

**Benthic macroinvertebrate community structure responses to multiple stressors in
mining-influenced streams of central Appalachia USA**

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ACADEMIC ABSTRACT

Headwaters are crucial linkages between upland ecosystems and navigable waterways, serving as important sources of water, sediment, energy, nutrients and invertebrate prey for downstream ecosystems. Surface coal mining in central Appalachia impacts headwaters by burying streams and introducing pollutants to remaining streams including excessive sediments, trace elements, and salinity. Benthic macroinvertebrates are widely used as indicators of biological conditions of streams and are frequently sampled using semi-quantitative methods that preclude calculations of areal densities. Studies of central Appalachian mining impacts in non-acidic streams often focus on biotic effects of salinity, but other types of pollution and habitat alteration can potentially affect benthic macroinvertebrate community (BMC) structure and perhaps related functions of headwater streams. Objectives were: 1) use quantitative sampling and enumeration to determine how density, richness, and composition of BMCs in non-acidic central Appalachian headwaters respond to elevated salinity caused by coal surface mining, and 2) determine if BMC structural differences among study streams may be attributed to habitat and water-quality effects in addition to elevated salinity. I analyzed BMC structure, specific conductance (SC, surrogate measure of salinity), and habitat-feature data collected from 15 streams, each visited multiple times during 2013-2014. BMC structure changed across seasonal samples. Total benthic macroinvertebrate densities did not appear to be impacted by SC during any months, but reduced densities of SC-sensitive taxa were offset by increased densities of SC-tolerant taxa in high-SC streams. Total richness also declined with increasing SC, whereas BMCs in high-SC streams were simplified and dominated by a few SC-tolerant taxa. Taxonomic replacement was detected in high-SC streams for groups of benthic macroinvertebrates that did not exhibit density or richness response, showing that taxonomic replacement could be a valuable tool for detecting BMC changes that are not evident from analyses using conventional metrics. Specific conductance, water-column selenium concentration, large-cobble-to-fines ratio of stream substrate, and relative bed stability were associated with changes in BMC structure. These results suggest multiple stressors are influencing BMCs in mining-influenced Appalachian streams. These findings can inform future management of headwater streams influenced by mining in central Appalachia.

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PUBLIC ABSTRACT

Humans rely on headwater streams to provide water of sufficient quantity and quality for use in fishing, swimming, and other designated purposes. In central Appalachia USA, surface coal mining impacts headwaters by burying streams and introducing various types of pollutants to remaining streams. Benthic macroinvertebrates, primarily stream insects, are widely used as indicators of stream-water quality because responses by certain groups of benthic macroinvertebrates to types and levels of pollution are known. Studies of central Appalachian coal mining impacts in non-acidic streams often focus on the effects of elevated salinity on benthic macroinvertebrate community (BMC) structure; however, other types of pollution and habitat alteration can also potentially affect BMC structure. Study objectives were to determine how BMCs in central Appalachian headwaters responded to elevated salinity caused by coal surface mining, and to determine if BMC structure differences among study streams may be caused by habitat and water-quality effects other than elevated salinity. I analyzed BMC, salinity, and habitat data collected from 15 streams, each visited up to six times during 2013-2014. I found that BMC structures varied seasonally, likely caused by natural life cycle variation of benthic macroinvertebrates. Streams in watersheds impacted by more areal coal mining disturbance tended to have higher levels of salinity, water selenium concentrations, and fine sediments, and fewer types of benthic macroinvertebrates although total BMC densities were comparable to those in less-disturbed streams. The study revealed that BMCs in mining-influenced streams were less diverse and were likely functioning less effectively than BMCs in forested reference streams. Also, the measurement and analysis methods used to characterize BMC structural responses to pollution influence assessments of stream condition. Understanding the responses of benthic macroinvertebrate structure to changes in habitat and water quality in mining-influenced streams can inform future stream management efforts.

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CHAPTER 1 – Introduction

Background

Headwaters are crucial linkages between upland ecosystems and navigable waterways (Freeman *et al.* 2007, Wipfli *et al.* 2007). Headwaters are defined generally as the upper branches (1st- and 2nd-order; Strahler 1957) of a stream drainage network (Gomi *et al.* 2002, Hill *et al.* 2014), where runoff and subsurface drainage are concentrated enough to create permanent channel structures with perennial or intermittent streams (Dietrich and Dunne 1993). Most of the global stream network length is comprised of headwaters, which generally represent 60-80% of stream length in a catchment (Downing *et al.* 2012), and are important sources of water, sediment, organic matter, nutrients, and invertebrate prey for downstream systems (Gomi *et al.* 2002, Richardson and Danehy 2007, Meyer *et al.* 2007). Headwater streams are typically relatively diverse (Wohl 2017) and isolated from each other geographically, which promotes genetic isolation and thus high biodiversity for a region (Gomi *et al.* 2002, Meyer *et al.* 2007). Given all the importance of headwaters, they are vulnerable and currently under significant scrutiny, particularly with policy implications (Biggs *et al.* 2017, Creed *et al.* 2017).

Surface coal mining in central Appalachia frequently begins by stripping vegetation and soil from the land surface, then removing up to 200 vertical meters of rock that composes the mountain ridges using explosives and heavy machinery to access the coal seams below (Bernhardt and Palmer 2011). The fractured rock, known as overburden or mine spoil, is increased in volume when removed such that, in central Appalachia, all cannot be replaced in the excavation during reclamation. Thus, excess overburden is often placed into adjacent valleys, burying headwater streams and creating valley fills composed of unweathered newly exposed rock (Merricks *et al.* 2007). Mine reclamation in central Appalachia often leaves shallow, compacted, homogenized soils and grasslands with altered biogeochemical and hydrological properties compared to pre-mining forests (Negley and Eshleman 2006, Bernhardt and Palmer 2011, Shrestha and Lal 2011). The combination of valley fills and surrounding mine lands impacts the headwater streams that drain them, often causing increased sediment loads (Bonta 2000), elevated specific conductance (SC, a surrogate measurement for salinity; Hartman 2005, Pond *et al.* 2008), and altered streamflow response (Negley and Eshleman 2006). The loss and alteration of headwater streams leads to loss of biodiversity and ecosystem functions and services and can potentially degrade downstream systems and human health (Hitt and Hendryx 2010, Palmer *et al.* 2010).

Bioassessments are used to evaluate biological condition (e.g. Burton and Gerritsen 2003) of headwater streams impacted by mining or other disturbances. Bioassessments are integral to Clean Water Act implementation and enforcement. Benthic macroinvertebrates are widely used in bioassessments because of their ubiquity, ability to integrate effects of short-term environmental variations, relative immobility ideal for indicating local conditions, ease of sampling and identification, and broad range of stressor tolerances (Barbour *et al.* 1999).

Bioassessments can be conducted in streams by sampling benthic macroinvertebrates using either quantitative or semi-quantitative methods. Quantitative sampling and enumeration of

benthic macroinvertebrates entails counting the entire sample of organisms taken from a strictly delineated area of stream bottom. This contrasts with semi-quantitative sampling, which relies on fixed-count subsampling (Barbour and Gerritsen 1996) that involves counting a fixed number of organisms from an area of stream bottom that is not strictly delineated. Quantitative sampling and enumeration provides advantages such as the ability to estimate areal densities and, when conducted by sampling larger numbers of organisms than a corresponding semi-quantitative fixed-count, to obtain more accurate estimates of benthic macroinvertebrate richness and an increased ability to detect rare organisms compared with semi-quantitative sampling and fixed-count subsampling (Courtemanch 1996). Additionally, a quantitative accounting of a taxon's density can indicate how close that taxon is to extirpation within the habitat type that is sampled, something that cannot be done using relative abundance as done with semi-quantitative sampling. However, quantitative sampling is generally much more time consuming and therefore more expensive than semi-quantitative sampling (Barbour and Gerritsen 1996). Consequently, semi-quantitative sampling is more widely used for rapid bioassessment procedures.

Community structure is essentially the composition of interacting populations of organisms that inhabit a location. Beta diversity can be defined generally as the variation in community structure among locations (Legendre *et al.* 2005). One way to measure beta diversity of a region with respect to a gradient of a stressor such as salinity is taxonomic replacement. Taxonomic replacement, which is “the change in community structure from one sampling unit to another along a spatial, temporal or environmental gradient” (Anderson *et al.* 2011), provides information about community response to that gradient. 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. For example, it is possible that two streams could have equal numbers of taxa (equal richness) but different taxonomic identities and therefore different community structures.

Stream habitat – stream characteristics including physicochemical attributes, substrate distribution, and channel stability – and flow regime are influenced by conditions in the channel and surrounding watershed (Hynes 1975, Black 1996, Allan 2004). Stream habitat and flow regime are principal drivers of benthic community structure (Hynes 1970, Gordon *et al.* 2004, Allan and Castillo 2007). Prior studies have found that surface mining operations affect the streamflow regime (Dickens *et al.* 1989, Messinger 2003, Negley and Eshleman 2006, Ferrari *et al.* 2009), physicochemical attributes (Johnson *et al.* 2010, Griffith *et al.* 2012), water chemistry (Lindberg *et al.* 2011, Cormier *et al.* 2013, Hopkins *et al.* 2013), sediment loading (Fox 2009), and ecological processes (Pond *et al.* 2008) of streams draining mining-influenced watersheds.

Justification

Numerous recent studies have found relationships between salinity and benthic macroinvertebrate community structure in streams of the central Appalachian coalfield (e.g. 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). Few of these studies, however, have focused on the potential for impacts by associated

stressors such as substrates, streamflow regimes, or temperatures altered by mining practices. Such alterations have been documented to affect certain benthic macroinvertebrate community elements in other systems (e.g. Duan *et al.* 2008, Statzner *et al.* 1988, Haidekker and Hering 2008); hence, it is reasonable to expect that similar stream characteristics altered by mining would also affect the benthic community in headwater streams of the central Appalachians.

Many of the prior salinity studies in Appalachian coalfield mine-impacted streams were conducted using semi-quantitative benthic macroinvertebrate sampling. Novel elements of my project relative to prior studies of mining influence in central Appalachian coalfield streams include use of quantitative sampling, analysis of community effects to benthic macroinvertebrates using methods that include evaluation of taxonomic replacement, and assessment of potential effects by mining-associated habitat alterations in addition to elevated salinity.

Objectives

The overall purpose of this dissertation research was to examine influences of salinity and other mining-impacted habitat stressors on benthic macroinvertebrate community structure using quantitative sampling of the benthic macroinvertebrate community.

Specific objectives were to:

- 1) Use quantitative sampling of benthic macroinvertebrates to assess variation of benthic macroinvertebrate community structure across a salinity gradient in headwater streams influenced by coal mining in central Appalachia, and
- 2) Determine if stressors or stressor-indicator variables other than salinity were associated with benthic macroinvertebrate community structure variation in a manner that indicates potential causality.

Dissertation overview

Chapter 1 provides a brief introduction to my dissertation research and presents objectives of my research.

Chapter 2 is a detailed account of methods and materials for fieldwork, lab analyses, and statistical analyses.

Chapter 3 focuses on Objective 1. In this chapter, I demonstrate impacts of salinity on benthic macroinvertebrate community structure and postulate potential effects on the functioning of headwater stream ecosystems. I use quantitative sampling and enumeration to enable calculations of benthic macroinvertebrate metrics such as density, richness, and taxonomic replacement. I also examine temporal variation of benthic macroinvertebrate community structure using data from six sampling events over the course of a year.

