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Selenium bioaccumulation from surface coal mining

Selenium Bioaccumulation Across Trophic Levels and Along a Longitudinal Gradient in Headwater Streams

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Abstract: Toxic effects of selenium (Se) contamination in freshwaters have been well-documented. However, study of Se contamination has focused on lentic and larger-order lotic systems, whereas headwater streams have received little scrutiny. In central Appalachia, surface coal mining is a common Se source to headwater streams, thus providing a useful system to investigate Se bioaccumulation in headwater food chains and possible longitudinal patterns in Se concentrations. Toward that end, we assessed Se bioaccumulation in 2 reference and 4 mining-influenced headwater streams. At each stream, we sampled ecosystem media, including streamwater, particulate matter (sediment, biofilm, leaf detritus), benthic macroinvertebrates, salamanders, and fish, every 400 m along 1.2 and 1.6 km reaches. We compared media Se concentrations within and among streams and evaluated longitudinal trends in media Se concentrations. Selenium concentrations in sampled media were higher in mining-influenced streams compared to reference streams. We found the highest Se concentrations in benthic macroinvertebrates; however, salamanders and fish bioaccumulated Se to potentially harmful levels in mining-influenced streams. Only one stream demonstrated dilution of streamwater Se with distance downstream, and few longitudinal patterns in Se bioaccumulation occurred along our study reaches. Collectively, our work provides a field-based assessment of Se bioaccumulation in headwater food chains, from streamwater to fish, and highlights the need for future assessments of Se effects in headwater streams and receiving downstream waters.

Keywords: trophic transfer, food chain, benthic macroinvertebrates, fish, salamanders

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INTRODUCTION

Contamination of aquatic systems by elevated concentrations of the trace element selenium (Se) is a global environmental concern because of its high potential for biological uptake and subsequent toxicity (Lemly 2004). Selenium can be released to freshwater environments from numerous sources, including agricultural irrigation, phosphate and coal mining, and coal fly-ash waste (Lemly 2004). Selenium is an essential micronutrient but can become toxic, particularly in egg-laying vertebrates, at only marginally elevated water-column concentrations (Janz et al. 2010). Selenium has been linked to toxic effects in fish (Lemly 2002) and aquatic birds (Ohlendorf et al. 1990), prompting the development of water- and tissue-based aquatic life ambient water quality criteria (WQC) by U.S. EPA (2016). However, uncertainty in the WQC arises from variability in Se bioaccumulation potential across freshwater systems in response to site-specific factors, including Se form and bioavailability (Simmons and Wallschläger 2005), food chain dynamics (Fan et al. 2002; Stewart et al. 2004), and organism movement (Janz et al. 2010). Further, study of Se bioaccumulation has largely focused on lentic and larger-order lotic systems and the biota that inhabit them, whereas headwater streams, which play an outsized role in ecosystem processes (Alexander et al. 2007), have received little scrutiny. Consequently, field-based assessments of Se bioaccumulation across freshwater environments, including headwater streams, are needed to ensure adequate protection of aquatic life.

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In central Appalachia, surface coal mining is a common source of Se contamination to headwater streams (U.S. EPA 2011), thus providing an ideal test system in which to investigate Se bioaccumulation in headwater ecosystems. Surface coal mining is the main driver of land-use change in this region (Sayler 2008) with associated impacts to hydrology and chemistry of headwater streams (Pond et al. 2008; Bernhardt and Palmer 2011). Surface-mining activities extract buried coal seams by removing overlaying strata of bedrock using explosives and earth-moving equipment (U.S. EPA 2011). Excess waste rock, or overburden, is often deposited into adjacent valleys of headwater streams, burying stream channels (Palmer et al. 2010). Accelerated weathering of mineral surfaces in deposited overburden releases dissolved major ions to receiving streams, leading to elevated streamwater salinity (Daniels et al. 2016; Clark et al. 2018). Likewise, water-soluble Se oxyanions (selenate and selenite) are commonly released from the fractured rocks and transported to receiving streams (Lussier 2003).

The primary pathway for Se uptake (and thus toxicity) in aquatic animals is dietary through food chains as opposed to direct uptake from the water column through the skin or gills (Hamilton 2004). Inorganic Se in the water column is transformed into organic forms by primary producers, bacteria, and fungi and then made available to higher trophic levels (Fan et al. 2002; Luoma and Presser 2009). Enrichment of Se from the water column into particulate matter (e.g., via adsorption onto sediments and organic detritus and incorporation into benthic microbial and algal communities) represents the largest increase in Se concentrations in aquatic systems (Presser and Luoma 2010). Smaller but significant increases in Se concentrations can occur through dietary pathways from particulate matter to primary-consumer macroinvertebrates (i.e., shredders, scrapers,

and collectors) and into higher trophic levels including predator macroinvertebrates (Whitmore et al. 2018) and fish (Presser 2013; Arnold et al. 2014). However, potential uptake of Se by fish and other aquatic life varies greatly and is driven by dietary preferences (Sappington 2002) and bioavailability of Se throughout the food web (Presser and Luoma 2010; Conley et al. 2013), highlighting the need for rigorous field-based assessments of Se bioaccumulation to inform management of Se-contaminated systems (Presser and Luoma 2010).

Selenium bioaccumulation in fish is a major concern for regulatory agencies because of maternal transfer into eggs (Conley et al. 2014) and resultant negative effects on reproductive success (Janz et al. 2010). Because Se bioaccumulation potential is determined by many factors, Se concentration in whole-body and egg/ovary tissue is considered by U.S. EPA (2016) as a preferred metric over water-column concentrations to assess potential Se toxicity in freshwater ecosystems. However, despite the focus on fish tissue in Se-bioaccumulation research in many lentic (e.g., Lemly 2002; Brandt et al. 2017) and high-order lotic systems (e.g., Muscatello et al. 2008; Presser 2013; Arnold et al. 2014), there has been limited study of Se bioaccumulation in fish inhabiting headwater streams.

In addition, Se bioaccumulation has not been well-studied in salamanders, which are ubiquitous in forested headwater streams of central Appalachia and many other regions. Salamanders are preyed upon by birds and mammals (Patnode et al. 2005), thereby performing an important ecological role by transferring energy between aquatic and terrestrial systems (Davic and Welsh 2004). Previous research has shown that riparian spiders, which consume contaminant-enriched aerial adults of aquatic insects,

can transfer aquatic contaminants to higher terrestrial trophic levels (i.e., arachnivoracious birds) (Walters et al. 2009). Similarly, it is possible that salamanders represent an important link in Se transfer from contaminated headwater streams into adjacent terrestrial systems. Further, salamanders become the dominant predatory vertebrate in the absence of well-developed fish assemblages and thus could replace fish tissue as a preferred metric to assess Se bioaccumulation and toxicity when fish are absent, as is common in headwater streams (Patnode et al. 2005).

