

Temporal dynamics of benthic macroinvertebrate communities and their response to elevated specific conductance in Appalachian coalfield headwater streams



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ABSTRACT

Coal mining in central Appalachia USA causes increased specific conductance in receiving streams. Researchers have examined benthic macroinvertebrate community structure in such streams using temporally discrete measurements of SC and benthic macroinvertebrates; however, both SC and benthic macroinvertebrate communities exhibit intra-annual variation. Twelve central Appalachian headwater streams with reference quality physical habitat and physicochemical conditions (except for elevated SC in eight streams) were sampled \leq fourteen times each between June 2011 and November 2012 to evaluate benthic macroinvertebrate community structure. Specific conductance was recorded at each sampling event and by in situ data loggers. Streams were classified by mean SC Level (Reference, 17–142 μ S/cm; Medium, 262–648 μ S/cm; and High, 756–1535 μ S/cm). Benthic macroinvertebrate community structure was quantified using fifteen metrics selected to characterize community composition and presence of taxa from orders Ephemeroptera, Plecoptera, and Trichoptera. Metrics were analyzed for differences among SC Levels and months of sampling. Reference streams differed significantly from Medium-SC and High-SC streams for 11 metrics. Medium-SC streams had the most metrics exhibiting significant differences among months. Relative abundances of Plecoptera and Trichoptera were not sensitive to SC, as the families Leuctridae and Hydropsychidae exhibited increased relative abundance (vs. reference) in streams with elevated SC. In contrast, Ephemeroptera richness and relative abundance were lower, relative to reference, in elevated-SC streams despite increased relative abundance of Baetidae. Temporal variability was evident in several metrics due to influence by taxa with seasonal life cycles. These results demonstrate that benthic macroinvertebrate communities in elevated-SC streams are altered from reference condition, and that metrics differ in SC sensitivity. The time of year when samples are taken influenced measured levels and differences from reference condition for most metrics.

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1. Introduction

The Appalachian coalfield covers >4.5 million hectares in West Virginia, Virginia, Tennessee, and Kentucky (USEPA, 2011a) and extends into adjacent states. Surface coal mining in Appalachia often occurs in upper watershed areas (USEPA, 2011a), influencing

water quality in headwater streams (Griffith et al., 2012) and in downstream reaches (Lindberg et al., 2011).

Mining activity exposes un-weathered rocks to water and accelerated weathering, increasing total dissolved solids (TDS) in mine-influenced waters relative to background levels (Hartman et al., 2005; Merricks et al., 2007; Pond et al., 2008; Fritz et al., 2010; Timpano et al., 2010, 2015; Lindberg et al., 2011). In mine-influenced Appalachian waters, Ca^{2+} , Mg^{2+} , SO_4^{2-} , and HCO_3^- are often the predominant major ions by mass (Pond et al., 2008; USEPA, 2011a,b; Timpano et al., 2015).

Elevated major ion concentrations can act as toxicants to sensitive freshwater taxa (Cañedo-Argüelles et al., 2013). Laboratory

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Table 1

Areas (mean \pm standard error; and range) for watershed catchments defined by the study reaches.

Stream type	n	Mean [†] (km ²)	Range (km ²)
Reference	4	4.4 \pm 2.6	0.8–12.3
Medium-SC	4	4.5 \pm 0.8	2.7–6.7
High-SC	4	2.7 \pm 0.6	1.1–4.2
Overall	12	3.9 \pm 0.9	0.8–12.3

[†] Mean values do not differ significantly by stream type ($p < 0.05$).

toxicity tests confirm that elevated TDS levels lead to increased mortality and impaired reproduction and growth in freshwater invertebrates, although effect concentrations depend on selected test organisms, ions comprising TDS, and test duration (Mount et al., 1997; Kennedy et al., 2003; Soucek and Kennedy, 2005; Merricks et al., 2007; Chapman et al., 2000; Kennedy et al., 2004; Echols et al., 2010; Kefford et al., 2012; Kunz et al., 2013). Several studies of Appalachian streams have demonstrated strong associations between specific conductance (SC; electrical conductivity at 25 °C), an easily measured water parameter that is highly correlated with TDS, and benthic macroinvertebrate community metrics, suggesting causality or influence (Green et al., 2000; Freund and Petty, 2007; Pond et al., 2008; Gerritsen et al., 2010; Bernhardt et al., 2012; Cormier et al., 2013a). However, a variety of effect levels have been observed in field studies of SC-biota relationships.

When assessing effects of anthropogenic stressors, water managers should be aware of potential confounding by temporal variation of the targeted community that occurs naturally (Resh and Rosenberg, 1989; Linke et al., 1999; Šporka et al., 2006; Leunda et al., 2009; Álvarez-Cabria et al., 2010). Benthic macroinvertebrate community structure is influenced by seasonal growth and emergence patterns (Butler, 1984; Linke et al., 1999) which are related to dynamic environmental variables such as thermal regimes (Vannote and Sweeney, 1980; Sweeney et al., 1986) and resource availability (Hawkins and Sedell, 1981; Murphy and Giller, 2000). During some seasons, emergence patterns may cause decreased richness, density, or absence of particular taxonomic groups (Šporka et al., 2006).

In the USA and elsewhere, government agencies enforce water-protection laws by monitoring benthic macroinvertebrate community structure and assessing multi-metric structural indices (Metcalf, 1989; Gerritsen et al., 2000; Burton and Gerritsen, 2003). Agencies have recognized temporal variation of community structure by establishing specific index periods for regulatory compliance sampling (e.g., Burton and Gerritsen, 2003). However, it is possible that multi-metric index scores may vary within individual index periods, or between periods occurring in different seasons. Hence, improved understanding of benthic macroinvertebrate temporal variation is important to water quality management. To help address this need, we quantified temporal dynamics of benthic macroinvertebrate communities in Virginia coalfield headwater streams with varying SC levels and analyzed relationships of community metrics to, and their variability with, SC level.

2. Materials and methods

2.1. Stream selection

Eight test streams with elevated SC and four reference streams (Fig. 1 and Table 1) were selected from first- and second-order streams in the Virginia (USA) coalfield (Timpano et al., 2015). The study area is within Ecoregion 69, Central Appalachians, which extends into coal-bearing regions of Kentucky, West Virginia, Pennsylvania, and Tennessee (Omernik, 1987). Reference sites were selected based on relative absence of anthropogenic impacts and

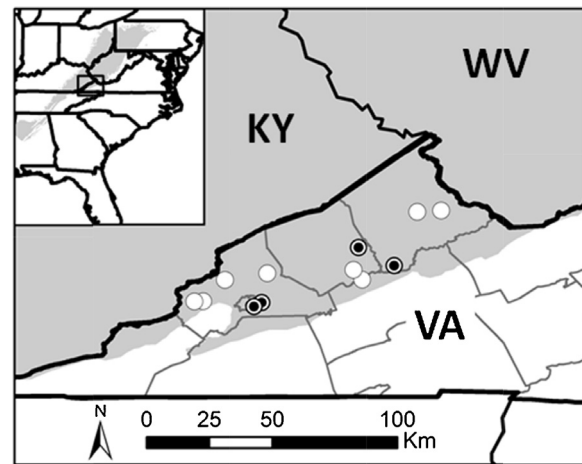


Fig. 1. Map of study streams in the coalfield of southwestern Virginia, USA, with state and county boundaries. Reference streams are shown as symbols with black centers and test streams as white symbols. The inset shows study-area location within eastern USA. The Appalachian coalfield's areal extent is denoted by gray background.

low levels of SC (Timpano et al., 2015). Test streams were selected to provide a continuum of SC levels and to differ from reference streams only in having elevated SC.