Chapter 4 focuses on Objective 2. In this chapter, I investigate potential causal influence by habitat features in addition to salinity on benthic macroinvertebrate community structure. This is achieved by examining existing evidence in scientific literature that supports my observations of associations between selected stressor-indicator variables in addition to salinity and benthic macroinvertebrate metrics in headwater streams influenced by mining.

Chapter 5 is a summary of main points from chapters 3 and 4, implications of the findings in this dissertation research, and recommendations to consider for future research, policy, and management regarding mining-influenced headwater streams of the central Appalachian coalfield.

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CHAPTER 2 – Detailed Methods and Materials

Field Methods

Stream selection

Field work was conducted from May 2013 to April 2014. Study streams (i.e. sample units) were a subset of 1st- and 2nd- order streams (Strahler 1957) selected for study by Timpano *et al.* (2015), including four reference streams and eleven mining-influenced streams along a gradient of specific conductance (SC) (Figure 2.1). The streams were in Wise, Dickenson, and Buchanan Counties in Virginia, and McDowell, Raleigh, and Kanawha Counties in West Virginia; all were within EPA Level IV Ecoregion 69d (Omernik 1987, Woods *et al.* 1999; Figure 2.1). 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, and watersheds were devoid of residences, major roads, commercial or industrial facilities other than surface coal mines and, for a few watersheds, small numbers of natural gas wells. Catchments for each stream sampling point were delineated using Spatial Analyst tools in ArcMap v.10 (ESRI Inc., Redlands, CA, USA) software with digital elevation rasters downloaded from the National Map (United States Geological Survey (USGS); <http://nationalmap.gov/>). Using these data, study streams were selected such that reference streams had similar catchment areas as mining-influenced streams (Table 2.1).

Benthic macroinvertebrate sampling

Benthic macroinvertebrates were sampled from 10-13 of the 15 study streams six times over twelve months (May 2013-April 2014) using a Hess sampler (Wildco; Yulee, FL, U.S.A.) with a 363µm Nitex mesh. Because of logistical and site-specific limitations (e.g. water level too low to sample), some streams were not sampled during some sampling periods. For each sample, three to six sub-samples were collected from run sections below randomly selected riffles. For collection of each sub-sample, a Hess sampler was driven into the streambed sediment, completely enclosing 0.086 m² of stream bottom as needed to prevent organisms from drifting in or out of the sampler. Sub-samples were placed in 500 mL containers, submersed in 95% ethanol, and stored at room temperature in the laboratory until processed.

Water chemistry sampling

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-min intervals. The HOBO U24-001 temporal stability and accuracy were evaluated by Maupin *et al.* (2012) and found to significantly drift upwards over time but exhibited the highest accuracy of the sensors tested and had within-type sensor differences of 10 µS cm⁻¹. During each of the six benthic sampling visits, a multimeter probe (YSI Professional Plus; Yellow Springs, OH, U.S.A) was placed near the logger and measured SC and stream temperature for use in calibrating the logger data.

Synoptic samples of stream water were collected near the conductivity logger from approximately 10 cm below the surface of flowing water at the time of benthic macroinvertebrate sampling using a syringe, and filtered streamside using a Fisherbrand PVDF cellulose ester disposable filter with a nominal pore size of 0.45 μm . Two 60-mL samples (one for metals analysis, one for back-up) were stored in Fisherbrand sample bags, put on ice, brought back to the lab and stored at 4°C until analyses. The samples were acidified to pH < 2 with 1+1 concentrated nitric acid in the field.

Streamflow measurements

Solinst Edge Leveloggers (Solinst Canada Ltd.) were installed in each stream in September 2013 and used to measure stage at 15-minute intervals. Leveloggers were located where water would cover the transducer during extremely low flows, usually near the edge of a pool, in straight sections of the channel. Stilling wells were used to stabilize flow. Barologgers (Solinst Canada Ltd.) were hung in trees on the stream banks to record atmospheric pressure simultaneously with the Leveloggers. Velocity, wetted width, and depth were measured six times between May 2013 and April 2014 along lateral transects at two-to-three points in each stream, from which mean discharge for the stream was calculated using the velocity-area method (Gore 2007). Wetted width was measured using a tape measure across each transect. Velocity was measured using an OTT Acoustic Digital Current meter (OTT Hydromet, Loveland, CO, USA) placed at approximately 0.6 of the depth at 10 equally-spaced points across the stream. Depth was also measured by meter stick at each point where velocity was measured. Stage was measured at the Leveloggers simultaneous to discharge component measurements.

Sediment measurements

During March 2014, a modified Wolman (1954) count of 200 pebbles was performed from lateral transects along a 100-m reach at each stream. Ten pebbles were collected at randomly spaced points on each of 20 transects. Transects 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. If the sediment encountered was too small to be measured with the meter stick it was simply recorded as “1”, indicating fines.

Geomorphic measurements

Relative bed stability is an index of the propensity of the streambed substrate to shift (Kaufmann *et al.* 1999), and its calculation is partially determined by using geomorphological parameters such as slope and hydraulic radius. Kaufmann *et al.* (1999) used mean thalweg depth to calculate bankfull hydraulic radius as part of the calculation of relative bed stability. The present study modified that method by calculating mean bankfull hydraulic radius (cross-sectional area/perimeter) from 11 cross-sections within a 150-m reach of each stream. The components for bankfull hydraulic radius were obtained by measuring the lateral perimeter between bankfull points on either side of the stream and bankfull cross-sectional area. Cross-sectional area was calculated by multiplying bankfull width by mean bankfull depth. Bankfull depths were measured by laying a PVC pipe across the stream where possible and measuring from the bottom

of the pipe to the bottom of the stream at 10 evenly-spaced points. The 10 measurements were averaged for mean bankfull depth. Where the stream was too wide to accommodate the pipe, a level was extended from bankfull points on either side of the stream to the edges of the stream and heights were measured from the end of the level to the water surface. Average depth of the water was calculated from 10 evenly-spaced points in the stream between bankfull points and added to the average of the two bankfull heights. Bankfull width was the distance between bankfull points. Mean slope for the reach was estimated by measuring slope over 30 m in each of the upstream and downstream directions with an Abney level and leveling rod at the water surface at each cross-section, then taking the arithmetic mean of all measurements. All geomorphic measurements were taken in June 2014.

Laboratory Methods

Benthic macroinvertebrate sub-samples were quantitatively counted and identified to genus when possible using dichotomous keys (Merritt *et al.* 2008, Stewart *et al.* 1993, Wiggins 1996). The families Chironomidae and Ceratopogonidae, and sub-class Oligochaeta were identified to those levels.

Water samples were analyzed for dissolved trace elements (Al, Ni, Fe, Mn, Se, Zn) using an inductively coupled plasma mass spectrometer (Thermo Electron X-Series ICP-MS, Thermo Fisher Scientific, Waltham, Massachusetts, USA) (APHA 2005). Method detection limits and method reporting limits for the April 2014 analyses are reported in Table 2.2.

Downloaded data from continuous conductivity loggers were converted to SC at 25 °C using HOBOWare software (Onset Computer Corp., Bourne, Massachusetts, USA) and calibration data measured at the data logger by the multimeter probe. The HOBOWare Conductivity Assistant (Onset Computer Corp., Bourne, Massachusetts, USA) was used with the multimeter probe calibration data to calibrate the loggers and adjust for drift. The multimeter probe was calibrated using a standard solution of KCl before each use as per APHA (2005). The dataset was checked for missing data, $SC < 0 \mu S \text{ cm}^{-1}$, extreme spikes, and other anomalies before data analyses.

Downloaded data from Levelloggers and Barologgers were converted to time-series records of stage height using Levellogger 4.3.0 software (Solinst Canada Ltd.). Barologger data were used to correct Levellogger transducer data for atmospheric pressure.

Data Analysis Methods

Jaccard dissimilarity

Dissimilarity matrices were square matrices composed of dissimilarity indices of stream pairs, created using the Jaccard method (McCune *et al.* 2002) with *vegdist* in the R package *vegan* (Oksanen *et al.* 2016). Jaccard dissimilarity was chosen over other methods such as Bray-Curtis, Gower, etc. because it represents simple taxonomic identity differences among streams instead of

abundance-based differences. Jaccard dissimilarity (D_J) was calculated for incidence (i.e. presence/absence) data:

$$D_J = \frac{b + c}{a + b + c}$$

Eq. 2.1

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 (but including Chironomidae, Ceratopogonidae, and Oligochaeta) were used to construct dissimilarity matrices for each sampling month. 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 streams has no taxa in common and $D_J = 1$. Many stream pairs at dissimilarity saturation within a matrix (e.g. many ones in a Jaccard dissimilarity matrix) can inhibit detection of correlation with predictor variables.

Selected benthic macroinvertebrate groups

Functional feeding groups (FFGs) were included as response variables because they perform ecosystem functions such as cycling of nutrients and organic matter (Cummins and Klug 1979, Vannote *et al.* 1980, Wallace and Webster 1996). The FFGs selected for this study were filterers, gatherers, predators, scrapers, and shredders (Wallace and Webster 1996). In addition, habit groups (e.g. clingers, sprawlers, burrowers, climbers, and swimmers as selected for this study) were assessed because they are associated with specific types of physical habitat and are thus potentially sensitive to differences in habitat such as substrate composition or mean stream velocity. Habit groups and FFGs were assigned to each taxon primarily according to tables in Merritt *et al.* (2008), though a few taxa had to be classified using other sources as necessary.

Taxonomic groups known to be sensitive to SC and other types of pollution (e.g. Ephemeroptera, Plecoptera, Trichoptera, or EPT) are widely employed in bioassessments (Plafkin *et al.* 1989, Wallace *et al.* 1996, Burton and Gerritsen 2003). Some families (e.g. Baetidae, Hydropsychidae, and Leuctridae) within the orders of EPT are pollution-tolerant and can be dominant under polluted conditions; thus, potentially affecting strongly responses of the groups in which they are members but for which most other members are pollution intolerant. For this reason, some groups (EPT orders, FFGs, and habits) were presented with and without the dominant pollution-tolerant families included in analyses.

Benthic macroinvertebrate metrics

Richness

Richness of sampled benthic macroinvertebrate communities in each stream was determined by counting number of taxa summed across three (Chapter 3) or six (Chapter 4) sub-samples.

Controlling for sample area (total sampled area = number of sub-samples x 0.086 m²) allowed for variation of number of individuals among samples to be dependent on organism density. Species-accumulation curves indicated that richness did not reach an asymptote after six sub-samples (Gotelli and Colwell 2001), so richness was expressed on a per-sampling-area basis (i.e. identical ordinate positions on a sample-based species-accumulation curve), which allows comparison among streams. Richness values did not include individuals from higher-order classification caused by immature or damaged specimens that could not be classified to genus. Richness was calculated for selected taxonomic, habit, and functional feeding groups, as well as overall.

Density

Density of benthic macroinvertebrate individuals in the sampled stream-bottom area was calculated for each stream as mean abundance of sub-samples divided by area of the Hess sampler (0.086 m²). Density values included all specimens regardless of condition, provided they could be identified at least to the classification level of interest. Classification into FFGs and habit groups was at the level of identification for a specimen. Density was calculated for taxonomic, habit, and FFGs, as well as overall.

Diversity

Simpson's Diversity (D) was calculated using the formula:

$$D = 1 / (\sum_{i=1}^S p_i^2)$$

Eq. 2.2

where the quantity in parentheses is the sum of squares of species frequencies (McCune *et al.* 2002, Jost 2006). Shannon's Diversity (H) was calculated using the formula:

$$H = - \sum_{i=1}^S p_i \ln p_i$$

Eq. 2.3

where p_i is the proportion of individuals belonging to taxon i (McCune *et al.* 2002, Jost 2006).