Downstream trends in ecosystem media Se concentrations are largely understudied but represent another important consideration for management of freshwater resources. Selenium water-column concentrations can decrease with distance downstream because of immobilization into particulate matter and subsequent trophic transfer, dilution by tributaries and groundwater, and/or via atmospheric volatilization (Simmons and Wallschläger 2005). However, bioaccumulated Se can also be carried downstream through passive drift (e.g., particulate matter and macroinvertebrates) and active movement (e.g., fish migration). In addition, stream redox conditions can affect Se bioaccumulation within stream networks by influencing Se speciation (Lemly 1999; Oram et al. 2010), and thus uptake by producers, where the reduced form selenite is most bioavailable. Consequently, Se contamination in headwater streams from upstream sources (e.g., where mining activities take place) may have significant, but largely unknown, implications for downstream ecosystems.

In the present study, we evaluated Se bioaccumulation at multiple trophic levels within and among 6 central Appalachian headwater stream reaches. These streams included both reference and surface mining-influenced streams, providing a range of

water-column Se concentrations. Our objectives were to: 1) assess how Se bioaccumulation may differ among ecosystem media types within each stream, with a particular focus on salamanders and fish; 2) compare Se concentrations among streams for each individual media type; and 3) evaluate longitudinal patterns in Se concentrations in ecosystem media within each stream reach.

METHODS

Stream selection

A subset of 6 central Appalachian headwater streams were selected from a set of 24 streams that have been monitored in other studies of surface-mining impacts on water quality and benthic macroinvertebrate community structure (e.g., Timpano et al. 2018a, 2018b). These 24 headwater streams are located in the coalfield region of southwestern Virginia and southern West Virginia, in U.S. EPA Level IV Ecoregion 69d (Omernik and Griffith 2014). Streams were identified to meet rigorous criteria for water chemistry and habitat quality and to isolate elevated levels of dissolved ions (i.e., salinity) as the stressor to aquatic life where other non-salinity stressors were minimized. The 6 study streams we selected met 3 additional criteria: 1) ≥ 1.2 km of stream reach without perennial tributaries, 2) forested riparian buffer throughout the stream reach, and 3) lack of features with reducing conditions (e.g., sediment-control ponds and wetlands) that could increase Se bioavailability by enabling selenite production. The 6 selected streams were then divided into 3 categories of 2 streams each based on historic water-column Se measurements: 1) reference; streams with no mining influence, 2) low-Se; mining-influenced streams with Se less than the lotic water-column WQC ($3.1 \mu\text{g Se L}^{-1}$) developed by U.S. EPA (2016), and 3) high-Se; mining-influenced streams with $\text{Se} > 3.1$

$\mu\text{g Se L}^{-1}$. Selected streams spanned a gradient of salinity (Table 1) and geographic area within the central Appalachian coalfield (Figure 1).

Stream delineation and sampling design

From June to August 2018, each study stream was sampled once for a full suite of ecosystem media, including streamwater, leaf detritus, biofilm, streambed sediment, primary-consumer and predator macroinvertebrates, salamanders, and fish. Streamwater was grab-sampled every 400 m. All other ecosystem media were collected within a 50-m reach upstream of each streamwater sample location. Four streams had 5 collection points along 1,600 m of stream length, and 2 streams had 4 collections points along 1,200 m of stream length. Collections of biofilm, leaf detritus, streambed sediment, and primary-consumer and predator macroinvertebrates were composited in the field at each sample location. Salamanders and fish captured from each sample location were analyzed individually. Ecosystem media collected at each sampling location within a stream were treated as stream-wise replicates for among-stream comparisons and individually for analysis of longitudinal trends.

Field and laboratory methods

Water-column sampling and analysis. At each sample location, stream surface water for Se analysis was collected as a grab sample from a well-mixed location downstream from a riffle. For analysis of total dissolved Se, streamwater was drawn into a sterile syringe and pushed through a 0.45 μm pore polyvinylidene fluoride filter into sterile polyethylene sample bags. Samples were acidified to $\text{pH} < 2$ by adding 0.5% (v/v) of a solution of trace-metal-grade concentrated nitric acid diluted 1:1 with deionized

water (U.S. EPA 1996a). Samples were refrigerated until analysis by ICP-MS (Thermo iCAP-RQ) (U.S. EPA 1996a).

We also analyzed collected streamwater for Se speciation, but only at one high-Se stream (H2) for initial assessment because of financial and operational constraints. For this analysis, unfiltered streamwater was stored in pre-labeled, sterile polyethylene sample bags and refrigerated until analysis. A custom method was developed for speciation analysis of selenite (SeIV) and selenate (SeVI) forms. Samples were filtered through 0.45 μm PTFE membranes before being injected for analysis. After filtration, the Se species were analyzed by first separating the species using an Agilent 1100 series HPLC system equipped with 2 Agilent Poroshell 120 EC-C18 columns. These columns were chosen because of their effective retention of analytes combined with low pressure builds. The isocratic separation utilized a flow rate of 0.5 mL/min of an aqueous buffer that was compatible with both the columns and the ICP-MS. The buffer was composed of 25 mM ammonium citrate, 5 mM tetrabutylammonium chloride, and 2% (v/v) methanol. The separated Se species were then detected at 78 m/z using an Agilent 7900 ICP-MS operating in helium collision cell mode to reduce or remove potential polyatomic interferences.

Collection of ecosystem media. All media were collected within a 50-m reach at each sampling location. The 50-m reach was further divided into 10-m sections for representative collection of streambed sediment. A composite sample of streambed sediment was collected using an acid-washed polypropylene scoop to sample representative hydrologic features (e.g., pool, run, and riffle) inside 10-m reach subsections. Collection was limited to a depth of 1 to 3 cm to isolate biologically active

sediments, where benthic organisms actively disturb sediment layers (e.g., excretion/ingestion, burrowing, and biodeposition) (U.S. EPA 2015). Biofilm, defined in the present study as accumulated mats of algae, fungi, and bacteria communities (Whitmore et al. 2018), was collected by scraping rocks with an acid-washed polystyrene knife. When epilithic biofilm was not present, biofilm accumulation on sandy substrates was collected using an acid-washed polypropylene scoop. All brown, submerged leaves found within each sample reach were collected. Green leaves were not collected because of their short incubation time in the stream environment. Benthic macroinvertebrates were sampled using a 0.3-m-wide D-frame kicknet and multi-habitat procedures (Barbour et al. 1999). Crustaceans and mollusks were removed from macroinvertebrate samples and were returned to the stream unharmed. Two species of salamanders, the northern dusky salamander (*Desmognathus fuscus*) and seal salamander (*Desmognathus monticola*), were collected with a dipnet by overturning logs and rocks at the streambank interface where they are commonly found. Predominately 2 species of fish, blacknose dace (*Rhinichthys obtusus*) and creek chub (*Semotilus atromaculatus*), were collected using barrel minnow traps baited with oyster crackers for approximately 3 hours. Other fish species collected in these traps included the mottled sculpin (*Cottus bairdi*), bluegill (*Lepomis macrochirus*), and silver shiner (*Notropis photogenis*). Biofilm, sediment, and leaf detritus were stored in light-excluding polyethylene bags, and the remaining media types were stored in clear polyethylene bags. Salamanders and fish were euthanized on site using a solution of Tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate. All media were kept on dry ice during transport and stored at -20 °C until analysis.

