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Multiple stressors influence benthic macroinvertebrate communities in central Appalachian coalfield streams

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Abstract Headwater streams impacted by surface coal mining in the central Appalachian region of the eastern USA have characteristics not shared by reference-quality streams. These include elevated salinity, often measured using specific conductance (SC) and cited as a primary stressor of benthic macroinvertebrate communities. The study objective was to assess influence by mining-origin stressors on benthic macroinvertebrate community structure in headwater streams. Stream habitat characteristics were measured and benthic macroinvertebrates were sampled from 12 central Appalachian streams, 9 of which were influenced by mining. Multiple benthic macroinvertebrate community metrics, including Ephemeroptera density, richness, and composition were correlated negatively with watershed mining extent and with SC. Predator density and scraper richness were correlated negatively with watershed mining, stream-water selenium, and SC. Clinger richness was correlated positively with stream substrate characteristics including large cobble-to-fines ratios and relative bed stability, and was correlated negatively with watershed mining and SC. Relationships of predator density and scraper richness with selenium concentrations, and relationships of clinger richness with stream substrate characteristics, are consistent with stress mechanisms revealed by prior studies. Improved understanding of how habitat features are altered by mining and influence community structure in headwater streams can inform water resource management in mining areas.

Keywords Salinity · Fine sediment · Selenium · Quantitative sampling · Habitat · Mining

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Introduction

Surface coal mining has affected landscapes and watersheds over large areas in the central Appalachian coalfield of eastern USA (Pericak et al., 2018). Appalachian mining operations fracture and remove rocks overlying the coal seams and use those materials to reconstruct landscapes. Results include severe alterations of geologic structure, topography, and hydrology (Evans et al., 2015; Ross et al., 2016; Nippgen et al., 2017). As a consequence, numerous attributes of streams draining such landscapes, including chemistry, rainfall response, and sediment loadings are often affected as well (Negley & Eshleman, 2006; Fox, 2009; Pond et al., 2008, 2014). Stream characteristics such as water chemistry, physical habitat characteristics such as substrate composition and water temperature, and flow regime, which are influenced by surface coal mining in Appalachian landscapes (Wiley et al., 2001; Miller & Zégre, 2014, Timpano et al., 2018b) are principal drivers of benthic macroinvertebrate community structure in flowing freshwater streams (Gordon et al., 2004; Allan & Castillo, 2007).

Benthic macroinvertebrate community structure is often altered in streams emerging from mining-influenced Appalachian landscapes. Community-structural metrics representing taxonomic- and functional-group composition are often associated with indicators of water salinity, such as specific conductance (SC); when characterized in multiple streams, community-structural metrics such as total taxa richness, Ephemeroptera richness and relative abundance, taxonomic diversity, and richness of scraper taxa become increasingly dissimilar to a reference condition with increasing SC. (e.g., Pond et al., 2008; Boehme et al., 2016; Timpano et al., 2018a). Such alterations are consistent with effects of stream salinization as occurs in response to surface coal mining (Cormier et al., 2013a, b) and to numerous other anthropogenic activities worldwide (Cañedo-Argüelles et al., 2013; Castillo et al., 2017).

However, stream habitat measures such as streambed sediment size distributions and channel embeddedness (Green et al., 2000; Chambers & Messinger, 2001; Howard et al., 2001; Pond, 2004; Hartman et al., 2005) and concentrations of trace elements including selenium (Pond et al., 2008, 2014) are also altered commonly in mining-influenced streams, and are also associated with community-structural alterations (e.g., Pond, 2004; Whitmore et al., 2018). Hence, it appears that a variety of stressors may be acting to influence stream communities in mining-influenced Appalachian streams, even when physical habitat features that are not directly influenced by upstream mining such as riparian canopy remain intact. Yet, elevated salinity, as indicated by SC, is often considered to be the primary driver of the benthic macroinvertebrate community-structural changes that occur in mining-influenced Appalachian streams (Pond et al., 2008; Cormier et al., 2013a).

In this study, we measured multiple characteristics of mining-influenced streams to determine if stressors other than or in addition to SC were correlated with alterations of benthic macroinvertebrate community structure. The following questions were addressed: (1) Are stressors in addition to SC associated with responses of specific taxonomic and functional groups comprising the sampled benthic macroinvertebrate communities? and (2) Are those associations consistent with what is known about the underlying response mechanisms? Understanding how various environmental variables are associated with and influence community structure in these systems can inform stream management and restoration in streams affected by Appalachian coal mining.

Materials and methods

Stream selection

The study streams (i.e., sample units) were a subset of 1st- and 2nd- order streams (Strahler, 1957) selected by Timpano et al. (2015), including three reference streams and nine mining-influenced streams along a gradient of SC (Fig. 1). The streams were in Wise, Dickenson, and Buchanan Counties in Virginia, and McDowell, Raleigh, and Kanawha Counties in West Virginia; all were within the EPA Ecoregion level IV (69d; Woods et al., 1996; Fig. 1). A 100-m reach of each stream within a forested area was selected for study. All stream sampling sites had canopy cover of $> 90\%$ during summer months, as measured by a densitometer. Watersheds of all streams were devoid of residences, major roads, commercial or industrial facilities other than surface coal mines and small numbers of natural gas wells. With the exception of mined areas, all stream watersheds were predominantly forested. Study streams were selected such that reference streams had a similar range of catchment area as mining-influenced streams. Reference streams were defined as best available, without mining disturbance but otherwise similar to the mining-influenced