Composition

Benthic macroinvertebrate community composition variables consisted of proportions of each taxonomic, FFG, and habit group within the total sampled communities. Ratios of FFG densities were also calculated to reflect the state of specific stream characteristics (see Table 3 in Cummins *et al.* 2005 for complete descriptions). Functional Riparian Corridor (FRC) was calculated as the ratio of shredders: collectors (gatherers + filterers); Production Rate (PR) was calculated as the ratio of scrapers: collectors; Substrate Stability (SS) is the ratio of scrapers + filterers: shredders + gatherers.

Taxonomic replacement

Taxonomic replacement was characterized as correlations among benthic macroinvertebrate group dissimilarities with SC differences. Three distance matrices, 1) Jaccard dissimilarity matrix (D_Y), 2) Euclidean distance matrix for SC differences (D_X), and 3) 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 is essentially a Pearson product-moment correlation between two distance matrices and it evaluates the following null hypothesis: “The distances among objects in matrix D_Y are not (linearly or monotonically) related to the corresponding distances in 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 differences among streams cannot be attributed to proximities of the streams to one another. 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 1,000 times (Legendre 2000, Oksanen *et al.* 2016).

Stressor-indicator variables

The definition for stressor or stressor-indicator variable (SIV), as used in this study, is a physical and/or chemical characteristic of a stream that is known to be altered in systems influenced by mining and has potential to impact benthic community structure. Many of the measured stream attributes in this study were not technically stressors having direct effects on benthic macroinvertebrate community structure (e.g. relative bed stability), but likely function through indirect effects, so they are referred to as SIVs.

Mining land use

Percent mined area in the catchment of each study stream was determined from analyses of Landsat satellite data as described by Li *et al.* (2015), and published in Timpano *et al.* (2017), which included cumulative percent mined area 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 mined area from 1980-2014. One catchment, however, required closer investigation to show that no additional mining occurred between 2011 and April 2014.

Streamflow

Rating curves were developed from stage measurements and discharge calculations conducted at the times of Levellogger data downloads and applied to the Levellogger stage records from February to April 2014 to produce time-series of discharge. The reason for the period of record was that for causality to be inferred, occurrence of the explanatory variable should have been before the benthic macroinvertebrate samples were collected – April 2014. Levellogger records for all the streams began in February 2014.

Unit-area discharge was chosen as a variable calculated as discharge divided by catchment area to minimize the confounding influence of varying catchment areas. Several statistics were calculated for discharge and unit-area discharge in order to assess potential SIVs involving discharge: high (95th quantile), low (5th quantile), median, high-low range, median-low range, and median-high range.

Flashiness for each stream was quantified by applying the Richards-Baker Flashiness Index (RBI; Baker *et al.* 2004) to discharge records from February to December 2014:

$$RBI = \frac{\sum_{i=1}^n |q_i - q_{i-1}|}{\sum_{i=1}^n q_i} \quad \text{Eq. 2.4}$$

where q = average daily flow ($\text{m}^3 \text{s}^{-1}$). In this case, a longer period of record was recommended (Baker *et al.* 2004), and it was assumed that the flashiness indices of the streams did not change significantly from the prior year.

Sediment

Variables chosen to represent sediment characteristics were quantiles of the sediment distributions (10, 16, 25, 50, 75, 84, and 90; expressed as D10, D16, etc.), proportion of sediment that occur in each size class (e.g. % fines, % gravel, % cobble) based on the Udden-Wentworth grain size classification system (Wentworth 1922, Blair and McPherson 1999), ratio of % small cobble (64-128 mm) to % fines (<2 mm) (SCF), ratio of % large cobble (128-255 mm) to % fines (<2 mm) (LCF), interquartile range, Simpson's diversity of size class proportions, and ratio of D84 to D50.

Relative Bed Stability

The log of relative bed stability (LRBS) was calculated as follows: $\log_{10} (D50/13.7 \cdot \text{mean bankfull hydraulic radius} \cdot \text{mean slope [rise/run]})$, where D50 was the median pebble size from pebble counts conducted in March 2014. As indicated in the Field Methods section, the method used in this study to calculate LRBS was modified from the method in Kaufmann *et al.* (1999), but the effects of that modification changed the calculation of mean bankfull hydraulic radius, not the above LRBS equation. Two components of mean bankfull hydraulic radius were modified: 1) mean thalweg depth was replaced by an average of bankfull hydraulic radii from 11 equidistant cross-sections, and 2) the roughness corrections were not applied because there was negligible large woody debris in all streams and not enough data were collected to calculate residual pool analyses (e.g. Kauffmann *et al.* 1999).

Spearman rank correlation

The statistical analysis technique most employed throughout this research was Spearman rank correlation. It was chosen because distributions of analyzed variables varied widely. Spearman correlations allow identical form of analysis despite those widely varying distributions (McDonald 2009). Spearman correlations were used in chapter 3 to assess responses of various

benthic macroinvertebrate metrics to SC, and in chapter 4 to assess responses of benthic macroinvertebrate metrics to multiple potential SIVs in addition to SC.

NMDS and PerMANOVA

Non-metric multidimensional scaling was used in chapter 3 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 Jaccard dissimilarity matrices with the *metaMDS* function in *vegan* (Oksanen *et al.* 2016). 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. Permutational multivariate analysis of variance (i.e. PerMANOVA; Anderson 2001), executed by the *adonis* function in R package *vegan* (Oksanen *et al.* 2016), was used to test the null hypothesis of no difference in assemblage structure with season, gradient of SC, and interaction of season and SC (Anderson 2001). Jaccard was the dissimilarity method used for the community data. Samples were classified into two levels of SC and analyzed with PerMANOVA to test patterns of “High SC” ($SC > 533 \mu S cm^{-1}$) and “Low SC” ($SC < 533 \mu S cm^{-1}$) groupings as indicated on the NMDS plots. The specific null hypothesis for the data grouped into sampling months and SC levels was “the centroids of the groups, as defined in the space of the chosen resemblance measure, are equivalent for all groups” (Anderson and Walsh 2013). The specific null hypothesis for the community structure response to continuous SC data was a correlation coefficient of zero. The interaction tested dependence of the effect of SC on the month of sampling. The function runs pseudo F-tests based on sequential sums of squares from permutations of the raw data to test significance (Oksanen *et al.* 2016).

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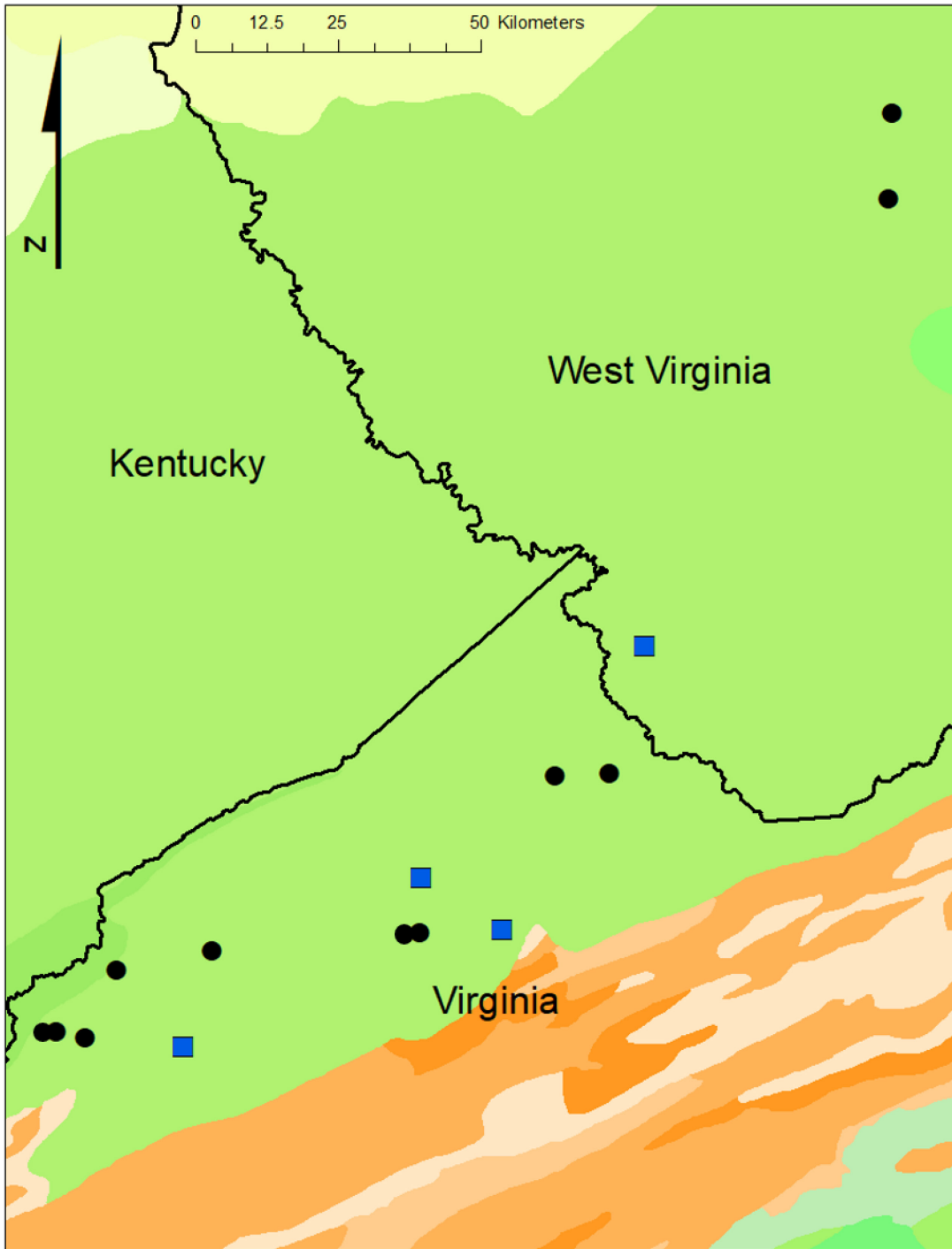


Figure 2.1. Study streams mapped on EPA Ecoregion level IV (69d) map (U.S. EPA). Black dots represent mining-influenced streams, and blue squares represent reference streams.

Table 2.1. Catchment areas of study streams.

Stream ID	Area (km ²)
<u>Reference</u>	
COP	0.82
CRO	2.31
EAS	2.39
HCN	5.91
<u>Mining-influenced</u>	
KUT	1.13
KEL	2.63
POW	2.69
MIL	2.75
RIC	4.24
LLC	4.34
GRA	4.38
RFF	4.61
FRY	5.66
SPC	6.73
ROC	7.21

Table 2.2. April 2014 analytes measured by mass spectrometry.

Analyte	Atomic mass*	Metallic Classification*	Method detection limit ($\mu\text{g L}^{-1}$)	Method reporting limit ($\mu\text{g L}^{-1}$)
Selenium	78.96	Non-metal	0.7	5.0
Manganese	54.94	Metal	0.1	1.0
Nickel	58.69	Metal	0.0	0.1
Aluminum	26.98	Metal	0.1	1.0
Iron	55.85	Metal	0.8	10.0
Zinc	65.39	Metal	0.4	10.0

*Denniston *et al.* 2004.

