

## Original Articles

# Using density, dissimilarity, and taxonomic replacement to characterize mining-influenced benthic macroinvertebrate community alterations in central Appalachia



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## ARTICLE INFO

## Keywords:

Specific conductance  
Salinity  
Total dissolved solids  
Quantitative sampling  
Coal mining  
Seasonality

## ABSTRACT

Elevated specific conductance (SC) in freshwater streams has been associated with alterations of benthic macroinvertebrate communities. Responses by dominant taxa to high salinity in streams can potentially complicate bioassessments. Taxonomic replacement is a method that can detect changes of community identities along an SC gradient as a supplement to conventional metrics that consider taxa richness or abundance. We assessed structural differences among benthic macroinvertebrate communities along an SC gradient in headwater streams influenced by coal mining in the central Appalachian coalfield. Benthic macroinvertebrate communities were sampled quantitatively in 15 southwestern Virginia streams up to six times over the course of a year. Sampled macroinvertebrates were identified to genus when possible and enumerated quantitatively, enabling determination of total-sample density and richness for various taxonomic and functional groups as assemblage structural response metrics. Multivariate methods were used to examine dissimilarities of taxonomic structure among samples along the SC gradient, and Spearman correlations were used to determine associations between SC and group response metrics for each sampling month. Dissimilarity analyses revealed distinct separations of assemblage structure between 1) spring and fall sampling months, and 2) low-SC and high-SC streams. Ephemeroptera richness and density declined with increasing SC, supporting findings by prior studies. Certain tolerant taxa were present at high densities seasonally, masking responses of other taxa within taxonomic and functional groups of which they were members. *Leuctra*, an SC-tolerant Plecoptera genus, increased in density with increasing SC, and comprised up to 85% of sample densities seasonally in high-SC streams. Richness displayed a strong negative response to SC regardless of season and was a robust indicator of salinity effects. Some functional feeding groups (including collector-gatherers and scrapers) showed seasonal declines in density and/or richness with increasing SC. Taxonomic replacement along an SC gradient was evident in several taxonomic and functional feeding groups during months when richness and density did not exhibit an SC response. Season and SC exerted strong influence over macroinvertebrate community structure in quantitative samples from mining-influenced headwater streams. Taxonomic and functional group composition metrics that do not consider taxonomic identity may not reflect clearly community structure alterations caused by responses of dominant taxa within those groups.

## 1. Introduction

Headwaters (1st- and 2nd-order streams) comprise 60–80% of total stream length in most basins; and the steep relief of mountain streams creates headwaters with high drainage densities and high spatial and temporal heterogeneity (Leopold et al., 1964; Benda et al., 2005). Headwater streams are also directly linked to riparian systems, with

higher edge-to-area ratios and lateral connectivity relative to lower reaches, which makes them rich sources of allochthonous organic matter and nutrients for lower reaches (Vannote et al., 1980; Richardson and Danehy 2007; Wipfli et al., 2007). Headwaters are prone to periodic extreme low flows that can isolate lengths of stream, limiting predator size and favoring specialists within the benthic macroinvertebrate community that are adapted to low-flow conditions

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<https://doi.org/10.1016/j.ecolind.2019.105535>

Received 15 September 2018; Received in revised form 26 June 2019; Accepted 28 June 2019

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(Richardson and Danehy, 2007; Larned et al., 2010; Leigh and Datry, 2017). Headwaters provide services such as colonist, energy, and nutrient exports (Meyer et al., 2007), and because of their unique and highly heterogeneous environment, serve as habitat for many species that do not occur elsewhere in river systems. Benthic macroinvertebrates are among the most diverse and ubiquitous life forms in lotic freshwaters, including headwaters (Strayer, 2006; Clarke et al., 2008), and they perform functions that are essential to ecosystem processes, both locally and downstream. Macroinvertebrates regulate in-stream primary production, process organic carbon, and aid nutrient cycling; and they are primary food for higher trophic-level organisms including many fish and other aquatic vertebrates (Wallace and Webster, 1996). Benthic macroinvertebrate communities are often monitored as indicators of lotic freshwater ecosystem health and condition (Plafkin et al., 1989; Barbour et al., 1999; Buss et al., 2015).

Elevated specific conductance (SC) has been associated with altered biotic condition in headwater streams draining coal surface mines in the central Appalachian region of the U.S.; multiple studies have found macroinvertebrate communities to be altered in streams with elevated SC from mining, relative to communities in streams draining relatively undisturbed watersheds with SC levels characteristic of natural background (Green et al., 2000; Chambers and Messinger, 2001; Howard et al., 2001; Kennedy et al., 2003; Pond, 2004; Hartman et al., 2005; Pond et al., 2008; Pond, 2012; Timpano et al., 2015; Boehme et al., 2016; Timpano et al., 2018). Certain benthic macroinvertebrate taxa (e.g. Ephemeroptera) are often depressed or absent in Appalachian mining-influenced streams with elevated SC (Hartman et al., 2005; Pond, 2010; Pond et al., 2014; Timpano et al., 2018). Macroinvertebrate communities are often monitored and assessed as indicators of aquatic ecosystem condition (Plafkin et al., 1989; Barbour et al., 1999).

Fracturing and removal of rock overlying coal seams causes expansion of volume. Valley fills are created when excess mine spoils are placed in adjacent valleys, burying headwater streams (Merricks et al., 2007). Valley fills, and other mined-landscape features are composed of mine rock fragments that, when exposed to water and atmospheric oxygen, undergo accelerated weathering reactions that generate water-soluble ions (Griffith et al., 2012). As water percolates through fractured mine rocks to emerge as headwater streams, concentrations of dissolved mineral constituents become elevated (Merricks et al., 2007; Pond et al., 2008). In mining-influenced Appalachian streams, elevated levels of total dissolved solids (TDS), individual major ions (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{SO}_4^{2-}$ ,  $\text{K}^+$ ,  $\text{HCO}_3^-$ ,  $\text{Na}^+$ ), and SC in the water column relative to natural background conditions are often observed (Pond et al., 2014; Timpano et al., 2015).

An alternative or supplemental measure of community differences caused by stressor effects – taxonomic turnover or replacement – is applied commonly in ecological studies (e.g. Qian and Ricklefs, 2007; Verleyen et al., 2009; Pellissier et al., 2013), but has rarely been applied to characterize effects of elevated SC on benthic macroinvertebrate communities in the Appalachian coalfield. Taxonomic replacement is a change in the taxonomic composition of a community that occurs along an environmental gradient; such changes may not be characterized by analyses of richness and abundance measures. For example, it is possible that two streams affected differentially by a stressor could have equal numbers of taxa (equal richness) but differing taxonomic identities and therefore differing community structures. Correlations between community dissimilarity and SC distance matrices can be calculated to quantify correspondence of community dissimilarities with differences of SC (Anderson et al., 2011). For example, a high positive correlation indicates that as the magnitude of stream-to-stream SC differences increases, the taxonomic composition of the communities being compared becomes increasingly dissimilar. Taxonomic replacement is a tool for detecting environmental gradient-driven taxonomic composition changes that could potentially be unaccounted for by other analyses of common community structural metrics, such as richness and

relative abundance measures.