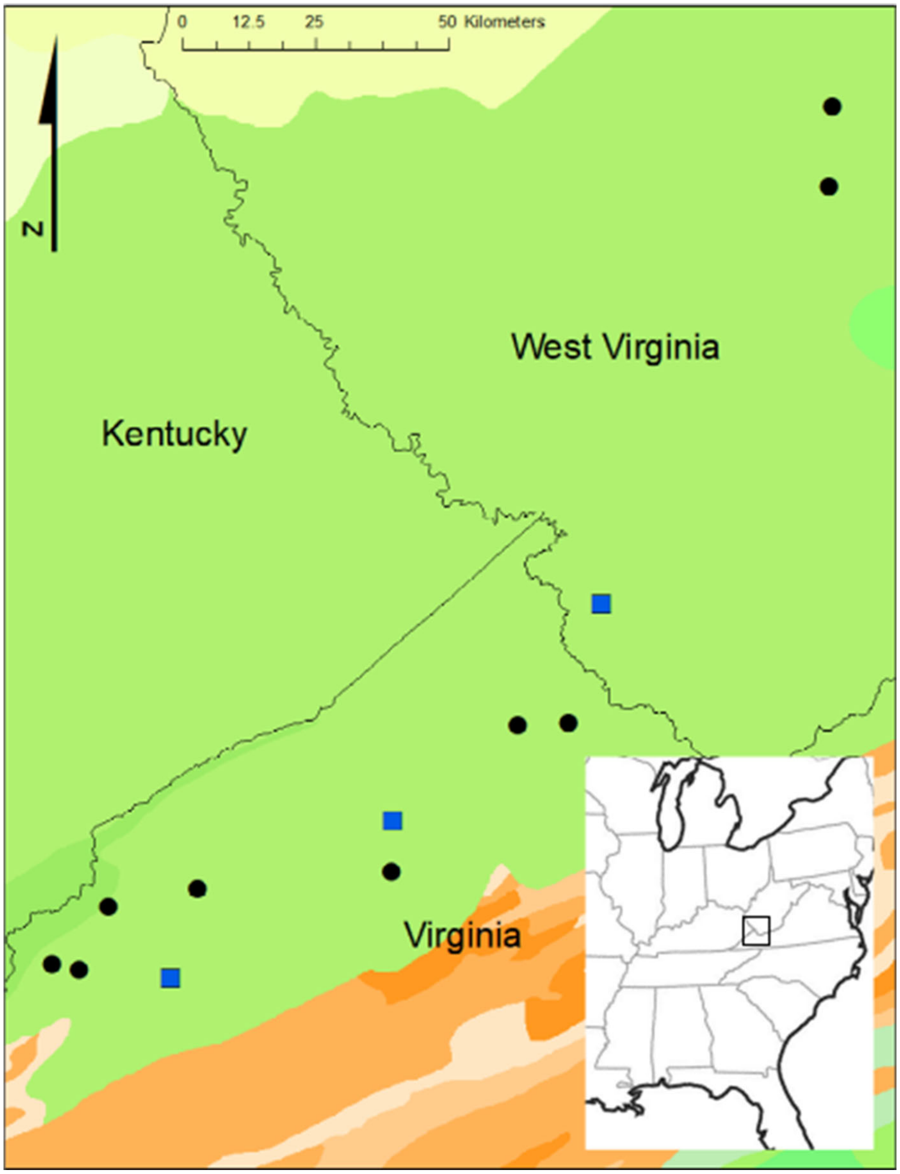


Fig. 1 Map of twelve study headwater streams in central Appalachia. Blue squares represent reference streams and black dots represent mining-influenced streams. Map colors represent level four ecoregions (U.S. EPA)

streams. All streams had pH > 7 as measured using a handheld multi-probe meter (YSI Professional Plus; Yellow Springs, OH, U.S.A.). Reference- and mining-influenced streams differed in major-ion composition (Supplementary data, Table S1; measured as reported by Timpano et al., 2018b) in a manner that is consistent with water-chemistry effects of ion release by coal-surface-mine spoils throughout central Appalachia (Pond et al., 2008, 2014; Daniels et al., 2016).

Data collection and processing

Benthic macroinvertebrates were sampled in April 2014, using a Hess sampler (Wildco; Yulee, FL, U.S.A.) with a 363-µm Nitex mesh. Six samples were collected from run habitat sections below randomly selected riffles in each stream; this sampling method was used because riffle sections were too shallow to obtain samples using the Hess sampler. For each sample collection, a Hess sampler was driven into the streambed sediment, completely enclosing 0.086 m^2 of stream bottom as needed to prevent organisms from drifting in or out. Samples were sorted quantitatively and insects were identified to the lowest practical taxonomic level (to genus level unless specimens were either too immature or damaged to key to genus) using keys provided in Merritt et al. (2008). As exceptions, regardless of condition of the specimen, Ceratopogonidae and Chironomidae were assigned to family level and Oligochaeta were assigned to class level.

Potential stressor variables are defined here as physical and chemical characteristics that are known to be altered in mining-influenced streams and have potential to impact benthic community structure.

Candidate stressor variables were selected based on previous studies that described impacts of mining land use on stream attributes. Candidate stressor variables used in this study included flow regime (e.g., Miller & Zégre, 2014), benthic sediment composition (e.g., Wiley et al., 2001), water temperature (e.g., Wiley et al., 2001), and water chemistry (e.g., Merriam et al., 2011) (Supplementary Data, Table S2).

Mined area, expressed as a fraction of watershed area ("mining"), was determined from Landsat satellite data as described by Li et al. (2015) and with reference to Timpano et al. (2017), which included cumulative percent area mined for all the catchments during the years 1980–2011 and 1980–2016. For most catchments, simple interpolation of the two values produced estimates for cumulative percent area mined from 1980 to 2014.