CHAPTER 3 - Detecting effects of elevated specific conductance on benthic macroinvertebrate communities in central Appalachia using quantitative sampling

Abstract

Elevated specific conductance (SC) in freshwater streams has been associated with alterations of benthic macroinvertebrate communities. The objective of my study was to use quantitative sampling to assess 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 coalfield streams six times over the course of a year in 2013-2014. 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 sampling months. Dissimilarity analyses revealed distinct separations of assemblage structure between 1) spring and fall sampling months, and 2) low-SC and high-SC streams. Ephemeroptera declined most markedly with increasing SC, supporting findings by prior studies. Certain tolerant taxa were present at high densities seasonally, masking responses of other taxa within taxonomic or 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 at high-SC streams. Richness metrics displayed a strong negative response to SC regardless of season and were robust indicators of salinity effects. In some cases, functional feeding groups showed declines in density and/or diversity in high-SC streams, relative to low-SC streams. 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. Implications of this study are that bioassessments could be supplemented with quantitative sampling and taxonomic replacement, and by understanding that seasonality and dominant taxa are important considerations for benthic macroinvertebrate sampling in the central Appalachian coalfields.

Introduction

Headwater contributions

Headwaters (1st- and 2nd-order streams; Strahler 1957) 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 (Richardson and Danehy 2007, Larned *et al.* 2010, Leigh and Datry 2017). Because of their unique and highly heterogeneous environment, headwaters serve as habitat for many species that do not occur elsewhere in river systems, and provide services such as colonist-, energy-, and nutrient exports (Meyer *et al.* 2007).

Elevated specific conductance in mining-influenced streams

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. (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). Excess mine spoils are generated when rock overlying coal seams is fractured and removed, which causes expansion of volume. Valley fills (VF) 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 weathering reactions that produce water-soluble ions (Griffith *et al.* 2012). As water percolates through fractured mine rocks, concentrations of dissolved mineral constituents become elevated (Merricks *et al.* 2007, Pond *et al.* 2008). In mining-influenced Appalachian streams, SC is often highly correlated with total dissolved solids (TDS) and individual major ions (e.g. Ca^{2+} , Mg^{2+} , SO_4^{2-} , K^+ , HCO_3^- , Na^+) in the water column (Pond *et al.* 2014, Timpano *et al.* 2015). 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).

Many studies reporting that benthic macroinvertebrate communities in mining-influenced Appalachian streams with elevated SC differ from those present in unmined reference streams have been conducted using semi-quantitative sampling (e.g. Green *et al.* 2000, Pond 2004, Timpano *et al.* 2015, Boehme *et al.* 2016). These studies compare community-structure metric values at elevated-SC streams to those at reference streams and/or determine that metric values are influenced by SC levels in the water column. However, simple metric comparisons such as relative abundance or richness from semi-quantitative samples may not fully capture differences at mining-influenced and reference streams. 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 not been applied to characterize effects of elevated SC on benthic macroinvertebrate communities in the Appalachian coalfield.

Quantitative sampling

Another limitation of most studies of benthic macroinvertebrate responses to mining disturbance conducted to date is their reliance on semi-quantitative collection of benthic macroinvertebrates, followed by subsampling a fixed number of individuals from a sample for taxonomic identification (e.g. Barbour *et al.* 1999, Burton and Gerritsen 2003, Klemm *et al.* 2002). Although such methods are commonly applied for bioassessment, they may not provide a complete accounting of the community at a given stream. For example, Boehme *et al.* (2016)

found that > 80% of individuals in semi-quantitative samples collected from high-SC streams were of three high-frequency taxa and questioned if taxonomic richness at those high-SC sampling streams had been fully characterized by such samples. Quantitative sampling, which accounts for all individuals inhabiting a defined area of stream bottom, can be more sensitive to presence of rare taxa and can provide a more accurate estimate of richness than semi-quantitative, fixed-count methods (Courtemanch 1996, Vinson and Hawkins 1996). Quantitative sampling also characterizes organism densities (i.e. numbers of organisms per unit-area of stream bottom), a fundamental ecosystem property. Unlike conventional semi-quantitative sampling, quantitative sampling can indicate if organism densities are altered in streams with elevated SC. Quantitative sampling techniques have been applied only rarely in studies characterizing biotic effects of elevated SC in streams of the Appalachian coalfield (Hartman *et al.* 2005).

Taxonomic replacement

Taxonomic replacement, which is “the change in community structure from one sampling unit to another along a spatial, temporal or environmental gradient” (Anderson *et al.* 2011), provides information in addition to richness and abundance measures. For example, it is possible that two streams could have equal numbers of taxa (equal richness) but different taxonomic identities and therefore different community structures. Dissimilarity coefficients (e.g. Jaccard coefficients that quantify taxonomic differences between each pair of streams) can be placed in a site-by-site dissimilarity (i.e. distance) matrix. A distance matrix composed of differences in SC between each pair of streams can also be constructed. Correlations between community dissimilarity and SC distance matrices can then 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

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 does not describe the types of food ingested, only the method by which food is ingested. Typically, five major FFGs are described in headwater streams: 1) collector-filterers (i.e. filterers) filter fine particulate organic matter (FPOM; <1mm) from the water column; 2) collector-gatherers (i.e. 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 (CPOM; >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).

Seasonality

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, Petty *et al.* 2010, Bernhardt *et al.* 2012, Pond 2012, Pond *et al.* 2014). 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.

Objective and questions

The objective of this study was to use quantitative sampling to assess structural differences among benthic macroinvertebrate communities along an SC gradient in headwater streams influenced by coal mining in central Appalachia. I 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 dissimilarity 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?

Secondary objectives were to determine if quantitative sampling and taxonomic replacement analysis could further inform these questions.

Methods

Stream selection

Study streams (i.e. sample units) were a subset of 1st- and 2nd- order streams (Strahler 1957) selected for study by Timpano *et al.* (2015) with the intent of minimizing influence of non-TDS stressors (Figure 3.1). Included were four reference streams and eleven mining-influenced streams along a gradient of increasing mean SC within a range of 24-1,437 $\mu\text{S}/\text{cm}$ among the streams for the period of January 2013 – May 2014. Streams are in Wise, Dickenson, and Buchanan counties in Virginia, and McDowell, Raleigh, and Kanawha counties in West Virginia, all within EPA Level IV Ecoregion 69d (Omernik 1987; Figure 3.1). 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 and, for a few watersheds, small numbers of natural gas wells with accompanying 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 downloaded from the National Map (United States Geological Survey (USGS); <http://nationalmap.gov/>). Using these data, study

streams were selected such that reference streams had a similar range of catchment area as mining-influenced streams.

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 and measured SC and stream temperature for use in calibrating the logger data to correct for drift and converting logger-recorded electrical conductivity to SC in HOBOWare Pro v.3.4.1 software (Onset Computer Corp., Bourne, Massachusetts, USA). The multimeter probe was calibrated using a standard solution of KCl 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 μ m Nitex mesh. Because of logistical and 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 to 13 of the 15 study streams were sampled. For each sample, three sub-samples were collected from run sections below randomly selected riffles. For collection of each sub-sample, a Hess sampler was driven into the streambed sediment, completely enclosing 0.086 m² of stream bottom as needed to prevent organisms from drifting in or out of the sampler.

Complete sub-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 keys provided in Merritt *et al.* (2008). As exceptions regardless of condition of the specimen, Chironomidae and Ceratopogonidae were identified at family level and Oligochaeta were identified at class level.

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 – 0.258m²) and density (number of individuals per square meter) of 1) total taxa; 2) FFGs (filterers, gatherers, predators, scrapers, and shredders); and 3) groups representing the taxa orders Ephemeroptera (E), Plecoptera (P), and Trichoptera (T), (EPT when considered collectively). Because preliminary data analyses found high densities of certain taxa, apparently SC-tolerant, at elevated-SC streams seasonally, those taxa densities were also included as response metrics for certain analyses. FFGs were chosen 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). Each

of the EPT orders contains a known SC-tolerant family. The families Baetidae (B), Hydropsychidae (H), and Leuctridae (L) in the orders Ephemeroptera, Trichoptera, and Plecoptera, respectively, have been found to be SC-tolerant in previous studies (Pond 2010, Pond 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. These tolerant taxa are also referred to as “dominant” throughout this study.

Richness was determined by summing number of taxa identified in the three sub-samples for each stream. Controlling for sample area (total sampled area = 0.258 m²) allowed for variation of number of individuals among samples to be dependent on organism density. Richness was expressed on a per-sampling-area (3 x 0.086 m² = 0.258 m²) basis (i.e. identical ordinate positions on a sample-based species-accumulation curve). Richness values did not include individuals from higher-order classification caused by immature or damaged specimens that could not be classified to genus. Density per square meter was calculated for each stream as mean abundance of the three sub-samples divided by area of the Hess sampler (0.086 m²). Simpson’s Diversity (Simpson’s D) was expressed as effective number of species (Hill 1973, Jost 2006) calculated using the formula:

$$D = 1 / (\sum_{i=1}^S p_i^2)$$

Eq. 3.1

where the quantity in parentheses is the sum of squares of species frequencies (Jost 2006).

“High-SC” and “low-SC” levels were classified by the median SC level (533 µS cm⁻¹) for all streams in all sample months (i.e., high-SC streams had mean SC values for the full study period > 533 µS cm⁻¹, and low-SC streams < 533 µS cm⁻¹). This threshold is resonant with other studies that use 500 µS cm⁻¹ as a criterion for reference condition (Burton and Gerritsen 2003) or “low” level of mining disturbance (Pond 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), which includes cumulative percent mined area for all the catchments.

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.

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. Bray-Curtis, which is a commonly-used dissimilarity method (McCune *et al.* 2002), detects changes of abundance along an SC gradient and can be susceptible to influence by dominant taxa. Jaccard dissimilarity (D_J) was calculated for incidence (i.e. presence/absence) data:

$$D_J = \frac{b + c}{a + b + c}$$

Eq. 3.2

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 (but including Chironomidae, Ceratopogonidae, and Oligochaeta) 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 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). 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. Permutational multivariate analysis of variance (i.e. PerMANOVA; Anderson 2001), executed by the *adonis* function in R package *vegan* (Oksanen *et al.* 2016), was used 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 classified into two levels of SC and analyzed with PerMANOVA to test patterns of “High SC” ($SC > 533 \mu S cm^{-1}$) and “Low SC” ($SC < 533 \mu S cm^{-1}$) groupings as indicated on the NMDS plots (Figure 2).

The specific null hypothesis for the data grouped into sampling months and SC levels was “the centroids of the groups, as defined in the space of the chosen resemblance measure, are equivalent for all groups” (Anderson and Walsh 2013). The specific null hypothesis for the community structure response to continuous SC data was a correlation coefficient of zero. The interaction tested dependence of the effect of SC on the month of sampling. The function runs pseudo F-tests based on sequential sums of squares from permutations of the raw data to test significance (Oksanen *et al.* 2016).

Taxonomic replacement was characterized as correlations among group dissimilarities with SC differences. Three distance matrices, 1) Jaccard dissimilarity matrix (D_J), 2) Euclidean distance matrix for SC differences (D_X), and 3) 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 is essentially a Pearson product-moment correlation between two distance

matrices, which evaluates the following null hypothesis: “the distances among objects in matrix D_Y are not (linearly or monotonically) related to the corresponding distances in D_X ” (Legendre and Legendre 2012, p 600). A partial Mantel test uses a third matrix to control for another variable. In this case, the third variable is geographic distance between streams. This is necessary so that community structural differences between streams cannot be 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 carried out 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 1,000 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.

Results and Discussion

Stream SC and macroinvertebrate response metric means

Mean SC for each sampling month and overall across the year was higher in high-SC samples than in low-SC samples (Table 3.1). Mean macroinvertebrate density did not differ between high-SC and low-SC samples in all months and overall. Mean macroinvertebrate richness was higher in low-SC samples than in high-SC samples overall and in all months except for May. Simpson’s D for macroinvertebrate communities was higher in low-SC samples than in high-SC samples overall and in four out of six months.

