## Environmental Toxicology

# Selenium Dynamics in Headwater Streams of the Central Appalachian Coalfield

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**Abstract:** Coal mining can cause selenium (Se) contamination in US Appalachian streams, but linkages between water-column Se concentrations and Se bioaccumulation within Appalachian headwater streams have rarely been quantified. Using elevated specific conductance (SC) in stream water as an indicator of mining influence, we evaluated relationships between SC and Se concentrations in macroinvertebrates and examined dynamics of Se bioaccumulation in headwater streams. Twenty-three Appalachian streams were categorized into 3 stream types based on SC measurements: 1) reference streams with no coal-mining history; 2) mining-influenced, high-SC streams; and 3) mining-influenced, low-SC streams. Selenium concentrations in macroinvertebrates exhibited strong positive associations with both SC and dissolved Se concentrations in stream water. At 3 streams of each type, we further collected water, particulate matter (sediment, biofilm, leaf detritus), and macroinvertebrates and analyzed them for Se during 2 seasons. Enrichment, trophic transfer, and bioaccumulation factors were calculated and compared among stream types. Particulate matter and macroinvertebrates in mining-influenced streams accumulated high Se concentrations relative to reference streams. Concentrations were found at levels indicating Se to be a potential environmental stressor to aquatic life. Most Se enrichment, trophic transfer, and bioaccumulation factors were independent of season. Enrichment factors for biofilm and sediments and bioaccumulation factors for macroinvertebrate predators varied negatively with water-column Se. Our results increase scientific understanding of Se bioaccumulation processes in Appalachian headwater streams. *Environ Toxicol Chem* 2018;37:2714–2726. © 2018 SETAC

**Keywords:** Benthic macroinvertebrates; Bioaccumulation; Bioconcentration; Conductivity; Enrichment; Trophic transfer

## INTRODUCTION

Environmental contamination by the trace element selenium (Se) is a global concern (Lemly 2004). Selenium is an essential micronutrient to many organisms including animals, algae, and bacteria (Mayland 1994; Janz et al. 2010). However, biotic exposures to elevated Se concentrations in water have caused lethal and sublethal effects, including pronounced effects in egg-laying vertebrates (Janz et al. 2010). Because of the propensity of Se to bioaccumulate, toxic effects may be observed in aquatic biota where water-column concentrations are only marginally elevated above essential levels (Janz et al. 2010).

Coal mining in central Appalachia, USA, is a driver of water chemistry changes within the region (US Environmental Protection Agency 2011). The mining process removes layers

of rock to uncover coal seams. When mining-disturbed rocks are exposed to rainfall, major ions are leached from unweathered rock surfaces and transported into headwater streams; this process causes increased specific conductance (SC) of receiving waters (Pond et al. 2008; Evans et al. 2014; Daniels et al. 2016). Likewise, mineral forms of Se contained in mine rock can oxidize to water-soluble selenite and selenate anions and may be transported into streams at elevated concentrations in association with the major ions that are primary contributors to elevated SC (Pond et al. 2008; Young et al. 2010). Laboratory studies have shown that release of Se and release of most major ions from mine rocks exhibit patterns of progressive leaching (Clark 2017). Elevated SC is an indicator of coal-mining activities, and multiple studies have connected it with degraded benthic macroinvertebrate communities in coal mining–influenced headwater streams (Pond et al. 2008, 2014; Timpano et al. 2015).

Unlike major ions, the dominant pathway for Se transfer to consumers is through the consumer food source, not directly through the water column (Presser and Luoma 2010). Bioaccumulation of Se in aquatic ecosystems is initiated when bacteria,

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algae, or plants take up dissolved inorganic Se from the water column (Stewart et al. 2010). This initial uptake is the most concentrating step of Se bioaccumulation within food webs and is often quantified by calculating an enrichment factor (EF), the ratio of Se concentration in biofilm and detritus (particulate matter) to the concentration of dissolved Se in the water column (DeForest et al. 2016; US Environmental Protection Agency 2016). Lesser but significant bioaccumulation of Se occurs through trophic transfers when biota consume particulate matter consisting of Se-enriched living or detrital particles. Additional bioaccumulation of Se can occur when primary consumers are consumed by predators (Presser and Luoma 2010).

Site-specific biogeochemical factors can affect Se bioaccumulation within aquatic systems (Presser and Luoma 2010). Community composition at all levels of the aquatic food web influences both enrichment processes and trophic transfers. Species differences in assimilation efficiencies, ingestion, and excretion rates may scale up to community-level differences in Se concentrations among aquatic systems (Presser and Luoma 2010). As a further complication in Appalachian mining-influenced streams that are often sulfate-enriched (Pond et al. 2008, 2014), elevated sulfate concentrations in the water column may inhibit Se uptake by biofilm (Williams et al. 1994; DeForest et al. 2017). Water residency times in aquatic systems influence chemical forms of Se, ecosystem enrichment, and retention of Se. Rapidly flowing streams limit reactivity time and can flush Se-enriched particles out of the stream system, restricting buildup of Se-enriched sediments and limiting bioaccumulation through detrital pathways. Selenate is typically the dominant form of Se in lotic environments, but in slower-moving lentic environments greater recycling of Se occurs, generally resulting in more biologically reactive selenite and organic forms of Se (Young et al. 2010). Hence, organisms generally bioaccumulate Se more efficiently in lentic systems than in lotic systems, and organisms within slow-moving, low-gradient streams may bioaccumulate Se more efficiently than those residing in high-gradient headwater streams (Lemly 1999; Orr et al. 2006).

Bioaccumulation pathways are crucial to understanding linkages between Se concentrations in stream water and potential toxicity to biota. Because of the complexity of these processes, site-specific studies are needed to inform appropriate resource management and environmental protection practices (Presser and Luoma 2010). Though studies have associated coal mining with elevated SC and Se in receiving waters (Pond et al. 2008, 2014; Lindberg et al. 2011), few studies in central Appalachia have examined Se dynamics within aquatic food webs (see Presser 2013). In the present study, our primary goal was to improve scientific understanding of Se dynamics in the headwater systems that dominate river networks in the region and are generally most proximate to mining-related geologic disturbances. Our primary objectives were to 1) assess the relationship between SC and Se bioaccumulation, both of which may be elevated downstream of coal mining and may impact aquatic communities in central Appalachian headwater streams, and 2) characterize Se bioaccumulation dynamics in mining-influenced and reference-quality headwater streams of central Appalachia.

# METHODS

## Stream selection

First- and second-order streams located in the central Appalachian coalfield of Virginia and West Virginia, USA, were previously selected to identify relationships between elevated major dissolved ion concentrations and coal mining (Timpano et al. 2015). Among the selected streams were 5 reference streams within forested watersheds that had minimal anthropogenic disturbance and 18 mining-influenced streams within watersheds subjected to surface mining. Concentrations of major ions (and SC as their proxy) ranged widely, but study streams were selected with the intent that environmental conditions other than SC would meet "reference-like" conditions (Supplemental Data, Table S1). Timpano et al. (2015) demonstrated elevated SC to be driving the decline in Ephemeroptera species richness and relative abundance in selected streams, a finding that is consistent with numerous other studies (e.g., Pond et al. 2008, 2014). In the present study SC is considered a surrogate for extent of mining influence and a stressor to aquatic invertebrates pervasive in the Appalachian coalfield (Cormier et al. 2013).

We conducted the present study in 2 major sampling efforts. In the summer of 2015, 23 streams were surveyed (Figure 1) to evaluate associations among SC, dissolved Se concentrations in stream water, and Se concentrations in tissue samples of selected aquatic fauna. In the fall of 2015 and spring of 2016, we intensively sampled 9 of the 23 headwater streams to more thoroughly evaluate Se dynamics in these stream ecosystems.

Timpano et al. (2015, 2018b) previously installed in situ SC data loggers in the present study streams, which have recorded SC at 15-min intervals. A yearly mean of SC readings recorded from July 2014 to June 2015 (hereafter referred to as "continuous SC") was used to separate mining-influenced streams into high-SC and low-SC stream classes. Streams with mean SC ≥75th percentile of our 23-stream sample were classified as high-SC, and mining-influenced streams with mean SC <75th percentile were classified as low-SC. Reference streams were defined as streams that had no history of mining within their watersheds and as a result had low SC. Dissolved Se concentrations and benthic macroinvertebrate community analyses were also available for all streams from a sampling event in April 2014 (Table 1).