Functional feeding groups (FFGs) are classifications of taxa based on morpho-behavioral adaptations for acquiring food (Cummins and Klug, 1979; Vannote et al., 1980; Wallace and Webster, 1996). This classification describes the method by which food is ingested. Typically, five major FFGs are described in headwater streams: 1) collector-filterers (a.k.a. filterers) filter fine particulate organic matter (< 1mm) from the water column; 2) collector-gatherers (a.k.a. gatherers) collect fine material from the substrate; 3) predators (often sub-divided into engulfers and piercers) consume prey organisms; 4) shredders break down coarse particulate organic matter (> 1mm) such as leaves and woody debris; and 5) scrapers (a.k.a. grazers) scrape biofilm from substrate (Cummins and Klug, 1979; Wallace and Webster, 1996). These FFGs are useful indicators of ecosystem function, but their study is often limited to measures of relative abundance (e.g. Cummins et al., 2005).

Prior studies on mining-induced SC effects in Appalachian streams have often been conducted only at single points in time, usually the spring season (e.g. Pond et al., 2008, 2014; Petty et al., 2010; Bernhardt et al., 2012; Pond, 2012). Some multi-metric indices were developed for application during both spring and fall (e.g. Burton and Gerritsen, 2003). However, both benthic macroinvertebrate communities and water-column SC are variable in time. Boehme et al. (2016) demonstrated in the coal-mining area of southwestern Virginia that both the measured values of several common bioassessment metrics, and their dependence on SC, vary with time-of-year. In the Appalachian coalfield, dependence of SC-induced community change on time-of-year has received limited study.

The objective of this study was to assess structural differences among benthic macroinvertebrate communities along an SC gradient in headwater streams influenced by coal mining in central Appalachia. We conducted quantitative sampling of macroinvertebrate taxa six times over the course of one year and analyzed community structural measures of density, richness, FFG composition, and taxonomic identity dissimilarities among streams to address the following questions:

- 1) Do abundance, richness, and taxonomic composition vary in response to SC?
- 2) Does community structural response to SC vary temporally over the course of one year?
- 3) Does taxonomic replacement provide information that conventional metrics do not detect in Appalachian coalfield streams?

## 2. Materials and methods

### 2.1. Stream selection

Study streams (i.e. sample units) were a subset of 1st- and 2nd-order streams selected with the intent of minimizing influence of non-TDS stressors (Timpano et al., 2015; Fig. 1). Included were four reference streams and eleven mining-influenced streams along an SC gradient ranging from 24 to 1437  $\mu\text{S}/\text{cm}$  (mean values, January 2013 – May 2014). Streams are in Wise, Dickenson, and Buchanan counties in Virginia, and McDowell, Raleigh, and Kanawha counties in West Virginia (Fig. 1).

A 100-m reach of each stream within a forested area was selected for study. All streams had canopy cover of > 90% during summer months as measured by a densitometer. Watersheds were devoid of residences, major roads, commercial or industrial facilities other than surface coal mines (mining-influenced streams only) and, for a few watersheds, small numbers of natural gas wells with unpaved access roads. Catchments for each stream were delineated using Spatial Analyst tools in ArcMap v.10 (ESRI Inc., Redlands, CA, USA) software with digital elevation rasters from USGS (2018). Study streams were selected such that reference streams and mining-influenced streams had similar ranges of catchment areas (0.82–5.91  $\text{km}^2$  and 1.31–7.21  $\text{km}^2$ , respectively), and mean values of catchment areas (0.82 and 1.31  $\text{km}^2$ ,

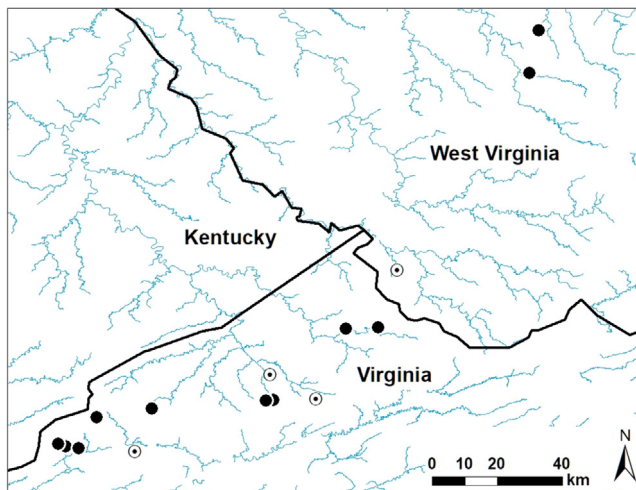


Fig. 1. Study stream locations in relationship to regional larger-order streams. Closed black circles represent mining-influenced streams ( $n = 11$ ), and open circles represent reference streams ( $n = 4$ ). All study streams and their upstream watersheds are within Level IV Ecoregion 69d (US EPA, 2018), which corresponds with a large segment of the Appalachian coalfield.

respectively) that did not differ statistically ( $\alpha = 0.05$ ).

## 2.2. Data collection

Each of the study streams was equipped with a HOBO Freshwater Conductivity Data Logger, model U24-001 (Onset Computer Corp., Bourne, MA, U.S.A.) that recorded electrical conductivity and temperature at 15-minute intervals. During each benthic sampling visit, a multimeter probe (YSI Professional Plus; Yellow Springs, OH, U.S.A.) was placed near the logger to measure SC and stream temperature for use in calibrating the logger data. Logger-recorded data were converted to SC using HOBOWare Pro v.3.4.1 software (Onset Computer Corp., Bourne, Massachusetts, USA). The multimeter probe was calibrated before each use as per APHA (2005). The arithmetic mean of SC over the period prior to a given benthic macroinvertebrate sample date (approximately two months, and generally the period since the prior benthic macroinvertebrate sample) was calculated and is referred to as SC in the text that follows.

Benthic macroinvertebrates were sampled in study streams six times over twelve months (May 2013–April 2014) using a Hess sampler (Wildco; Yulee, FL, U.S.A.) with a 363  $\mu\text{m}$  Nitex mesh. Because of site-specific limitations (e.g. water level too low to sample), some streams were not sampled during some sampling periods. For each of the six sampling events, 10–13 of the 15 study streams were sampled (Table 1). In each stream at each sampling date, three samples were collected from run sections below randomly selected riffles. For collection of each sample, a Hess sampler was driven into the streambed sediment, completely enclosing 0.086  $\text{m}^2$  of stream bottom as needed to prevent organisms from drifting in or out of the sampler.

Complete samples were sorted quantitatively and identified to the lowest practical taxonomic level (mostly to genus unless specimens were either too immature or damaged to key to genus) using a dissecting microscope and Merritt et al. (2008). As exceptions, Chironomidae and Ceratopogonidae were identified at family level and Oligochaeta were identified at class level.

## 2.3. Data analyses

All statistical analyses were completed using R (R Core Team 2016). Primary response variables were genus-level richness (number of taxa per sample area – 0.258  $\text{m}^2$ ) and density (number of individuals per square meter) of total taxa, FFGs (filterers, gatherers, predators,

scrapers, and shredders), and groups representing the taxa orders Ephemeroptera (E), Plecoptera (P), and Trichoptera (T) (EPT when considered collectively). The families Baetidae (B), Hydropsychidae (H), and Leuctridae (L) in the orders Ephemeroptera, Trichoptera, and Plecoptera, respectively, have also been found to be SC-tolerant in previous studies (Pond, 2010, 2012; Bernhardt et al., 2012; Boehme et al., 2016). Hence, for certain analyses, the metrics for which B, H, and L comprised a relatively high proportion of density were analyzed both including and excluding the tolerant taxa, which we also refer to as dominant; densities for these taxa were also included as response metrics. Functional feeding groups were selected because they represent taxa groups that perform ecosystem functions such as cycling of nutrients and organic matter (Cummins and Klug, 1979; Vannote et al., 1980; Wallace and Webster, 1996). Ephemeroptera, Plecoptera, and Trichoptera were selected because they are among the most disturbance-sensitive orders and are often used to assess stream condition (e.g. Plafkin et al., 1989; Wallace et al., 1996; Barbour et al., 1999; Burton and Gerritsen, 2003).