Macroinvertebrate community structural effects by SC and season

The NMDS plots demonstrate that macroinvertebrate community structural differences occurred in response to SC and season (Figure 3.2). The final stress for the NMDS was 0.16, represented in three dimensions, which indicates an acceptable fit (McCune *et al.* 2002). Samples grouped into distinct sampling months and seasons. May 2013 and April 2014 overlapped despite being the most distant in terms of time between sampling, likely because they are seasonally similar. Likewise, September and November overlapped. Samples also grouped by SC levels, based on the plot of axes 1 and 2.

PerMANOVA tests (Table 3.2) rejected the null hypothesis of no difference in macroinvertebrate assemblage structure for Season and SC grouped levels (i.e. “High-SC” and “Low-SC”), confirming the patterns in the NMDS (Figure 3.2). Assemblage structure was also significantly related to SC gradient. Adjusted R^2 was higher for Season ($R^2 = 0.25$, $P = 0.0002$) than for SC gradient ($R^2 = 0.07$, $P = 0.0002$), indicating that seasonality was more important for structuring benthic macroinvertebrate community than was SC. The interaction effect between Season and SC gradient was significant ($R^2 = 0.07$, $P = 0.036$), indicating that the relationships between SC gradient and taxonomic dissimilarities varied temporally with sampling month. Taxonomic replacement of the total communities in Table 3.5 provides further evidence that the relationships

between SC gradient and taxonomic dissimilarities do not remain constant across sample months. 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.

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*, *Acroneuria*, and *Maccaffertium* were predominantly found in low-SC streams, and *Tipula* and *Prosimulium* were predominantly found 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 persisted at elevated SC.

Pond *et al.* (2013) also found clustering 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 samples 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 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).

Responses of macroinvertebrates to SC

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 3.3 and 3.4). 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 $< 25 \text{ m}^{-2}$), which suggests SC as a limiting factor (Figure 3.3). Two samples (in May and November; approximately $1,000 \mu\text{S cm}^{-1}$), had much higher Ephemeroptera densities than other samples at similar SC levels (Figure 3.3). These higher Ephemeroptera densities were composed of only three genera: *Ameletus* (345 m^{-2}) in November; and *Baetis* and *Heterocloeon* (of the tolerant family Baetidae; 349 and 39 m^{-2} , respectively) in May. *Ameletus* was found in samples with SC as high as $1,856 \mu\text{S cm}^{-1}$. Pond (2010) supports classification of *Baetis* as an SC-tolerant mayfly genus in Kentucky. Kay *et al.* (2001) found Baetidae in Australian agriculture-impacted rivers with conductivity as high as $19,600 \mu\text{S cm}^{-1}$, though the

Baetidae genera were not identified. Ephemeroptera comprised up to 60% of total density seasonally in low-SC streams. Because Ephemeroptera densities declined with increasing SC while total density did not vary significantly with SC during any sampling month, the expectation is that another taxon or other taxa replaced Ephemeroptera at higher SC levels.

Ephemeroptera sensitivity could be related to their physiology. 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. 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 spent on growth and other functions, leading to osmotic stress.

Gatherer and scraper richness were also negatively correlated with SC in several sampling months (Table 3.4) related to, in part, the large proportion of Ephemeroptera taxa within these FFGs in low-SC samples. Gatherers had a range of 55-67% Ephemeroptera taxa, and scrapers had a range of 40-53% Ephemeroptera taxa in low-SC streams in all months for which the gatherer or scraper richness correlations with SC were significant (Table 3.4). Some non-Ephemeroptera scrapers were also found to exhibit negative correlations with SC across several sampling months, such as Trichoptera *Goera* and *Neophylax*, and Coleoptera *Optioservus*, *Oulimnius*, and *Psephenus*. In France, Piscart *et al.* (2005) considered the possibility that the scraper decline that they observed 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 $\mu\text{S cm}^{-1}$, approximately 5x the maximum levels observed in this study.

Some macroinvertebrate groups with densities that were not correlated with SC in any sampling month showed strong negative responses 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 3.3). Boehme *et al.* (2016) reported similar findings in an Appalachian coalfield that included some of these same streams. Plecoptera density was correlated strongly and positively with SC in July and September, but P-L was 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 3.4), indicating that increased densities of apparently SC-tolerant EPT taxa counteracted loss of more SC-sensitive taxa in high-SC samples.

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 3.3-3.5). 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 (T-H) richness and density, however, were negatively correlated with SC (Tables 3.3 and 3.4). 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 LC₅₀ SC values of 6,400-26,000 $\mu\text{S cm}^{-1}$ for Trichoptera, levels far higher than in my 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 $\mu\text{S cm}^{-1}$) for 4 h. In my 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 3.3 and 3.4). *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 report 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.

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 3.3).

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 among reference and high-SC streams. Other studies (Bernhardt *et al.* 2012, Pond 2012) found an opposite pattern with Leuctridae decreasing in high-SC streams 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 3.3), 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 3.3), 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 3.3). 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 $SC > 1,000 \mu S \text{ cm}^{-1}$ 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. $> 1,000/\text{m}^2$ in most cases) from late spring to mid-summer (i.e. April, May, and July), and was diminished (e.g. $< 1,000/\text{m}^2$) 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 systems (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 characterized as 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 at high-SC streams.

Hydropsychidae were apparently dominant in November, when both Trichoptera and filterers each showed varying responses contingent on Hydropsychidae inclusion or exclusion in the analysis (Table 3.3). 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 vs. SC was not significant in November.

Hydropsychidae are known to be tolerant to elevated salinity. When characterizing effects of oilfield brine on lotic systems in eastern Kentucky, Short *et al.* (1991) found the greatest density of *Cheumatopsyche* at SC of 5,118 $\mu\text{S cm}^{-1}$, with lower densities at lower levels of SC. Also working in eastern Kentucky, Pond (2012) found more *Cheumatopsyche* in mined streams than in any other land use category and classified Hydropsychidae taxa as “increasers”, meaning that the taxa were more frequently observed at the mining-influenced streams, which had elevated SC, than at reference streams. Rutherford and Kefford (2005) found *Cheumatopsyche* at a salinity of 25.9 g L⁻¹ (40,533 $\mu\text{S cm}^{-1}$).

Functional feeding groups

Certain 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 3.3). 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 was primarily in response to loss of mayfly scrapers (see Appendix D). Four Ephemeroptera genera found in low-SC streams, all in the family Heptageniidae (i.e. *Cinygmula*, *Leucrocuta*, *Maccaffertium*, and *Stenonema*), were not observed in high-SC streams. Scraper richness was correlated negatively with SC (Table 3.4), leaving mostly armored caddisflies and 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 is highly preferred by a certain predator. For example, Hitt *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, whose preferred prey are Heptageniidae and Baetidae (both in order Ephemeroptera).

McEvoy and Goonan (2003) contend that salinity is a driver for restructuring of communities, and that 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 3.1) caused by reduction of richness (Table 3.4) 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.

Taxonomic replacement

For some group-season combinations not exhibiting decline in density (Table 3.3) or richness (Table 3.4) with SC, taxonomic replacement was detected (Table 3.5). The 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 3.5), but not significant density (Table 3.3) or richness (Table 3.4) 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 (see Appendix D). Diptera, the order that contains Simuliidae, are salt-tolerant organisms in high-saline systems draining agriculture in southwestern Australia (Kay *et al.* 2001).

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 300-1,000 $\mu\text{S cm}^{-1}$, values corresponding to the range of salinity in this study. They also found that at SC levels $> 1,000 \mu\text{S cm}^{-1}$, the rate of change towards dominance of salt-tolerant taxa began to slow, suggesting that there is a critical range for taxonomic replacement.

Summary and Conclusions

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.

Ephemeroptera was the most salt-sensitive taxonomic group, as Ephemeroptera richness and density declined markedly as SC increased. Ephemeroptera density was variable at low-SC streams, but that variability was reduced at 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.

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.

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 apparent effect by the major ions comprising the salinity monitored as SC on community composition when other metrics such as richness or density did not. Finally, many of the community structural responses to SC could be traced to a few highly responsive taxonomic groups (e.g. Ephemeroptera during all seasons, Leuctra and Hydropsychidae during their seasons of dominance); responses to elevated SC, however, were not confined to these groups.

Conclusions

Overall changes of benthic macroinvertebrate density, relative to reference 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 appeared to be driven primarily by presence of salt-tolerant and dominant taxa (e.g. *Leuctra* and Hydropsychidae), and of salt-sensitive taxa (e.g. Ephemeroptera). Consideration of density metrics offers information beyond what can be realized from relative-abundance metrics. For example, *Leuctra* relative abundance was high in high-SC samples, but without estimates of density we could not determine if that high relative abundance was caused by high density of *Leuctra*, low densities of other taxa, or some combination. Density metrics also provide

information about absolute abundances, and therefore can indicate how close a taxonomic group may be to extirpation (e.g. Ephemeroptera in high-SC streams).

Apparent effects by salinity in these systems include reduced diversity. 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, perhaps related to a dearth of salt-tolerant taxa in the regional pool to replace sensitive taxa at higher salinities and the presence of salt-tolerant dominants. Reduced biodiversity can potentially lead to ecosystem vulnerabilities to natural and anthropogenic disturbances.

Assessment of taxonomic replacement provides additional insights into the nature of benthic macroinvertebrate community structural change that occurs in response to increasing salinity. In some cases where richness or density responses to SC were not detected, community composition was nonetheless altered. 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.

Seasonality is an important consideration for accurately characterizing stream condition in central Appalachian headwaters. Composition of benthic macroinvertebrate communities, as sampled, appears to vary seasonally. 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.

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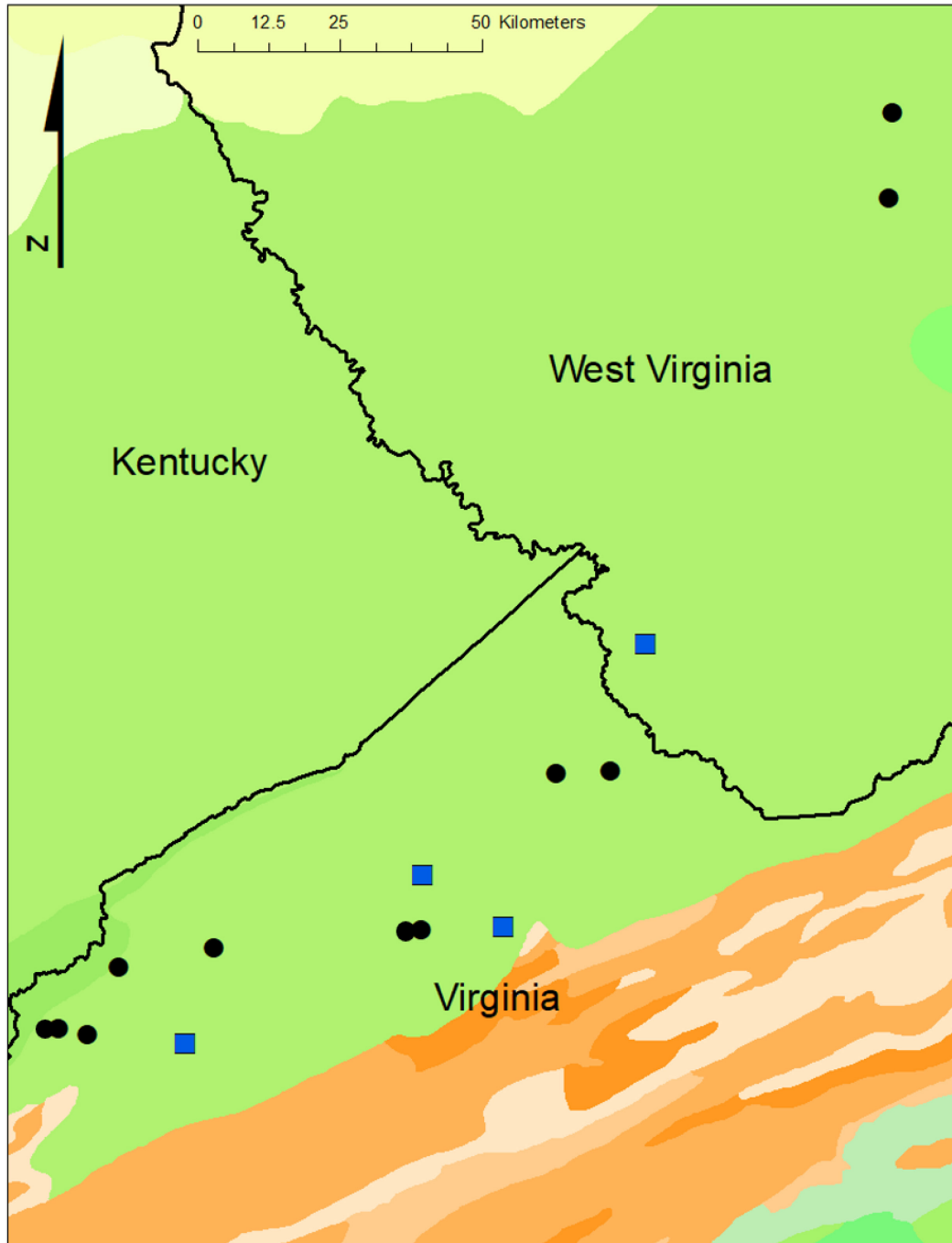