We sampled water and macroinvertebrates at all 23 sites during an initial stream survey. For further study of Se bioaccumulation dynamics, a subsample of 3 reference, 3 low-SC, and 3 high-SC streams was selected. Geographical proximity was also considered in selection of the 9 streams. Because only 2 high-SC streams were located in southwestern Virginia, a third high-SC stream located in southern West Virginia was selected to maintain a balanced study among stream types despite its lack of geographic proximity (Figure 1).

## Initial 23-stream survey

In July and August 2015, we collected stream water and benthic macroinvertebrates, including crayfish from the family Cambaridae and 2 dragonfly nymph families (Gomphidae and
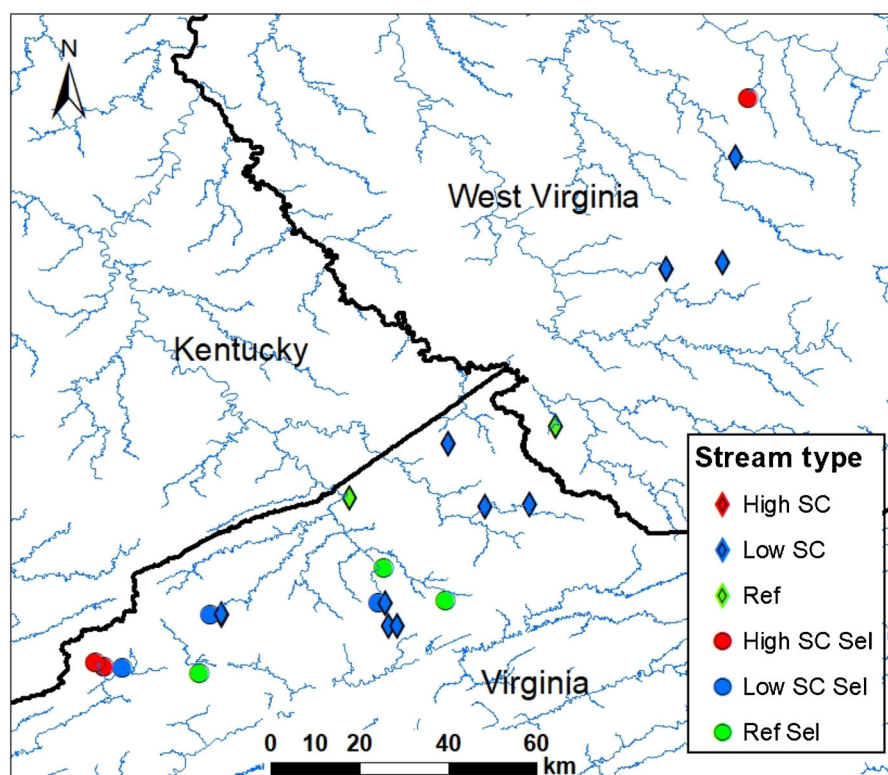
**FIGURE 1:** Location of 23 streams selected for initial sampling, including 9 streams selected for further sampling in central Appalachia USA. Ref = reference; SC = specific conductance; Sel = selected for further sampling.

**TABLE 1:** Specific conductance (SC), dissolved selenium, and selected benthic macroinvertebrate metrics from 23 headwater streams in central Appalachia USA

| Site[a] | Stream type | Continuous SC (µS/cm)[b] | Dissolved Se (µg/L)[c] | Total taxa richness[d] | Shannon Diversity Index[d] | % Ephemeroptera[d] |
|---|---|---|---|---|---|---|
| EAS | Reference | 25 | <2.5 | 25 | 2.7 | 36.8 |
| MCB | Reference | 51 | <2.5 | 33 | 2.8 | 31.7 |
| HCN | Reference | 61 | <2.5 | 22 | 2.3 | 23.8 |
| CRO | Reference | 63 | <2.5 | 31 | 2.8 | 49.2 |
| COP | Reference | 120 | <2.5 | 25 | 2.6 | 40 |
| GRA | Low-SC | 185 | <2.5 | 23 | 2.4 | 39.6 |
| RFF | Low-SC | 314 | <2.5 | 26 | 2.5 | 23.1 |
| SPC | Low-SC | 316 | <2.5 | 24 | 2.3 | 24.5 |
| FRY | Low-SC | 344 | <2.5 | 22 | 2.2 | 21.8 |
| HUR | Low-SC | 365 | <2.5 | 20 | 2.3 | 26.4 |
| CRA | Low-SC | 430 | <2.5 | 25 | 2.4 | 33.8 |
| RUT | Low-SC | 530 | <2.5 | 16 | 1.8 | 3.7 |
| MIL | Low-SC | 551 | <2.5 | 14 | 1.4 | 0 |
| ROL | Low-SC | 563 | <2.5 | 16 | 2 | 1.4 |
| LLE | Low-SC | 572 | <2.5 | 19 | 1.5 | 9.8 |
| BIR | Low-SC | 591 | <2.5 | 11 | 1.1 | 1.9 |
| LAB | Low-SC | 634 | 4.5 | 20 | 2.3 | 18.1 |
| ROC | Low-SC | 674 | 20.5 | 17 | 1.5 | 6.2 |
| KEL | High-SC | 762 | 4.0 | 10 | 1.4 | 7 |
| LLW | High-SC | 1090 | 3.8 | 16 | 1.6 | 2.3 |
| KUT | High-SC | 1143 | 8.4 | 11 | 1.6 | 0.5 |
| LLC | High-SC | 1165 | 14.5 | 23 | 2 | 0.5 |
| RIC | High-SC | 1346 | <2.5 | 19 | 2.1 | 0.5 |

[a] For further information on site codes, see Whitmore (2016).
[b] Annual mean value of specific conductance calculated using data from in situ continuous conductivity meter, July 2014 to June 2015. Mean concentrations for sites where 1 or both observations were less than the minimum reportable level are listed as <2.5.
[c] Mean of April 2014 and Summer 2015 Se concentrations in stream water filtered through a 0.45-µm pore membrane.
[d] Water Se and benthic macroinvertebrates sampled April 2014 (Timpano et al. 2018a, 2018b).

Cordulegastridae). These larger-bodied taxa were selected because they were easily sampled to provide sufficient biomass for analysis of Se. Macroinvertebrate samples from each stream were composited and transported on dry ice to the laboratory, where they were stored at –20 °C.

## Se-dynamics study

To describe the processes of Se bioaccumulation linking dissolved Se to body-burden Se in macroinvertebrates, we applied ecosystem-scale modeling concepts described by Presser and Luoma (2010). Using this methodology, we quantified EFs and trophic transfer factors (TTFs) as ratios between particulate matter and dissolved Se and between consumers and their food. In selecting media for sampling, we sought community constituents that are representative of a typical headwater stream food web in central Appalachia (Figure 2).

There are differences between the intended use of the ecosystem-scale model and our application of that model for the present study. The ecosystem-scale model developed by Presser and Luoma (2010) was designed to link dissolved Se concentrations and toxic concentrations of Se in a selected vertebrate species within an impacted system. Quantifications of bioaccumulation processes are most precise when individual species are used in food-web models to control for differing assimilation efficiencies and ingestion rates among species. The present study sampled reference streams as well as mining-influenced streams to enable comparisons of Se concentrations and bioaccumulation processes within streams of varying levels of mining influence. Invertebrate community-composition differences among stream types prevented us from selecting one taxon group that was present in sufficient quantities at all streams. Hence, we composited sampled macroinvertebrates by trophic level to approximate the community present at each stream.

## Stream delineation

At each selected stream, we delineated reaches 100 m in length centered approximately on in situ SC meters installed in streams. When necessary, study reaches were shifted upstream to avoid having downstream segments located below roadways or tributaries. Reaches were further divided into 10-m sub-reaches to facilitate evenly distributed collection of ecosystem media.

## Field collection and laboratory processing

The water-column and benthic macroinvertebrate collection and analysis procedures described in the present section were conducted for the initial 23-stream survey, and all procedures were conducted for the 9-stream Se-dynamics study.