Lab processing of ecosystem media. All media were quickly thawed for laboratory processing and preparation for microwave digestion. Streambed sediment was hand-pressed through a stainless-steel 1-mm sieve (U.S. EPA 2001) and then homogenized by thoroughly mixing. Epilithic biofilm was gently rinsed of sediment and organic debris using deionized water. Sandy biofilm samples were serially decanted to reduce the amount of mineral sediments in the sample (Whitmore et al. 2018). Leaf material was gently washed with deionized water to remove sediment and other organic debris and was dried in the oven at 65 °C for ≥ 5 days to ensure complete drying. After drying, leaf mid-stems were removed, and the remaining leaf material was ground in a ball mill for 1 min at a vibration frequency of 25 sec⁻¹ and homogenized (Whitmore et al. 2018). Benthic macroinvertebrates were identified to family and separated into primary-consumer and predator taxa. In cases where organisms belonged to a family consisting of both primary-consumer and predator functional feeding groups, individuals were further identified to genus (Merritt et al. 2008). Individuals from the genera *Pteronarcys* and *Tipula* were separated from the other primary-consumer taxa and analyzed separately because of their disproportionately large body sizes. Salamanders and fish were identified to species and measured for total length. Stomach contents of fish were removed to avoid contamination from recent ingestion of oyster crackers used for baiting. Individual salamanders and fish were homogenized in a blender. All processed media were stored in 15-ml sterile polypropylene vials and re-frozen at -20 °C. Samples were lyophilized for at least 4 days to ensure complete drying and then ground to a fine powder using a mortar and pestle.

Acid digestion and Se analysis. Dry-weight Se concentrations of lyophilized media were analyzed using microwave acid digestion methods developed from U.S. EPA (1996b) and Whitmore et al. (2018). Samples were weighed and placed in Teflon vessels along with 10 ml of trace-metal grade concentrated nitric acid (70% HNO₃). Vessels were sealed and placed in a microwave digestion system (Mars 6 Express, CEM Corp). Vessels were heated to 200 °C over 15 minutes, held at 200 °C for 20 minutes, and allowed to cool overnight. Then, the contents of each vessel were poured into a 50-ml volumetric flask, brought up to volume with deionized water, and shaken vigorously to ensure complete mixing. Samples were allowed to settle for ≥ 5 hours and then the supernatant was poured into sterile scintillation vials. Aliquots of this solution underwent a quantitative 1:10 dilution before analysis for Se by ICP-MS (Thermo iCAP-RQ).

Samples were analyzed in batches of 20 with 2 replicates of certified reference material (Tort-3, National Research Council, Canada) and one blank exposed to the same laboratory procedures. All blanks were below the instrument detection limit for Se (0.07 $\mu\text{g Se L}^{-1}$), and recovery of Se in reference material ($10.34 \pm 0.45 \mu\text{g Se g}^{-1}$ dry weight) was within the certified range (9.9–11.9 $\mu\text{g Se g}^{-1}$ dry weight). Lab duplicates were made for every ten samples and averaged 9.4% difference.

Data analysis

We tested for differences among ecosystem media within each stream (Objective 1) and among streams (Objective 2) using one-way ANOVA. If the result of the ANOVA test was statistically significant, Tukey's honestly significant difference was used to test all pairwise comparisons. Assumptions of normality and homoscedasticity were tested using the Shapiro-Wilk test and Levene test, respectively. Assuming that body length is

correlated with age for salamanders and fish, we conducted linear models of whole-body Se concentrations and length for each species of salamander and fish collected at each stream to assess the potential influence of age (and thus exposure duration) on whole-body Se concentrations.

To further assess bioaccumulation differences in ecosystem media and among study streams, we used an ecosystem-scale modelling approach using methods described by Presser and Luoma (2010). Enrichment factors (EF) were calculated as the ratio of Se concentrations in living- and non-living particulate matter (i.e., leaf detritus, sediment, and biofilm) to Se dissolved in the water column. Dissolved water-column Se concentrations less than the instrument detection ($<0.07 \mu\text{g Se L}^{-1}$) were treated as half the detection limit ($0.035 \mu\text{g Se L}^{-1}$). Dissolved water-column Se concentrations $>0.07 \mu\text{g Se L}^{-1}$ but less than the reporting limit ($0.50 \mu\text{g Se L}^{-1}$) were analyzed as instrument-reported values. Animal Se concentrations were modelled using Trophic Transfer Factors (TTF) calculated by dividing the Se concentration in an animal by its food source. We critically note that this ecosystem-scale modelling approach is most precise when food chains are modelled with individual species and the diets of animals are known. However, we sampled reference and mining-influenced streams, which are known to differ in macroinvertebrate communities (Pond et al. 2008, Timpano et al. 2015, 2018a), and thus composited macroinvertebrate samples based on trophic level (i.e., primary consumer and predator). In addition, diets of the salamanders and fish collected are variable and include both aquatic macroinvertebrates and other food sources (Jenkins and Burkhead 1993; Felix and Pauley 1996).

To address Objective 3, we assessed possible longitudinal patterns in Se bioaccumulation in each stream using simple linear regressions of media Se concentrations from each sampling location versus distance downstream. We also conducted a simple linear regression between Se speciation proportions (percent selenate and percent selenite) and distance downstream for the high-Se stream (H2) where speciation analysis was conducted. For each linear model, we performed a global validation of model assumptions as well as separate evaluations of skewness, kurtosis, and heteroscedasticity. RStudio software (RStudio, Boston, MA) and R version 3.4.1 (R Core Team 2018) were used for all statistical analyses, with a significance threshold of $p = 0.05$.