Richness was determined by summing number of taxa identified in the three samples for each stream and sampling date. Richness was expressed on a per-sampling-area ( $3 \times 0.086 \text{ m}^2 = 0.258 \text{ m}^2$ ) basis. Richness values did not include individuals from higher-order classifications caused by immature or damaged specimens. Density per square meter was calculated for each stream and sampling date as mean abundance of the three samples divided by area of the Hess sampler (0.086  $\text{m}^2$ ). Simpson's Diversity (Simpson's D) was expressed as effective number of species (Hill, 1973; Jost, 2006) calculated as per Jost (2006).

An SC gradient was used in correlative analyses, but for Permutational multivariate analysis of variance (PerMANOVA) testing the streams were divided into high and low categories of SC levels in addition to a gradient. High-SC and Low-SC levels were classified by the median SC level (533  $\mu\text{S cm}^{-1}$ ) for all streams in all sample months (i.e. high-SC streams had mean SC values for the full study period  $> 533 \mu\text{S cm}^{-1}$ , and low-SC streams  $< 533 \mu\text{S cm}^{-1}$ ). This threshold is resonant with other studies that use 500  $\mu\text{S cm}^{-1}$  as a criterion for reference condition (Burton and Gerritsen, 2003) or “low” level of mining disturbance (Pond et al., 2008). Additionally, low-SC streams had a range of 0–4% mining land use, whereas high-SC streams had a range of 23–65% mining land use. Percent mined area for each catchment is provided by Timpano et al. (2017).

Dissimilarity matrices were created using the Jaccard method (McCune et al., 2002) with *vegdist* in the R package *vegan* (Oksanen et al., 2016). Jaccard dissimilarity method was chosen to detect changes of taxonomic identity along an SC gradient. Jaccard dissimilarity ( $D_j$ ) was calculated for incidence (i.e. presence/absence) data:

$$D_j = (b + c)/(a + b + c)$$

where  $a$  = number of shared taxa (i.e. taxa found in both samples),  $b$  = number of taxa found in sample 1 but not sample 2,  $c$  = number of taxa found in sample 2 but not sample 1 (Baselga, 2012). Genus-level taxa where determined, and Chironomidae, Ceratopogonidae, and Oligochaeta taxa, were used to construct Jaccard dissimilarity matrices.

Dissimilarity matrices were tested for dissimilarity saturation (Tuomisto et al., 2012) by calculating percentage of maximum dissimilarities (ones) in the upper triangle of each matrix. Dissimilarity saturation occurs when a pair of sites has no taxa in common and  $D_j = 1$ . Many site pairs at dissimilarity saturation within a matrix (e.g. many ones in a Jaccard dissimilarity matrix) can inhibit detection of correlation with predictor variables.

Non-metric multidimensional scaling (NMDS) was used to visually examine dissimilarities in benthic community structure among streams across sampling months. Number of dimensions was chosen based on inspection of a scree plot (stress vs. number of dimensions; McCune et al., 2002). Ordinations were produced from the Jaccard dissimilarity matrices with the *metaMDS* function in *vegan* (Oksanen et al., 2016).

**Table 1**  
Stream-type monthly and overall means and standard errors, and overall minima and maxima for specific conductance (SC) and selected macroinvertebrate metrics.<sup>1</sup>

	13-May	13-Jul	13-Sep	13-Nov	14-Jan	14-Apr	Overall	Min	Max
SC ( $\mu\text{S cm}^{-1}$ )									
Low-SC	226*	244*	331*	345*	166*	184*	247*	21	542
± SE	63	65	71	86	48	66	28		
High-SC	807	1057	1043	1034	799	926	941	562	1856
± SE	103	169	178	188	93	120	58		
Density									
Low-SC	2607	1683	1019	2906	3372	5034	2789	539	13,849
± SE	580	284	237	614	333	1793	381		
High-SC	4486	2030	1032	1951	2653	4492	2793	477	7891
± SE	1203	338	251	577	642	833	343		
Richness									
Low-SC	30	28*	26*	38*	48*	39*	35*	17	52
± SE	3	3	2	3	2	3	2		
High-SC	24	17	17	22	29	24	22	11	38
± SE	3	2	1	2	3	2	1		
Simpson's D									
Low-SC	7*	5	9*	10	11*	7*	8*	1	19
± SE	2	2	1	2	1	1	1		
High-SC	2	2	4	5	6	3	4	1	9
± SE	0	0	1	1	1	1	0		
n									
Low-SC	5	7	5	6	6	6	35		
High-SC	5	5	5	6	7	7	35		

<sup>1</sup> SC = specific conductance; threshold for separating low-SC and high-SC streams was  $533 \mu\text{S cm}^{-1}$ ; SE = standard error. Density is represented as individuals per  $\text{m}^2$ . Richness is number of taxa. n is number of observations (i.e. streams). Low-SC mean values with asterisks are significantly different ( $p < 0.05$ ) from their high-SC counterparts.

Starting configuration was random as default in *metaMDS*, and a solution was reached within 100 iterations. Stress was calculated using Kruskal's monotone regression (Kruskal, 1964). Final configuration of the ordination was rotated so that Axis 1 was the principal axis (i.e. explained the most variation).

Sampling months (i.e. season) and SC values were superimposed on the ordinations to visualize response patterns of community structure to season and SC. A PerMANOVA analysis was executed by the *adonis* function in R package *vegan* (Oksanen et al., 2016) to test the null hypothesis of no difference in assemblage structure with season, gradient of SC, and interaction of season and SC (Anderson, 2001). Samples were analyzed with PerMANOVA to test patterns of High-SC and Low-SC groupings as indicated on the NMDS plots (Fig. 2).

Spearman rank correlations were used to analyze associations of richness and density metrics with SC for each sampling month. All identified taxa were included in the Spearman correlation analyses.

Taxonomic replacement was characterized as correlations among group dissimilarities with SC differences. Three distance matrices, Jaccard dissimilarity matrix ( $D_Y$ ), Euclidean distance matrix for SC differences ( $D_X$ ), and Euclidean distance matrix for geographic coordinates ( $D_Z$ ), were used to determine community taxonomic replacement response to SC (Anderson et al., 2011). The Mantel test was applied to evaluate the null hypothesis: distances in matrix  $D_Y$  are not related to corresponding distances in matrix  $D_X$  (Legendre and Legendre, 2012, p 600). A partial Mantel test uses a third matrix to control for another variable, in this case geographic distance between streams. This is necessary so that community structural differences between streams are not attributed to spatial dependency (i.e. Tobler's first law of geography, Tobler, 1970). The partial Mantel test evaluates the following null hypothesis: the partial correlation between  $D_Y$  and  $D_X$ , while controlling for  $D_Z$ , is zero (Legendre, 2000). The partial Mantel test was conducted using the *mantel.partial* function in the *vegan*

package in R. All plots of relationships between matrices were inspected for linearity. Significance was assessed using a permutation method with the rows and corresponding columns in the dissimilarity matrix randomly permuted 1000 times (Legendre, 2000; Oksanen et al., 2016). This procedure was then repeated for each sub-group (i.e. groups within FFG and EPT) to determine which group dissimilarities were associated with differences of SC. The results were tabulated for each sample month.