2.2. Field and laboratory methods

Stream reaches of 100 m with optimum benthic macroinvertebrate riffle habitat were sampled during base flow conditions. Each stream was sampled in June, August, September, October, and November of 2011; and in January, March, April, May, June, August, September, October, and November of 2012. During some months, some streams were not sampled because of low flows. A total of 151 benthic macroinvertebrate samples were collected.

Habitat quality was recorded at each sampling event using Rapid Bioassessment Protocols (Barbour et al., 1999). During each field visit, water temperature, dissolved oxygen, SC (referred to as discrete SC), and pH were measured by a calibrated Hanna Instruments 9828 Probe (Hanna Instruments, Smithfield, RI). Onset HOB0 U-24 (Onset Computer Corporation, Bourne, MA) freshwater conductivity loggers were installed in each sampling reach between April and November, 2011, and recorded SC at fifteen-minute intervals until the end of the study period.

During each sampling visit, single grab samples of water were filtered with a nominal pore size of 0.45 μ m using either (1) acid-rinsed cellulose ester filters and stored in acid-rinsed polypropylene bottles (June–August 2011) or (2) disposable syringe filters and stored in disposable sample bags (September 2011–November 2012). Water samples were transported to the laboratory on ice and stored at 4 °C until analysis. Samples to be analyzed for dissolved metals were amended with 1 + 1 nitric acid to decrease the pH to <2 for preservation (APHA, 2005). Samples were analyzed for Ca²⁺, Mg²⁺, K⁺, Na⁺, Cl⁻, SO₄²⁻, TDS, and total alkalinity using methods consistent with APHA (2005) and described by Timpano et al. (2015); and HCO₃⁻ was calculated from alkalinity and pH measurements (APHA, 2005).

Benthic macroinvertebrates were collected using Virginia Department of Environmental Quality (VDEQ) (2008) protocols for single-habitat riffle-run sampling. A composite sample (approximately 2 m² of substrate) was made from six riffle samples per stream using a 0.3 m-wide D-frame kick-net with 500 μ m mesh size. No mollusks or crayfish were retained. Samples were preserved in 95% ethanol until quantified in the laboratory. Benthic macroinvertebrates were sub-sampled in the laboratory to obtain

a standard random 200 ($\pm 10\%$) specimen count following VDEQ Standard Operating Procedures (VDEQ, 2006) as per U.S. EPA Rapid Bioassessment Protocols (Barbour et al., 1999). Sub-samples were identified to the lowest practical taxonomic level: usually genus but in some cases family (Chironomidae, most Simuliidae, some Ceratopogonidae) or subclass (Oligochaeta) using Smith (2001) and Merritt et al. (2008).

2.3. Metric selection

Five genus-level richness metrics (Total; Ephemeroptera; Plecoptera; Trichoptera; and combined Ephemeroptera, Plecoptera, and Trichoptera, EPT) were calculated as numbers of taxa observed within each sample. Relative abundances for Ephemeroptera, Plecoptera, Trichoptera, and combined EPT were also calculated as proportions of individuals within each sample. Dominant taxa for EPT orders were apparent in some streams, so three additional relative-abundance metrics (%Baetidae, %B; %Hydropsychidae, %H; and %Leuctridae, %L) were calculated. Ephemeroptera, Plecoptera, and Trichoptera were emphasized because these three orders are commonly considered as sensitive indicators of community condition (Burton and Gerritsen, 2003) and have been found to exhibit differing patterns and degrees of TDS sensitivity (Pond, 2010; 2012). A composite metric, %EPT excluding Hydropsychidae, Baetidae, and Leuctridae (%EPT – HBL) was calculated to explore how exclusion of seemingly tolerant taxa would characterize macroinvertebrate community response to elevated SC. Genus-level Simpson Diversity (Simpson, 1949) and Percent Top 2 Dominant taxa were calculated to examine differences in community diversity and evenness, respectively, among SC Levels. Chironimidae were not excluded from Simpson Diversity.

2.4. Data analysis

Benthic macroinvertebrate data were entered into VDEQ's genus-level Ecological Data Assessment System (EDAS) (VDEQ, 2010), which calculated most biotic metrics; other metrics (%L and %EPT – HBL) were calculated manually. Genus-level richness metric calculations included taxa identified to family-level when representative genera from that family were not observed. Immature or damaged specimens unidentifiable to genus level were excluded from richness and diversity metrics to avoid double-counting but were included in abundance metrics.

Both continuous and temporally discrete conductivity data for each stream were summarized as means of SC values recorded over the study period. Using continuous conductivity means, the four reference streams were assigned to one SC Level (17–142 $\mu\text{S}/\text{cm}$), and the eight non-reference streams were divided into two groups of four stratified by SC mean: Medium-SC (262–648 $\mu\text{S}/\text{cm}$), and High-SC (756–1535 $\mu\text{S}/\text{cm}$). Both temporally discrete and continuous conductivity mean values produced identical stream groupings.

Data were analyzed using JMP Pro 10.0.0 (SAS Institute, Cary, NC) with test level of $\alpha = 0.05$, unless otherwise noted. Individual habitat parameter scores, overall habitat score, water temperature, dissolved oxygen, SC, and pH were evaluated for differences among SC Levels using mixed models, with Month and Site ID as random effects and SC Level as the fixed effect. Treating Site ID as a random effect accounted for correlation among repeated measures from the same site (Jiang 2007). Standard Least Squares and the Restricted Maximum Likelihood approaches were employed. Biotic metrics were analyzed for differences among SC Levels by month using mixed models with SC Level, Month, and their interaction as fixed effects, and Site ID as a random effect. A Bonferroni Correction ($p = 0.05/15 = 0.003$) was used to avoid false positive results, as the dataset was evaluated using 15 biotic metrics, some of which were closely related (Bland, 1995). Mixed model residuals were checked

for normality using the Shapiro–Wilkes test ($\alpha = 0.05$); residuals for all models were found to be distributed normally. For each metric, mean values were tested for unequal variance among SC Levels using the Brown–Forsythe test. Tukey's HSD was used to determine significant differences among SC Levels for each month and across months for each SC Level ($\alpha = 0.05$). For variables with unequal variances among SC Levels, mixed-model results for SC Level differences were confirmed by applying Welch's ANOVA to test for significant differences among the means ($\alpha = 0.05$).