*Water column.* We collected water samples for analysis of dissolved Se at approximately midreach and downstream of riffle habitat, to ensure vertical mixing. Water was filtered in the field using a 0.45-μm-pore filter membrane, followed by addition of trace metal–grade nitric acid to pH <2 and storage in Nasco Whirl-Pak® sample bags on ice until transport back to the laboratory, where they were stored at 4 °C. Acidified samples were analyzed on an inductively coupled plasma mass spectrometer (ICP-MS; Perkin-Elmer; Presser and Luoma 2010).
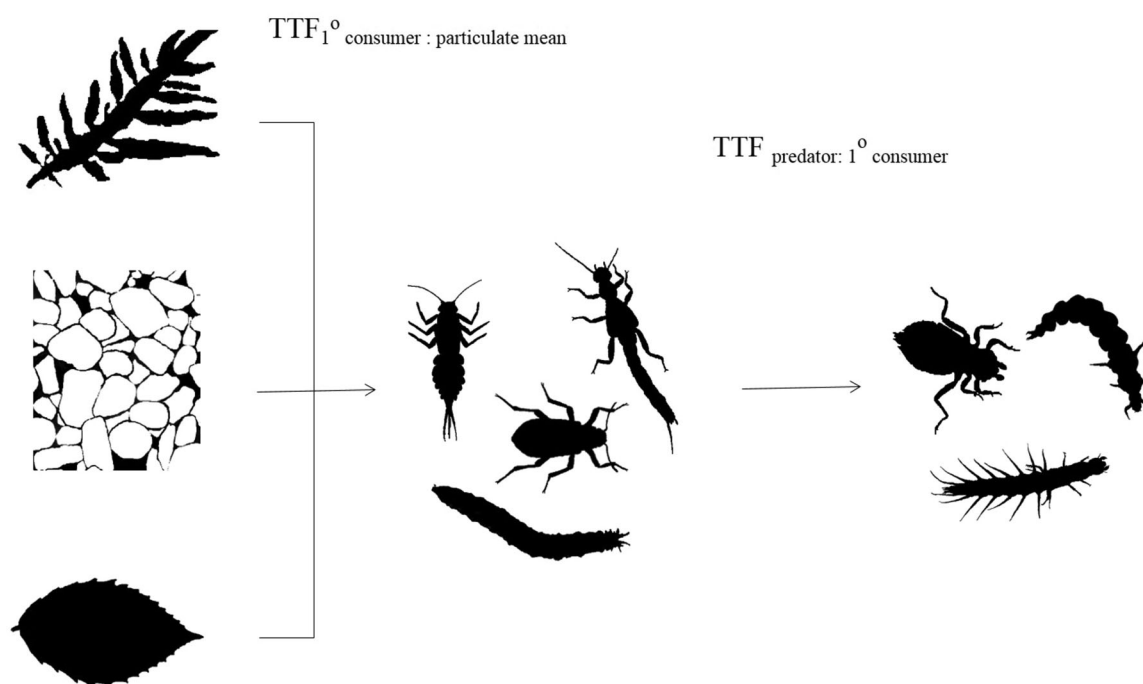


$$\text{TTF}_{1^o \text{ consumer : particulate mean}}$$

$$\text{TTF}_{\text{predator: } 1^o \text{ consumer}}$$

**FIGURE 2:** Example food web of a headwater stream. Biofilm, sediment, and leaf detritus represent material at the bottom of the food chain. Macroinvertebrate communities are separated into primary consumer and predator taxa groups. 1° = primary; TTF = trophic transfer factor.

**Stream-bed sediment.** At 5 evenly spaced intervals in 3 lateral transects per 10-m subreach, stream-bed sediment was collected using an acid-washed plastic scoop. Loss of fines was limited by selecting for sampling areas of reduced flow behind naturally occurring barriers such as in-stream cobbles. Sediment sub-samples were combined into a composite sample in light-excluding Fisherbrand Whirl-Pak bags. During the sampling process, we selected recently deposited, biologically active sediment by restricting sample depth to 1 to 3 cm (US Environmental Protection Agency 2001). Sediment samples were transported on dry ice and stored at 4 °C. After removing extraneous materials such as leaves and twigs, thawed samples were hand-pressed through a clean 1-mm stainless-steel sieve into a stainless-steel collection bowl. We homogenized and stored sediments <1 mm in Corning brand sterile vials after discarding sediments ≥1 mm and mixed, refroze, and lyophilized samples for approximately 120 h to ensure complete drying.

**Biofilm.** Biofilm, defined in the present study as mats of accumulated algae, fungi, and bacteria communities, was scraped from rock substrate using a plastic knife. Three rocks supporting the largest apparent accumulations of biofilm were selected for sampling within each reach. All visible biofilm accumulated on a selected rock was collected. During the fall sample period, epilithic biofilm was not available for collection in all streams, but biofilm accumulation could be seen on sediments; in these cases, we sampled sandy substrate by scooping the full visible extent of sediment surfaces carrying biofilm accumulation within the reach. We placed composite biofilm samples in light-excluding Fisherbrand Whirl-Pak bags and transported them on dry ice back to the laboratory for storage at 4 °C (Casey 2005; Orr et al. 2006). Biofilm samples, particularly samples collected from sandy deposits, contained large quantities of sand and silt. Sand and silt portions of samples were reduced by serial shaking and decanting (Bell and Scudder 2007), followed by refreezing samples at 4 °C. We then lyophilized samples for approximately 120 h and ground dried samples using a mortar and pestle.

**Leaf detritus.** Leaf detritus originating from the tree canopy bordering each stream was collected from leaf packs within the stream. Leaves were completely senescent, fully submerged in water, and not covered by sediments. We combined composite leaf samples for each stream, placed them in light-excluding Fisherbrand Whirl-Pak bags, and transported them on dry ice back to the laboratory, where they were briefly thawed, identified to tree species when possible, and agitated lightly in deionized water to remove excess sediment. After drying leaves at 65 °C for ≥5 d, leaf midveins were removed, and the remaining leaf matter was ground using a ball mill.

**Benthic macroinvertebrates.** Benthic macroinvertebrates were collected following multihabitat sampling procedures using a 0.3-m-wide D-frame kicknet with 500-μm mesh (Barbour et al. 1999). Net contents were emptied into plastic tubes, and macroinvertebrates were removed from debris using stainless-steel tweezers and placed into plastic containers filled with stream water. Sampling continued until a volume of macro-invertebrates appearing as sufficient in mass for Se analysis was collected. For the 23-stream initial survey, we preferentially extracted crayfish from the family Cambaridae and dragonflies from the families Cordulegastridae and Gomphidae for subsequent Se analysis. They were stored, transported, and processed.

For the Se-dynamics study, all collected macroinvertebrates were retained. Because of differences in average macroinvertebrate taxa sizes among streams, numbers of individuals collected ranged from 482 to 1199 per sample. Crayfish (Cambaridae) were also collected from streams by targeting optimal habitat and selecting 5 or fewer individuals from each stream that were comparable in size within and among streams. We transported macroinvertebrates in Fisherbrand Whirl-Pak bags on dry ice back to the laboratory, where they were thawed, identified to family, and separated into primary consumer and predator taxa (Merritt and Cummins 1996; Poff et al. 2006). In cases where the family taxon group contained both predacious and nonpredacious genera as specified in Merritt and Cummins (1996), individuals were identified to genus. We refroze samples, followed by lyophilization for approximately 120 h to ensure complete drying, and then ground samples with a mortar and pestle.

## Acid digestion and analysis for Se

We used methods adapted from US Environmental Protection Agency (2007) for acid digestion in a microwave digestion system (MarsExpress; CEM). Macroinvertebrates sampled from 23 streams were digested using 10 mL of trace metal–grade nitric acid (70% $HNO_3$). To improve Se recovery in the 9-stream study, subsequent digestions were completed using 5 mL of trace metal–grade nitric acid (70% $HNO_3$) and 1.5 mL of hydrogen peroxide (30% $H_2O_2$). Samples were analyzed in triplicate when sufficient mass was available. An ICP-MS was used to determine Se concentration in final solutions.