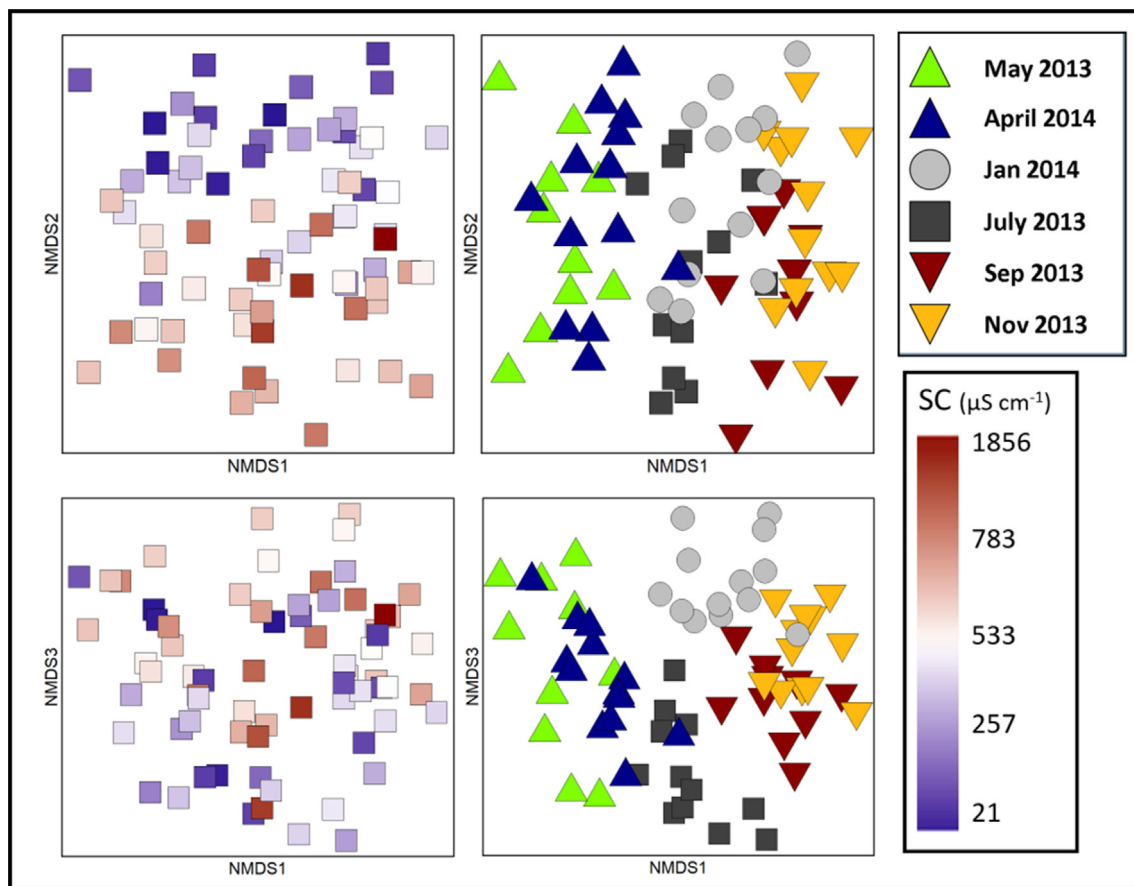
### 3. Results and discussion

#### 3.1. Stream SC and macroinvertebrate response metric means

Mean SC for each sampling month and overall across the year were higher in high-SC streams than in low-SC streams (Table 1). Mean macroinvertebrate density did not differ between high-SC and low-SC streams in any month or overall. Mean macroinvertebrate richness was higher in low-SC streams than in high-SC streams overall and in all months except May. Simpson's D for macroinvertebrate communities was higher in low-SC streams than in high-SC streams overall and in four out of six sampling months.

#### 3.2. Macroinvertebrate community structural effects by SC and season

The NMDS plots demonstrate that macroinvertebrate community structural differences occurred in response to SC and season (Fig. 2). The final stress for the NMDS was 0.16, represented in three dimensions, which indicates an acceptable fit (McCune et al., 2002). Samples were separated and grouped into distinct sampling months and seasons. May 2013 and April 2014 sampling dates overlapped despite being the most distant in time between sampling months, likely because they are seasonally similar. Likewise, September 2013 and November 2013 are



**Fig. 2.** Non-metric multidimensional scaling (NMDS) of benthic macroinvertebrate communities in Appalachian headwater streams based on Jaccard dissimilarity indices. Left panels are overlaid with specific conductance values, with breakpoints in legend specified as quartiles (median SC =  $533 \mu\text{S cm}^{-1}$ ). Right panels are overlaid with sampling months. Stress = 0.16.

seasonally similar and overlapped, but were separated from seasonally dissimilar May 2013 and April 2014 samples. Samples also separated and grouped by SC levels, based on the plot of axes 1 and 2.

PerMANOVA tests (Table 2) rejected the null hypothesis of no difference in macroinvertebrate assemblage structure for sampling-month and SC grouped levels (i.e. High-SC and Low-SC), confirming the patterns in the NMDS (Fig. 2). Assemblage structure was also related significantly to SC gradient. Adjusted  $R^2$  was higher for sampling month ( $R^2 = 0.25$ ,  $P = 0.0002$ ) than for SC gradient ( $R^2 = 0.07$ ,  $P = 0.0002$ ), indicating that seasonality was more important for structuring benthic macroinvertebrate community samples than was SC. The interaction effect between sampling month and SC gradient was significant

**Table 2**

PerMANOVA results for benthic macroinvertebrate community structure response to A) specific conductance (SC) gradient, sampling month, and interaction of SC gradient and sampling month, and B) grouping of samples into high-SC and low-SC levels.

A.	df	SS	MS	F	R2	P(> F)
SC gradient	1	1.1597	1.1597	6.1843	0.07	0.0002
Sampling month	5	4.4093	0.8819	4.7028	0.25	0.0002
SC $\times$ Sampling month	5	1.2104	0.2421	1.2910	0.07	0.0358
Residuals	58	10.8762	0.1875		0.62	
Total	69	17.6556			1.00	
B.	df	SS	MS	F	R2	P(> F)
SC level	1	1.5170	1.5170	6.3919	0.09	0.0002
Residuals	68	16.1386	0.2373		0.91	
Total	69	17.6556			1.00	

( $R^2 = 0.07$ ,  $P = 0.036$ ), indicating that the relationships between SC gradient and taxonomic dissimilarities varied temporally. Another interpretation of the interaction was that the variation of taxonomic dissimilarities across sampling months depended on SC values in streams, implying that SC affects taxonomic identities of benthic macroinvertebrates and/or affects the life cycles of taxa in the streams. Working in some of these same streams, Boehme et al. (2016) found interactions among effects by sampling months and SC for multiple macroinvertebrate community metrics.