Figure 3.1. Study streams mapped on EPA Ecoregion level IV (69d) map (U.S. EPA). Black dots represent mining-influenced streams, and blue squares represent reference streams.

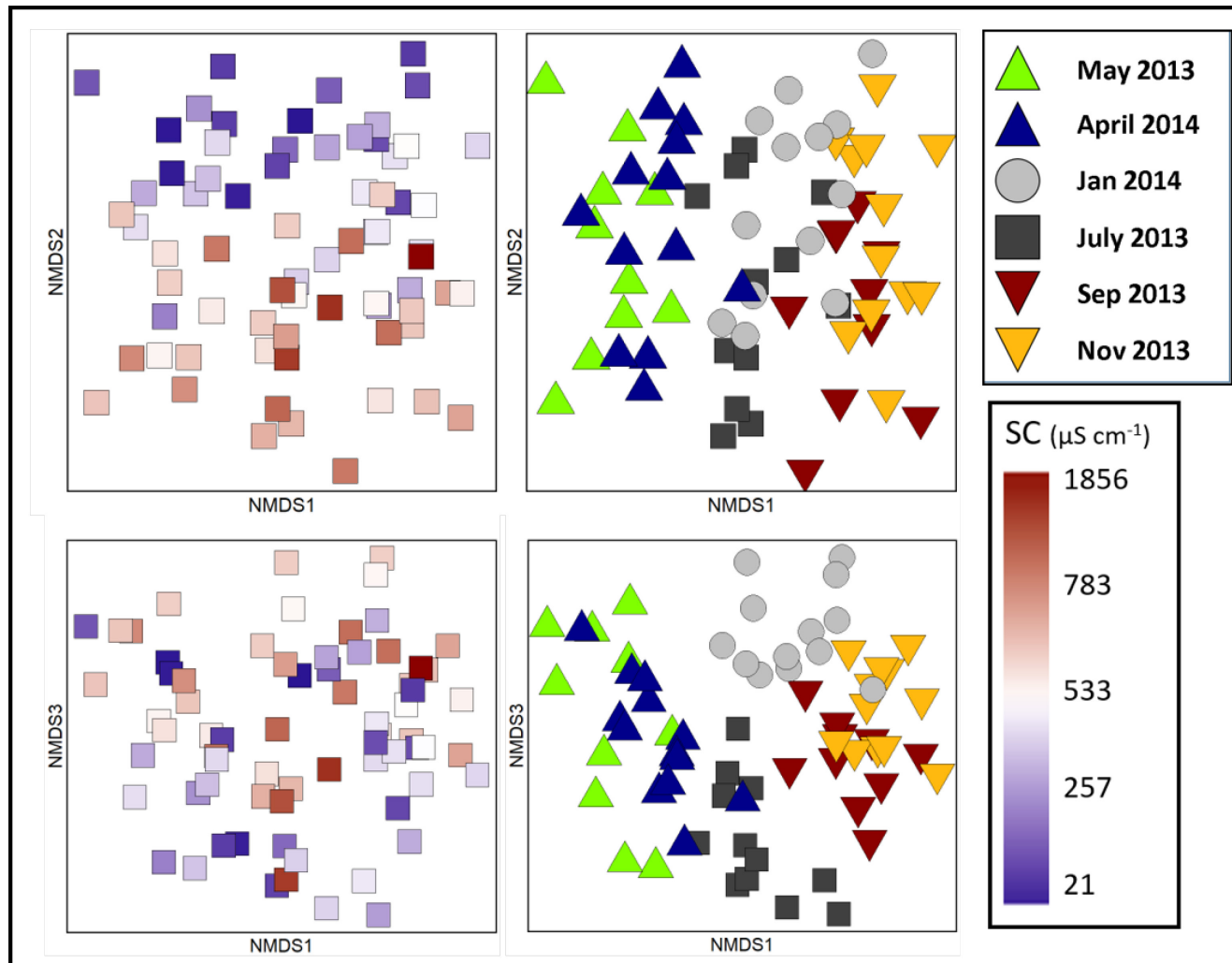


Figure 3.2. NMDS of benthic macroinvertebrate communities in headwater streams of central Appalachia based on Jaccard dissimilarity indices. Left panels are overlaid with specific conductance values, with breakpoints in legend specified as quartiles (median SC = 533 $\mu\text{S cm}^{-1}$). Right panels are overlaid with sample months. Stress = 0.16.

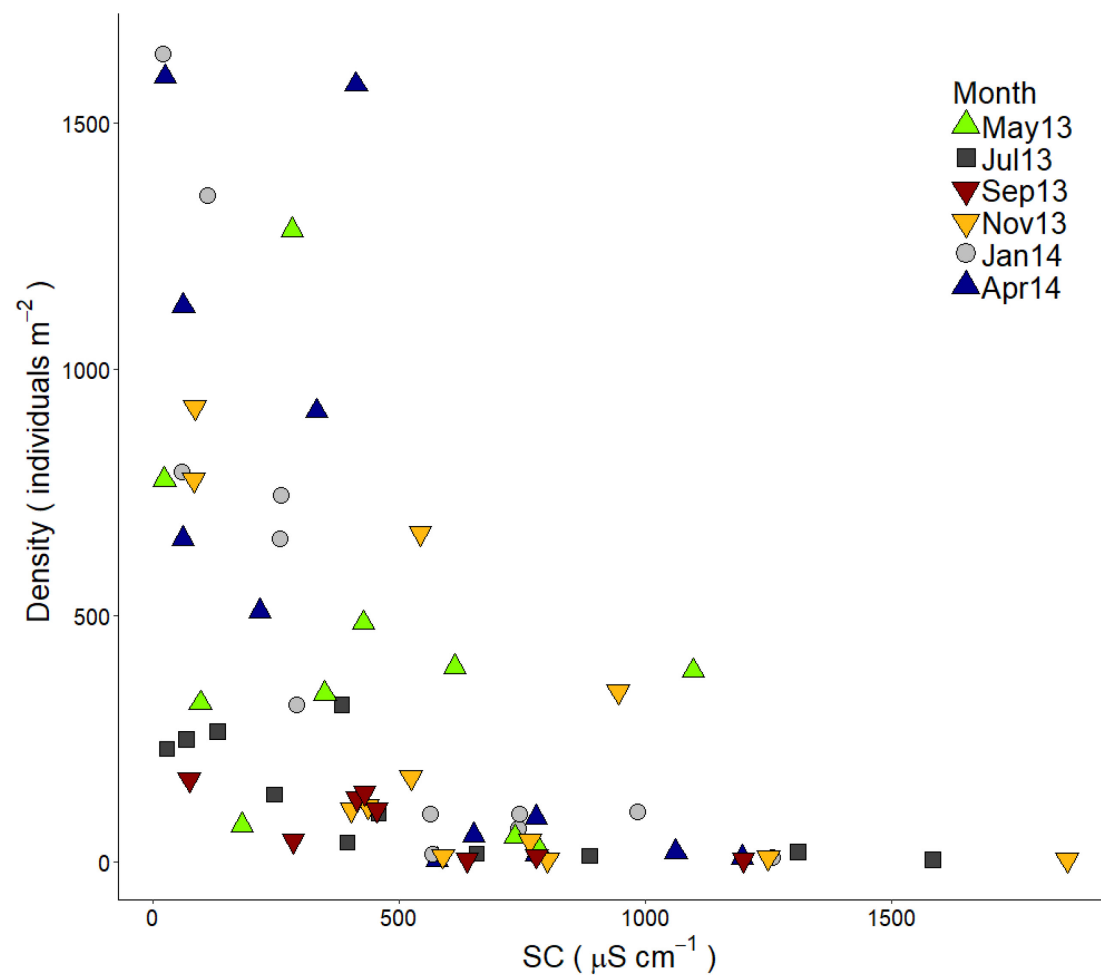


Figure 3.3. Effect of specific conductance in headwater streams of central Appalachia on Ephemeroptera density for all months.

Table 3.1. Stream-type means and standard error for month-macroinvertebrate metric combinations, overall means, and range of values (min, max) for entire study period.

SC ($\mu\text{S cm}^{-1}$)	May13	Jul13	Sep13	Nov13	Jan14	Apr14	Overall	Min	Max
Low-SC	226*	244*	331*	345*	166*	184*	247*	21	542
$\pm\text{SE}$	63	65	71	86	48	66	28		
High-SC	807	1057	1043	1034	799	926	941	562	1856
$\pm\text{SE}$	103	169	178	188	93	120	58		
Density									
Low-SC	2607	1683	1019	2906	3372	5034	2789	539	13849
$\pm\text{SE}$	580	284	237	614	333	1793	381		
High-SC	4486	2030	1032	1951	2653	4492	2793	477	7891
$\pm\text{SE}$	1203	338	251	577	642	833	343		
Richness									
Low-SC	30	28*	26*	38*	48*	39*	35*	17	52
$\pm\text{SE}$	3	3	2	3	2	3	2		
High-SC	24	17	17	22	29	24	22	11	38
$\pm\text{SE}$	3	2	1	2	3	2	1		
Simpson's D									
Low-SC	7*	5	9*	10	11*	7*	8*	1	19
$\pm\text{SE}$	2	2	1	2	1	1	1		
High-SC	2	2	4	5	6	3	4	1	9
$\pm\text{SE}$	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		

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 m^2 ; Richness is number of taxa; n is number of observations (i.e. streams);

Low-SC values with asterisks are significantly different from their high-SC counterparts.

Table 3.2. PerMANOVA results for benthic macroinvertebrate community structure response to A) specific conductance (SC) gradient, season, and interaction of SC gradient and season, 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
Season	5	4.4093	0.8819	4.7028	0.25	0.0002
SC x Season	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	

Table 3.3. Spearman rank correlation coefficients between macroinvertebrate group density and specific conductance for each month-taxonomic group combination.