Solinst Edge Leveloggers (Solinst Canada Ltd.) were installed in each stream, paired with Barologgers (Solinst Canada Ltd.) placed on stream banks in September 2013 to measure stage at 15-min intervals. Six times during the study, stream velocity, wetted width, and water depth were measured along lateral transects at two-to-three points in each stream, from which discharge was calculated using the velocity-area method (Gore, 2007). Stage was measured at the Leveloggers simultaneous to measurements of discharge components. Rating curves were developed from stage measurements and discharge calculations and applied to the Levelogger stage records from February to April 2014 to produce time-series of discharge. Unit-area discharge was calculated as discharge divided by basin area. Several statistics were calculated for discharge and unit-area discharge: high (95th percentile), low (5th percentile), median, high-low range, median-low range, and median-high range. Flashiness for each stream was quantified by applying the Richards-Baker Flashiness Index (Baker et al., 2004) to discharge records from February to December 2014.

In March 2014, a modified Wolman (1954) count of 200 pebbles was performed from lateral transects along a 100-m reach in each stream. Ten pebbles were collected from each of 20 transects, which were randomly chosen across run sections of the streams. Only pebbles within the wetted channel were collected and measured. Measurement of each pebble size was taken as the length of the "B-axis" (i.e., 2nd longest axis) of the pebble using a meter stick.

Variables chosen to represent sediment characteristics were percentiles of the sediment distributions (10, 16, 25, 50, 75, 84, and 90; expressed as D_{10}, D_{16}, etc.), proportion of sediment that occurs in each size class (e.g., % fines, % gravel, % cobble) based on the Udden-Wentworth grain-size classification system (Wentworth, 1922; Blair & McPherson, 1999), ratio of % small cobble (64–128 mm) to % fines (< 2 mm), ratio of % large cobble (128–255 mm) to % fines (< 2 mm) (LCF), interquartile range of sediment distributions, Simpson's diversity of size-class proportions, and ratio of D_{84} to D_{50}.

Relative bed stability is an index of the propensity of the streambed substrate to shift (Kaufmann et al., 1999). Kaufmann et al. (2008) derived a roughness-corrected index of relative bed stability for regional stream surveys. They used mean thalweg depth to arrive at bankfull hydraulic radius. The present study modified that method by measuring and calculating mean bankfull hydraulic radius from 11 cross-sections within a 150-m reach of each stream. Mean slope for the reach was estimated by measuring slope with an Abney level and leveling rod at the water surface at each cross-section, then taking the arithmetic mean of all measurements. Measurements were taken in June 2014. The log of relative bed stability (LRBS) was calculated as follows:

$$\mathrm{LRBS} = \log_{10}(D_{50}/13.7 * R_{\mathrm{bf}} * S)$$

where D_{50} was the median pebble size from pebble counts conducted in March 2014, R_{bf} was mean bankfull hydraulic radius, and S was mean slope expressed as rise/run.

Each of the streams was equipped with a HOBO conductivity logger (Onset Computer Corp., Bourne, MA) set to sample electrical conductivity and temperature every 15 min, from which SC was calculated using Onset HOBOware Pro v.3.4.1 software. At the time of logger-data download, water temperature, dissolved oxygen, SC, and pH were measured using a handheld multi-probe meter (YSI Professional Plus; YSI Incorporated, Yellow Springs, Ohio) near the conductivity logger. These in situ measurements were used in HOBOware to calibrate the conductivity loggers and adjust for logger-data drift. The multimeter probe was calibrated using a standard solution of KCl before each use as per APHA (2005). The variable "SC" as used in the analyses of this study was mean SC calculated from the time-series SC data for the period of mid-January–mid-April 2014. Stream temperature minimum, maximum, median, mean, interquartile range, and total range were calculated for the periods of April 2013–April 2014 (Annual), June–August 2013 (Summer), and November 2013–February 2014 (Winter).

Samples of stream water were collected from the thalweg of each stream at the time of benthic macroinvertebrate sampling, filtered, and acidified with nitric acid to $\mathrm{pH} < 2$ streamside. Concentrations of nickel, manganese, selenium, zinc, aluminum, and iron were measured using inductively coupled plasma mass spectrometry (Perkin-Elmer, Norwalk, CT). For each analyte, a method detection limit (MDL) and method reporting limit (MRL) were determined by the instrument operator. Analyte concentrations measured at $< \mathrm{MRL}$ but $\geq \mathrm{MDL}$ were modeled using the instrument-measured values.

Data analyses

Response variables were genus-level richness determined by counting numbers of taxa identified in the six samples (total sampled area = 0.516 m^2) for each stream, densities (number of individuals per square meter), Shannon and Simpson diversities, evenness (% top 2, 3, and 5 dominant taxa), and compositions (fraction of total, expressed as percent). Densities were calculated for each stream as mean abundance of benthic macroinvertebrates in the six samples divided by area of the Hess sampler (0.086 m^2).

Benthic macroinvertebrate response metrics were selected for analysis based on groups whose characteristics describe ecological relationships (Supplementary Data, Table S3). Functional feeding groups (FFGs; e.g., shredders, filterers, scrapers, gatherers, predators) represent ecosystem functions such as nutrient spiraling and organic matter processing (Vannote et al., 1980; Wallace & Webster, 1996). Taxonomic groups (e.g., Ephemeroptera, Plecoptera, Trichoptera, or EPT) known to be sensitive to SC and other types of pollution are widely employed in bioassessments (Plafkin et al., 1989; Wallace et al., 1996). Some families (e.g., Baetidae, Hydropsychidae, and Leuctridae) within the orders of EPT are pollution-tolerant, can be dominant seasonally, and may respond to stressors differently from other taxa of groups in which they are members. For this reason, some groups were presented with and without the dominant families included in analyses to refine determination of group responses.

A Spearman rank correlation matrix with the candidate stressor variables and response metrics was constructed. Benthic macroinvertebrate response metrics of interest were chosen based on having significant correlations ($P < 0.05$) with SC. Stressor variables retained for further analysis were those that were correlated ($P < 0.05$) with mining and were judged to best represent stressor variables with similar and redundant mechanisms.