Coefficient of variation (CV), calculated by dividing the standard deviation (σ) by the mean (μ), can be used to compare variability of measures that are not expressed on common scales. For each SC Level, we calculated modified CVs for individual metrics as $\sigma_{\text{MSCL}}/\mu_{\text{M}}$ where σ_{MSCL} = standard deviation of the metric means for that SC Level ($n = 14$), and μ_{M} is the overall metric mean ($n = 42$, 14 metric means for each of 3 SC Levels). Modified CVs were evaluated to compare month-to-month variability across metrics nominally due to lack of a known test for making such comparisons statistically. Among SC Levels, individual metric CVs were compared using the Brown–Forsythe test for unequal variance, as stated above.

In data interpretations, we apply the term “trends” to describe benthic macroinvertebrate metrics exhibiting ≥ 3 months of continuously increasing or decreasing values, with significant differences between first and last months. We also use the terms “increase” and “decrease” to describe such trends.

3. Results and discussion

3.1. Physical habitat and physicochemical parameters

No differences among SC Levels were detected for individual physical habitat parameters, total habitat scores, stream temperature, or dissolved oxygen (Table 2), which suggests effects of potentially confounding physical habitat stressors were minimized as intended. Reference pH (7.7 ± 0.5) was nominally lower than pH in Medium- (8.1 ± 0.3) and High-SC streams (8.2 ± 0.3) (Table 2) but within USEPA reference recommendations ($6.5 < \text{pH} < 9$; USEPA 1986), suggesting pH was not a primary influence on differences among community metrics. Mean temporally discrete SC measurements differed among Reference ($58 \pm 49 \mu\text{S}/\text{cm}$), Medium-SC ($449 \pm 188 \mu\text{S}/\text{cm}$), and High-SC ($1070 \pm 361 \mu\text{S}/\text{cm}$) streams. Mean continuous SC was $69 \pm 55 \mu\text{S}/\text{cm}$ in reference streams, lower than the $423 \pm 204 \mu\text{S}/\text{cm}$ and $1021 \pm 361 \mu\text{S}/\text{cm}$ values recorded in Medium- and High-SC streams, respectively (Table 2). Reference stream SC levels were similar to regional reference levels reported by Pond (2010, 2012), Lindberg et al. (2011), Bernhardt et al. (2012), Cormier et al. (2013a), and Timpano et al. (2015).

Specific conductance exhibited a seasonal pattern in all three stream types. SC tended to be highest in late summer and fall: August through October in 2011, and August through November in 2012 (Fig. 2). This pattern was consistent with that found by Cormier et al. (2013a,b) in West Virginia streams, also within the Appalachian coalfield. Seasonal variation of water quality may contribute to seasonal patterns of altered benthic macroinvertebrate community structure in disturbed watersheds (e.g., Helms et al., 2009) but our analyses were not structured to investigate such influence.

Major-ion proportions by mass were altered in Medium- and High-SC streams, relative to Reference (Table 3). Reference stream water was dominated by HCO_3^- whereas mining-influenced streams had higher proportions of SO_4^{2-} (Table 3). These differences are similar to findings by others (e.g., Pond et al., 2008; Cormier et al., 2013a; Timpano et al., 2015). Similarity of ionic composition in our study streams to that of streams in other studies

Table 2
Mean (± 1 SE) habitat scores and physicochemical parameter values, with ranges, by specific conductance (SC) Level.

Parameter [†]	Reference streams	Medium-SC streams	High-SC streams
Substrate/cover	17 \pm 1 (15–19) [‡]	17 \pm 1 (13–19)	16 \pm 1 (13–19)
Embeddedness	16 \pm 2 (10–19)	16 \pm 2 (12–19)	16 \pm 1 (13–18)
Velocity/depth regime	16 \pm 3 (10–20)	16 \pm 3 (10–20)	16 \pm 2 (12–20)
Sediment deposition	15 \pm 3 (7–19)	16 \pm 1 (13–19)	14 \pm 2 (10–18)
Flow status	15 \pm 4 (6–20)	15 \pm 3 (8–20)	15 \pm 2 (10–19)
Channel alteration	20 \pm 1 (17–20)	20 \pm 0 (19–20)	20 \pm 0 (19–20)
Riffle frequency	17 \pm 1 (16–19)	17 \pm 1 (15–19)	17 \pm 1 (15–19)
Bank stability	16 \pm 1 (13–19)	17 \pm 2 (12–20)	16 \pm 2 (11–18)
Bank vegetative cover	18 \pm 2 (15–20)	19 \pm 2 (11–20)	19 \pm 2 (16–20)
Riparian vegetation width	19 \pm 1 (16–20)	18 \pm 3 (8–20)	19 \pm 1 (16–20)
Total habitat score	171 \pm 10 (146–186)	171 \pm 11 (139–191)	167 \pm 7 (156–189)
Discrete temperature ($^{\circ}$ C)	13.1 \pm 5.0 (3.7–21.2)	13.5 \pm 5.0 (2.5–20.8)	13.0 \pm 4.2 (1.9–20.2)
Discrete SC (μ S/cm)	58 \pm 49 (13–175) a	449 \pm 188 (72–845) b	1070 \pm 361 (498–1795) c
Conductivity logger SC (μ S/cm)	69 \pm 55 (1–221) a	423 \pm 204 (1–2579) b	1021 \pm 394 (6–2281) c
pH	7.7 \pm 0.5 (6.7–8.4) a	8.1 \pm 0.3 (7.4–8.6) b	8.2 \pm 0.3 (7.5–8.7) b
Dissolved oxygen (mg/L)	8.6 \pm 1.4 (5–10.9)	9.5 \pm 1.5 (6.6–12.9)	9.4 \pm 1.1 (7.5–12.5)

[†] Individual habitat metrics are on a scale of 1–20, total habitat score from 1 to 200.

[‡] Values followed by different letters are significantly different among stream types ($\alpha = 0.05$).

Table 3
Mean relative proportion of major-ions by mass (% of major-ion sum), for each specific conductance (SC) Level.

SC level	n	Cl ⁻	SO ₄ ²⁻	HCO ₃ ⁻	Ca ₂ ⁺	K ⁺	Mg ²⁺	Na ⁺	Major ion sum (mg/L)
Reference	45	7b [†]	20a	46c	15b	3b	5a	5b	39
Medium	54	2a	33b	35b	15b	1a	5a	10c	260
High	60	1a	54c	19a	13a	1a	9b	2a	831

[†] For each ion, means followed by different letters are significantly different among SC Levels ($\alpha = 0.05$).