Group	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	-0.69*	-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	-0.87***	-0.94***	-0.30	-0.75**	-0.56*
E	-0.36	-0.83**	-0.86**	-0.73**	-0.84***	-0.81**
E-B	-0.77**	-0.87***	-0.89***	-0.69*	-0.91***	-0.87***
P	0.54	0.66*	0.70*	0.22	0.52	0.30
P-L	0.07	-0.80**	-0.78**	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	-0.66*	-0.32	-0.30
Filterer	0.59	0.12	0.64*	0.62*	0.24	0.28
Filterer-H	0.43	0.16	-0.01	0.02	-0.35	-0.26
Gatherer	-0.07	-0.66*	0.03	-0.40	-0.47	-0.63*
Predator	-0.47	-0.79**	-0.49	-0.48	-0.29	-0.02
Scraper	-0.37	-0.81**	-0.33	-0.41	-0.70**	-0.45
Shredder	0.58	0.70*	0.68*	0.37	0.54	0.26
Shredder-L	0.08	-0.85*	-0.94***	0.16	0.26	0.37
Baetidae	-0.16	-0.47	-0.33	-0.30	-0.13	-0.17
Hydropsychidae	0.36	0.12	0.64*	0.62*	0.38	0.45
Leuctridae	0.58	0.70*	0.82**	0.77**	0.55	0.30

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

E = Ephemeroptera, P = Plecoptera, T = Trichoptera, B = Baetidae, H = Hydropsychidae, L = Leuctridae

Table 3.4. Spearman rank correlation coefficients between macroinvertebrate group richness and specific conductance for each month-taxonomic group combination.

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

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

E = Ephemeroptera, P = Plecoptera, T = Trichoptera, B = Baetidae, H = Hydropsychidae, L = Leuctridae

Table 3.5. Pearson correlation coefficients from partial Mantel tests for Jaccard dissimilarity vs. specific conductance difference in macroinvertebrate groups, controlling for geographic coordinates.

Group	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

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

E = Ephemeroptera, P = Plecoptera, T = Trichoptera, B = Baetidae, H = Hydropsychidae, L = Leuctridae

CHAPTER 4 - Multiple stressors associated with benthic macroinvertebrate communities in central Appalachian coalfield streams

Abstract

Headwater streams impacted by surface coal mining and valley fill in the central Appalachian region of the eastern U.S. have characteristics not shared by reference-quality streams in the region. These include elevated salinity, often measured using the surrogate specific conductance (SC) and often cited as a primary stressor of benthic macroinvertebrate communities. The objective of this study was to determine if benthic macroinvertebrate community composition differences from reference condition in mining-influenced streams could be attributed to potential stressors other than SC. Stream habitat characteristics were measured and calculated, and benthic macroinvertebrates were sampled from multiple central Appalachian streams. Multiple benthic macroinvertebrate community metrics, including Ephemeroptera density, richness, and composition were correlated strongly and negatively with SC. Predator density and scraper richness were correlated strongly and negatively with water selenium concentrations. Clinger richness was correlated strongly and positively with large cobble-to-fines ratios in the stream substrate and relative bed stability. These correlations are consistent with known mechanisms revealed by prior studies. Benthic macroinvertebrate responses to surface mining influences in central Appalachian streams may involve a complex interaction among multiple stressors.

Introduction

Headwater streams impacted by surface coal mining and valley fill in the central Appalachian region of the eastern U.S. have characteristics not shared by reference-quality streams in the region. Appalachian mining operations fracture and remove rocks overlying the coal seams. The expanded volume of rock and debris (i.e. spoil) caused by fracturing often exceeds what is needed to reconstruct terrain in the mining disturbance, so excess spoil is placed or dumped into the adjacent valleys. Valley fills are composed of rock fragments of varying size and often bury natural headwater streams; their construction changes the topography (Bonta *et al.* 1997, Bernhardt and Palmer 2011). Mining operations also change the biogeochemistry, porosity, and hydraulic conductivity of soils and underlying geologic materials (Johnson and Skousen 1995, Negley and Eshleman 2006, Simmons *et al.* 2008). These alterations affect streamflow (Dickens *et al.* 1989, Messinger 2003, Negley and Eshleman 2006, Ferrari *et al.* 2009), physicochemical attributes (Johnson *et al.* 2010, Griffith *et al.* 2012), water chemistry (Lindberg *et al.* 2011, Cormier *et al.* 2013, Hopkins *et al.* 2013), sediment loading (Fox 2009), and the ecology (Pond *et al.* 2008) of streams draining these disturbed catchments.

Stream habitat – stream characteristics including water quality and physicochemical attributes, and sediment size distribution and stability – is influenced by the flow regime (Cobb *et al.* 1992, Newbury and Gaboury 1993) and by the surrounding watershed

(Frissell *et al.* 1986). Stream habitat and flow regime are principal drivers of benthic macroinvertebrate community structure (Hynes 1970, Gordon *et al.* 2004, Allan and Castillo 2007). Thus, when stream habitat is altered, the expectation is that the benthic macroinvertebrate community structure will be altered as well. When multiple aspects of habitat are altered, community structure can be expected to reflect those alterations accordingly.

Unweathered rock disturbed by mining and exposed to the ambient environment on surface mines and within valley fills can weather rapidly as water and oxygen contact rock surfaces, reacting chemically with the minerals present and releasing dissolved ions and trace elements to waters that drain into the streams below (Griffith *et al.* 2012). Specific conductance (SC), a measure of water solution conductance of electricity at a standard temperature (Hem 1985), is often used as a surrogate for salinity (Hem 1985, Leland *et al.* 2001, Atkinson and Mabe 2006). Elevated SC has been associated with altered biotic condition in headwater streams draining coal surface mines in the central Appalachian region of the U.S. (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).

Potential stressors are defined here as physical and chemical characteristics of a stream that are known to be altered in systems influenced by mining and have potential to impact benthic community structure. Some stressors cannot be measured easily or directly but can be represented by stressor-indicator variables (SIVs). Prior studies have addressed stressors through causal assessment, treating stressors other than SC and total dissolved solids (e.g. habitat quality, deposited sediment, pH, selenium, catchment area, settling ponds, and trace metals) as factors that confound biotic influence by SC in mining-influenced streams (e.g. Suter and Cormier 2013, U.S. EPA 2010). Few studies have been conducted to address the impacts of stressors other than SC in mining-influenced alkaline streams of central Appalachia.

In this study, I measured multiple characteristics of mining-influenced streams to determine if SIVs in addition to SC were associated with benthic macroinvertebrate community composition. The following specific questions were addressed: 1. Are there SIVs in addition to SC associated with responses of specific taxonomic and functional groups comprising the sampled benthic macroinvertebrate community? 2. Are those associations consistent with what is known about the underlying response mechanisms? Understanding how various environmental variables relate to each other and shape community structure in these systems can provide guidance for stream management and restoration in streams affected by Appalachian coal mining.

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 (MI) streams along a gradient of SC (Figure 4.1). Mean values of SC for the study streams ranged from 24-1,437 $\mu\text{S}/\text{cm}$ for the period of January 2013 to May 2014. 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; Omernik 1987; Figure 4.1). 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 densiometer, and watersheds were devoid of residences, major roads, commercial or industrial facilities other than surface coal mines and small numbers of natural gas wells. Study streams were selected such that reference streams had a similar range of catchment area as mining-influenced streams. All streams had pH > 7 as measured using a handheld multi-probe meter. Streams differed in ionic composition between reference and mining-influenced streams. Dominant aqueous ions in the reference streams were calcium and bicarbonate, whereas the dominant aqueous ions in the mining-influenced streams were calcium and sulfate (Timpano *et al.* 2018).

Data collection and processing

Benthic macroinvertebrates

Benthic macroinvertebrates were sampled in April 2014, using a Hess sampler (Wildco; Yulee, FL, U.S.A.) with a 363 μm Nitex mesh. Six sub-samples were collected from run habitat sections below randomly selected riffles. For each sub-sample collection, a Hess sampler was driven into the streambed sediment, completely enclosing 0.086 m² of stream bottom as needed to prevent organisms from drifting in or out. Complete sub-samples were sorted quantitatively and identified to the lowest practical taxonomic level (to genus level unless specimens were either too immature or damaged to key to genus) using a dissecting microscope and keys provided in Merritt *et al.* (2008). As exceptions, regardless of condition of the specimen, Ceratopogonidae and Chironomidae were keyed to family level and Oligochaeta were keyed to class level.

Candidate SIVs

Candidate SIVs were selected based on previous studies that described impacts of mining land use on stream attributes such as flow regime (e.g. Miller and Zegre 2014), benthic sediment distribution (e.g. Wiley *et al.* 2001), water temperature (e.g. Wiley *et al.* 2001), and water chemistry (e.g. Merriam *et al.* 2011). The complete list of candidate SIVs assessed for effects on benthic macroinvertebrate community structure is provided in Table 4.1.

Mining land use

Mined area, expressed as a fraction of watershed area, was determined from analyses of Landsat satellite data as described by Li *et al.* (2015), and with reference to Timpano *et al.* (2017), which included cumulative percent mined area 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 mined area from 1980-2014.

Streamflow

Solinst Edge Leveloggers (Solinst Canada Ltd.) were installed in each stream in September 2013 to measure stage at 15-minute 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 mean 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 quantile), low (5th quantile), 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 (Table 4.1).

Sediment

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 quantiles of the sediment distributions (10, 16, 25, 50, 75, 84, and 90; expressed as D10, D16, etc.), proportion of sediment that occur in each size class (e.g. % fines, % gravel, % cobble) based on the Udden-Wentworth grain size classification system (Wentworth 1922, Blair and 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 D84 to D50 (Table 4.1).

Relative Bed Stability

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:

$$\text{LRBS} = \log_{10} (\text{D50}/13.7 * \text{mean bankfull hydraulic radius} * \text{mean slope} [\text{rise/run}])$$

where D50 was the median pebble size from pebble counts conducted in March 2014.

Water Chemistry

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 minutes, from which SC was calculated using Onset HOBOWare Pro v.3.4.1 software. At the time of sampling, 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 loggers. These *in-situ* measurements were used to calibrate the conductivity loggers and adjust for logger data drift in HOBOWare. The multimeter probe was calibrated using a standard solution of KCl before each use as per APHA (2005).

Samples of stream water were collected from one point in the thalweg of the stream at the time of benthic macroinvertebrate sampling using a syringe, and filtered streamside using a Fisherbrand PVDF cellulose ester disposable filter with a nominal pore size of 0.45µm. Two 60-mL samples (one for metals analysis and one for backup) were stored in Fisherbrand sample bags, put on ice, brought back to the lab and stored at 4°C until analyses. Samples were acidified to pH < 2 with 1+1 concentrated nitric acid in the field. An ICP-MS (Perkin-Elmer, Norwalk, CT) was used to measure concentrations of nickel, manganese, selenium, zinc, aluminum, and iron (Table 4.1). For each of the analytes, a method detection limit (MDL) and method reporting limit (MRL) were calculated. The analyte concentrations measured at < MRL but ≥ MDL were modeled using the instrument-measured values.

Water Temperature

Stream temperature data downloaded from conductivity loggers was used to calculate minimum, maximum, median, mean, interquartile range, and total range for the periods of April 2013 – April 2014 (Annual), June – August 2013 (Summer), November 2013 – February 2014 (Winter)(Table 4.1).

Data analyses

Benthic macroinvertebrate metrics

Response variables were genus-level richness (number of taxa per sample area), densities (number of individuals per square meter), and compositions (fraction of total, expressed as percent).

Richness was determined by counting the number of taxa identified in the six sub-samples (total sampled area = 0.516 m²) for each stream. Density was calculated for each stream as the mean abundance of benthic macroinvertebrates in the six sub-samples divided by the area of the Hess sampler (0.086 m²).

