.2	0.7	4			63.6	2.4	3			-	-	1		
CRO	**28.7**	**1.2**				**23.3**	**-**				**10.1**	**1.3**				**28.4**	**16.8**				**492.7**	**33.6**			
Fall	27.4	-	1								11.9	-	1			10.0	0.0	2			536.5	-	1		
Spring	29.2	1.0	3			23.3	-	1			9.4	0.4	3			40.7	0.2	3			478.1	20.4	3		
EAS	**38.8**	**5.1**				**18.9**	**-**				**8.1**	**1.2**				**14.9**	**4.1**				**357.0**	**18.0**			
Fall	30.0	-	1								10.3	-	1			10.2	-	1			339.8	-	1		
Spring	41.0	1.2	4			18.9	-	1			7.5	0.2	4			17.3	0.6	2			365.6	14.3	2		

Table E-13. Mean cadmium concentration (µg g^{-1} dry wt.) in particulate matter subsamples by stream and season.

	sediment					biofilm					leaf detritus				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**0.15**	**0.02**				**0.45**	**0.19**				**0.17**	**0.12**			
KEL	**0.15**	**0.02**				**0.26**	**0.07**				**0.14**	**0.02**			
Fall	0.14	0.01	2			0.20	0.03	3	0	3	0.15	0.01	3	0	3
Spring	0.17	0.00	3			0.32	0.01	3	0	3	0.12	0.01	3	0	3
KUT	**0.14**	**0.02**	**5**			**0.41**	**0.08**				**0.11**	**0.04**			
Fall	0.14	0.03	2			0.49	0.01	3	0	3	0.08	0.01	3	0	3
Spring	0.14	0.01	3			0.34	0.02	3	0	3	0.14	0.02	3	0	3
LLC	**0.16**	**0.03**	**6**			**0.66**	**0.13**				**0.26**	**0.19**			
Fall	0.14	0.00	3			0.55	0.04	3			0.09	0.00	3	0	3
Spring	0.19	0.01	3			0.78	0.01	3			0.43	0.03	3	0	3
Low-Se	**0.12**	**0.08**				**0.23**	**0.13**				**0.11**	**0.04**			
BIR	**0.22**	**0.01**				**0.40**	**0.04**				**0.16**	**0.02**			
Fall	0.22	0.01	3			0.42	0.01	3	0	3	0.15	0.01	3	0	3
Spring	0.22	0.00	3			0.37	0.04	3	0	3	0.18	0.01	3	0	3
MIL	**0.06**	**0.00**				**0.15**	**0.04**				**0.10**	**0.01**			
Fall	0.06	0.00	3	0	3	0.19	0.02	3	0	3	0.10	0.00	3	0	3
Spring	0.06	0.00	3	0	3	0.11	0.01	3	0	3	0.10	0.00	3	0	3
RFF	**0.07**	**0.01**				**0.14**	**0.01**				**0.07**	**0.01**			
Fall	0.07	0.01	3	0	3	0.13	0.00	3	0	3	0.06	0.00	3	0	3
Spring	0.07	0.01	3	0	3	0.14	0.01	3	0	3	0.08	0.00	3	0	3
Reference	**0.06**	**0.03**				**0.18**	**0.11**				**0.10**	**0.03**			
COP	**0.05**	**0.01**				**0.10**	**0.00**				**0.07**	**0.02**			
Fall	0.05	0.00	3	0	3	0.10	0.00	3	0	3	0.09	0.00	3	0	3
Spring	0.04	0.00	3	0	3	0.09	0.00	3	0	3	0.05	0.02	3	1	3
CRO	**0.04**	**0.01**				**0.17**	**0.03**				**0.10**	**0.02**			
Fall	0.04	0.00	3	0	3	0.20	0.00	3	0	3	0.08	0.00	3	0	3
Spring	0.03	0.00	3	0	3	0.15	0.01	3	0	3	0.12	0.01	3	0	3
EAS	**0.10**	**0.02**				**0.39**	**0.02**				**0.13**	**0.02**			
Fall	0.12	0.01	3	0	2						0.13	0.03	3	0	3
Spring	0.09	0.00	3	0	3	0.39	0.02	3	0	3	0.13	0.01	3	0	3

Table E-14. Mean cadmium concentration (µg g-1 dry wt.) in macroinvertebrate subsamples by stream and season.