Results

Benthic macroinvertebrate metrics correlated with potential stressor variables

Twenty-three of 68 benthic macroinvertebrate metrics were correlated with SC (Table 1); all were also correlated with mining. Most of the correlations were negative except for (1) shredder-minus-Leuctridae, (2) Plecoptera-minus-Leuctridae, (3) percent sprawlers, and (4) percent shredders, which had positive correlations with SC and with mining.

Eight of 87 potential stressor variables were significantly correlated with mining; four of those potential stressor variables (Table 2; Fig. 2) were selected for further study: SC, selenium (Se), large cobble-to-fines ratio (LCF), and log relative bed stability (LRBS). The other four potential stressor variables (percent fines, percent cobble, D_{16}, and D_{25}) were not selected for further analysis because they were redundant or similar in mechanism (i.e., loss of habitat; alteration of substrate size distribution) with LCF. Also, LCF exhibited more significant correlations with selected benthic macroinvertebrate metrics

Table 1 Spearman correlations between selected benthic macroinvertebrate metrics and selected stressors in headwater streams influenced by mining in central Appalachia

Group[a]	Mining	SC[c]	Se[c]	LCF[c]	LRBS[c]
Density					
E-B[b]	− 0.81**	− 0.87***	− 0.69*	0.73**	0.71*
E	− 0.75**	− 0.83**	− 0.70*	0.73**	0.61*
Swimmer	− 0.65*	− 0.68*	− 0.78**	0.71**	0.58*
Shredder-L[b]	0.89***	0.67*	0.62*	− 0.61*	− 0.65*
Predator	− 0.70*	− 0.63*	− 0.89***	0.45	0.53
P-L[b]	0.87***	0.60*	0.41	− 0.71**	− 0.78**
Richness					
Gatherer	− 0.89***	− 0.80**	− 0.72**	0.64*	0.67*
E-B[b]	− 0.73**	− 0.79**	− 0.80**	0.70*	0.63*
E	− 0.74**	− 0.78**	− 0.78**	0.73**	0.65*
EPT	− 0.77**	− 0.70*	− 0.77**	0.82**	0.77**
EPT-BHL[b]	− 0.75**	− 0.66*	− 0.69*	0.79**	0.72**
Total	− 0.79**	− 0.65*	− 0.77**	0.81**	0.87***
Scraper	− 0.75**	− 0.63*	− 0.78**	0.62*	0.70*
Swimmer	− 0.64*	− 0.62*	− 0.58*	0.69*	0.55
Clinger	− 0.76**	− 0.62*	− 0.77**	0.82**	0.83***
Diversity					
Simpson	− 0.81**	− 0.59*	− 0.61*	0.89***	0.75**
Shannon	− 0.87***	− 0.58*	− 0.55	0.80**	0.75**
Composition					
% E-B[b]	− 0.84***	− 0.87***	− 0.68*	0.77**	0.72**
% E	− 0.80**	− 0.83**	− 0.68*	0.80**	0.66*
% Sprawler	0.88***	0.71**	0.44	− 0.65*	− 0.71**
% Swimmer	− 0.64*	− 0.68*	− 0.68*	0.69*	0.58*
% Shredder	0.84***	0.68*	0.38	− 0.65*	− 0.72**

[a] *E* Ephemeroptera, *P* Plecoptera, *B* Baetidae, *H* Hydropsychidae, *L* Leuctridae

[b] Minus sign in group name indicates exclusion of dominant taxon (B, H, and/or L)

[c] *SC* specific conductance, *Se* water-column selenium, *LCF* large cobble-to-fines ratio, *LRBS* log relative bed stability

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

Table 2 Stressor variable ranges across all study streams

Stressor	Min	Max	Unit
Mining	0	65	%
Specific conductance	24	1,445	μS cm^{-1}
Selenium	0.7[a]	15.7	μg l^{-1}
Large cobble: fines	0.4	2.1	ratio
Log relative bed stability	− 1.10	− 0.39	ratio