RESULTS

Within-stream comparisons of media Se concentrations

Selenium bioconcentrated from water into particulate matter and then further increased into macroinvertebrates in all mining-influenced streams (Figure 2, Table 2). Reference streams showed a similar pattern; however, biofilm Se concentrations were comparable to macroinvertebrate concentrations (Figure 2). Among particulates, biofilm had the highest mean Se concentration in 5 streams, with Se concentrations significantly higher than in sediment in 5 streams and higher than in leaf detritus in 3 streams. In all streams except R2, macroinvertebrates had the highest mean Se concentrations compared to all other media, although such differences were not significantly different in some cases. Only 2 streams (R1 and H2) showed a significant difference between primary-consumer and predator macroinvertebrate Se concentrations (Figure 2).

Despite their higher trophic status, salamanders and fish consistently had lower Se concentrations than macroinvertebrates, and to a greater extent in mining-influenced streams. Salamanders had significantly lower Se concentrations than macroinvertebrates in all but one study stream (i.e., TTFs <1.0; Table 2). Fish Se concentrations were also typically lower than macroinvertebrate concentrations (Table 2) but were higher compared to salamander concentrations; however, the magnitude and significance of differences varied by fish species. For example, in H1, Se concentrations in 2 of the 3 collected fish species (*S. atromaculatus* and *R. obtusus*) were significantly lower than in macroinvertebrates, but all 3 fish species had significantly higher Se concentrations than salamanders (Figure 2E). Although we did not observe significant differences in Se concentrations between salamander species within streams, Se concentrations varied among fish species in some streams. In H1, Se concentrations in *C. bairdi* were approximately 70% higher than *R. obtusus* and 80% higher than *S. atromaculatus*, which themselves did not differ significantly (Figure 2E). Last, we did not observe significant relationships between body length and whole-body Se concentrations for each collected salamander and fish species at each stream (data not shown).

To illustrate the general pattern of Se transfer observed across trophic levels and relative to streamwater Se concentrations, we constructed boxplots of Se concentration by media type and stream type (Figure 3). We pooled data by stream type and pooled biofilm, streambed sediment, and leaf detritus into an aggregate media group called “Particulate”, which represents the dietary source of Se for primary consumers. We also pooled vertebrate species data into “Salamander” and “Fish” groups. Data show increasing concentrations from streamwater to particulate matter and then to

macroinvertebrates. Again, however, top trophic levels (i.e., fish and salamanders) did not show higher concentrations than macroinvertebrates, where macroinvertebrates generally had the highest Se concentrations in each stream.

Among-stream comparisons of media Se concentrations

Selenium concentrations for all ecosystem media were nominally higher in mining-influenced streams than in reference streams, and media Se concentrations generally increased with water-column Se concentration (Figure 3). Water-column Se concentrations were $<0.07 \mu\text{g Se L}^{-1}$ for all sample locations in both reference streams (Figure 4A), whereas Se concentrations in low-Se streams concentrations were approximately 6 times greater than this detection limit. Selenium concentrations in high-Se streams concentrations were significantly higher and approximately 20 times greater than Se concentrations in low-Se stream concentrations.

In contrast to water-column concentrations, streambed sediment Se concentrations were less variable among study streams (Figure 4B); however, L2 had significantly higher concentrations compared to the other 5 study streams, and both high-Se streams were significantly greater than R2. Selenium concentrations in the other particulate matter (i.e., biofilm and leaf detritus) generally increased with water-column Se with significant differences detected between high-Se streams and reference streams (Figure 4C, D).

Primary-consumer and predator macroinvertebrate Se concentrations were approximately 12 and 7 times higher in high-Se streams than in reference streams, respectively (Figure 5A, B). Salamander Se concentrations were more similar among

streams with statistical differences only being observed for *D. fuscus* between three mining-influenced streams (all but L2) and R2, and for *D. monticola* between high-Se streams and reference streams (Figure 5C, D). Fish showed stronger differences among streams than salamanders (Figure 5E, F). *R. obtusus* Se concentrations in H1 were approximately 4 times higher than in R2. *S. atromaculatus* Se concentrations were approximately 9 times higher in H2 than in R2.

Longitudinal patterns in media Se concentrations

We found few significant patterns in ecosystem media Se concentrations along longitudinal gradients within our study streams. Reference streams had no significant longitudinal patterns. The low-Se stream (L2) had one significant association with distance downstream: decreasing leaf-detritus Se concentration ($p = 0.016$). High-Se streams had one relatively weak and one relatively strong decreasing longitudinal pattern in water-column Se (H1 $p = 0.091$, H2 $p = 0.045$), but with no associated trends in other media concentrations, except for decreasing leaf detritus Se concentrations in H1 ($p = 0.023$).

As an assessment of a potential driver of downstream differences in Se bioaccumulation, we analyzed stream H2 for Se speciation within the water column in each of its 5 sample locations. We observed a longitudinal pattern, wherein total Se decreased with distance downstream ($p = 0.065$), %selenate increased ($p = 0.053$), and %selenite decreased ($p = 0.053$) (Table 3). Selenate ranged from 95.10 to 96.48% of total Se, and selenite ranged from 3.52 to 4.90% of total Se.

DISCUSSION

Our results show that Se concentrations increase from the water column into particulate matter and to macroinvertebrates (Figures 2, 3; Table 2). Salamanders and fish had lower whole-body Se concentrations than macroinvertebrates (Figure 2, 3) but bioaccumulated Se to potentially toxic levels in streams where water-column Se concentrations exceeded U.S. EPA's lotic water-column WQC. Differences among streams were evident (Figures 3–5) where ecosystem media collected from mining-influenced streams had higher Se concentrations than media collected from reference streams. Lastly, there was little evidence of longitudinal patterns in Se concentrations within individual streams along our study reach lengths (1–2 km) with no perennial tributaries. Although our work is focused on one specific Se source and region (surface mining in central Appalachia), it is one of the few published field-based assessments (see Whitmore et al. 2018) of Se bioaccumulation, including in fish and salamanders, in headwater streams.

Comparisons of media Se concentrations within and among streams

Surface coal mining can increase concentrations of dissolved Se in headwater streams in central Appalachia (U.S. EPA 2011), with subsequent bioaccumulation into ecosystem media (Presser 2013; Arnold et al. 2014, 2017; Whitmore et al. 2018). Despite significant differences in water-column Se concentrations among stream types (Figure 4A), the magnitude of difference in streambed sediment Se concentrations across our study streams was relatively small and comparable to results found in other central Appalachian headwater streams by Whitmore et al. (2018). It is likely that in the fast-moving headwater streams studied here, immobilization of Se into streambed sediment is

limited by water residence time (Lemly and Smith 1987). It also is possible that differences of streambed sediment composition (i.e., mineral vs. organic fraction) among streams may have influenced our results. In contrast to streambed sediment, Se concentrations in biofilm and leaf-detritus Se concentrations were significantly elevated in mining-influenced streams, suggesting that bioaccumulation of Se into leaf detritus and biofilm may be a critical step for subsequent trophic transfer to primary-consumer macroinvertebrates.