	Pteronarcys					*Tipula*					prey - *Tipula* - *Pteror*					predator					*Cambaridae*				
	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl	mean	sd	n	n < mdl	n < mrl
High-Se	**0.39**	**0.08**				**0.55**	**0.17**				**0.95**	**0.17**				**0.34**	**0.11**				**0.64**	**0.18**			
KEL						**0.38**	**0.01**				**0.79**	**0.11**				**0.29**	**0.06**				**0.68**	**0.05**			
Fall											0.63	-	1	0	1	0.22	0.01	2	0	2	0.61	-	1		
Spring						0.38	0.01	3	0	3	0.85	0.01	3			0.33	0.01	3	0	3	0.70	0.02	3		
KUT						**0.48**	**0.02**				**1.00**	**0.13**				**0.29**	**0.02**				**0.45**	**0.06**			
Fall											1.19	-	1			0.27	-	1	0	1	0.39	0.00	2	0	2
Spring						0.48	0.02	3	0	3	0.94	0.03	3			0.30	0.02	3	0	3	0.49	0.01	3	0	3
LLC	**0.39**	**0.08**				**0.74**	**0.06**				**1.08**	**0.15**				**0.45**	**0.12**				**0.85**	**0.02**			
Fall	0.45	-	1	0	1						1.17	0.06	2			0.64	-	1			0.87	-	1		
Spring	0.33	-	1	0	1	0.74	0.06	4			0.92	-	1			0.39	0.02	3	0	3	0.84	0.02	3		
Low-Se	**0.14**	**0.01**	**6**			**0.32**	**0.09**				**1.25**	**0.87**				**0.37**	**0.27**				**0.27**	**0.07**			
BIR						**0.44**	**0.01**				**2.53**	**0.78**				**0.73**	**0.37**				**0.32**	**0.06**			
Fall											3.43	-	1			1.15	-	1			0.38	-	1	0	1
Spring						0.44	0.01	3	0	3	2.08	0.01	2			0.52	0.02	2	0	1	0.30	0.05	4	0	4
MIL						**0.27**	**0.01**				**0.98**	**0.26**				**0.25**	**0.05**				**0.27**	**0.11**			
Fall											0.52	-	1	0	1	0.31	-	1	0	1	0.35	-	1		
Spring						0.27	0.01	5	0	5	1.09	0.04	4			0.22	0.01	2	0	2	0.19	-	1		
RFF	**0.14**	**0.01**				**0.25**	**0.00**				**0.65**	**0.10**				**0.25**	**0.05**				**0.21**	**0.03**			
Fall	0.15	0.00	2	0	2						0.50	-	1			0.30	0.01	3	0	3	0.23	0.00	2	0	2
Spring	0.14	0.00	4	0	3	0.25	0.00	2	0	2	0.69	0.02	3			0.20	0.00	3	0	3	0.18	0.01	2	0	2
Reference	**0.43**	**0.28**				**0.35**	**0.11**				**2.11**	**1.88**				**0.80**	**0.64**				**0.73**	**0.72**			
COP	**0.23**	**0.01**				**0.30**	**0.01**				**0.74**	**0.08**				**0.42**	**0.10**				**0.26**	**0.09**			
Fall											0.88	-	1			0.56	-	1	0	1	0.24	0.09	3	0	3
Spring	0.23	0.01	3	0	3	0.30	0.01	3	0	2	0.70	0.01	4			0.37	0.01	3	0	3	0.33	-	1	0	1
CRO	**0.21**	**0.00**				**0.30**	-				**0.84**	**0.30**				**0.47**	**0.01**				**0.36**	**0.02**			
Fall	0.21	-	1	0	1						0.40	-	1	0	1	0.48	0.02	2	0	2	0.39	-	1	0	1
Spring	0.21	0.00	3	0	3	0.30	-	1	0	1	0.99	0.02	3			0.47	0.01	3	0	3	0.34	0.01	3	0	3
EAS	**0.72**	**0.17**				**0.55**	-				**4.51**	**0.51**				**1.85**	**0.21**				**1.84**	**0.16**			
Fall	0.41	-	1	0	1						3.59	-	1			1.62	-	1			2.00	-	1		
Spring	0.80	0.03	4	0	4	0.55	-	1	0	1	4.73	0.06	4			1.96	0.10	2			1.76	0.10	2		