## Quality assurance/quality control

Certified reference material (TORT-2 and TORT-3; National Research Council of Canada) was digested in triplicate in all laboratory batches. Recovery of Se (mean = $9.0 \pm 0.49$ μg Se g$^{-1}$ dry wt) was less than the certified range (9.9–11.9 μg Se g$^{-1}$ dry wt) when 10 mL $HNO_3$ was used. Recovery of Se improved in the 9-stream survey when we used a combination of $HNO_3$ and $H_2O_2$ and was within the range of certified values (mean = $11.2 \pm 0.39$ μg Se g$^{-1}$ dry wt). Concentrations in blanks of deionized water run in parallel with samples were near instrumental detection limits and ranged from –0.17 to 1.27 μg Se L$^{-1}$. Percentage of difference among replicate samples averaged 7.3%.

## Data processing

We analyzed subsamples of each media type in replicates of 3 from each stream when sufficient material was available. Mean blank concentrations run in parallel with subsamples were

subtracted from subsample concentrations. Concentrations of Se below the instrument detection limit ($0.51\,\mu g\,Se\,L^{-1}$) were replaced with concentrations that were half the detection limit ($0.255\,\mu g\,Se\,L^{-1}$) for calculation of EFs and TTFs. The $0.255\,\mu g\,Se\,L^{-1}$ estimated concentrations are within the range of measured Se concentrations in reference and near-reference streams within the study area (Supplemental Data, Table S2). Selenium concentrations in replicate subsamples were averaged for statistical analyses.

The experimental design employed in the Se-dynamics study addressed the site-specific nature of Se bioaccumulation by quantifying major processes in bioaccumulation at each stream (Presser and Luoma 2010). The EFs were calculated for each stream and particulate media type (biofilm, leaf detritus, and sediment) by dividing particulate-phase Se concentrations by water-column Se concentrations. The TTFs were calculated for each stream and particulate media type by dividing macro-invertebrate primary-consumer Se concentrations by particulate-phase Se concentrations. We derived a second-level TTF by dividing macroinvertebrate predator Se concentrations by primary-consumer Se concentrations. Bioaccumulation factors (BAFs) were calculated as concentration ratios of primary consumers to stream water and of predators to stream water. Cambaridae family members were excluded from most calculations of Se trophic transfer because of their large body size in comparison with other macroinvertebrates and the different sampling methods used to collect Cambaridae samples.

## Statistical analysis

R Studio (RStudio) and JMP®, Ver 11 and 13 (SAS Institute, 1989–2007), software were used to perform statistical analyses. To meet assumptions of normality and homoscedasticity, data were $log_{10}$-transformed before statistical analysis. Multiple Se concentrations in water-column and leaf-detritus samples were below detection limits, resulting in left-censored data that did not meet assumptions of normality after log transformation. Therefore, leaf-detritus data were transformed using nonparametric aligned-rank transformation (Wobbrock et al. 2011).

Using data from the initial 23-stream survey, we constructed 2 regression models with water-column measures (SC and Se, respectively) and taxon as fixed effects to predict macroinvertebrate whole-body Se concentrations. The 12-mo mean SC and the mean of dissolved Se concentrations in stream water collected in April 2014 and summer 2015 (Table 1) were used as numeric predictors. Water Se concentrations measured at less than the minimum reportable level ($2.5\,\mu g\,L^{-1}$) but at or above the minimum detectable level (MDL) were modeled using the instrument-measured values. Water Se concentrations measured at $\leq$MDL ($0.7\,\mu g\,L^{-1}$ for 4/14, $0.5\,\mu g\,L^{-1}$ for summer 2015) were set at $0.5\,\mu g\,L^{-1}$ for modeling purposes. Modeling was conducted using Ln-transformations of water-column SC, water-column Se, and macroinvertebrate Se values. Model residuals versus predicted plots were inspected for outliers and bias, and model residuals were checked for normality using the Shapiro-Wilk technique and for homoscedasticity using the Levene and Bartlett tests ($\alpha = 0.05$).

To determine effect of stream type (reference, low-SC, and high-SC), season (fall and spring), and their interaction on Se concentrations in ecosystem media and calculated EF and TTF ratios from the subset of 9 streams, we used a 2-way analysis of variance (ANOVA) on transformed data sets. Models generating insignificant interaction terms ($p \geq 0.05$) were rerun using only the main effects (stream type and season). When interaction terms were significant, individual post hoc models for each season were run for interpretive purposes. Where ANOVA revealed significant differences among stream types, we compared stream types using Tukey's honestly significant difference.

To evaluate how EFs, TTFs, and BAFs may have varied with water Se concentrations, we also determined if these relationships fit the following model form:

$$Ln\,(bioaccumulation\,factor) = f[Ln\,(water\,Se),\,sampling\,season\\(random)]$$

We also modeled macroinvertebrate-Se relationships to presumed dietary Se sources using similar model forms:

$$Ln\,(TTF_{1°\,consumer:\,particulate\,mean}) = f[Ln\,(particulate\,mean\,Se,\\sampling\,season\,(random)]$$

$$Ln\,(TTF_{predator:1°\,consumer}) = f[Ln\,(1°\,consumer\,Se,\\sampling\,season\,(random)]$$

Particulate mean Se is the mean value of Se concentrations for the 3 particulate matter forms. All statistical analyses were interpreted for significance at $\alpha = 0.05$.

# RESULTS

## Initial 23-stream survey

Annual mean SC was lower in all reference streams than in any mining-influenced stream (Table 1). The water-column Se values used for modeling were correlated positively with annual mean SC (Spearman $\rho = 0.80$, $p < 0.0001$).

Body-burden Se concentrations in macroinvertebrates exhibited a positive response to annual mean SC in stream water (Figure 3, left). The SC-prediction model (adjusted $R^2 = 0.66$, $p < 0.0001$) is

$$Se_m = exp[-2.277 + 0.618 \times Ln\,(SC) + C_{t\text{-}SC}]$$

where $Se_m$ is macroinvertebrate concentration of Se (micrograms per gram), exp is the inverse of the natural log, SC is water specific conductance (microsiemens per centimeter), and $C_{t\text{-}SC}$ is a taxon-specific constant for the SC-prediction model (–0.530 for Cambaridae, 0.152 for Cordulegastridae, and 0.378 for Gomphidae).

Macroinvertebrate body-burden Se concentrations also exhibited a positive response to water Se (Figure 3, right). The water-Se prediction model (adjusted $R^2 = 0.83$, $p < 0.0001$) is

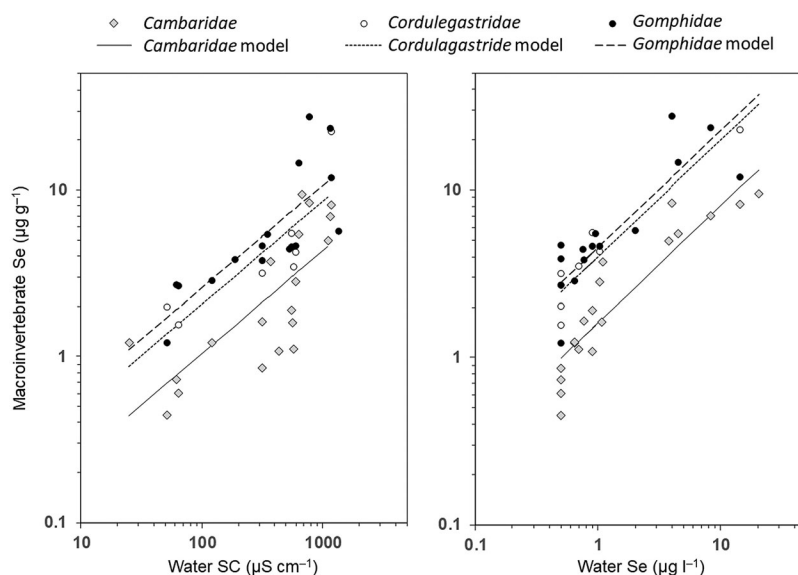$$Se_m = exp[1.129 + 0.698 \times Ln\,(Se) + C_{t\text{-}Se}]$$

**FIGURE 3:** Relationships of 1-yr mean specific conductance (SC) in stream water (left) and water-column selenium (Se) concentrations (right) to Se in tissue samples of Gomphidae, Cordulegastridae, and Cambaridae collected from 23 streams in central Appalachia. Models represented in the figure are detailed in the text. Because 1 or more targeted benthic macroinvertebrate taxa, Gomphidae, Cordulegastridae, or Cambaridae, were not found in most streams, each stream is represented by 1 to 3 taxa groups. Six of 23 sites had mean Se concentrations exceeding 3.1 μg L$^{-1}$, the monthly mean level recommended by the US Environmental Protection Agency (2016) as a freshwater ambient chronic water quality criterion for Se.

where Se is water concentration of dissolved Se (micrograms per liter) and $C_{t-Se}$ is a taxon-specific constant for the Se-prediction model (–0.652 for Cambaridae, 0.257 for Cordulegastridae, and 0.395 for Gomphidae).