Enrichment of Se from the water-column to particulate matter is the largest increase in Se concentrations in aquatic food chains, with rates highly dependent on water-column Se concentration and speciation (Presser and Luoma 2010). Enrichment factors for particulate matter in the present study were higher in reference streams than mining-influenced streams (Table 2). DeForest et al. (2007) and Whitmore et al. (2018) observed similar patterns where water-column Se increases were associated with relative decreases in enrichment of Se into particulate matter. Enrichment factors from high-Se streams in the present study are within range of others found in streams with similar water-column Se concentrations (Presser and Luoma 2010; Presser 2013; Kuchapski and Rasmussen 2015; Whitmore et al. 2018).

Macroinvertebrates had the highest mean Se concentrations in most of our study streams (Figures 2, 3) and showed significant bioaccumulation in mining-influenced streams (Figure 5A, B). We did not find significantly higher Se concentrations in predator macroinvertebrates than in primary-consumer macroinvertebrates for any mining-influenced streams (e.g., mean TFFs ranged from 1.2 to 0.73 in these streams; Table 2). Our finding of comparable Se concentrations in primary-consumer and predator

macroinvertebrates is counter to similar studies of Se dynamics in central Appalachia (Presser 2013; Whitmore et al. 2018), which found higher Se concentrations in predator macroinvertebrates relative to their prey. Selenium bioaccumulation rates vary among aquatic organisms as a result of dietary preferences, physiological requirements, and ability to assimilate and process Se (Stewart et al. 2010). Consequently, we suspect that differences in taxon composition across our composite macroinvertebrate samples could be a cause of this discrepancy.

We also found higher Se concentrations in salamanders and fish in mining-influenced streams compared to reference streams, although the difference was smaller than observed in macroinvertebrates. In addition, we found no associations between body length (as an indicator of age) of fish and salamanders and Se concentrations, suggesting that age did not affect whole-body Se concentrations of fish and salamanders collected in this study. However, we note that larval and juvenile fish and salamanders, which may vary in Se concentration compared to adults (Lemly 2002), were not sampled in this study. In all streams, Se concentrations were lower in salamanders and fish than in macroinvertebrates (Figures 2, 5). Presser (2013) also found lower Se concentrations in *S. atromaculatus* compared to composite macroinvertebrate samples in mining-influenced streams in southern West Virginia, USA. We suspect that lower Se concentrations in these higher trophic levels are primarily a result of their varied feeding habits. Benthic macroinvertebrates feed exclusively on aquatic media (e.g., leaf detritus, suspended particulates, and biofilm) that are exposed to Se in the water column, whereas salamanders and fish can consume a variety of food (i.e., not exclusively macroinvertebrates), including, for salamanders, terrestrial media not exposed to Se

(Jenkins and Burkhead 1993; Felix and Pauley 1996). In addition, salamanders and fish are more mobile than macroinvertebrates. Consequently, salamanders and fish may be feeding in upstream tributaries with low water-column Se, or in downstream reaches with lower concentrations of Se as a result of dilution from tributary or groundwater inputs. We note that organism movement, while possibly decreasing Se tissue concentrations, represents a Se pathway for further bioaccumulation in downstream waters (e.g., in larger piscivorous fish) and in terrestrial ecosystems (e.g., from salamanders to birds), representing likely, but largely unknown, cascading effects from Se-contamination in headwater systems.

Although the number of fish species sampled was limited, we observed some variability in Se concentrations among species collected from the same stream. Differences in Se concentrations among fish species likely reflect different feeding habits (Besser et al. 1996; Janz et al. 2010; Presser 2013). For example, higher whole-body Se concentrations in the benthic insectivore *C. bairdi* compared to the omnivorous *R. obtusus* and *S. atromaculatus* in mining-influenced streams (Figure 2E) may be explained by higher Se concentrations in their macroinvertebrate food source compared to other aquatic media consumed by omnivorous fish (e.g., algae, zooplankton, terrestrial insects, and other fish). However, we also note that differences in mobility among sampled fish species may also be contributing to differences in Se concentrations (Burger et al. 2001). Our results indicate that in mining-influenced headwater streams, elevated Se concentrations in macroinvertebrates are significant contributors to Se concentration in fish tissue, particularly the insectivorous *C. bairdi*, and that considering feeding habits is important when selecting fish species for Se monitoring in freshwater systems.

Longitudinal patterns in Se bioaccumulation

We found few strong longitudinal patterns of Se concentration in individual ecosystem media, suggesting that, within headwater stream reaches of similar size (1–2 km) and hydrology, Se bioaccumulation is influenced by long-term water-column Se concentrations and possible hydraulic and organismal movement of bioaccumulated material. Further, the observed absence of dilution in Se concentrations with distance downstream suggests headwater streams can transport Se below the contamination original source. However, our study reaches were limited in scale, and more work is needed to understand downstream impacts of Se contamination in headwater streams, particularly where downstream dilution of water-column Se may be more pronounced.

Downstream changes in Se speciation may also influence bioaccumulation dynamics. However, we only observed moderately significant longitudinal patterns in Se speciation in the one high-Se stream evaluated, where the proportion of total Se as selenate increased with concordant decreases in selenite. Loss of the more-reduced and more-bioavailable selenite may be attributable to oxidation and/or preferential biological uptake (Simmons and Wallschläger 2005). These results, while being some of the few for Se speciation in headwater streams, are limited to one stream over a short distance. Additionally, our sampled reach was selected to avoid reducing environments, such as wetlands and settling ponds, where selenite proportions and thus Se bioavailability may be increased. Last, our sampling was limited to surface water, yet pore water with lower oxygen in streambed sediments may also have higher proportions of selenite and thus increased Se bioavailability in locations where Se enrichment in particulate matter largely

occurs. Hence, the role of Se speciation and thus stream redox conditions in bioaccumulation remains an important knowledge gap.