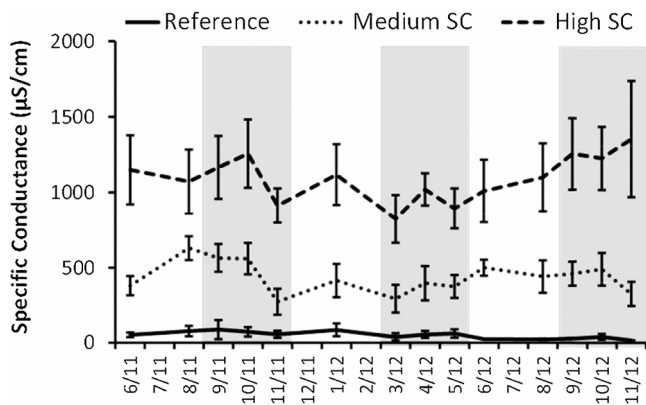


Fig. 2. Mean temporally discrete specific conductance (SC) by SC Level and Month. Error bars are 1 standard error from the mean. Shaded boxes denote March–May and September–November periods.

indicates potential for general application of our findings to similar streams in central Appalachia.

3.2. Benthic macroinvertebrate community metrics

3.2.1. Total Richness, Evenness, Diversity

Total Richness was highest in Reference (27 \pm 1.0) and lowest in High-SC streams (15 \pm 0.9) (full study period means, Table 4). Over the full study period, Total Richness at High-SC sites was reduced by 38–51%, relative to Reference. Similarly, in West Virginia, Pond et al. (2008) found Total Richness during spring (March–May) to be 30–50% lower in mining-influenced streams with spring SC > 500 μ S/cm relative to streams in unmined watersheds. Percent Top 2 Dominant was lowest, and Simpson Diversity highest in Reference streams (Table 4).

Within SC Levels, Total Richness did not differ among months for both High-SC and Reference streams. However, Medium-SC streams exhibited an increasing trend in Total Richness from

June 2011 through March 2012 and a decreasing trend from March 2012 through June 2012 (Fig. 3A). Seasonal life history patterns for Ephemeroptera and Plecoptera Richness, described below, contributed to these trends. Other studies have found benthic macroinvertebrate community richness to vary temporally. In northern Spain, Álvarez-Cabria et al. (2010) reported Total Richness to peak in fall; and in central Europe, Šporka et al. (2006) observed summer months to reflect low richness for taxa that exhibit summer emergence. Also in Spain, García-Criado et al. (1999) observed family-level richness differences between coal mining-impacted and un-impacted sites to be greatest in winter and smallest in summer.

Total Richness, Simpson Diversity, and %Top 2 Dominant all demonstrate increasing deviation from Reference with increasing SC. Modified CV for Simpson Diversity was lower for Reference streams than for Medium- and High-SC streams (Fig. 4), indicating lower month-to-month variation for Reference streams.

3.2.2. Ephemeroptera

Ephemeroptera metrics in Medium- and High-SC streams were altered from Reference. Mean Ephemeroptera Richness was highest in Reference streams (6 \pm 0.3) and lowest in High-SC streams (1 \pm 0.3) (Table 4), a finding similar to those of other studies in mining-influenced Appalachian streams (e.g., Pond, 2004; Pond et al., 2008; Cormier et al., 2013a). *Eurylophella*, *Seratella*, *Hexagenia*, *Leucrocuta*, and *Isonychia* were only observed in Reference streams, whereas *Heterocleon*, *Attenella*, *Drunella*, *Ephemerella*, *Ephemera*, *Cinygmula*, *Epeorus*, *Maccaffertium*, *Stenacron* were only observed in Reference and Medium-SC streams. Except for Baetidae during most months, Heptageniidae in March 2012, and Leptophlebiidae in October and November 2012, Ephemeroptera were absent from High-SC streams. Similarly during spring (March–May) in West Virginia, few Ephemeroptera genera were present in streams with SC > 500 μ S/cm (Pond et al., 2008).

Ephemeroptera Richness did not differ among months in Reference and High-SC streams (Table 4). Although Ephemeroptera Richness in Reference streams peaked in spring, multiple

Table 4Mean macroinvertebrate metrics by specific conductance (SC) Level, with *p*-values for effects of SC Level, month of sampling (Month), and SC Level × Month interaction.

Dependent variable	SC Level <i>p</i> -value [†]	Month <i>p</i> -value	SC Level × Month <i>p</i> -value	Reference Mean ± SE [‡]	Medium-SC Mean ± SE	High-SC Mean ± SE
Total Richness	<0.0001	0.0002	0.8849	27 ± 1.0 a	21 ± 0.9 b [*]	15 ± 0.9 c
%Top 2 Dominant	<0.0001	<0.0001	0.7691	37 ± 2.5 a	52 ± 2.4 b	63 ± 2.4 c
Simpson Diversity	0.0004	<0.0001	0.0738	0.9 ± 0.02 a	0.8 ± 0.02 b	0.7 ± 0.02 c [*]
Ephemeroptera Richness	<0.0001	<0.0001	0.0246	6 ± 0.3 a	3 ± 0.3 b [*]	1 ± 0.3 c
Plecoptera Richness	0.0021	<0.0001	<0.0001	7 ± 0.5 a	5 ± 0.5 b [*]	3 ± 0.5 b
Trichoptera Richness	0.0179	0.0066	0.7953	6 ± 0.3	6 ± 0.3	4 ± 0.3
EPT Richness	<0.0001	<0.0001	0.1733	19 ± 0.7 a	14 ± 0.7 b [*]	9 ± 0.7 c
%Ephemeroptera	<0.0001	<0.0001	<0.0001	25 ± 1.5 a [*]	10 ± 1.4 b [*]	5 ± 1.4 b [*]
%Baetidae	0.7461	<0.0001	0.2203	4 ± 1.6 [*]	6 ± 1.5 [*]	5 ± 1.5 [*]
%Plecoptera	0.0002	<0.0001	0.0132	17 ± 3.2 a	39 ± 3.0 b [*]	45 ± 3.0 b [*]
%Leuctridae	0.0013	<0.0001	<0.0001	6 ± 3.6 a	21 ± 3.5 b [*]	33 ± 3.5 c [*]
%Trichoptera	0.2195	<0.0001	<0.0001	26 ± 2.9	33 ± 2.9	30 ± 2.9 [*]
%Hydropsychidae	0.0282	<0.0001	<0.0001	18 ± 2.4 [*]	28 ± 2.4 [*]	28 ± 2.4 [*]
%EPT	0.0002	<0.0001	0.0164	68 ± 1.6 a	82 ± 1.5 b [*]	81 ± 1.5 b [*]
%EPT – HBL	0.0006	<0.0001	0.1514	39 ± 3.0 a [*]	26 ± 2.8 b [*]	14 ± 2.8 c [*]

^{*} Metric exhibited significant differences among months for this SC Level (individual month-to-month differences are specified by Boehme, 2013).

[†] Bonferroni Correction to reduce likelihood of false positive results: $\alpha = 0.05/15 = 0.003$.

[‡] For each dependent variable, means followed by different letters are significantly different among SC Levels ($\alpha = 0.05$, with Bonferroni Correction).