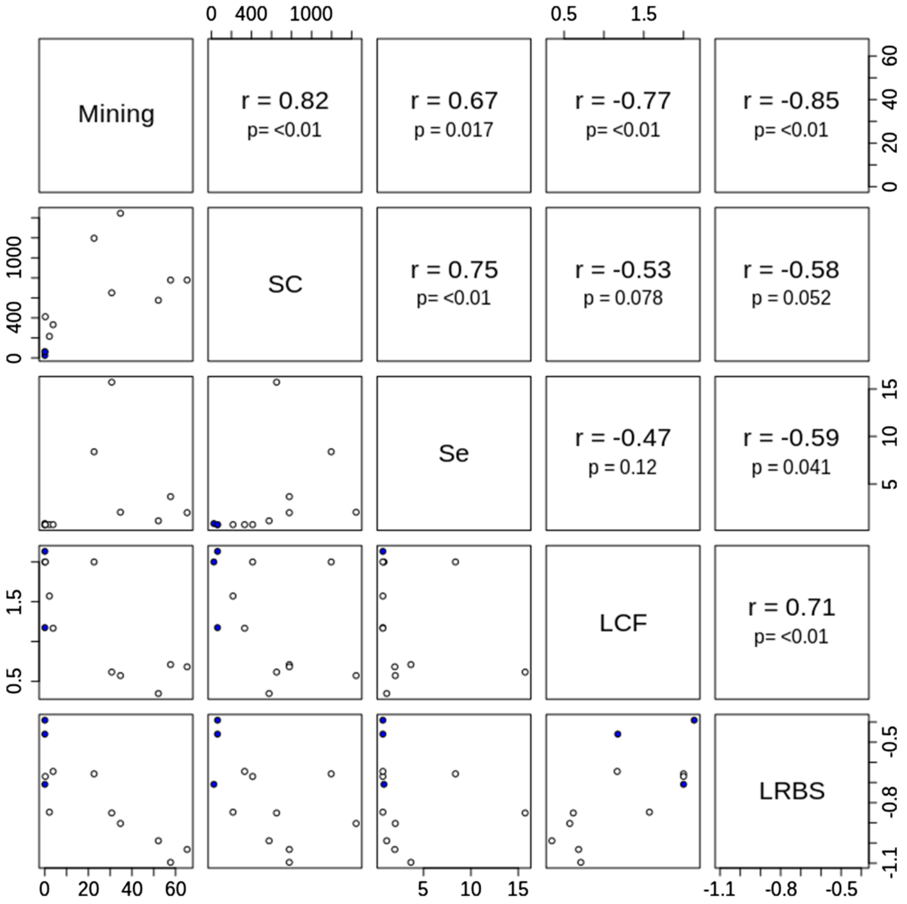
[a] Method detection limit

than the non-selected potential stressor variables. Specific conductance and Se were correlated with one another but were not correlated with LCF or LRBS (Fig. 2).

Six of the streams had mining areas comprising $< 4\%$ of watershed area (i.e., "low-mining"), whereas the other six had mining areas ranging from 23 to 65% of watershed area (i.e., "high-mining"). Five of the streams, including one of the reference streams, had LCF ratios < 1, indicating substrates with percent fines greater than percent large cobbles. All streams were considered "impaired" (LRBS $<$ 0.2) according to relative bed stability categories of Kaufmann et al. (1999), with two of these streams considered "highly impaired" (LRBS $< -$ 1.0).

Generally, streams with low mining had nominally different levels of the selected stressor variables compared with high-mining streams (Fig. 2). Most (5 out of 6) of the high-mining streams were characterized by SC > 575 μS cm^{-1}, Se > 1.0 μg l^{-1}, LCF < 0.7, and LRBS $< -$ 0.85. One stream had higher LCF (2.0) and LRBS (− 0.66) than all other streams, and its SC and Se concentration were 2nd highest of all the streams in this study. All low (0–4%)

Fig. 2 Spearman correlation matrix of stressor variables influencing benthic macroinvertebrate community metrics, with scatterplots in the lower triangle, and Spearman rho coefficients (*r*) and *P* values in the upper triangle. Units for axes are as follows: Mining = %, specific conductance (SC) = µS cm^{-1}, selenium concentration in water (Se) = µg l^{-1}, large cobble-to-fines ratio (LCF), and log of relative bed stability (LRBS) = unitless ratios



mining streams were characterized by SC < 412 µS cm^{-1}, Se < 0.8 µg l^{-1}, LCF > 1.2, and LRBS > − 0.85.

Benthic macroinvertebrate relationships with selected stressor variables

Leuctra and Chironomidae were the most abundant of the 98 benthic macroinvertebrate taxa collected, comprising 29–77% of the total density among the 12 study streams. Taxon richness ranged from 41 to 62 in the low-mining streams and from 22 to 38 in the high-mining streams. EPT richness comprised a mean (± SD) of 66% (± 4%) and 47% (± 10%) of the total community richness in low-mining and high-mining streams, respectively.

Among observed taxa, *Leuctra*, Chironomidae, and Oligochaeta were ubiquitous, occurring in all streams. Other common taxa differed between low-mining and high-mining streams. In low-mining streams, *Acentrella*, *Ephemerella*, *Drunella*, and *Cinygmula* (all Ephemeroptera) were among the most common genera, whereas in high-mining streams *Amphinemura* (Plecoptera), *Oulimnius* (Coleoptera), *Diplectrona* (Trichoptera), and *Chelifera* (Diptera) were among the most common genera.

Ephemeroptera density was correlated strongly with SC (Table 1; Fig. 3a). Ephemeroptera richness was correlated strongly with SC (Fig. 3b) and Se. Percent Ephemeroptera was correlated strongly with SC (Fig. 3c) and mining. Gatherer richness also was correlated strongly with SC and with mining. Gatherers were composed of 13–57% and 0.2–3% represented by Ephemeroptera, whereas Ephemeroptera comprised 54–75% and 0–38% total density, in low-mining/low-SC and high-mining/high-SC streams respectively.

Predator density was correlated strongly and negatively with Se (Table 1; Fig. 3d). At the stream with the highest Se concentration (15.7 µg l^{-1}), 10