Assessment of potential toxicity

Benthic macroinvertebrates are often overlooked in Se toxicology research yet have been shown in the present study, others in central Appalachia (Presser 2013; Arnold et al. 2014; Whitmore et al. 2018), and in other regions (Fan et al. 2002; Muscatello et al. 2008; Kuchapski and Rasmussen 2015) to bioaccumulate Se to levels exceeding current WQC for fish tissue (U.S. EPA 2016). Whitmore et al. (2018) documented macroinvertebrate Se concentrations ranging from 12.43 to 33.42 $\mu\text{g Se g}^{-1}$ dry weight in similar high-Se streams, which are comparable to concentrations observed in the present study (9.88–28.63 $\mu\text{g Se g}^{-1}$ dry weight). Arnold et al. (2014) also found elevated Se concentrations in macroinvertebrates ($10.1 \pm 0.2 \mu\text{g Se g}^{-1}$ dry weight) from a mining-influenced reach of the Mud River, West Virginia, USA. In a review of internal, dietary, and aqueous Se concentrations associated with toxicity in 29 different macroinvertebrate species, DeBruyn and Chapman (2007) observed sublethal effects at tissue concentrations ranging from 1–30 $\mu\text{g Se g}^{-1}$ dry weight. When food resources were limited, Conley et al. (2011) showed reduced fecundity and survivorship in laboratory mayflies (*Centroptilum triangulifer*, now *Neocloeon triangulifer*) when they were fed periphyton containing Se concentrations of $\geq 4.2 \mu\text{g Se g}^{-1}$ dry weight and $\geq 11.9 \mu\text{g Se g}^{-1}$ dry weight, respectively. Selenium concentrations in biofilm from our high-Se streams (5.3–12.6 $\mu\text{g Se g}^{-1}$ dry weight) and low-Se streams (2.0–7.1 $\mu\text{g Se g}^{-1}$ dry weight) are comparable to periphyton concentrations found by Conley et al. (2011) to adversely affect mayflies. In addition, Drover et al. (2019) and Pond et al. (2008) found significant negative correlations of

Ephemeroptera and Scraper Richness metrics with dissolved water-column Se concentrations in central Appalachian headwater streams; however, we note that potential toxicity associated with increased major ion concentrations may be a contributing factor to these negative correlations (Timpano et al. 2015, 2018a). Nonetheless, these studies and macroinvertebrate Se concentrations observed here suggest that elevated Se concentrations as a result of surface coal mining in headwater streams may be causing toxicity to macroinvertebrates. Future toxicity research in these and other Se-contaminated freshwaters is needed to identify toxicity thresholds to macroinvertebrates.

Selenium toxicity to salamanders has also been largely understudied, particularly in headwater ecosystems where they are common and functionally important species. Patnode et al. (2005) estimated a Se toxicity reference value (TRV) of $4.0 \mu\text{g Se g}^{-1}$ dry weight and found reduced species diversity and abundance in salamander assemblages in Appalachian headwater streams with concentrations exceeding this value. Approximately 26% of salamanders collected from our high-Se streams and 6% of salamanders collected from our low-Se streams exceeded this TRV. These results indicate that Se may be causing toxicity to salamanders in streams influenced by surface mining and point to similar research needs in other headwater systems vulnerable to Se contamination. In addition, bioaccumulation of Se into whole-body salamander tissue may be a possible source of Se transfer from aquatic to terrestrial environments and suggests that salamanders could be used to monitor Se in headwater streams where fish assemblages are absent, which is common in headwater streams.

In contrast to macroinvertebrates and salamanders, Se toxicity in fish is broadly recognized, where Se bioaccumulation in fish tissue can reduce reproductive success

(Janz et al. 2010). In response, U.S. EPA (2016) established a whole-body fish tissue WQC of $8.5 \mu\text{g Se g}^{-1}$ dry weight. In our study, fish individuals collected from reference and low-Se streams did not exceed this WQC. However, numerous fishes in our 2 high-Se streams had whole-body Se concentrations that exceeded this criterion, including 5 *C. bairdi* individuals (100% of the fish collected), 4 *S. atromaculatus* (27%), one *R. obtusus* (8%), 4 *N. photogenis* (57%), and one *L. macrochirus* (50%). Our results indicate that Se is bioaccumulating to levels exceeding established whole-body tissue WQC in some fish species, particularly the benthic insectivore *C. bairdi*, in mining-influenced headwater streams that have water-column Se concentrations above the lotic water-column WQC. Other fish species, such as the omnivorous *S. atromaculatus* and *R. obtusus*, may not be as affected despite elevated water-column Se concentrations. Selenium WQC were developed mainly based on observed toxicity in piscivorous and omnivorous gamefish species found in larger waters (e.g., *Salmo trutta*, *Esox Lucius*, and *Micropterus salmoides*) as opposed to the smaller-sized fish species, some of which are benthic insectivores, that are more common in headwater streams. Fish-tissue Se concentrations are currently preferred to water-column measurements of Se and will likely take precedent in regulatory matters (U.S. EPA 2016). With such emphasis on fish-tissue Se, a better understanding of differences in both bioaccumulation and toxicity potential among a greater variety of species, especially those with varied dietary habits, could improve development and application of Se criteria to protect headwater streams.

conclusionS

Selenium enrichment from water column to biofilm and leaf detritus and subsequently to benthic macroinvertebrates is an important pathway for Se trophic

transfer in headwater streams of central Appalachia. Macroinvertebrates, salamanders, and fish can bioaccumulate Se to potentially toxic levels in headwater streams with elevated water-column Se, especially when water-column Se exceeds U.S. EPA criteria. Notably, Se concentrations varied among fish species, likely related to differences in dietary habits. Further, we found little evidence of decreasing Se concentrations among sampled media with distance downstream, indicating that headwaters streams likely transport Se well beyond the contamination source. Collectively, our findings emphasize the importance of including multiple forms of heterotrophic aquatic life in field-based assessments of Se bioaccumulation and trophic transfer, highlighting important considerations for Se management in headwater systems and other freshwaters vulnerable to Se contamination.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (emailaddress).

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Figures

Figure 1: Location of the 6 central Appalachian headwater streams for selenium (Se) bioaccumulation study. Stream categories are based on U.S. EPA (2016) Se lotic water quality criterion and biannual (autumn and spring) water-column Se concentrations measured from 2013 to 2018 (Cianciolo 2019).

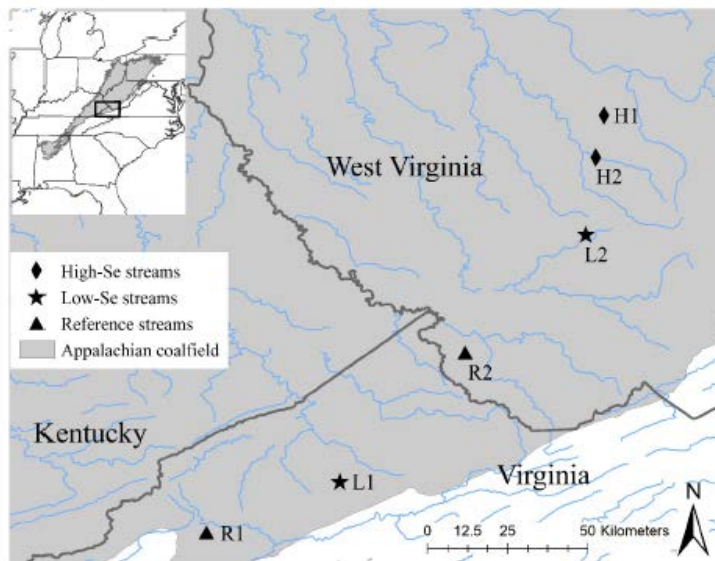


Figure 2: Boxplots of selenium (Se) concentrations in ecosystem media within (A) reference stream 1; (B) reference stream 2; (C) low-Se stream 1; (D) low-Se stream 2; (E) high-Se stream 1; and (F) high-Se stream 2. Note different y-axis ranges reflecting stream differences in Se media concentrations. Letters represent significant differences in media Se concentration within each stream (Tukey HSD, $p < 0.05$). SD = streambed sediment; LF = leaf detritus; B = biofilm; PC = primary-consumer macroinvertebrates; PD = predator macroinvertebrates; DM = *Desmognathus monticola*; DF = *Desmognathus fuscus*; RO = *Rhinichthys obtusus*;

SA = *Semotilus atromaculatus*; CB = *Cottus bairdi*; NP = *Notropis photogenis*; LM = *Lepomis macrochirus*.