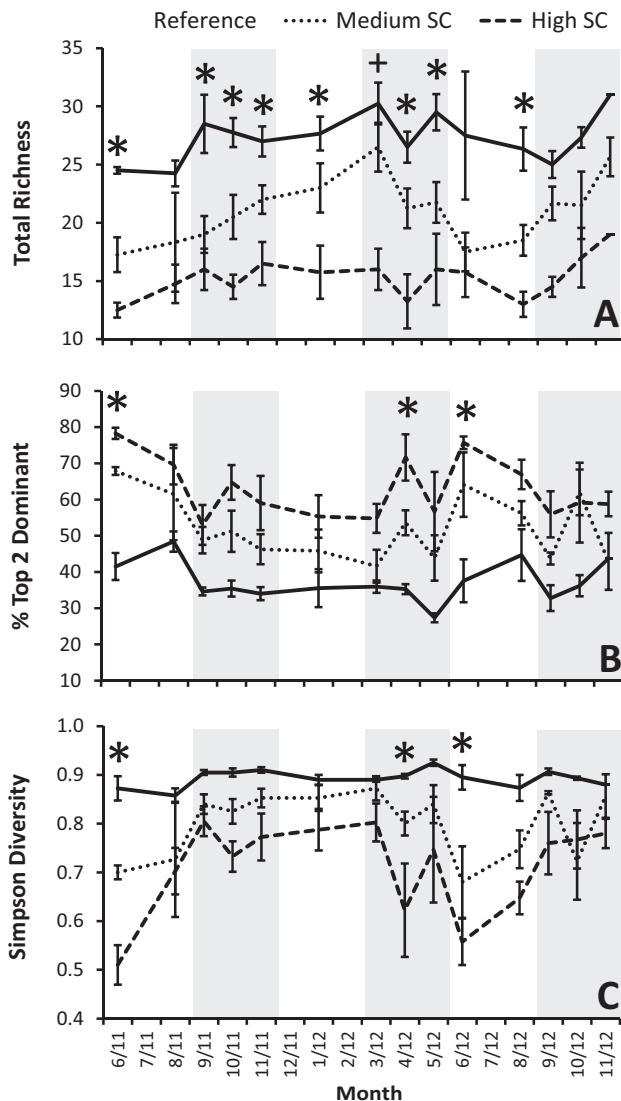


Fig. 3. Mean genus-level. (A) Total Richness, (B) %Top 2 Dominant, and (C) Simpson Diversity by Month and specific conductance (SC) Level. Error bars are 1 standard error from the mean. Shaded boxes denote March–May and September–November periods. * Reference is significantly different from High-SC; and + Reference and Medium-SC are significantly different from High-SC ($\alpha = 0.05$) for the designated sampling month.

Ephemeroptera taxa were observed during other seasons. In Medium-SC streams, a relatively high month-to-month variance (Fig. 4) was accompanied by an increasing trend from October 2011 when *Baetis*, *Maccaffertium*, and *Stenonema* were present, through March 2012 when substantially more genera (including *Ameletus*, *Acerpenma*, *Baetis*, *Drunella*, *Ephemerella*, *Epeorus*, and *Stenonema*) were collected. Medium-SC Ephemeroptera Richness then declined between March and September 2012, as *Ameletus* and Ephemerellidae were not observed between May and November 2012.

Most of the observed Ephemeroptera genera have one seasonal life cycle per year (Poff et al., 2006) with adults emerging, mating, and laying eggs in spring (Merritt et al., 2008). Consequently, mayfly nymph abundance is generally highest in spring, prior to emergence, and lowest in fall when early instar nymphs are too small for capture by 500 μm -mesh nets or are not present in riffle habitats. Reference and Medium-SC streams exhibited the expected pattern, with increased Ephemeroptera relative abundance and richness during spring months and comparatively low relative abundance and richness during summer months. Percent Ephemeroptera was highest in Reference streams (25 ± 1.5) and lowest in High-SC streams (5 ± 1.4) (Table 4). Reference-stream %Ephemeroptera levels during spring months, 38–54%, were similar to the 25–50% levels reported for spring samples from eastern Kentucky and West Virginia reference streams (Pond et al., 2008; Pond, 2010). Other studies have found reduced relative abundance of Ephemeroptera in mining-influenced Appalachian streams with elevated SC (Kennedy et al., 2003; Hartman et al., 2005; Pond, 2010; Cormier et al., 2013b). Baetidae comprised the majority of Ephemeroptera in our Medium- and High-SC streams (60% and 96%, respectively), whereas Reference Ephemeroptera assemblages included only 17% Baetidae, on average. High observation frequencies for Baetidae in elevated-SC streams were also noted by Pond (2010) and by Garcia-Criado et al. (1999) in Spain.

The significant interaction detected between SC Level and Month (Table 4) occurred due to altered Ephemeroptera community composition in elevated-SC streams, as emergence timing varies among taxa (Brittain, 1982). Peak %Ephemeroptera was earliest in Reference streams (March) when Ephemerellidae and Heptageniidae abundance peaked; these genera were less abundant in Medium-SC streams and almost entirely absent from High-SC streams. Then, Medium-SC %Ephemeroptera peaked in April with increased presence of Ephemerellidae and Baetidae. Finally, High-SC %Ephemeroptera peaked in May due to high relative abundance by Baetidae (Fig. 5A).

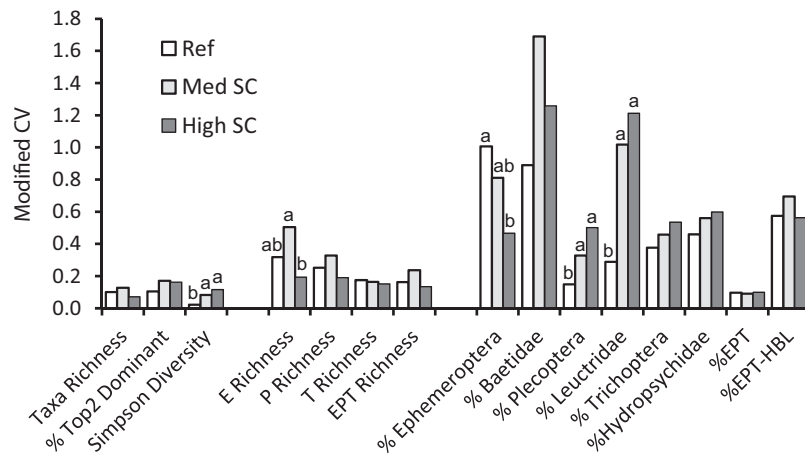


Fig. 4. Modified coefficients of variation (CV) for benthic macroinvertebrate community metrics. For each metric, bars with different letters have unequal month-to-month variances across the study period ($\alpha = 0.05$).