For both models, the water variables and macroinvertebrate taxa were highly significant as predictors of macroinvertebrate body-burden Se ($p \leq 0.0001$), and residual analyses demonstrated that the models satisfied standard regression assumptions. Also, for both models the taxon-specific constants for Cordulegastridae and Gomphidae did not differ significantly from one another, but both differed significantly from the Cambaridae constant.

## Se-dynamics study

Dissolved Se concentrations in stream water exhibited a significant interaction effect between season and stream type ($F_{2,15} = 5.4$, $p = 0.045$; Figure 4A and B). Fall and spring Se concentrations in 2 of 3 reference streams were below the instrument detection limit. Mean water-column Se concentrations in high-SC streams were 15 times higher than mean concentrations in low-SC streams in the fall and 5 times higher in the spring. Mean water Se concentrations in high-SC streams were 20 times and 15 times higher than water concentrations, estimated by managing <MDL concentrations as stated in *Methods*, in reference streams in the fall and spring, respectively. Mean water Se concentrations in low-SC streams were also higher than in reference streams in spring, but our post hoc model did not show significant differences in stream water Se between low-SC and reference streams in the fall.

Selenium concentrations in sediment and biofilm did not vary by season (sediment: $F_{1,16} = 0.31$, $p = 0.59$; biofilm: $F_{1,15} = 0.76$,

$p = 0.40$) but did differ significantly among stream types (sediment: $F_{2,15} = 36.5$, $p < 0.001$; biofilm: $F_{2,14} = 28.9$, $p < 0.001$). All 3 stream types differed for these media with the exception of biofilm Se in reference streams in comparison to low-SC streams (Figure 4C and D). Mean Se concentrations in sediment were approximately 3 times higher in high-SC streams than in low-SC streams and were 3 times higher in low-SC streams than in reference streams. Mean Se concentrations in biofilm in high-SC streams were approximately 3.5 times higher than in low-SC streams and approximately 1.5 times higher in low-SC streams than in reference streams.

Leaf-detritus Se concentrations exhibited a significant interaction between season and stream type ($F_{2,15} = 10.4$, $p = 0.011$). Mean values generally followed patterns observed in stream water and other particulate media (Figure 4E and F). Leaf-detritus Se concentrations in reference streams differed from high-SC streams in both the spring and fall. However, whereas post hoc models for the fall showed that leaf-detritus Se concentrations in low-SC streams were 5.9 times higher than those in reference streams ($p = 0.004$), post hoc models for the spring indicated that high-SC streams were 16.3 times higher than low-SC streams ($p < 0.001$). No other post hoc pairwise comparisons were significant.

Mean tissue Se concentrations did not differ by season but did differ among stream types for all benthic macroinvertebrate consumer groups: primary consumers (season: $F_{1,16} = 1.6$, $p = 0.22$; stream type: $F_{2,15} = 84.0$, $p < 0.001$), predators (season: $F_{1,16} = 0.36$, $p = 0.56$; stream type: $F_{2,15} = 103.5$, $p < 0.001$), and Cambaridae (season: $F_{1,16} = 0.31$, $p = 0.59$; stream type: $F_{2,15} = 105.4$, $p < 0.001$; Figure 4G–I). Mean Se concentrations were approximately 4 times higher in primary consumers, predators, and Cambaridae in high-SC streams than in low-SC streams and approximately 4 times higher in primary
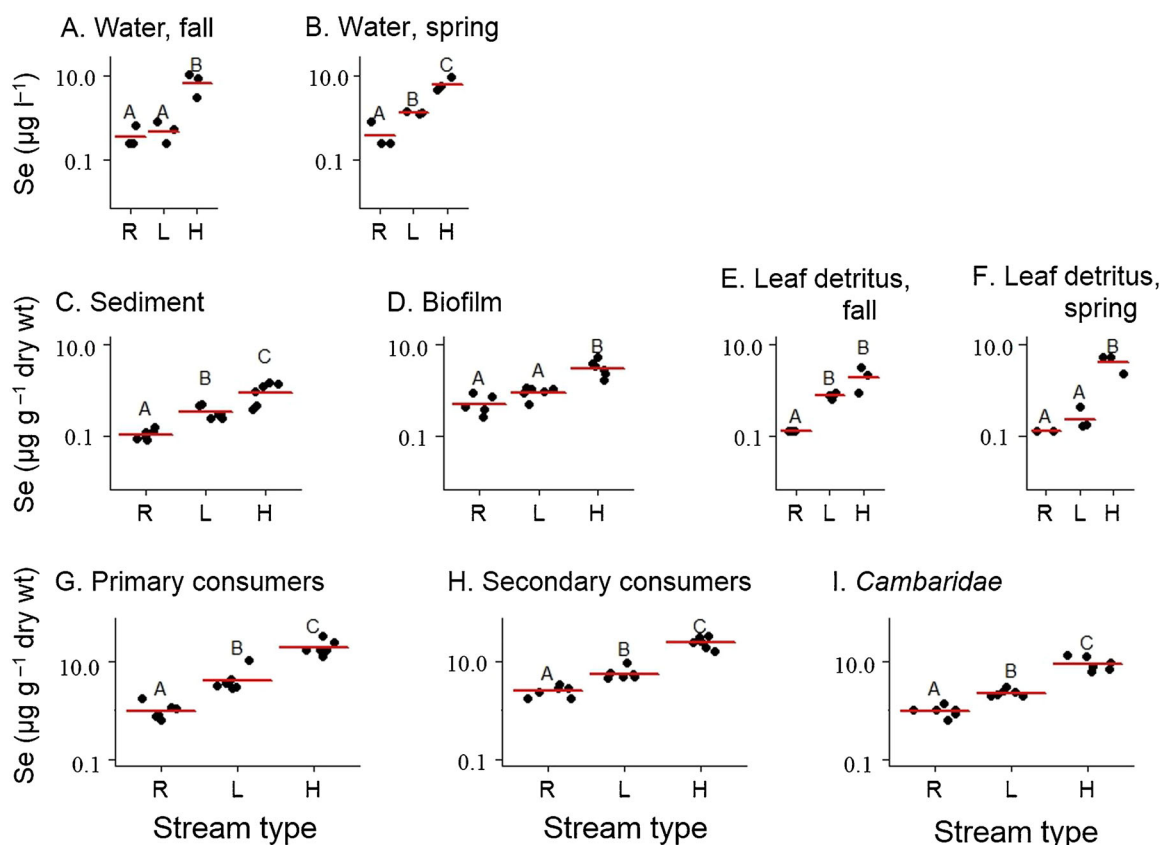
**FIGURE 4:** Selenium (Se) concentrations in (**A**) water column, fall; (**B**) water column, spring; (**C**) sediment; (**D**) biofilm; (**E**) leaf detritus, fall; (**F**) leaf detritus, spring; (**G**) primary consumers; (**H**) predators; and (**I**) Cambaridae in reference, low-SC, and high-SC headwater streams of the central Appalachian coalfield. Horizontal lines indicate mean values, and letters indicate significant differences among stream types for each medium. H = high-SC; L = low-SC; R = reference.

consumers and approximately 2 times higher in predators and Cambaridae in low-SC streams than in reference streams.

## Enrichment, trophic transfer, and bioaccumulation

Enrichment factors describing associations between water-column and sediment Se ($EF_{sediment}$) did not differ by season ($F_{1,16} = 1.5$, $p = 0.24$) or stream type ($F_{2,15} = 3.6$, $p = 0.055$; Figure 5A). Differences in associations between water-column and biofilm Se ($EF_{biofilm}$) values were not detected between seasons ($F_{1,15} = 3.6$, $p = 0.081$) but were detected among stream types ($F_{2,14} = 4.90$, $p = 0.026$), where reference stream values were 4 times higher than high-SC stream values (Figure 5B). A significant interaction effect between season and stream type was detected for associations between water-column and leaf-detritus ($EF_{leaf\ detritus}$) Se values ($F_{2,15} = 7.5$, $p = 0.008$). Mean $EF_{leaf\ detritus}$ values for low-SC streams were higher nominally than mean $EF_{leaf\ detritus}$ values for other stream types in fall and lower nominally than other stream types in the spring, but analyses did not reveal significant differences by stream type in either season (fall: $F_{2,15} = 4.06$, $p = 0.08$; spring: $F_{2,15} = 3.78$, $p = 0.09$; Figure 5C and D).