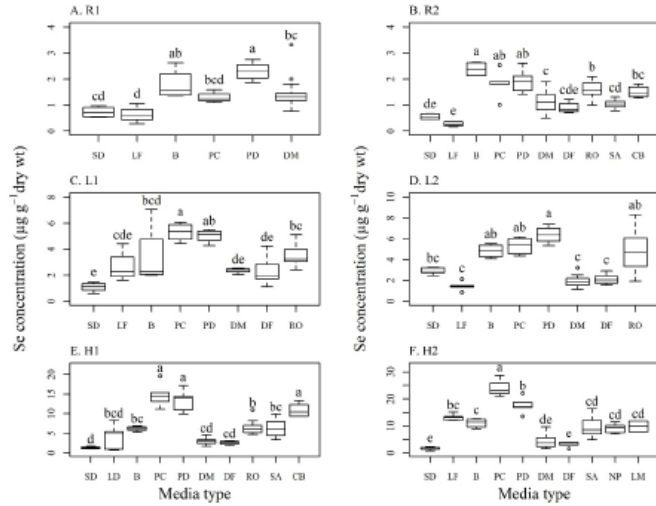


Figure 3: Boxplots of selenium (Se) concentrations in ecosystem media grouped by media type and stream type. The particulate media type combines Se concentrations in biofilm, streambed sediment, and leaf detritus. Salamander and fish media types combine Se concentrations in multiple species as appropriate.

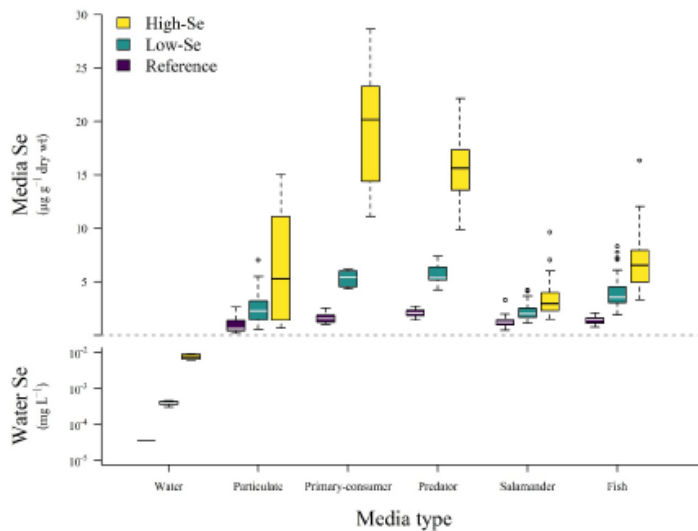


Figure 4: Boxplots of selenium (Se) concentrations in (A) water column; (B) streambed sediment; (C) leaf detritus; and (D) biofilm among streams collected at 4 or 5 sampling locations per stream. Letters represent significant differences in media Se concentrations among streams (Tukey HSD, $p < 0.05$). R1 = reference stream 1; R2 = reference stream 2; L1 = low-Se stream 1; L2 = low-Se stream 2; H1 = high-Se stream 1; H2 = high-Se stream 2.

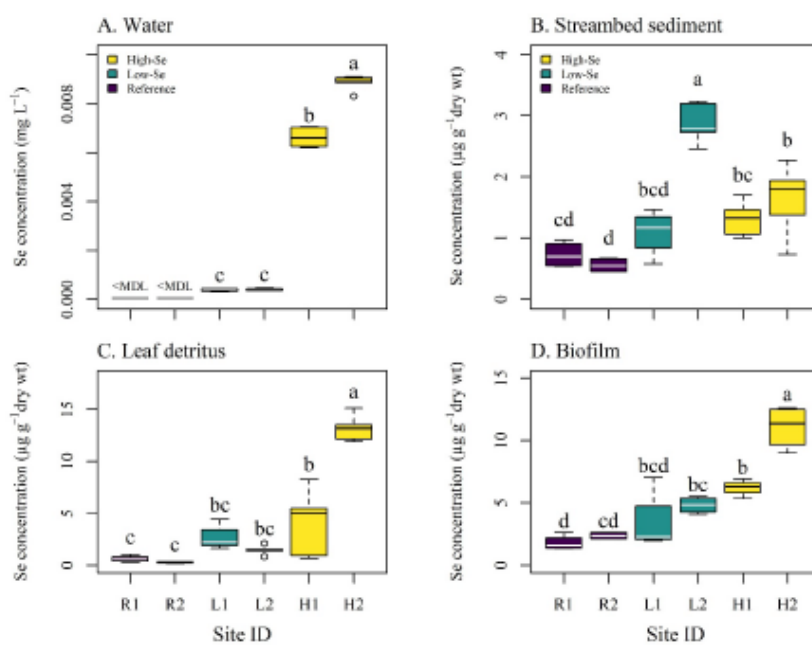


Figure 5: Boxplots of selenium (Se) concentrations in (A) primary-consumer macroinvertebrates; (B) predator macroinvertebrates; (C) *Desmognathus fuscus*; (D) *Desmognathus monticola*; (E) *Rhinichthys obtusus*; and (F) *Semotilus atromaculatus* among study streams collected at 4 or 5 sampling locations per stream. Letters represent statistically significant differences in Se concentrations among streams (Tukey HSD, $p < 0.05$). R1 = reference stream 1; R2 = reference

stream 2; L1 = low-Se stream 1; L2 = low-Se stream 2; H1 = high-Se stream 1; H2 = high-Se stream 2.