3.2.3. Plecoptera

Plecoptera metrics also exhibited both SC-Level and seasonal effects. Plecoptera Richness was higher in Reference streams (7 ± 0.5) than in Medium- (5 ± 0.5) and High-SC streams (3 ± 0.5) (Table 4). *Alloperla*, *Haploperla*, *Sweltsa*, *Soyedina*, *Clioperla*, *Cultus*, *Diploperla*, *Isoperla*, *Malirekus*, *Pteronarcys*, *Taenionema*, and *Taeniopteryx* were all present in multiple reference samples but not observed in High-SC streams. Similarly, Plecoptera Richness was negatively correlated with SC in West Virginia streams (Pond et al., 2008) and, in eastern Kentucky, was lower in mining-influenced streams with elevated SC than in reference streams (Pond, 2012).

Although Plecoptera Richness did not differ among months in Reference and High-SC streams, Medium-SC seasonal contrasts were striking (Fig. 5B). Medium-SC Plecoptera Richness peaked in January–February 2012 when *Allocapnia*, *Paracapnia*, *Alloperla*, immature *Nemouridae*, *Ostrocerca*, *Shipsa*, *Peltoperla*, *Acroneuria*, *Isoperla*, *Oemopteryx*, *Taenionema*, and *Taeniopteryx* were all observed; and was higher throughout winter and early spring than in both Junes. Medium-SC %Plecoptera also varied seasonally. In March 2012, *Amphinemura* comprised 72% of the Plecoptera observed in Medium-SC streams, Capniidae were not present, *Leuctra* occurred in relatively low abundance, but no other notable differences were present relative to January composition. In contrast, *Leuctra* represented 90 and 96% of the Plecoptera in Medium-SC streams in June 2011 and June 2012, respectively.

Percent Plecoptera was higher in High-SC (45 ± 3.0) and Medium-SC (39 ± 3.0) streams relative to Reference (17 ± 3.2) (Table 4). During most months, %Plecoptera in Medium- and High-SC streams was comprised primarily of Leuctridae, which were 54, and 73%, on average, of Plecoptera in those stream types respectively, but only 34% in Reference streams. Relative abundances of *Leuctridae*, *Nemouridae*, and *Capniidae* were also greater in Medium- and High-SC streams, and *Taeniopterygidae* was greater in Medium-SC streams relative to Reference streams. Our results are similar to eastern Kentucky findings of higher relative abundances for *Amphinemura*, *Perlesta*, and *Allocapnia* in elevated-SC streams, relative to reference (Pond, 2012).

Month-to-month variances for both %Plecoptera and %Leuctridae were greater in Medium- and High-SC streams than in Reference streams (Fig. 4). Both findings reflect high seasonal variability of *Leuctra*, the sole genus collected from the family Leuctridae, as %Leuctridae declined to <10% in all three stream types during October and November of both years.

Similarly, in West Virginia, Pond et al. (2008) observed strong influence by *Hydropsychidae* and *Amphinemura* on %EPT where SC > 500 $\mu\text{S}/\text{cm}$; and, hence, found %EPT as less-sensitive to

elevated SC than other metrics. In our study, mean relative abundances for *Nemouridae* were also higher in Medium- and/or High-SC than in Reference streams in January (12%, 9%, and 3%), March (25%, 26%, and 3%); and November 2012 (22%, 3%, and 1%, respectively).

3.2.4. Trichoptera

Trichoptera Richness did not differ by Month or SC Level (Table 4). *Hydropsychidae* was the dominant Trichoptera family in Reference streams, followed by *Philopotamidae* and *Rhyacophilidae* during most months. In our study, Trichoptera taxa with increased relative abundance (compared with Reference) in Medium- and High-SC streams were *Hydropsychidae* (*Ceratopsyche*, *Cheumatopsyche*, and *Diplectrona*), a finding similar to Pond (2010, 2012) in eastern Kentucky and Bernhardt et al. (2012) in West Virginia. In our study, there were no other notable differences in Trichoptera genera among SC Levels.

Seasonal patterns were evident in percent composition metrics among all SC Levels, with both %Trichoptera and %Hydropsychidae being higher during the fall months (September–November 2011 and September–October 2012) than in winter and spring months (January–May 2012), especially in Medium- and High-SC streams. *Hydropsychidae* comprised >89% of Trichoptera in High-SC streams during June through November of both years.

Month-to-month variability for Trichoptera richness was generally low relative to other metrics; and neither Trichoptera Richness nor %Trichoptera variance differed by SC Level (Fig. 4).

3.2.5. EPT

The EPT Richness metric was highest in Reference (19 ± 0.7) and lowest in High-SC streams (9 ± 0.7) (Table 4). Similarly, decreased EPT Richness in elevated-SC mining-influenced streams has been found in Spain (García-Criado et al. (1999) and West Virginia (Pond et al., 2008).

In our study, EPT Richness did not vary among months for Reference or High-SC streams, but Medium-SC streams showed increasing trends over two periods (June 2011–March 2012, and June–November 2012) separated by a decreasing trend (Fig. 5D). Seasonal changes of *Ephemeroptera* and *Plecoptera* taxa causing the Medium-SC trends are described above, but the presence of additional EPT taxa muted seasonal variation of the EPT Richness metric in Reference streams. Our finding of decreased EPT Richness during summer relative to other seasons, although significant only for Medium-SC streams, is similar to findings by Johnson et al. (2012) in Kentucky and to nominal differences observed by Šporka et al. (2006) in Spain.