Trophic transfer factors did not differ significantly by season, but differences among stream types were detected for trophic transfer of Se from sediment to primary consumers ($TTF_{1°\ Consumer:sediment}$) (season: $F_{1,16} = 0.41$, $p = 0.53$; stream type: $F_{2,15} = 6.9$, $p = 0.008$) and from biofilm to primary consumers ($TTF_{1°\ Consumer:biofilm}$; season: $F_{1,15} = 0.063$, $p = 0.81$; stream type: $F_{2,14} = 6.0$, $p = 0.014$). Mean $TTF_{1°\ Consumer:sediment}$ values were >2.5 times higher in high-SC streams than reference streams (Figure 5E), and mean $TTF_{1°\ Consumer:biofilm}$ values were 3 times higher in high-SC streams than in reference streams ($p = 0.012$; Figure 5F). No differences among stream type were detected in trophic transfer of Se between leaf detritus and macroinvertebrate primary consumers ($TTF_{1°\ Consumer:leaf\ detritus}$; season: $F_{1,15} = 0.18$, $p = 0.69$; stream type: $F_{2,14} = 0.53$, $p = 0.60$; Figure 5G). Second-level TTFs from benthic macroinvertebrate primary consumers to predators ($TTF_{predator:1°\ Consumer}$) were significantly different among stream types (season: $F_{1,15} = 2.6$, $p = 0.13$; stream type: $F_{2,15} = 17.8$, $p < 0.001$). Mean values of $TTF_{predator:1°\ Consumer}$ were nearly 2 times higher in reference streams than in low-SC streams and 2 times higher in reference streams than in high-SC streams (Figure 5H). Ratio of macroinvertebrate primary-consumer Se concentrations to water-column Se concentrations ($BAF_{1°\ Consumer:water}$) did not differ between seasons or among stream types (season: $F_{1,15} = 2.28$, $p = 0.15$; stream type: $F_{2,15} = 1.09$, $p = 0.36$), nor did the ratio of macroinvertebrate-predator Se concentrations to water-column Se concentrations
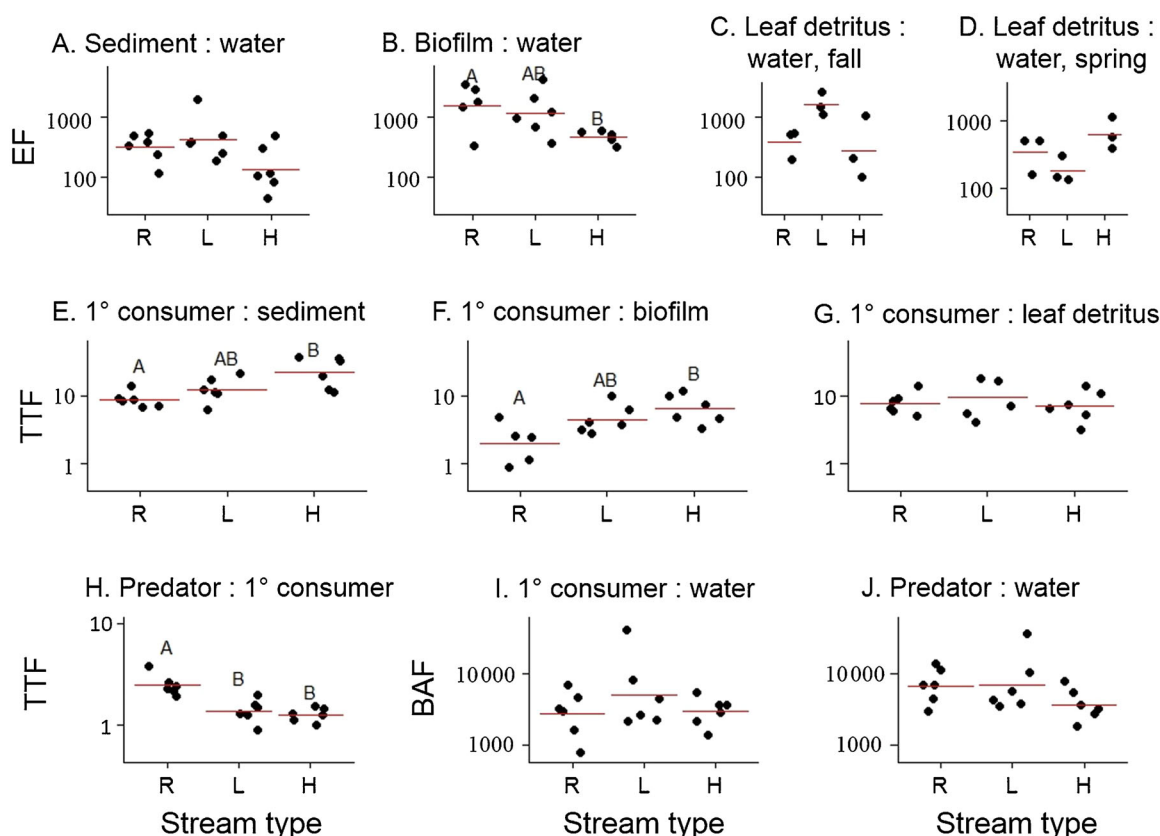
**FIGURE 5:** Selenium enrichment factors (EFs), trophic transfer factors (TTFs), and bioaccumulation factors (BAFs) in reference (R), low-SC (L) and high-SC (H) headwater streams in the central Appalachian coalfield: (A) $EF_{sediment}$; (B) $EF_{biofilm}$; (C) $EF_{leaf\ detritus}$, fall; (D) $EF_{leaf\ detritus}$, spring; (E) $TTF_{1°\ consumer:sediment}$; (F) $TTF_{1°\ consumer:biofilm}$; (G) $TTF_{1°\ consumer:leaf\ detritus}$; (H) $TTF_{predator:1°\ consumer}$; (I) $BAF_{1°\ consumer:water}$; and (J) $BAF_{predator:water}$. Horizontal lines indicate means by stream type, and letters indicate significant differences among stream types for each medium. H = high-SC; L = low-SC; R = reference.

differ between seasons or among stream types (season: $F_{1,15} = 1.74$, $p = 0.21$; stream type: $F_{2,15} = 1.78$, $p = 0.20$; $BAF_{predator:water}$; Figure 5I and J).

## Se-dynamics summary model

We found no difference in enrichment from the water column to the mean of all particulate matter by season or by stream type (season: $F_{1,15} = 1.02$, $p = 0.33$; stream type: $F_{2,15} = 1.30$, $p = 0.30$; Figure 6 and Table 2). In addition, there were no differences in trophic transfer ratios from particulate-matter mean Se concentrations to primary-consumer macroinvertebrates (season: $F_{1,15} = 1.15$, $p = 0.30$; stream type: $F_{2,15} = 3.54$, $p = 0.06$). The second-level TTF quantified in the present study, from primary consumers to predators, differed by stream type ($p < 0.001$), with reference streams having significantly higher TTFs than both low-SC and high-SC streams (Figure 5H). The EFs, TTFs, and BAFs for individual streams are in Supplemental Data, Table S3.