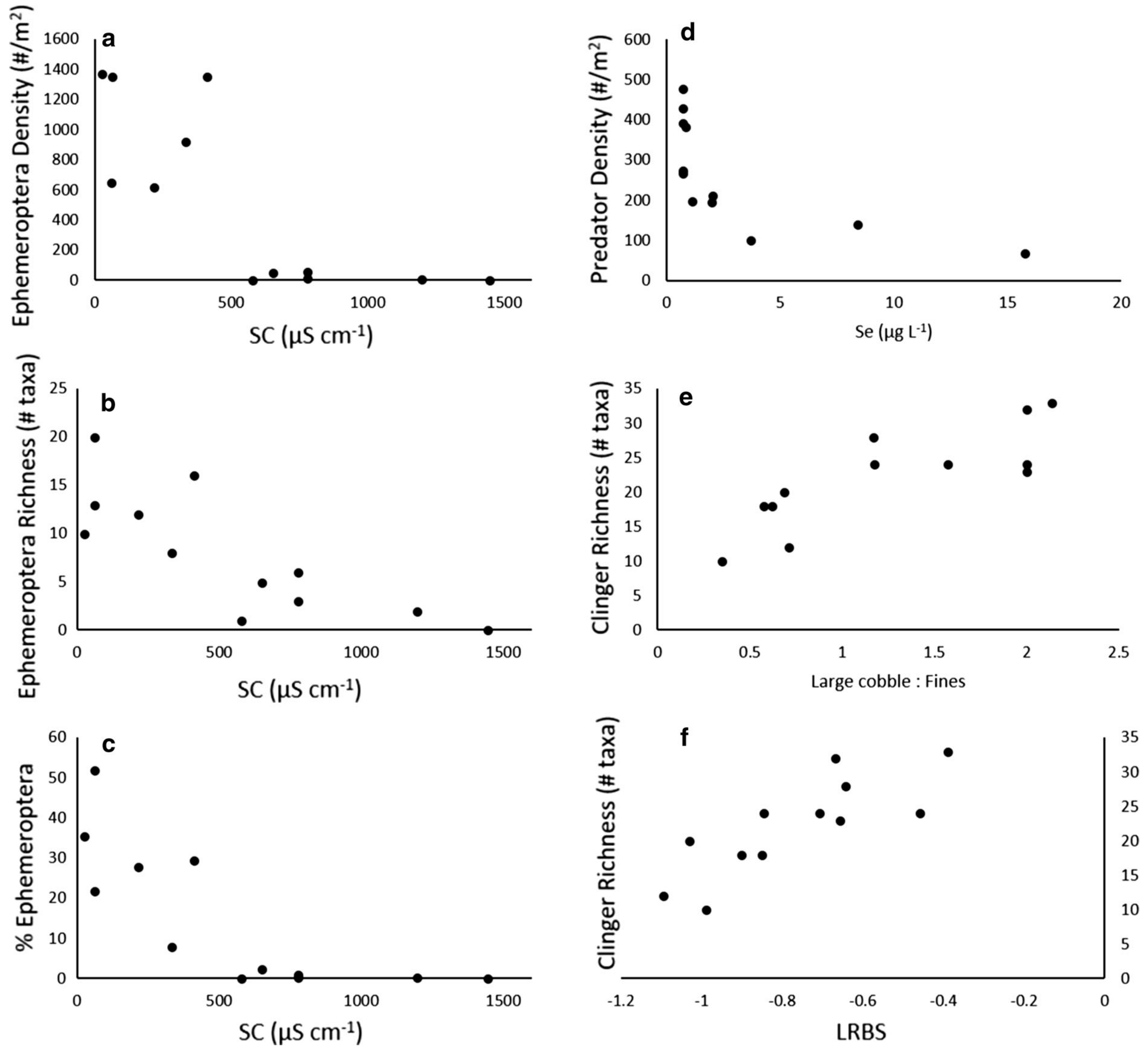


Fig. 3 Scatterplots of selected stressor variables versus selected benthic macroinvertebrate metrics. Ephemeroptera density, richness, and relative abundance were all negatively correlated with specific conductance (**a**–**c**); Predator density was negatively correlated with water-column selenium (**d**); Clinger richness was positively correlated with large cobble-to-fines ratio and log relative bed stability (**e**, **f**)

predator taxa were found, with a combined density of 50 individuals m^{-2}. In contrast, streams with Se < 3.1 µg l^{-1} (EPA ambient water-quality criterion, 30-day exposure; U.S. EPA, 2016) had a mean (± SD) of 14 (± 3) predator taxa, with a mean (± SD) density of 240 (± 83) individuals m^{-2}. Predator density was not correlated with either of the substrate stressor variables (LCF and LRBS).

Scraper richness was also strongly and negatively correlated with Se (Table 1). Only one scraper genus, the riffle beetle *Optioservus*, maintained higher densities (141 m^{-2} and 83 m^{-2}) at the two streams with the highest Se concentrations. All other scraper taxa were present at densities < 20 m^{-2} and most scrapers had densities < 5 m^{-2} at the streams with Se concentration > 3.1 µg l^{-1}.

Shredder-minus-Leuctridae and Plecoptera-minus-Leuctridae densities were correlated strongly and positively with mining. Leuctridae was dominant in every stream, so use of Leuctridae-exclusion metrics allowed for patterns of non-Leuctridae taxa to emerge. Percent sprawler and percent shredder were correlated strongly and positively with mining and were not correlated with Se.

Clinger, EPT, and total richness were correlated strongly with the two substrate stressor variables, LCF and LRBS (Table 1). Although there was evidence of clinger response to LCF (Fig. 3e), there was no evidence of burrower response. Clinger taxa richness decreased at a rate of approximately 1.2 taxa per percent fines increase. Taxa richness from the total population decreased at a rate of approximately 1.8 taxa per percent fines increase.

Discussion

Potential causal pathways

Results are interpreted to identify apparent linkages among selected stressor variables and benthic macroinvertebrate community metrics (Fig. 4) as indicated by presence of significant correlations and support derived from peer-reviewed scientific studies. This section focuses on identification and evaluation of those linkages, culminating in increased understanding of how specific stressors appear to affect specific community components in our study streams. Such apparent linkages are described as potential causal pathways.