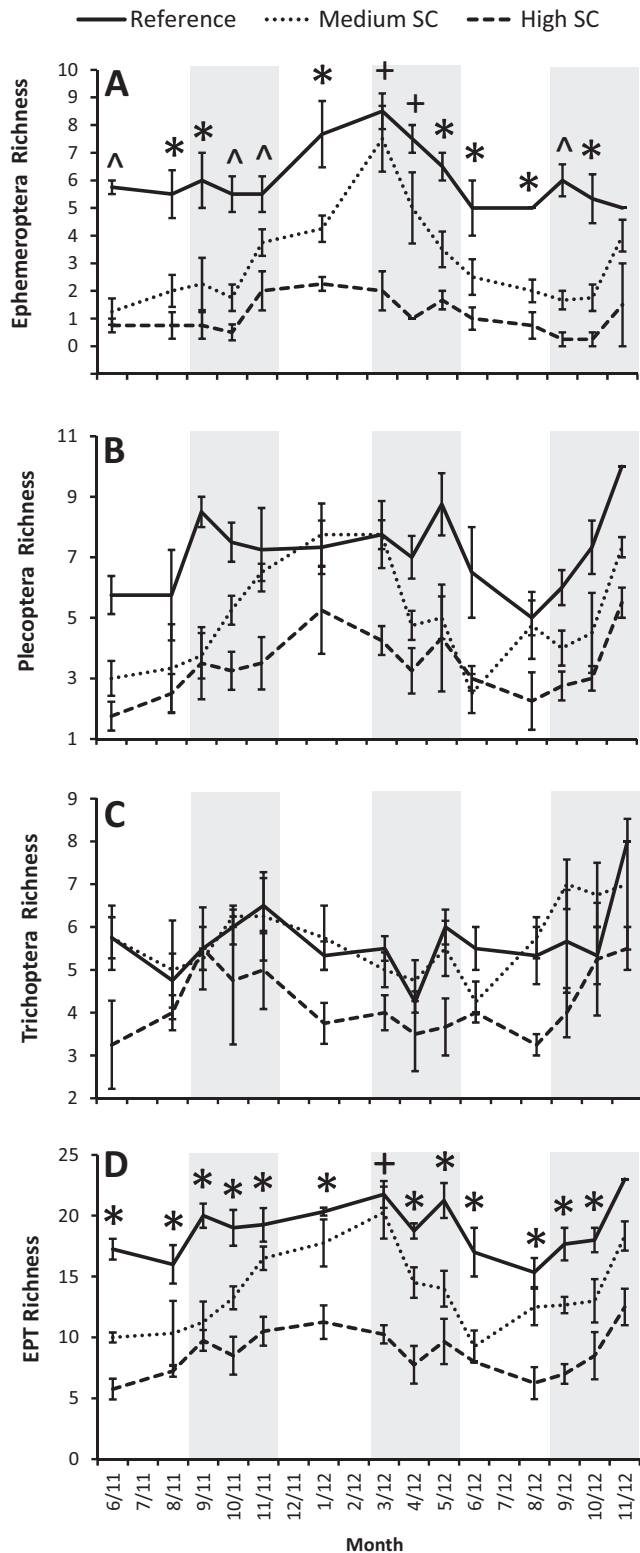


Fig. 5. Mean genus-level. (A) Ephemeroptera Richness, (B) Plecoptera Richness, (C) Trichoptera Richness, and (D) EPT Richness by Month and specific conductance (SC) Level. Error bars are 1 standard error from the mean. Shaded boxes denote March–May and September–November periods. * Reference is significantly different from High-SC; + Reference and Medium-SC are significantly different from High-SC; ^ Reference is significantly different from Medium- and High-SC ($\alpha = 0.05$) for the designated sampling month.

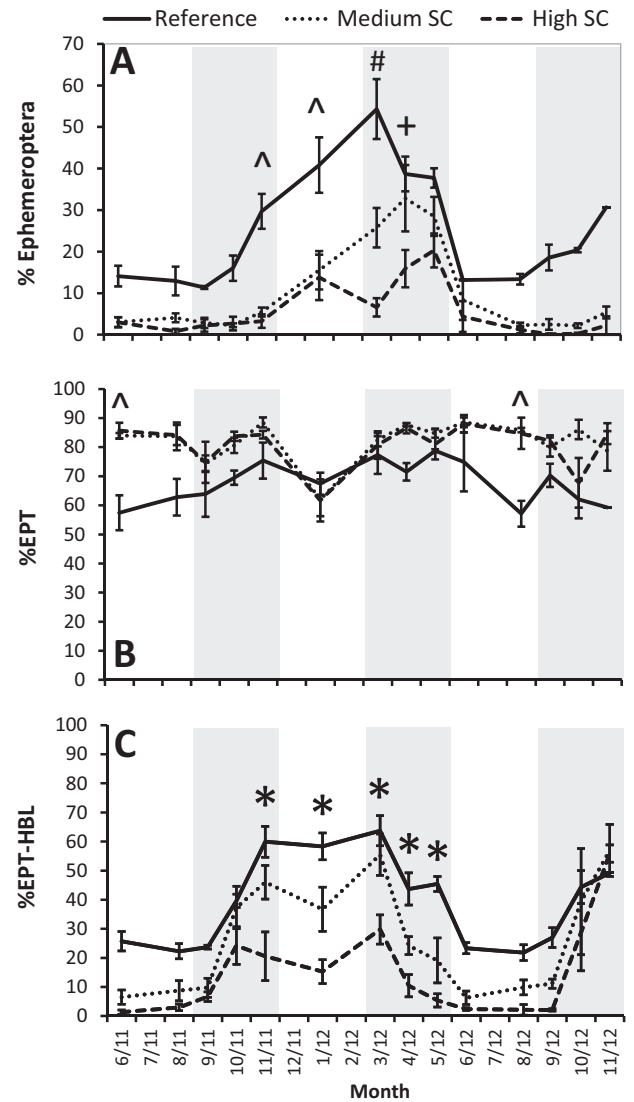


Fig. 6. Mean (A) %Ephemeroptera; (B) %EPT (Ephemeroptera, Plecoptera, and Trichoptera); and (C) %EPT–HBL (EPT less Hydropsychidae, Baetidae, and Leuctridae) by Month and specific conductance (SC) Level. Error bars are 1 standard error from the mean. Shaded boxes denote March–May and September–November periods. * Reference is significantly different from High-SC; + Reference and Medium-SC are significantly different from High-SC; ^ Reference is significantly different from Medium- and High-SC; and # Reference, Medium-SC, and High-SC are all significantly different from one another ($\alpha = 0.05$) for the designated month.

Percent EPT is a common bioassessment metric that is expected to decline with increasing stress in wadeable streams (Barbour et al., 1999). Here, %EPT was higher in Medium- (82 ± 1.5) and High-SC streams (81 ± 1.5) than in Reference streams (68 ± 1.6) (Table 4); and, hence, did not respond to elevated SC as expected. This result occurred because %EPT, as an aggregate metric prone to masking responses of sensitive taxa by inclusion of relatively tolerant taxa, was influenced by increased abundance of seemingly SC-tolerant families (Hydropsychidae, Baetidae, and Leuctridae) in elevated-SC streams.

3.2.6. Percent Ephemeroptera, Plecoptera, and Trichoptera less Hydropsychidae, Baetidae, and Leuctridae (%EPT–HBL)

Percent EPT–HBL was highest in Reference streams (39 ± 3.0) and lowest in High-SC streams (14 ± 2.8) (Table 4); and exhibited strong seasonal patterns for all SC Levels (Fig. 6C). Decreased relative abundance of Hydropsychidae in October–April and

Leuctridae in September–March corresponded with presence by taxa with fast seasonal life cycles (rapid growth, and emergence during winter/spring (Poff et al., 2006) such as Nemouridae, Taeniopterygidae, Capniidae, and several Ephemeroptera families which were reduced or not observed in Medium- and High-SC streams. Hence, winter and spring months exhibited significant %EPT – HBL differences between Reference and High-SC streams. Month-to-month variability was higher for %EPT – HBL than for %EPT at all SC Levels (Fig. 4). Similarly, in West Virginia, Pond et al. (2013) reported significant variation across seasons for %EPT less *Cheumatopsyche*.

A striking feature of our Medium- and High-SC stream communities was the increased presence of three taxonomic groups, Hydropsychidae, Baetidae, and Leuctridae, all of which exhibited strong seasonal patterns of occurrence and exerted strong influence on compositional metrics. It is unclear whether absolute abundances of Hydropsychidae, Leuctridae, and Baetidae increased with increasing SC or if their relative abundances increased due to reduced presence by other taxa. It is possible that the relative dominance of these three taxonomic groups may be influencing richness metrics in Medium- and High-SC streams by causing reduced probability for observation of non-HBL taxa in fixed-count samples. However, our findings of reduced Total Richness in elevated-SC streams are consistent with other studies (Green et al., 2000; Pond et al., 2008, 2014; Gerritsen et al., 2010; Timpano et al., 2015).