## Relationships of EFs, TTFs, and other BAFs to water and dietary Se sources

Two of the 3 EFs evaluated ($EF_{sediment}$ and $EF_{biofilm}$) exhibited strong negative relationships with water Se ($p < 0.001$;

Supplemental Data, Figure S1A and B). Two BAFs ($BAF_{predator:water}$ and $BAF_{Cambaridae:water}$, the ratio of Cambaridae Se concentrations to water Se concentrations) also exhibited strong
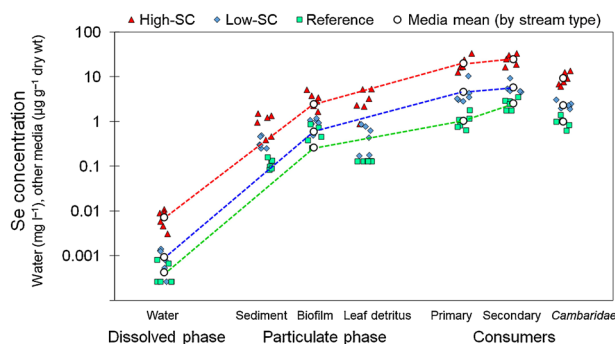


**FIGURE 6:** Selenium (Se) concentrations for each stream across 2 seasons in all media by stream type. Media are ordered to illustrate Se pathways of enrichment from water column to particulate matter, bioaccumulation pathways from particulate-phase media to benthic macroinvertebrate primary consumer taxa, and from benthic macroinvertebrate primary consumer taxa to predator taxa. Media mean concentrations for particulate-phase media are averaged by stream type. Dotted lines connect mean concentrations of media by stream type. Cambaridae Se concentration data are shown but not included in the illustration of Se pathways. SC = specific conductance.

**TABLE 2:** Derived factors describing selenium dynamics in mining-influenced and reference headwater streams in central Appalachia USA (mean values ± standard errors)

| Factors[a,b] | Reference | Low-SC[c] | High-SC[c] |
|---|---|---|---|
| $EF_{sediment}$ | 343 ± 63 | 591 ± 269 | 186 ± 70 |
| $EF_{biofilm}$ | 1952 ± 534 | 1542 ± 559 | 470 ± 41 |
| $EF_{leaf\ detritus}$ Fall | 399 ± 103 | 1675 ± 404 | 448 ± 300 |
| Spring | 387 ± 113 | 195 ± 58 | 707 ± 227 |
| $EF_{particulate\ mean}$ | 903 ± 204 | 1013 ± 386 | 411 ± 85 |
| $TTF_{1°\ consumer:particulate\ mean}$ | 4.4 ± 1.1 | 7.4 ± 1.4 | 8.1 ± 0.7 |
| $TTF_{predator:1°\ consumer}$ | 2.6 ± 0.3 | 1.4 ± 0.1 | 1.3 ± 0.1 |
| $BAF_{1°\ consumer:water}$ | 3323 ± 889 | 9963 ± 6141 | 3185 ± 566 |
| $BAF_{predator:water}$ | 7588 ± 1623 | 10 630 ± 5231 | 4149 ± 901 |

[a] Factors included are enrichment factors (EFs), summary EFs and trophic transfer factors (TTFs), and bioaccumulation factors (BAFs).
[b] Summary EF and TTF values calculated using an average of all particulate media collected at each stream. Significant differences for EFs, BAFs, and $TTF_{predator:1°\ consumer}$, when present, are designated in Figure 4; $TTF_{1°\ consumer:particulate\ mean}$ values do not differ significantly from one another.
[c] Mining-influenced streams designated as having low specific conductance (Low-SC) and high specific conductance (High-SC) based on 12-mo mean SC collected July 2014 to June 2015.

negative relationships with water Se ($p < 0.01$; Supplemental Data, Figure S1E and F). Two TTFs exhibited significant but weaker and contrasting relationships with water Se: $TTF_{1°\ Consumer:sediment}$ (positive, $p = 0.0140$) and $TTF_{predator:1°\ Consumer}$ (negative, $p = 0.0462$; Supplemental Data, Figure S1C and D). None of the other factors evaluated ($EF_{leaf\ detritus:water}$, $TTF_{1°\ Consumer:biofilm}$, $TTF_{1°\ Consumer:leaf\ detritus}$, and $TTF_{1°\ Consumer:water}$) exhibited statistically significant relationships with water Se, nor did first-level $TTF_{1°\ Consumer:particulate\ mean}$ exhibit a significant relationship with particulate mean Se. The second-level $TTF_{predator:1°\ Consumer}$ exhibited a strong negative relationship with primary-consumer Se concentrations ($p < 0.0001$; Supplemental Data, Figure S2).

## DISCUSSION

### SC of stream water and Se concentrations in stream ecosystem media

Headwater stream ecosystems in mined watersheds displayed increased water-column and macroinvertebrate Se concentrations relative to reference streams, likely caused by Se inputs from watershed coal mines, including both active mines and areas where mining had been completed. The present survey of 23 headwater streams showed strong positive associations among water-column SC, Se concentrations in water, and Se concentrations in macroinvertebrates. The more in-depth study of Se dynamics in a subset of these streams corroborated the covariance of SC in water with Se concentrations in macroinvertebrates as well as in other environmental media within the headwater streams studied.

Selenium concentrations in water, biofilm, and sediment in the reference streams of the present study were within or close to accepted background levels in freshwater environments (US Department of the Interior 1998), whereas Se concentrations in the particulate media of nonreference streams were within range or similar to Se concentrations collected in coal mining–influenced streams elsewhere in Appalachia (Presser and Luoma 2010; Presser 2013; Arnold et al. 2017), in Alberta (Casey 2005), and in the Canadian Rockies (Kuchapski and Rasmussen 2015).

Lower Se concentrations in particulate media, particularly in sediment, may be influenced by the relatively high-gradient morphology of the present study streams, resulting in less Se accumulation through sediment and detrital pathways (Orr et al. 2006). Because water conveyance in headwater streams is relatively fast, studies from other regions suggest that headwater stream ecosystems may not bioaccumulate Se to the same degree as slower-moving lentic and lower-gradient lotic systems (Lemly 1999); but previous studies in central Appalachia have not tested this expectation.

We were unable to find comparable studies measuring Se enrichment in leaf detritus. Microbial communities on leaf detritus have a large influence on nutrient-cycling dynamics within headwater streams and are important as food sources for benthic macroinvertebrate communities (Vannote et al. 1980). We expect that elevated Se concentrations of leaf detritus in mining-influenced streams resulted from microbial biofilm uptake of water-column Se, but we were unable to measure the microbial biofilm concentrations separately from the detrital leaf tissue. Significantly elevated Se concentrations in leaf detritus collected from mining-influenced streams suggest that uptake of Se by microbial biofilm on leaf detritus may be an important pathway of Se bioaccumulation.

Selenium concentrations in benthic macroinvertebrates collected from reference and low-SC streams were also similar to concentrations in benthic macroinvertebrates collected from reference and mining-influenced streams in other studies (Casey 2005; Presser 2013; Arnold et al. 2014; Kuchapski and Rasmussen 2015). In their analysis of Se contamination in aquatic environments in 29 field studies, Presser and Luoma (2010) reported Se concentrations in macroinvertebrates from a wide range of aquatic habitats. Among the field studies compiled, macroinvertebrates collected from lentic habitats were up to 5 times higher in Se concentration than Se concentrations found in the present study. However, field studies of stream habitats yielded macroinvertebrate Se concentrations notably lower than the mean Se concentration in macroinvertebrates of the present high-SC streams. Benthic macroinvertebrate Se concentrations in high-SC streams of the present study were 2 times higher on average than those of Arnold et al. (2014) and above maximum Se

concentrations in aquatic insects reported by Presser (2013); both of those studies were conducted in streams influenced by coal mining in West Virginia and included streams of higher order than the headwater streams of the present study.

Findings by Conley et al. (2009) and Arnold et al. (2014) suggest that Se concentrations in headwater stream particulate media and macroinvertebrates in the present study may be an environmental stressor to both invertebrates and their vertebrate consumers. Evaluation of the toxic effect of Se on consumers within the food webs was beyond the scope of the present study, but Se concentrations in other studies evaluating toxicity can be compared with the present results. Conley et al. (2009) reported reduced fecundity when laboratory mayflies (*Centroptilum triangulifer*, now *Neocloeon triangulifer*) were fed with a food source containing $\geq 4.2\,\mu g\,Se\,g^{-1}$ dry weight, a value within the range of particulate media concentrations observed in the present high-SC streams. Decreased survivorship and body mass were not observed unless food source Se concentrations were $\geq 11.9\,\mu g\,Se\,g^{-1}$ dry weight (Conley et al. 2009), approximately twice the highest particulate concentrations observed in the present study. Arnold et al. (2014) reported increased occurrence of Se-related fish deformities in a stream with benthic macroinvertebrate Se concentrations less than half the mean benthic macroinvertebrate Se concentration for the high-SC streams of the present study. Other studies of high-SC streams in Appalachia have found water Se concentrations higher than any water Se concentrations that we observed (Pond et al. 2008, 2014; Lindberg et al. 2011), suggesting potential for even greater body burdens in macroinvertebrates in those streams, as well as higher risks of adverse effects.