Pathways among stressor variables

Mining (% of watershed area) was correlated with all other selected stressor variables and was considered causal to those stressor variables (Fig. 2). Mining was positively correlated with SC and Se, as also found by previous studies (Bryant et al., 2002; Pond et al., 2008; Lindberg et al., 2011; Cormier et al., 2013b). There is ample scientific evidence that accelerated weathering of rock fractured and distributed by Appalachian surface mining is the cause of elevated SC and Se that are commonly found in mining-influenced Appalachian streams (e.g., Lindberg et al., 2011; Griffith et al., 2012; Daniels et al., 2016; Clark et al., 2018). Streams draining surface mines and valley fills often carry higher fine-sediment loads than in unmined watersheds (Bonta, 2000; Wiley et al., 2001), similar to our findings of negative correlation between mining and LCF. Relative bed stability is based on the ratio of actual substrate size to expected substrate size given geomorphological measurements (Kaufmann et al., 1999), meaning that an unstable stream will have finer sediments than would a more stable stream of its size,

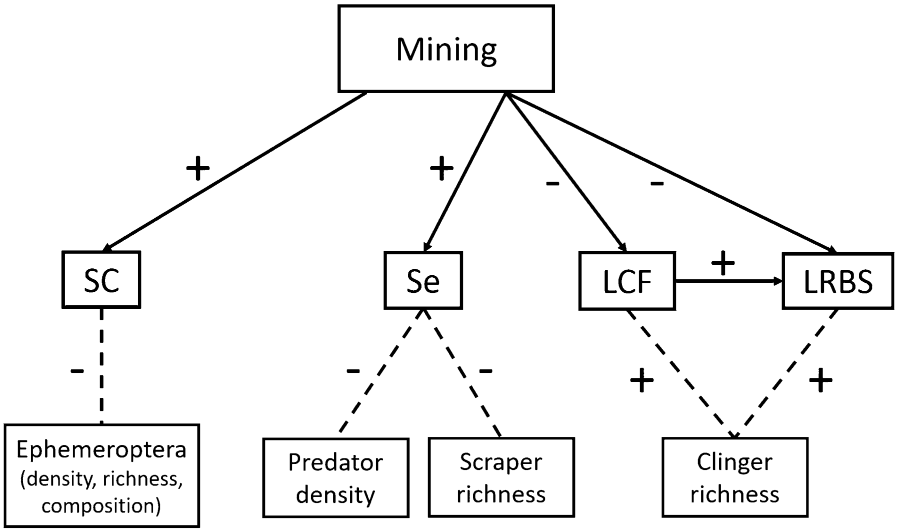


Fig. 4 Potential causal pathways among selected stressor variables and selected benthic macroinvertebrate metrics in headwater streams in central Appalachia. Signs next to arrows indicate correlation directions (positive or negative). Dotted lines indicate that causal logic, although supported by literature, is more tenuous for biotic pathways than for abiotic pathways. Stressor variables are specific conductance (SC), selenium concentration in water (Se), large cobble-to-fines ratio (LCF), and log of relative bed stability (LRBS)

slope and shape. The positive correlation between LCF and LRBS (Fig. 2) is consistent with the observation that larger substrate is generally more stable than finer substrate.

Responses by benthic macroinvertebrates

Mining

Mining, expressed here as a fraction of watershed area mined, is a catchment-scale stressor variable that captures, and likely causes, cumulative effects of the other stressor variables in this study. Results of this study support findings by Bruns (2005), who also found EPT and total richness to be inversely related to the watershed fraction affected by mining. Pond et al. (2008) reported lower Total, EPT, and Ephemeroptera richness, percent Ephemeroptera, and Shannon diversity values in streams draining mined watersheds, relative to unmined references.

Specific conductance

Ephemeroptera decline has been associated with increasing SC in recent studies of the impacts of surface coal mining (e.g., Pond, 2010; Timpano et al., 2018a). Osmotic stress has been suggested as a mechanism for effects of elevated levels of major ions on benthic macroinvertebrates (e.g., Pond et al., 2008; Cañedo-Argüelles et al., 2013). Ephemeroptera are highly susceptible to osmotic stress (e.g., Hassell et al., 2006), possibly because of relatively large exchange epithelial surfaces on the gills of some taxa (Buchwalter et al., 2003). 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 (Pond, 2010). 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 utilized for growth and other functions, leading to osmotic stress. Ephemeroptera density decreased to zero in some high-SC streams, providing supporting evidence for the hypothesis that water solutions of the major ions that are commonly elevated in alkaline mining-influenced Appalachian streams (i.e., calcium, magnesium, sulfate, and bicarbonate, as per Pond et al., 2008, 2014; and this study) can function as a toxicant for Ephemeroptera.

Selenium

Elevated levels of Se in the water column at levels observed in this study do not necessarily translate into Se toxicity because dietary Se, not direct exposures to the water column, is the most common mechanism of Se toxicity; and because there are numerous pathways for Se transfer and accumulation in the aquatic environment (Fan et al., 2002; Sappington, 2002). Prior studies have documented that benthic macroinvertebrates can bioaccumulate Se in mining-influenced streams that are contaminated by elevated Se (Wayland & Crosley, 2006; Arnold et al., 2014; Whitmore et al., 2018) and suggest that elevated water-column Se can cause toxic effects to benthic macroinvertebrates via food-chain bioaccumulation mechanisms (Debruyn & Chapman, 2007; Conley et al., 2009).

Predator density was strongly and negatively correlated with water-column Se concentrations (Table 1; Fig. 3d), a finding that is consistent with a Se bioaccumulation mechanism as cause for reduced predator densities in high-SC streams. Several studies have shown that Se bioaccumulation occurs via transfer from the water column to primary producers (Presser & Barnes, 1984; Besser et al., 1989); from primary producers to primary consumers (Conley et al., 2009); and from primary consumers to higher trophic levels (e.g., macroinvertebrate predators; Dubois & Hare, 2009), concentrating Se in tissue to 100–30,000 × greater than water-column levels (Lemly, 1999; Mason et al., 2000; Swift, 2002). Whitmore et al. (2018) found macroinvertebrate predator whole-body Se concentrations to be ~ 10,000 × water-column levels in mining-influenced Appalachian streams.