APPENDIX F: SE TISSUE CONCENTRATIONS AND CONDUCTIVITY

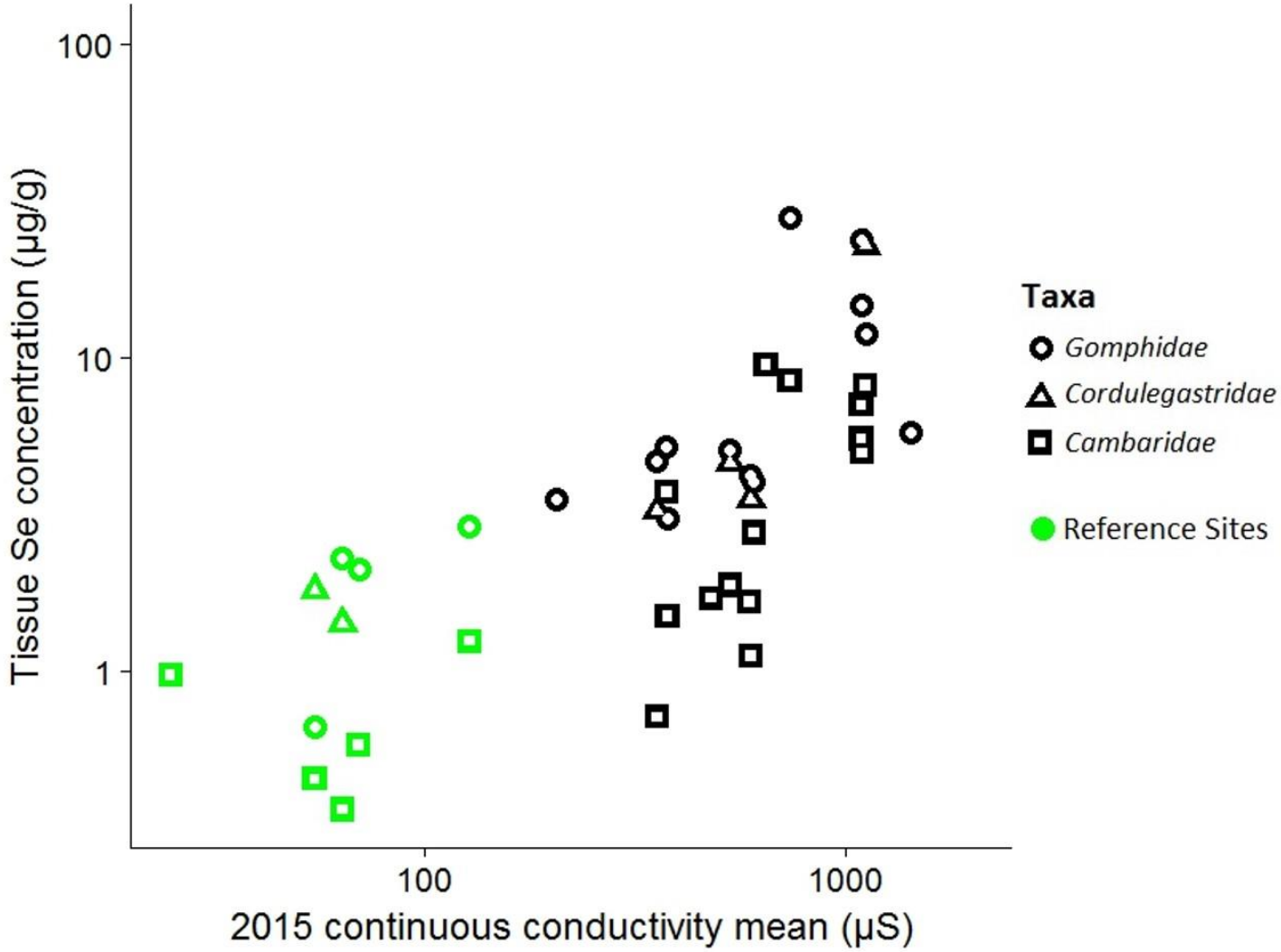

Figure F-1: Mean conductivity readings in 2015 measured with an *in-situ* continuously reading conductivity meter plotted against Se concentrations in macroinvertebrate tissues collected July – August 2015.