## Modeling relationships

Although the prediction models for macroinvertebrate Se concentrations in the present study are very strong (Figure 3), their potential for more general application is not clear, especially the SC-prediction model. Clearly, it is water-column Se that is driving the bioaccumulation of Se that we observed. Although both the major ions that produce SC and water Se originate as geochemical releases from the mining-disturbed rocks, we are unaware of scientific information to support a contention that SC and Se should be highly correlated in mining-influenced Appalachian waters universally. Clark (2017) demonstrated that, although all major ions except $HCO_3^-$ demonstrate general patterns of release similar to Se in mine-spoil leachates, Se concentrations tend to decline more rapidly, suggesting that stream water SC and Se concentrations, even if correlated among multiple streams immediately following a mining disturbance, would decouple with time. Ziemkiewicz et al. (2011) reported a similar finding from a study comparing $SO_4^{2-}$ and Se releases from coal waste in West Virginia.

Although the macroinvertebrate tissue concentrations of Se appear more strongly linked to stream water Se concentrations than to SC, the utility for broader application of the Se-prediction model is also in question. We had only 2 observations, separated by more than 1 yr, of Se water-column concentrations for each study stream. It is clear the SC varies

seasonally in mining-influenced streams of the Appalachian coalfield (Timpano et al. 2018b); hence, it is reasonable to expect that Se varies seasonally as well and that parameters of models linking water-column and macroinvertebrate Se concentrations will vary with time of water sampling and perhaps with other factors that we did not measure, such as water Se concentrations in weeks or months prior to environmental media sampling. Nonetheless, both modeled relationships for the present study streams were highly significant.

## Enrichment, trophic transfer, and bioconcentration of Se

In the present study, EFs and second-level TTFs for Se are consistent with those of previous studies (Supplemental Data, Tables S3 and S4). Reported EF values for Se in the present study are similar to values found in a higher-order mining-influenced Appalachian stream (1104; Presser and Luoma 2010) and in other recent literature (Casey 2005; Presser 2013; Kuchapski and Rasmussen 2015). In addition, mean second-level trophic transfer of Se from primary-consumer macroinvertebrates to predator macroinvertebrates in the present study were less variable than first-level TTFs and within the range of values reported for primary to secondary trophic transfer of Se in other systems in central Appalachia (Presser and Luoma 2010). In contrast, first-level TTFs from particulate media to primary consumers for Se in the present study, although within the range of other reported values, are higher and more variable than most other reported values.

Similarities between EFs, TTFs, and BAFs at reference and low-SC streams, excepting $TTF_{predator:1° \, Consumer}$ (Figure 5), indicate that Se dynamics in streams with less elevated major ion concentrations are similar to Se dynamics in unimpacted streams. Selenium dynamics differed more frequently between high-SC and reference stream types. A number of factors may be contributing to these EF and TTF differences among stream types. Among those factors are water Se concentrations. DeForest et al. (2007, 2016) reported that EFs for Se tend to decrease as exposure increases, an observation that is consistent with our findings for $EF_{sediment}$ and $EF_{biofilm}$ (Supplemental Data, Figure S1A and B) and with findings by Kuchapski and Rasmussen (2015) and Arnold et al. (2017). For our data, $EF_{leaf\,detritus}$ also exhibited a weak negative relationship with water Se, which, although not statistically significant ($p = 0.24$), did not contradict the pattern demonstrated by the other 2 particulate-matter forms. Such results may indicate that algae and microbes are regulating uptake of Se as needed to meet their metabolic needs and that, hence, lower levels of enrichment occur at higher concentrations of water Se (DeForest et al. 2007). The negative relationships of BAFs for predator macroinvertebrates and for Cambaridae ($BAF_{predators}$ and $BAF_{Cambaridae}$) with water Se (Supplemental Data, Figure S1E and F) are also consistent with the findings by the DeForest et al. (2007) meta-analysis of multiple Se bioaccumulation studies. In addition, heightened concentrations of sulfate, such as in the present high-SC streams (Timpano et al. 2018b), may inhibit Se uptake by algae and microbes (Williams et al. 1994; DeForest et al. 2017). The

negative relationships of bioaccumulation factors for predator macroinvertebrates and for Cambaridae (BAF$_{predators}$ and BAF$_{Cambaridae}$) with water Se (Supplemental Data, Figure S1 E and F) are also consistent with the findings by the DeForest et al. (2007) meta analysis of multiple Se bioaccumulation studies; as is the negative relationship of TTF$_{predator:1o\ consumers}$ to primary consumers' Se concentrations (Supplementary Data, Figure S2).

In contrast to particulate-matter EFs and 2 BAFs (BAF$_{predators}$ and BAF$_{Cambaridae}$), TTFs did not exhibit consistent relationships with water Se. Differences among TTFs may be attributed to variation in benthic macroinvertebrate community composition among the present study streams. Taxa groups differ in rates at which they ingest Se-enriched food and in efficiency at which they assimilate Se into their tissue (Luoma and Rainbow 2005). Because of these differences, shifts in community assemblages may scale up to produce differences in Se bioaccumulation ratios within a consumer community (Presser and Luoma 2010). Benthic macroinvertebrate community differences among the present streams may be driven by Se contamination, major ion concentration differences (Timpano et al. 2018a), and perhaps other inherent differences among streams that were not quantified. Moreover, the ratio of Se concentrations in macroinvertebrate primary consumers to dissolved water-column Se concentrations (BAF$_{1°\ Consumer:water}$) did not differ among stream types (Figure 5) and did not vary with water Se.

These results suggest that the Se dynamics of particulate-matter enrichment and predator bioaccumulation in Appalachian headwater streams are dependent on Se concentrations in the water column and, where water Se and water SC are highly correlated, may vary in association with water SC. The results also suggest that EFs and TTFs developed from the present study may be incorporated into a predictive tool connecting dissolved water-column Se to Se concentrations in macroinvertebrates in the central Appalachian coalfield.

## CONCLUSIONS

Sediment, biofilm, and leaf detritus comprise a significant portion of organic particulate matter in headwater streams and can become significantly enriched in Se when geologic disturbances caused by coal mining release Se in discharge waters, elevating water-column Se concentrations in streams receiving runoff or groundwater from the disturbed areas. Selenium-enriched organic particulate matter is bioavailable to benthic macroinvertebrate consumers, resulting in elevated Se concentrations in benthic macroinvertebrate tissue in these streams. Though high-gradient streams such as those in the present study are often considered to be at low risk for Se toxicity, the present results suggest that headwater stream ecosystems can accumulate Se at levels similar to those found in low-gradient streams.

Further, the high levels of macroinvertebrate Se we observed in high-SC streams support the hypothesis that elevated Se can be a costressor with SC to benthic macroinvertebrate populations. Additional study is needed to determine if 1) these high concentrations of Se are causing reduced viability in invertebrate and vertebrate populations in these ecosystems, 2) headwater stream Se enrichment processes are significantly affecting Se

dynamics downstream, and 3) Se bioaccumulation and toxicity in benthic macroinvertebrates contribute to reductions of richness that are commonly observed in high-SC coal mining–influenced streams in the central Appalachian coalfield.

Although particulate-matter Se-enrichment and macroinvertebrate predator Se-bioaccumulation processes varied inversely with water Se, the lower ratios of Se uptake occurring in association with elevated Se were not sufficient to offset the effects of mining influence because all sampled ecosystem media exhibited significantly elevated Se concentrations with higher levels of water SC, an indicator of mining influence. Our results indicate that the water-column concentration of dissolved Se is the primary driver of Se bioaccumulation in central Appalachian headwater streams. The largest disparity between the present results and findings in other systems was the relatively high first-level TTF values in our study, suggesting that trophic transfer to primary consumers in Appalachian headwater streams is of particular importance. The EF and TTF values developed in the present study can serve as a preliminary model for establishing linkages between water-column Se concentrations and consumer tissue concentrations in other Appalachian headwater streams.

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