The %EPT – HBL metric was calculated to explore how exclusion of seemingly tolerant taxa would characterize macroinvertebrate community response to elevated SC; and how such characterization would compare with that provided by %EPT, which is commonly used in bioassessment (Barbour et al., 1999). The average proportion of %EPT comprised of HBL taxa increased with SC Level, from <30% for Reference to 66% in Medium-SC and >80% in High-SC streams. In contrast to the %EPT metric, %EPT – HBL demonstrates increasing deviation from reference conditions with increasing SC (Table 4) and strong seasonality (Fig. 6C). Pond et al. (2013) observed that exclusion of the Hydropsychidae genus *Cheumatopsyche* increased %EPT sensitivity to SC in West Virginia.

3.3. Summary, context, and significance

Eleven of 15 benthic macroinvertebrate community structure metrics differed among SC Levels (Table 4). Other studies also found presence of benthic macroinvertebrate genera (Cormier et al., 2013a,b) and community metrics (Green et al., 2000; Hartman et al., 2005; Pond et al., 2008, 2014; Gerritsen et al., 2010; Bernhardt et al., 2012; Timpano et al., 2015) to vary among Appalachian streams in association with elevated SC. Phenological patterns and their influence on community metrics were important to our findings. Others observing seasonal taxonomic shifts have attributed them to various causal factors, including life cycle phenologies (Butler, 1984; Johnson et al., 2012), seasonal changes in food resources (Vannote et al., 1980; Hawkins and Sedell, 1981; Álvarez-Cabria et al., 2011), and seasonal patterns of physicochemical variables such as stream temperature (Vannote and Sweeney, 1980). Others have observed these factors are related given that taxa emergence patterns may be synchronized with environmental conditions that vary with seasonal influence (Vannote et al., 1980).

Our study is unique in demonstrating strong temporal patterns for metrics used for biological assessment in central Appalachian coalfield streams, and that such patterns occur both in streams with elevated SC and in reference streams. Although Timpano et al. (2015) demonstrated seasonal differences in the response of a multimetric index to elevated SC, no prior studies in the Appalachian coalfield have shown seasonal patterns similar to those demonstrated here. These patterns are consistent with those

demonstrated in other regions (Šporka et al., 2006; Álvarez-Cabria et al., 2010).

Our findings demonstrate that, for certain benthic macroinvertebrate community metrics, the magnitude of deviation from reference condition varied within and among seasons as well as by SC Level. Ephemeroptera exhibited the greatest variability, with more month-to-month differences among SC Levels than other metrics (Fig. 5A), and generally higher modified CVs than other richness and relative abundance metrics, respectively, for both Reference and Medium-SC streams (Fig. 4). Lower intra-annual variation of aggregate metrics is expected, as they summarize the SC response of many taxa with overlapping life cycles, allowing incoming taxa to substitute for outgoing taxa throughout the year. Total Richness was lower and %Top 2 Dominant higher in elevated-SC streams relative to Reference. Hence, influences by seasonal growth and emergence cycles of remaining taxa other metrics were accentuated, therefore increasing those metrics' temporal variability. This suggests timing of sampling is important to characterize effects of SC on benthic macroinvertebrate communities, consistent with findings of Linke et al. (1999), Šporka et al. (2006), Álvarez-Cabria et al. (2010), Kosnicki and Sites (2011), and Johnson et al. (2012) in other regions. Although our study did not quantify effect thresholds, our Medium-SC streams (mean SC: 262–648 $\mu\text{S}/\text{cm}$) spanned a range of biotic-effect thresholds found by other studies (295 $\mu\text{S}/\text{cm}$, Cormier et al., 2013a; 426 $\mu\text{S}/\text{cm}$, Green et al., 2000; 500 $\mu\text{S}/\text{cm}$, Pond et al., 2008; 501 $\mu\text{S}/\text{cm}$, Freund and Petty, 2007; 560 $\mu\text{S}/\text{cm}$ in fall (Timpano et al., 2015) and our High-SC streams (mean SC: 756–1535 $\mu\text{S}/\text{cm}$) exceeded all but one biotic-effect threshold (903 $\mu\text{S}/\text{cm}$ in spring, Timpano et al., 2015).

4. Conclusions

Benthic macroinvertebrate community structure in Medium- and High-SC streams was altered, relative to Reference streams, as indicated by increases in seemingly tolerant genera and decreases in sensitive taxa including most Ephemeroptera genera relative to Reference, and by the 11 of 15 metrics showing significant differences among SC Levels. Our results support previous findings that elevated major-ion concentrations and TDS (measured by proxy as SC) are associated with alterations in benthic macroinvertebrate communities in mining-influenced Appalachian headwater streams, including those lacking significant influence from degraded physical habitat or other physicochemical conditions.

In our study, richness metrics were generally more sensitive to SC than aggregate and relative abundance metrics. In our study's Medium- and High-SC streams, %EPT was dominated by a few seemingly SC-tolerant taxa and thus reflected neither the diverse community structure indicative of reference streams, nor the EPT taxa losses and highly altered EPT-taxa distributions present in elevated-SC streams. This finding illustrates the importance of considering multiple levels of taxonomic resolution when evaluating benthic macroinvertebrate communities in elevated-SC streams. An alternate %EPT metric formulation, %EPT – HBL, which excluded the seemingly SC-tolerant EPT taxa Hydropsychidae, Baetidae, and Leuctridae, exhibited significant alteration in elevated-SC streams and may be better suited than %EPT to discriminate effects by elevated major ions on benthic macroinvertebrate communities in central Appalachian mining-influenced streams.

Reference streams showed less temporal variability than Medium-SC streams for the metrics analyzed, suggesting that moderate elevation in SC may accentuate natural temporal variability by decreasing Total Richness and evenness throughout the year, enabling dominant taxa (e.g., Leuctridae, Hydropsychidae, Baetidae, Capniidae, Nemouridae, Taeniopterygidae) to have greater

influence on bioassessment metrics. High SC streams also showed increased temporal variability for certain metrics (e.g. %Plecoptera) but also decreased variability for others (%Ephemeroptera), relative to Reference streams.

Both benthic macroinvertebrate community structure and magnitudes of deviation from reference condition for certain metrics vary temporally in elevated-SC streams; hence, time of sampling is an important consideration for bioassessment in central Appalachian coalfield streams. Reliance on one or a few aggregate metrics from samples collected infrequently during the year may not adequately characterize deviation from reference condition in mining-influenced streams where elevated major ions are present. Considering the extent of surface coal mining and its known influence on SC levels in headwater streams, our study results can support more effective monitoring and management of water resources in central Appalachia.

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