Ephemeroptera richness was also correlated strongly and negatively with water-column Se concentrations (Table 1). Conley et al. (2009) reported reduction in fecundity for the mayfly *Centroptilum triangulifer* (McDunnough, 1931) in mesocosms with water-column Se concentrations within the range reported in this study. *C. triangulifer* is classified in the scraper FFG. Scraper richness in this study also responded strongly and negatively to water-column Se concentrations (Table 1), perhaps related to what could potentially be a ~ 1,000-fold increase in Se concentration from the water column to biofilm (Conley et al., 2011; Whitmore et al., 2018).

Large cobble-to-fines ratio

Sediment substrate is an important reach-scale determinant of benthic community structure (Rabeni et al., 2005; Larsen et al., 2011). Excessive deposition of fine sediments among gravels and cobbles causes embeddedness and habitat reduction, which in turn causes reduction in density and diversity of benthic macroinvertebrate taxa (Lenat et al., 1981; Duan et al., 2008).

Certain taxa of benthic macroinvertebrates exhibit substrate composition preferences (Erman & Erman, 1984; Waters, 1995). For example, burrowers are found in finer sediment (Rabeni et al., 2005; Larsen et al., 2011), whereas clingers occur in more stable and larger substrate (Rabeni et al., 2005; Pollard & Yuan 2010). Clinger richness was correlated strongly and positively with LCF in this study (Table 1; Fig. 3e), which implies that more clinger taxa occur in streams with higher percent cobble and/or lower percent fines. This could be associated with any combination of the stability or ample available interstitial (Waters, 1995; Bo et al., 2007) and surface space of the larger cobble in comparison to the finer sand and gravel. Rabeni et al. (2005) found significant decreases in clinger richness with increasing percent fines. Bo et al. (2007) reported 75% of Heptageniidae taxa (a clinger) in gravel-filled traps ($> 70\%$ gravel), and in higher abundances than in sand-filled traps ($> 70\%$ sand). Burrowers in this study did not appear to respond, either in density or richness, to incrementally higher proportions of fine sediment among streams. This was possibly because the relative increases of fine sediment were not enough to promote more habitat for burrowers in the runs sampled, or because another stressor was confounding the response. However, the response by clingers suggests that 15.5% fines (maximum % fines, Table 2) was sufficient to limit clinger taxa richness (Fig. 3e). Bryce et al. (2010) reported optimum proportions of sand and fines (≤ 2 mm) for 8 EPT taxa (all clingers) ranging from 7.3 to 11.4% based on similar pebble count methodology as employed in this study.

Relative bed stability

An LRBS value of 0.2 indicates that the streambed is stable; increasing departure from this value indicates increasing instability (Kaufmann et al., 1999). A negative LRBS value can result from a streambed that is composed of high proportions of fine sediment and/or has high bed shear stress (Kaufmann et al., 2008) and indicates that the streambed is subject to shift at flows smaller than bankfull. All streams in this study had negative LRBS values, including reference streams (Fig. 3f). In general, streams with lower percent mining and higher LCF had higher LRBS (Fig. 2). Clinger-, EPT-, and Total richness were strongly correlated with all three of these environmental variables, likely because lower LRBS is associated with finer bedload and embeddedness, leaving less habitat for those taxa that need large substrate for either protection from high velocities (McClelland & Brusven, 1980) or collection of food (Waters, 1995). Low LRBS values also indicate potential for entrainment and movement of fine sediments, as either suspension or saltation load, which can lead to abrasion (Vogel, 1994) and increased drift (Culp et al., 1986).

Those taxa found in streams with low LRBS are expected to have adaptations that accommodate shifting substrate habitat. Of the taxa found in the two streams with LRBS values rated as "highly impaired" based on Kaufmann et al. (1999), the most common were *Leuctra*, Chironomidae, *Amphinemura*, and Oligochaeta. Most of the remaining taxa had relatively low (< 50 m^{-2}) densities. Chironomidae and Oligochaeta are generally classified as burrowers, and *Leuctra* and *Amphinemura* are sprawlers (Merritt et al., 2008). Neither of these habits requires use of large particle size and stable substrates. In contrast, Ephemeroptera swimmers and clingers such as *Ephemerella*, *Habrophlebiodes*, *Ameletus*, *Diphetor*, and *Drunella* were among the most common taxa in the two streams with highest LRBS. Both swimmers and clingers prefer large stable substrates where they can seek refuge in the spaces between and underneath the substrate (Merritt et al., 2008).

Conclusions

Numerous studies have found that benthic macroinvertebrate communities are altered in Appalachian mining-influenced streams with high SC. This research confirms those findings and supports suggestions from prior researchers that elevated major-ion concentrations in mining-influenced streams are a significant factor associated with such alterations.

However, this research revealed other mechanisms that may contribute to the benthic macroinvertebrate community alterations occurring in high-SC streams. We found that predator and scraper densities were depressed in high-SC streams. Predators and scrapers also appeared to respond strongly and negatively to elevated Se in the water column. This consistency with a documented mechanism suggests that Se bioaccumulation may be influencing alterations in benthic macroinvertebrate communities in these streams.

These findings also revealed that elevated fine sediments in substrates of streams influenced by mining may be altering communities in those streams. Evidence for this mechanism is most direct for clinger taxa, which are depressed in streams with substrates characterized by high amounts of fine sediments and potential instability—headwater stream responses that are often associated with mining activity.

Results suggest mining influences multiple habitat- and water-quality attributes of headwater streams, and that those attributes may influence the benthic macroinvertebrate community structure in specific ways. The ability to draw linkages between habitat characteristics, in addition to elevated SC, and specific benthic macroinvertebrate community structure alterations can aid stream management. Improved understanding of how multiple habitat features are altered by mining and, in turn, influence community structure in headwater streams can inform water resource management in mining areas.

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Data availability Data are posted for public access at VTechData: D. Drover, Multiple stressors influence benthic macroinvertebrate communities in central Appalachian coalfield streams. https://doi.org/10.7294/4aq8-0955.

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