Separation of samples by SC level indicated that low-SC streams were more similar to one another than they were to high-SC streams based on taxonomic composition, suggesting that characteristic taxa differed for low-SC streams and high-SC streams. For example, *Ephemera*, *Stenonema*, *Pteronarcys*, and *Paraleptophlebia* were found exclusively in low-SC streams, and *Boyeria* were found exclusively in high-SC streams. *Psephenus*, *Acronuria*, and *Maccaffertium* were found predominantly in low-SC streams, and *Tipula* and *Prosimulium* were found predominantly in high-SC streams. Similarly, Cormier et al. (2013) report a characteristic set of macroinvertebrate genera extirpated at low SC and another characteristic set that occurred at elevated SC.

Pond et al. (2013) also found clustering of taxa within spring and summer assemblages using NMDS for Appalachian coalfield streams, and Linke et al. (1999) reported seasonal benthic macroinvertebrate community structure differences between summer and winter samples in Ontario, Canada using a clustering method. Clustering of sampled taxa by season is likely a result of varying seasonal life cycles of benthic insects (Hynes, 1970). For example, most mayflies emerge as adults in the spring or early summer (Brittain, 1982; Merritt et al., 2008), whereas stoneflies have longer life cycles, sometimes taking multiple

**Table 3**

Spearman correlation coefficients between macroinvertebrate group density and specific conductance for each sampling month-taxonomic/functional group combination.

Group <sup>1</sup>	May 13	Jul 13	Sep 13	Nov 13	Jan 14	Apr 14
Total	0.50	0.12	0.40	-0.13	0.08	0.01
Total-BHL	0.07	<b>-0.69*</b>	-0.43	-0.48	-0.52	-0.48
EPT	0.49	0.49	0.59	0.03	0.10	-0.08
EPT-BHL	-0.47	<b>-0.87***</b>	<b>-0.94***</b>	-0.30	<b>-0.75**</b>	<b>-0.56*</b>
E	-0.36	<b>-0.83**</b>	<b>-0.86**</b>	<b>-0.73**</b>	<b>-0.84***</b>	<b>-0.81**</b>
E-B	<b>-0.77**</b>	<b>-0.87***</b>	<b>-0.89***</b>	<b>-0.69*</b>	<b>-0.91***</b>	<b>-0.87***</b>
P	0.54	<b>0.66*</b>	<b>0.70*</b>	0.22	0.52	0.30
P-L	0.07	<b>-0.80**</b>	<b>-0.78**</b>	0.13	0.07	0.37
T	0.18	-0.06	0.44	0.38	0.31	0.26
T-H	-0.13	-0.55	-0.43	<b>-0.66*</b>	-0.32	-0.30
Filterer	0.59	0.12	<b>0.64*</b>	<b>0.62*</b>	0.24	0.28
Filterer-H	0.43	0.16	-0.01	0.02	-0.35	-0.26
Gatherer	-0.07	<b>-0.66*</b>	0.03	-0.40	-0.47	<b>-0.63*</b>
Predator	-0.47	<b>-0.79**</b>	-0.49	-0.48	-0.29	-0.02
Scraper	-0.37	<b>-0.81**</b>	-0.33	-0.41	<b>-0.70**</b>	-0.45
Shredder	0.58	<b>0.70*</b>	<b>0.68*</b>	0.37	0.54	0.26
Shredder-L	0.08	<b>-0.85*</b>	<b>-0.94***</b>	0.16	0.26	0.37
Baetidae	-0.16	-0.47	-0.33	-0.30	-0.13	-0.17
Hydropsychidae	0.36	0.12	<b>0.64*</b>	<b>0.62*</b>	0.38	0.45
Leuctridae	0.58	<b>0.70*</b>	<b>0.82**</b>	<b>0.77**</b>	0.55	0.30

\*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .<sup>1</sup> E = Ephemeroptera, P = Plecoptera, T = Trichoptera, B = Baetidae, H = Hydropsychidae, L = Leuctridae.

years to reach adult stage (Hynes, 1970; Brittain, 1990). Life cycles also differ among genera within the same families and can be affected by temperature and other habitat variables (e.g. Hynes, 1976; Brittain, 1982).

### 3.3. Responses of macroinvertebrates to SC: Density and richness

#### 3.3.1. Strong responses

Among all taxonomic groups of macroinvertebrates, Ephemeroptera had the strongest responses to SC, a finding that is consistent with previous studies of stream systems in the central Appalachian region (Green et al., 2000; Hartman et al., 2005; Pond et al., 2008; Pond, 2010; Bernhardt et al., 2012; Boehme et al., 2016) and supported in other parts of the world (Gallardo-Mayenco, 1994; Piscart et al., 2005; Hassell et al., 2006). Ephemeroptera richness and density were negatively correlated with SC in most sampling timeframes (Tables 3 and 4). Variability of Ephemeroptera densities in low-SC samples indicates effects by non-SC factors; but densities were relatively low in high-SC samples (68% of high-SC samples had Ephemeroptera densities < 25 m<sup>-2</sup>), which suggests SC as a limiting factor (Fig. 3).

**Table 4**

Spearman correlation coefficients between macroinvertebrate group richness and specific conductance for each sampling month-taxonomic/functional group combination.

Group <sup>1</sup>	May 13	Jul 13	Sep 13	Nov 13	Jan 14	Apr 14
Total	-0.31	<b>-0.62*</b>	<b>-0.62*</b>	<b>-0.66*</b>	<b>-0.67*</b>	<b>-0.71**</b>
Total-BHL	-0.38	<b>-0.65*</b>	<b>-0.63*</b>	<b>-0.73**</b>	<b>-0.68*</b>	<b>-0.64*</b>
EPT	-0.60	<b>-0.82**</b>	<b>-0.77**</b>	<b>-0.76**</b>	<b>-0.71**</b>	<b>-0.73**</b>
EPT-BHL	<b>-0.80**</b>	<b>-0.86***</b>	<b>-0.80**</b>	<b>-0.80**</b>	<b>-0.74**</b>	<b>-0.70**</b>
E	<b>-0.80**</b>	<b>-0.87***</b>	<b>-0.87**</b>	<b>-0.89**</b>	<b>-0.85***</b>	<b>-0.83***</b>
E-B	<b>-0.78*</b>	-0.52	<b>-0.77*</b>	<b>-0.93***</b>	<b>-0.89***</b>	<b>-0.73*</b>
P	-0.49	<b>-0.60*</b>	-0.62	<b>-0.62*</b>	<b>-0.56*</b>	<b>-0.69**</b>
T	-0.22	-0.46	-0.33	-0.16	-0.32	-0.06
T-H	-0.23	-0.32	-0.43	<b>-0.65*</b>	-0.35	-0.02
Filterer	0.34	0.06	-0.22	0.29	-0.02	-0.35
Filterer-H	0.48	0.08	-0.01	0.02	-0.04	-0.36
Gatherer	-0.59	<b>-0.87***</b>	-0.34	<b>-0.83***</b>	<b>-0.86**</b>	<b>-0.76**</b>
Predator	-0.10	-0.50	-0.34	-0.49	<b>-0.67*</b>	-0.01
Scraper	-0.59	<b>-0.75**</b>	<b>-0.75*</b>	<b>-0.75**</b>	<b>-0.71**</b>	<b>-0.61*</b>
Shredder	-0.11	0.01	<b>-0.75*</b>	-0.04	-0.04	-0.09

\*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .<sup>1</sup> E = Ephemeroptera, P = Plecoptera, T = Trichoptera, B = Baetidae, H = Hydropsychidae, L = Leuctridae.