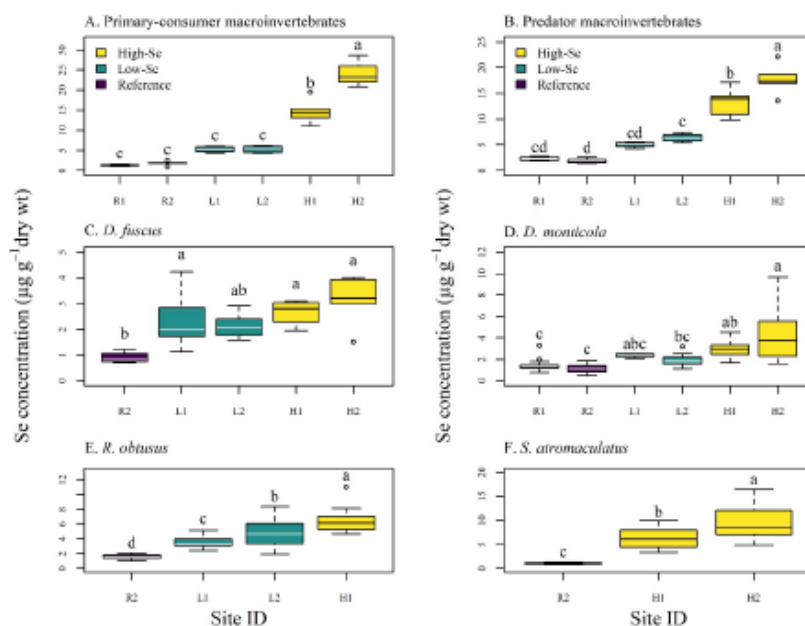


Table 1: Watershed characteristics of six central Appalachian USA headwater streams selected for selenium bioaccumulation study

Stream name	Site ID	Stream type	Study reach length (m)	Annual mean specific conductivity ^a (µs cm ⁻¹)	Water-column Se ^b (µg L ⁻¹)	Watershed area (km ²)
Eastland Creek	R1	Reference	1200	26	<0.5	2.42
Hurricane Branch	R2	Refer	1600	67	<0.5	5.96

		ence				
Fryingpan Creek	L1	Low-Se	1200	382	<2.5	5.66
Crane Fork	L2	Low-Se	1600	425	<2.5	9.77
Left Fork of Long Fork of Coal Fork	H1	High-Se	1600	1218	8.5	4.34
Rockhouse Creek	H2	High-Se	1600	719	11.8	7.13

^aAnnual mean specific conductance calculated using data collected at 30-minute intervals from 2011 to 2018 (Cianciolo 2019).

^bMedian selenium (Se) water-column concentration from biannual monitoring of water chemistry conducted from 2013 to 2018 (Cianciolo 2019). Selenium concentrations from reference streams were below the instrument detection limit ($0.5 \mu\text{g L}^{-1}$) and Se concentrations from low-Se streams were above detection but below the reporting limit ($2.5 \mu\text{g L}^{-1}$) for all samples.

Table 2: Enrichment Factors (EF) and Trophic Transfer Factors (TTF) describing selenium dynamics in reference and mining-influenced streams in central Appalachia USA (mean values \pm SE)

Factor ^b	Stream ID ^a					
	R1	R2	L1	L2	H1	H2
EF _{sediment}	21000 \pm	16000 \pm	2800 \pm	7400 \pm 610	200 \pm 16	180 \pm 30
	3000	1400	360			
EF _{leaf detritus}	18000 \pm	7500 \pm	6900 \pm	3700 \pm 450	610 \pm	1500 \pm
	4600	1100	1400			
EF _{biofilm}	51000 \pm	68000 \pm	9800 \pm	12000 \pm	940 \pm 65	1300 \pm
	8400	4000	4500	1100		120
EF _{particulate mean}	30000 \pm	27000 \pm	6500 \pm	7300 \pm 890	540 \pm 61	940 \pm 79
	3700	3300	1500			
TTF _{<i>Tipula</i>: leaf detritus}			2.5 \pm 0.34	4.3 \pm 0.70		2.4 \pm
						0.16
TTF _{<i>Pteronarcys</i>: leaf detritus}			0.95 \pm		5.8 \pm	
	1.1 \pm 0.32	2.8 \pm 0.51	0.15		2.63	

TTF _{primary consumer: particulate} mean	1.3 ± 0.24	2.2 ± 0.49	2.4 ± 0.30	2.0 ± 0.41	4.5 ± 0.85	2.9 ± 0.16
TTF _{predator: primary consumer}	1.8 ± 0.14	1.1 ± 0.20	1.0 ± 0.02	1.2 ± 0.10	0.90 ± 0.04	0.73 ± 0.04
TTF _{<i>D. fuscus</i>: macroinvertebrate} mean		0.58 ± 0.01	0.45 ± 0.03	0.37 ± 0.02	0.18 ± 0.02	0.14 ± 0.02
TTF _{<i>D. monticola</i>: macroinvertebrate} mean	0.83 ± 0.10	0.64 ± 0.13	0.47 ± 0.01	0.36 ± 0.07	0.25 ± 0.02	0.21 ± 0.04
TTF _{<i>R. obtusus</i>: macroinvertebrate} mean		0.87 ± 0.08	0.69 ± 0.05	0.71 ± 0.08	0.42 ± 0.04	0.52 ^c
TTF _{<i>S. atromaculatus</i>: macroinvertebrate} mean		0.89 ± 0.07			0.53	0.43 ± 0.06
TTF _{<i>C. bairdi</i>: macroinvertebrate} mean		0.56 ± 0.05			0.74 ± 0.04	0.62
TTF _{<i>N. photogenis</i>: macroinvertebrate} mean						0.42
TTF _{<i>L. macrochirus</i>: macroinvertebrate} mean						0.39

^aStudy streams separated into categories, reference (R), low-Se (L), and high-Se (H), based on biannual measurements of dissolved water-column selenium (Se) concentrations from 2013 to 2018 (Cianciolo 2019).

^bParticulate mean calculated as an average of leaf detritus, biofilm, and streambed sediment Se concentrations. Macroinvertebrate mean calculated as an average of primary-consumer- and predator Se concentrations. Trophic transfer factors for *Pternarcys* and *Tipula* were calculated using leaf detritus Se concentrations.

^cValues without standard error have n=1 (i.e., fish species was only collected from one sample location within a stream). Missing values represent streams where ecosystem media used to calculate specific TTFs were not collected.

Table 3: Water-column selenium speciation at sample locations within stream H2

Distance downstream (m)	Se as sum of species ($\mu\text{g L}^{-1}$)	%Selenate	%Selenite ^a
0	7.77	95.10	4.90
400	7.82	95.54	4.46
800	7.66	95.73	4.27

1200	7.53	95.56	4.44
1600	6.85	96.48	3.52

^aSelenite concentrations were below the instrument reporting limit ($1.00 \mu\text{g L}^{-1}$), but above the detection limit ($0.3 \mu\text{g L}^{-1}$) and should be considered estimated values.