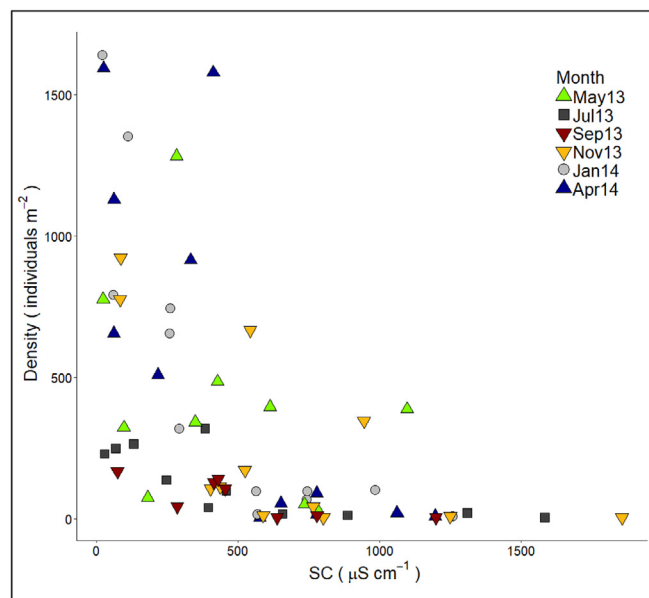


Fig. 3. Relationship of Ephemeroptera density with specific conductance (SC) in Appalachian headwater streams for all sampling months.

Ephemeroptera, which have evolved structures and cellular-level strategies adapted to a hypotonic environment (Nowghani et al., 2017), are prominent aquatic-community components in the naturally dilute streams of central Appalachia. In hypertonic environments such as our high-SC streams, the ability to excrete excess ions is much reduced (Wichard et al., 1973) and requires expenditure of energy (Griffith, 2017) otherwise used for growth and other functions, leading to osmotic stress.

Gatherer and scraper richness were also negatively correlated with SC in several sampling months (Table 4) related to, in part, the large proportion of Ephemeroptera taxa within these FFGs in low-SC samples. Ephemeroptera comprised 55–67% of gatherer taxa, and 40–53% of scraper taxa in low-SC streams in all months for which the gatherer or scraper richness correlations with SC were significant (Table 4). Some non-Ephemeroptera scrapers such as Trichoptera *Goera* and *Neophylax*, and Coleoptera *Optioservus*, *Oulimnius*, and *Psephenus* also declined in density with increasing SC during several sampling months. In central Appalachia, Pond et al. (2014) found that scraper relative abundances declined in response to elevated mining-origin SC during the spring season; and that declining scrapers included several Ephemeroptera taxa. In France, Piscart et al. (2005) considered the possibility that an observed scraper decline was due to a salinity-caused decrease in periphyton, but it is unknown whether the salinity was high enough to affect periphyton in the streams in this study. Short et al. (1991) did not observe scrapers in Kentucky streams with  $SC > 10,000 \mu S cm^{-1}$ , approximately  $5\times$  the maximum levels observed in this study. Drover (2018) found that SC-associated scraper declines in these same streams during April 2014, were correlated negatively with water selenium concentrations and suggested that elevated selenium in streams with elevated SC may be causative of those declines; however, selenium concentration data are not available for other months of this study.

Some macroinvertebrate groups with densities that were not correlated with SC in any sampling month showed strong negative responses to SC when dominant taxa were excluded from analyses. For example, EPT-BHL density was negatively correlated with SC in four out of six sampling months, though EPT density was not correlated with SC in any sampling month (Table 3). Boehme et al. (2016) reported similar findings in an Appalachian-coalfield study that included some of these same streams. Similarly, Plecoptera density was correlated strongly and positively with SC in July and September, but P-L was

Table 5

Taxonomic replacement for each sampling month represented by Pearson correlation coefficients from partial Mantel tests for Jaccard dissimilarity vs. specific conductance difference in macroinvertebrate groups, controlling for geographic coordinates.

Group <sup>1</sup>	May 13	Jul 13	Sep 13	Nov 13	Jan 14	Apr 14
Total	0.36*	0.30*	0.16	0.26*	0.47**	0.28*
Total-BHL	0.44**	0.48**	0.13	0.28*	0.54**	0.32*
EPT	0.44**	0.20	0.20	0.13	0.54**	0.40**
EPT-BHL	0.48**	0.31*	0.32*	0.16	0.50**	0.39**
E	0.14	0.38*	0.33*	0.27	0.59**	0.52**
E-B	0.35	0.56**	0.42**	0.33*	0.50**	0.43**
P	0.04	0.15	0.07	0.23	0.36**	0.10
T	0.29	0.25	0.08	0.02	0.03	0.05
T-H	0.25	0.11	0.17	0.01	0.04	0.06
Filterer	0.48**	0.10	0.04	0.07	0.01	0.04
Filterer-H	0.38*	0.16	0.00	0.02	0.00	0.07
Gatherer	0.09	0.11	0.14	0.43**	0.64**	0.49**
Predator	0.19	0.03	0.04	0.07	0.19	0.01
Scraper	0.25*	0.40**	0.03	0.01	0.11	0.09
Shredder	0.07	0.18	0.27*	0.02	0.39**	0.19

\*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ .

<sup>1</sup> E = Ephemeroptera, P = Plecoptera, T = Trichoptera, B = Baetidae, H = Hydropsychidae, L = Leuctridae.

correlated strongly and negatively with SC in the same two months. In July and September, *Acroneuria* and *Paracapnia*, respectively, were the only individual Plecoptera genera significantly correlated negatively with SC. *Paracapnia* was the only non-Leuctridae shredder genus significantly correlated negatively with SC, and only in September. Total richness and EPT richness were each negatively correlated with SC in five out of six sampling months (Table 4), indicating that increased densities of apparently SC-tolerant EPT taxa counteracted loss of more SC-sensitive taxa in high-SC samples.

### 3.3.2. Weak/no response

Several macroinvertebrate groups exhibited limited response to SC. Spearman correlations detected few relationships between SC and Trichoptera or between SC and filterers (Tables 3–5). Even when dominant Hydropsychidae taxa were excluded, the remaining Trichoptera taxa were not significantly correlated to SC in most cases. Few Trichoptera taxa were sensitive to SC. Only in November did Trichoptera taxa exhibit response to SC. Because Hydropsychidae occurred at elevated densities at high-SC streams in November, overall Trichoptera densities did not exhibit a response to SC despite the negative correlation of other Trichopteran taxa densities with SC (i.e. Hydropsychidae masked the response of Trichoptera density to SC). Non-Hydropsychidae Trichoptera taxa observed at high-SC streams in November were *Rhyacophila*, *Neophylax*, *Pycnopsyche*, and *Cernotina*, though *Neophylax* was found in diminished numbers in high-SC streams compared with low-SC streams. These observations suggest that many Trichoptera taxa are SC-tolerant in these headwater streams. Other studies have also found Trichoptera to be less sensitive to elevated SC than Ephemeroptera or Plecoptera. Pond (2012) found that Trichoptera relative abundance was not sensitive to SC in Kentucky. Clements and Kotalik (2016) also found Trichoptera to be relatively tolerant to elevated SC caused by multiple salt solutions in a mesocosm study. Working with simulated seawater and Australian taxa, Kefford et al. (2003) reported 72-h LC<sub>50</sub> SC values of 6400–26,000  $\mu S cm^{-1}$  for Trichoptera, levels far higher than in our streams. Williams and Williams (1998) found *Rhyacophila dorsalis*, collected from a stream in Wales, to be tolerant of simulated tidal inundation up to 17.5 ppt salinity (28,420  $\mu S cm^{-1}$ ) for 4 h. In our study, filterers were primarily composed of Trichoptera, so in most cases filterer patterns followed those of Trichoptera.

Predators also exhibited limited response to SC. Predator density

and richness were each correlated negatively with SC during only one month (i.e. July and January, respectively; Tables 3 and 4). *Rhyacophila* was among the predator taxa with the highest densities across most streams in all sampling months.

Total and EPT densities were not correlated with SC in any sampling months. Hartman et al. (2005) likewise reported no differences in total or EPT densities between reference and valley-fill-influenced streams with elevated SC, although some groups (e.g. Ephemeroptera, Coleoptera, Odonata, scraper, shredder) had lower densities in valley-fill-influenced streams compared to reference streams in their study.

### 3.3.3. Dominance

Numerically-dominant taxa shaped responses to SC by several groups, but this dominance varied seasonally. Hydropsychidae and Leuctridae were both dominant taxa within their respective orders, Trichoptera and Plecoptera, and their densities were correlated positively with SC seasonally (Table 3).

Leuctridae was dominant seasonally in total assemblages as well as within the Plecoptera order and the shredder group. In July, Leuctridae comprised < 30% of total density in low-SC streams, but > 75% of total density in high-SC streams. In all mining-influenced streams in July, relative abundance of Leuctridae was > 50%. Boehme et al. (2016) found similar relative abundances of Leuctridae seasonally in reference and high-SC streams in the central Appalachian coalfield. Other studies (Bernhardt et al., 2012; Pond, 2012) found an opposite pattern with Leuctridae decreasing in high-SC streams in the central Appalachians relative to reference streams, although the sampling times in Pond (2012) did not overlap with periods in this study when Leuctridae density was positively correlated with SC.

In this study during July, correlation of Leuctridae density with SC was strongly positive (Table 3), whereas correlation of Total-L density with SC was negative ( $\rho = -0.63$ ,  $p < 0.05$ ) and correlation of Total density with SC was non-significant (Table 3), suggesting that Leuctridae effectively masked the density response of all other taxa during that sampling month. The positive response of Leuctridae to SC also masked negative density responses by other Plecoptera and shredder taxa in July and September (Table 3). Both the Plecoptera and shredder density metrics were positively correlated with SC, but Leuctridae-excluded analog metrics were negatively correlated with SC during those two sampling months. For example, *Paracapnia* density was negatively correlated with SC in September (Spearman  $\rho = -0.90$ ;  $p = 0.0003$ ), but the positive response of Leuctridae densities to increasing SC more than offset that density loss. At SC > 1000  $\mu\text{S cm}^{-1}$  in September, *Leuctra* (the sole genus in Leuctridae in this study) was the only shredder genus observed.

Leuctridae dominance, and consequent masking of SC responses, varied seasonally. For example, Leuctridae density was highest (e.g. > 1000/m<sup>2</sup> in most cases) from late spring to mid-summer (i.e. April, May, and July), and was diminished (e.g. < 1000/m<sup>2</sup>) from September to January. Hydropsychidae displayed an opposing pattern, with densities generally increasing from September to peak density in November, then diminishing again between January and April. Boehme et al. (2016) found similar patterns with Hydropsychidae relative abundances being highest in fall, whereas Leuctridae relative abundances declined in fall relative to peak-density months in late spring through mid-summer.

In November, Capniidae genera were dominant within the Plecoptera and shredder groups. By January, *Amphinemura* density tracked *Leuctra* density (Pearson correlation between *Amphinemura* and *Leuctra*:  $r = 0.91$ ;  $P < 0.0001$ ) along the SC gradient with similar density magnitudes. *Amphinemura* also had the second-highest density across all streams in May and April.

Others have found *Leuctra* to be dominant in acidic streams (e.g. Townsend et al., 1983; Simpson et al., 1985; Groom and Hildrew, 1989), and some have proposed release from competition to be a factor (Groom and Hildrew, 1989). The streams in this study are circum-

neutral to alkaline, but it is possible that the same mechanism (i.e. release from competition caused by extirpation of competing taxa) is at least partially responsible for *Leuctra* dominance in high-SC streams.

Hydropsychidae were dominant in November, when both Trichoptera and filterers each showed varying responses contingent on Hydropsychidae inclusion in or exclusion from the analysis (Table 3). Trichoptera density was not significantly correlated with SC, but Trichoptera-H density and richness were negatively correlated with SC. A similar pattern was evident with filterer density having positive correlation with SC, but filterer-H density was not correlated significantly in November.

Hydropsychidae are known to be tolerant to elevated salinity (e.g. Short et al., 1991; Rutherford and Kefford, 2005; Pond, 2012). When characterizing effects of oilfield brine on lotic systems in eastern Kentucky, Short et al. (1991) found the greatest density of *Cheumatopsyche* (family Hydropsychidae) at SC of 5118  $\mu\text{S cm}^{-1}$ , with lower densities at lower levels of SC. Also working in eastern Kentucky, Pond (2012) found more *Cheumatopsyche* in streams influenced by mining than by any other land use and observed Hydropsychidae taxa more frequently in mining-influenced streams with elevated SC than in reference streams. In a summary of data for Australian rivers, Rutherford and Kefford (2005) found that *Cheumatopsyche* occurs at salinities as high as 25.9 g L<sup>-1</sup> (> 40,000  $\mu\text{S cm}^{-1}$ ).

### 3.3.4. Functional feeding groups

Several FFG richness metrics were also correlated negatively with SC during some sampling months. In some cases, loss of key taxa could potentially affect various trophic processes. In January, scraper densities were correlated negatively with SC (Table 3). January is a month when the canopy is open, so more autochthonous carbon is likely available than in leaf-on months (Cummins and Klug, 1979; Vannote et al., 1980). Lower scraper densities in higher-SC streams occurred primarily due to loss of mayfly scrapers. Four Ephemeroptera genera found in low-SC streams, all in the family Heptageniidae (*Cinygmula*, *Leucrocuta*, *Maccaffertium*, and *Stenonema*), were not observed in high-SC streams. Scraper richness was correlated negatively with SC (Table 4), leaving mostly armored caddisflies, armored riffle-beetles, and two tolerant mayflies, *Ameletus* and *Plauditus*, as scrapers in high-SC streams. The scrapers dominant in high-SC streams during January were primarily armored. Armored insects have been shown to be less susceptible to predation than their unarmored counterparts, thus algal carbon transfer could be effectively truncated at the level of primary consumers in streams with high SC (McNeely et al., 2007). Another way that higher trophic processes can be affected by high SC is simply by reduction of available prey, especially prey that are highly preferred by a certain predator. For example, Hiitt et al. (2016) suggest that loss of mayflies in high-SC waters of eastern Kentucky may be causing reductions of obligate invertivores such as the Kentucky Arrow Darter (*Etheostoma spilotum*), whose preferred prey, Heptageniidae and Baetidae, are both in the order Ephemeroptera.

McEvoy and Goonan (2003) contend that salinity is a driver for restructuring of communities, and that, in their study region of South Australia, salt-tolerant species will replace extirpated salt-sensitive species and their functions. That is feasible if there are enough salt-tolerant species in the regional pool to replace those lost by extirpation of salt-sensitive species in high-SC waters, and if replacement taxa function similarly to those lost. In these systems, there is a net loss of diversity (Table 1) caused by reduction of richness (Table 4) and increased dominance by salt-tolerant taxa (i.e. reduced evenness) with increasing SC, suggesting potential for functional depletion.

High diversity within FFGs is important because different taxa consume different qualities, types, and sizes of food (Wallace and Webster, 1996). For example, some caddisflies erect nets of varying shapes and sizes to capture specific ranges of particle sizes (Georgian and Wallace, 1981). Hydropsychidae utilize food of higher-quality (e.g. animals) than some detritivores (Wallace and Webster, 1996).



Shredders also discriminate among various types and qualities of leaf litter on which they feed (Graca, 2001). Thus, redundancy of taxa within a functional group does not necessarily mean functional redundancy (Rosenfeld, 2002), and loss of taxa from an FFG may result in reduced functional capacity.

### 3.4. Responses of macroinvertebrates to SC: Taxonomic replacement

For some group-sampling month combinations not exhibiting decline in density (Table 3) or richness (Table 4) with SC, taxonomic replacement was detected (Table 5). Occurrence of replacement when richness and density are not significantly altered shows an effect of SC on community structure that is not detected by conventional metrics. For example, Total, Total-BHL, and EPT groups all demonstrated significant correlation of taxonomic replacement with SC in May, the only sampling month when richness of these groups was not negatively correlated with SC. Hence, these groups demonstrated sensitivity to SC during all sampling months.

As another example, filterer and filterer-H showed significant correlation of taxonomic replacement with differences in SC in May (Table 5), but not significant density (Table 3) or richness (Table 4) relationships with SC; this finding implies filterer niches remained filled at the high-SC streams but with some differing taxa than those occurring at low-SC streams, potentially changing the size, type, and quality of food being filtered (Wallace et al., 1977; Wallace and Merritt, 1980). The hydropsychids *Diplectrona*, *Ceratopsyche*, and/or *Cheumatopsyche* were present in every sample during May. However, among the non-Hydropsychidae taxa, there was a distinct replacement of relatively sensitive caddisfly and mayfly genera in low-SC samples with blackfly (family: Simuliidae) genera in high-SC samples.

Chambers and Messinger (2001) also found replacement of pollution-sensitive with pollution-tolerant benthic macroinvertebrate taxa in mining-influenced streams of central Appalachia. Working in Queensland, Australia, Horrigan et al. (2005) reported macroinvertebrate communities that shifted from salt-sensitive to salt-tolerant taxa as SC values approached 300–1000  $\mu\text{S cm}^{-1}$ , values corresponding to the range of salinity in this study. They also found that at SC levels > 1000  $\mu\text{S cm}^{-1}$ , the rate of change towards dominance of salt-tolerant taxa began to slow, suggesting that there is a critical range for taxonomic replacement.

### 3.5. Summary

Several salient patterns within benthic macroinvertebrate communities were evident in the headwater streams of this study. Both SC and season appear to be drivers of community assemblage structure. Further, community structural response to SC varied across sampling months.

Overall changes of benthic macroinvertebrate density, relative to low-SC levels, were not found within sampled communities affected by elevated SC, suggesting that density losses of SC-sensitive taxa were offset by density gains of tolerant taxa. Density-metric responses to SC were driven primarily by responses of salt-tolerant and dominant taxa (e.g. *Leuctra* and Hydropsychidae), and of salt-sensitive taxa (e.g. Ephemeroptera).

Dominant taxa, apparently salt-tolerant, were prevalent in most selected taxonomic groups and FFGs seasonally. Seasonally-dominant Leuctridae and Hydropsychidae, both positively correlated with SC during seasons of their dominance, masked responses to SC by other taxa within their respective taxonomic groups and FFGs during those seasons. Excluding dominant taxa from structural metrics in some cases elucidated those masked responses and revealed that other group members were responding negatively even as the overall metric responded positively to SC because of the positive response by a dominant and salt-tolerant member of the group.

Ephemeroptera was the most salt-sensitive taxonomic group, as

Ephemeroptera richness and density declined markedly as SC increased. Ephemeroptera density was variable in low-SC streams, but that variability was reduced in higher-SC streams by density depletion. Functional groups with high representations of Ephemeroptera (scrapers and gatherers) showed significant negative responses to SC during most sampling months as a direct consequence of Ephemeroptera sensitivity.

In contrast, Trichoptera and filterers showed limited response to SC. This was partially caused by the masking effect by the dominant and salt-tolerant Hydropsychidae, but even non-Hydropsychidae Trichoptera densities exhibited negative response to SC in November.

Some FFGs were depleted of taxa at elevated SC, potentially leaving important functional niches unfilled and/or reducing energy and nutrient processing efficiency. Within shredder and filterer FFGs, dominant and salt-tolerant taxa were in some instances the only taxa present in streams with elevated SC. Several FFGs showed significant increase or decrease of richness, taxonomic replacement, and/or density in response to SC. Some of the alterations occurring as negative responses could have detrimental consequences to trophic transfer of energy and nutrients; such as in the case of scrapers, where armored taxa, which may not transfer energy and nutrients efficiently to the next-highest trophic level, replaced unarmored scrapers at elevated SC.

Taxonomic replacement occurred seasonally in several groups, in some cases revealing an effect by the major ions comprising the salinity monitored as SC on community composition when other metrics such as richness or density did not. Consequences of replacement are particularly important within FFGs. Differing taxa process food differently so replacement of salt-sensitive taxa by tolerant taxa does not necessarily equate to redundancy of function even within FFGs.

## 4. Conclusions

Consideration of density metrics offers information beyond what can be realized from relative-abundance metrics. Density metrics also provide information about absolute abundances, and therefore can indicate how close a taxonomic group may be to extirpation.

Organism densities did not respond to elevated SC, but community structure responded strongly. Community simplification (e.g. decreasing taxa richness combined with increased unevenness resulting from dominants) with increasing SC appeared to be the principal response in these systems.

Assessment of taxonomic replacement provides additional insights into the nature of benthic macroinvertebrate community structural change that occurs in mining-influenced Appalachian headwater streams as a response to increasing salinity. Taxonomic and functional group composition metrics that do not consider taxonomic identity may not reflect clearly community structure alterations caused by responses of dominant taxa within those groups.

Seasonality is an important consideration for accurately characterizing stream condition in central Appalachian headwaters. Temporal variability of density and taxonomic replacement responses to SC underscores the importance of sampling frequencies and timing when conducting bioassessments in mining-influenced central Appalachia headwaters.

## Declaration of Competing Interest

None.

## Acknowledgements

Funding: This work was supported by the Appalachian Research Initiative for Environmental Science (ARIES). We thank Megan Underwood, Beth Boehme, Liz Sharp, Kyle Dost, Lindsey Nolan, Sam Hays, and Janelle Salapich for field and laboratory assistance. Special thanks to Tony Timpano who scouted the streams, installed the

conductivity loggers, and provided advice throughout the project. Thanks to Patricia Donovan for preparing Fig. 1, and to Bryan Brown for advising on multivariate statistics.

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