Benthic macroinvertebrate response metrics were selected for analysis (Table 4.2) based on groups whose characteristics describe ecological relationships. 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 and Webster 1996). Ratios of FFG group densities were also calculated to reflect the state of specific stream characteristics (see Table 3 in Cummins *et al.* 2005 for complete descriptions). Functional Riparian Corridor (FRC) was the ratio of shredder: collectors (gatherer + filterer); Production Rate (PR) was the ratio of scraper: collectors; Substrate Stability (SS) was the ratio of scraper + filterer: shredder + gatherer. Habit groups (e.g. clingers, sprawlers, burrowers, climbers, swimmers) are associated with specific types of physical habitat and are thus potentially sensitive to differences in habitat such as substrate composition or mean stream velocity. 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, Burton and Gerritsen 2003). Some families (e.g. Baetidae, Hydropsychidae, and Leuctridae) within the orders of EPT are pollution-tolerant, can be dominant seasonally (see Chapter 3), 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.

Plausible SIV pathways

A Spearman rank correlation matrix with the candidate SIVs and response metrics was constructed. Benthic macroinvertebrate response metrics of interest were chosen based on having significant correlations ($p < 0.05$) with SC. The SIVs retained for further analysis were those that were correlated ($p < 0.05$) with mining and with most of the response metrics that were significantly correlated with SC.

Results

Benthic macroinvertebrate metrics correlated with SC

Twenty-three out of 68 benthic macroinvertebrate metrics were correlated with SC (Table 4.3); all were also correlated with mining. Most of the correlations were negative except for shredder-Leuctridae, Plecoptera-Leuctridae, percent sprawlers, percent shredders, and Functional Riparian Corridor, which had positive correlations with SC and with mining.

Selected SIVs

Eight out of 87 SIVs were significantly correlated with mining; four of those SIVs (Figure 4.2, Table 4.4) were selected for further study: SC, selenium (Se), large-cobble-to-fines ratio (LCF), and log relative bed stability (LRBS). The other four SIVs (percent fines, percent cobble, D16, and D25) were not selected because they were redundant 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 than the non-selected SIVs. SC and Se were correlated with each other but were not correlated with LCF or LRBS.

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 – 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 different levels of the selected SIVs compared with high-mining streams (Figure 4.2). Most (5 out of 6) of the streams with high (23-65%) mining were characterized with $SC > 575 \mu S cm^{-1}$, $Se > 1.10 \mu g L^{-1}$, $LCF < 0.71$, and $LRBS < -0.850$. One stream was exceptional with higher LCF (2.00) and LRBS (-0.657), though its SC and Se concentration were 2nd highest of all the streams in this study. All low (0-4%) mining streams were characterized by $SC < 412 \mu S cm^{-1}$, $Se < 0.820 \mu g L^{-1}$, $LCF > 1.15$, and $LRBS > -0.850$.

Benthic macroinvertebrate relationships with selected SIVs

Leuctra and Chironomidae were the most abundant of the 98 taxa collected, comprising 29-77% of the total density among the 12 study streams. Taxon richness ranged from 41-62 in the low-mining streams and from 22-38 in the high-mining streams. EPT richness comprised a mean($\pm$ SD) of 66.2%($\pm$ 4.4) and 47.2%($\pm$ 10.2) of the total community richness in low-mining and high-mining streams, respectively.

Among observed taxa, *Leuctra*, Chironomidae, and Oligochaeta were ubiquitous 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 (Figure 4.3A, Table 4.3). Ephemeroptera richness was correlated strongly with SC (Figure 4.3B, Table 4.3) and Se (Figure 4.4C, Table 4.3). Percent Ephemeroptera was correlated strongly with SC (Figure 4.3C, Table 4.3) and mining. Gatherer richness also was correlated strongly with SC and with mining. Gatherers were composed of 13-57% and 0.2-3% 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 (Figure 4.4A, Table 4.3). At the stream with the highest Se concentration ($15.73 \mu\text{g L}^{-1}$), 10 predator taxa were found, with a combined density of 50 individuals m^{-2} . In contrast, streams with $\text{Se} < 3.1 \mu\text{g L}^{-1}$ (EPA ambient water quality criterion, 30-day exposure; U.S. EPA 2016) had a mean($\pm$ SD) of 14($\pm$ 3) predator taxa, with a mean($\pm$ SD) density of 240($\pm$ 83) individuals m^{-2} . Predator density was not correlated with either of the substrate variables (LCF and LRBS).

Scraper richness was also strongly negatively correlated with Se (Figure 4.4B, Table 4.3). 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 $< 20 \text{ m}^{-2}$ and most scrapers had densities $< 5 \text{ m}^{-2}$ at the streams with Se concentration $> 3.1 \mu\text{g L}^{-1}$.

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 variables, LCF and relative bed stability (Figures 4.5 and 4.6, Table 4.3). Although there was evidence of clinger response to LCF, there was no evidence of burrower response. Clinger taxa decreased at a rate of approximately 1.2 taxa per percent fines increase. Taxa 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 SIVs and benthic macroinvertebrate community metrics (Figure 4.7) 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 SIVs appear to impact specific community components in our study streams. Such apparent linkages are described as potential causal pathways.

Pathways among SIVs

Mining was correlated with all other selected SIVs and was considered causal to those SIVs (Figures 4.2 and 4.7). 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.* 2013). There is ample scientific evidence that the accelerated weathering of rock fractured and distributed by Appalachian surface mining is the cause of elevated SC and Se that is commonly found in mining-influenced Appalachian streams (e.g. Lindberg *et al.* 2011; Griffith *et al.* 2012). Streams draining surface mines and valley fills have been found to carry higher fine sediment loads than in unmined watersheds (Curtis 1971, Dickens *et al.* 1985, Bonta 2000, Wiley *et al.* 2001), similar to my finding 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, slope and shape. The positive correlation between LCF and LRBS (Figures 4.2 and 4.7) 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, is a catchment-scale SIV that captures, and likely causes, cumulative effects of all the other SIVs in this study. It is useful to consider relationships between land-use and benthic community structure from a whole-catchment perspective, because land-use variables including area subjected to mining can be generated using geographic information systems.

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 (Green *et al.* 2000, Kennedy *et al.* 2003, Hartman *et al.* 2005, Pond *et al.* 2008, Pond 2010, Bernhardt *et al.* 2012, Boehme *et al.* 2016). Osmotic stress has been suggested as a mechanism (e.g. Hart *et al.* 1991, Pond *et al.* 2008, Cañedo-Argüelles *et al.* 2013) for effects of SC on benthic macroinvertebrates. 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. 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 spent on growth and other functions, leading to osmotic stress. In some high-SC streams in this study, Ephemeroptera density dropped to zero, providing supporting evidence for the causal hypothesis that SC is a toxicant for Ephemeroptera.

Gatherer richness was also correlated strongly and negatively with SC. Ephemeroptera taxa comprised high percentages of gatherer richness in the low-SC streams, but comprised low percentages in high-SC streams, where the few gatherer taxa were comprised primarily of tolerant non-Ephemeroptera taxa such as Chironomidae, Oligochaeta, *Antocha*, and *Chelifera*. In the high-SC streams, only 5-8 gatherer taxa were present, and 60% to 100% were non-Ephemeroptera taxa.

Selenium

Elevated levels of Se in the water column do not necessarily translate into Se toxicity, because there are many species with varying degrees of sensitivity to Se and numerous pathways for Se transfer and accumulation in the aquatic environment (Fan *et al.* 2002, Sappington 2002). For example, volatile methylated Se vaporizes from the water column into the atmosphere (Fan *et al.* 2002). One of the possible pathways for non-volatile Se to become toxic is to oxidize to selenite or selenate and transform into organoselenium as selenoamino acids. From there it can catalyze generation of oxyradicals within cells and cause oxidative stress (Fan *et al.* 2002). It can also substitute for sulfur in proteins, thus altering the tertiary structures and functions of the proteins (Maier and Knight 1994). Prior studies have documented that benthic macroinvertebrates can bioaccumulate Se in mining-influenced streams that are contaminated by Se (Wayland and Crosley 2006, Arnold *et al.* 2014, Whitmore 2016) and suggest that elevated Se can cause toxic effects to benthic macroinvertebrates (Debruyn and Chapman 2007, Conley *et al.* 2009).

Predator density was strongly and negatively correlated with water-column Se concentrations (Figure 4.4A, Table 4.3), a finding that is consistent with a Se bioaccumulation mechanism as cause for reduced predator densities in high-SC streams. Though only water-column Se was measured in this study, several studies have shown

that Se bioaccumulates from the water column to sediments (Besser *et al.* 1989) and primary producers (Presser and Barnes 1984) to primary consumers (Conley *et al.* 2009) to secondary consumers (i.e. predators; Dubois and Hare 2009), concentrating Se in tissue to 100 - 30,000× greater than water-column levels (Saiki *et al.* 1993, Lemly 1999, Mason *et al.* 2000, Swift 2002). Whitmore (2016) found macroinvertebrate predator whole-body Se concentrations to be ~10,000x water-column levels in mining-influenced Appalachian streams.

Ephemeroptera richness was also correlated strongly and negatively with water-column Se concentrations (Figure 4.4C, Table 4.3). Conley *et al.* (2009) reported reduction in fecundity for the mayfly *Centroptilum triangulifer* 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 (Figure 4.4B, Table 4.3), 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 2016).

Large cobble-to-fines ratio

Sediment substrate is an important reach-scale determinant of benthic community structure (Rabeni and Minshall 1977, Waters 1995, 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, Waters 1995, Duan *et al.* 2008).

Certain taxa of benthic macroinvertebrates exhibit substrate composition preferences (Erman and Erman 1984, Richards *et al.* 1993, 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 and Yuan 2010). Clinger richness was correlated strongly and positively with LCF in this study (Figure 4.5A, Table 4.3), 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 (the maximum percentage of fines was 15.5%), or because another stressor was confounding the response. However, the response by clingers suggests that 15.5% fines was a high enough percentage to limit clinger taxa richness. Bryce *et al.* (2010) reported optimum proportions of sand and fines ($\leq 2\text{mm}$) for 8 EPT taxa (all clingers) ranging from 7.3-11.4% 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. In general, streams with lower percent mining and higher LCF had higher LRBS (Figure 4.2). 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 and Brusven 1980) or collection of food (Hynes 1970, Waters 1995). Low LRBS values also indicate potentials for entrainment and movement of fine sediments, as either suspension or saltation load, which can lead to abrasion (Vogel 1994) and increased drift (White and Gammon 1976, 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”, the most common were *Leuctra*, Chironomidae, *Amphinemura*, and Oligochaeta. Most of the remaining taxa had relatively low ($< 50 \text{ m}^{-2}$) 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, in the two streams in this study with the highest LRBS, the most common taxa included all Ephemeroptera swimmers and clingers such as *Ephemerella*, *Habrophlebiodes*, *Ameletus*, *Diphetor*, and *Drunella* in addition to *Leuctra* and Chironomidae, which were generally dominant among all streams.

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 are a significant factor associated with this alteration.

However, this research reveals that other mechanisms may contribute to the benthic macroinvertebrate community alterations that were observed in high-SC streams. I 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. Few Se-toxicity studies focus on benthic macroinvertebrates, and of those that do, many analyze taxonomic groups such as

Ephemeroptera. This study suggests that there is potential in future Se-toxicity research on functional groups such as predators and scrapers.

This study also reveals 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 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 potentially provide guidance for stream management. Improved understanding of how multiple habitat features are altered by mining and, in turn, impact community structure in headwater streams can inform water resource management in mining areas.

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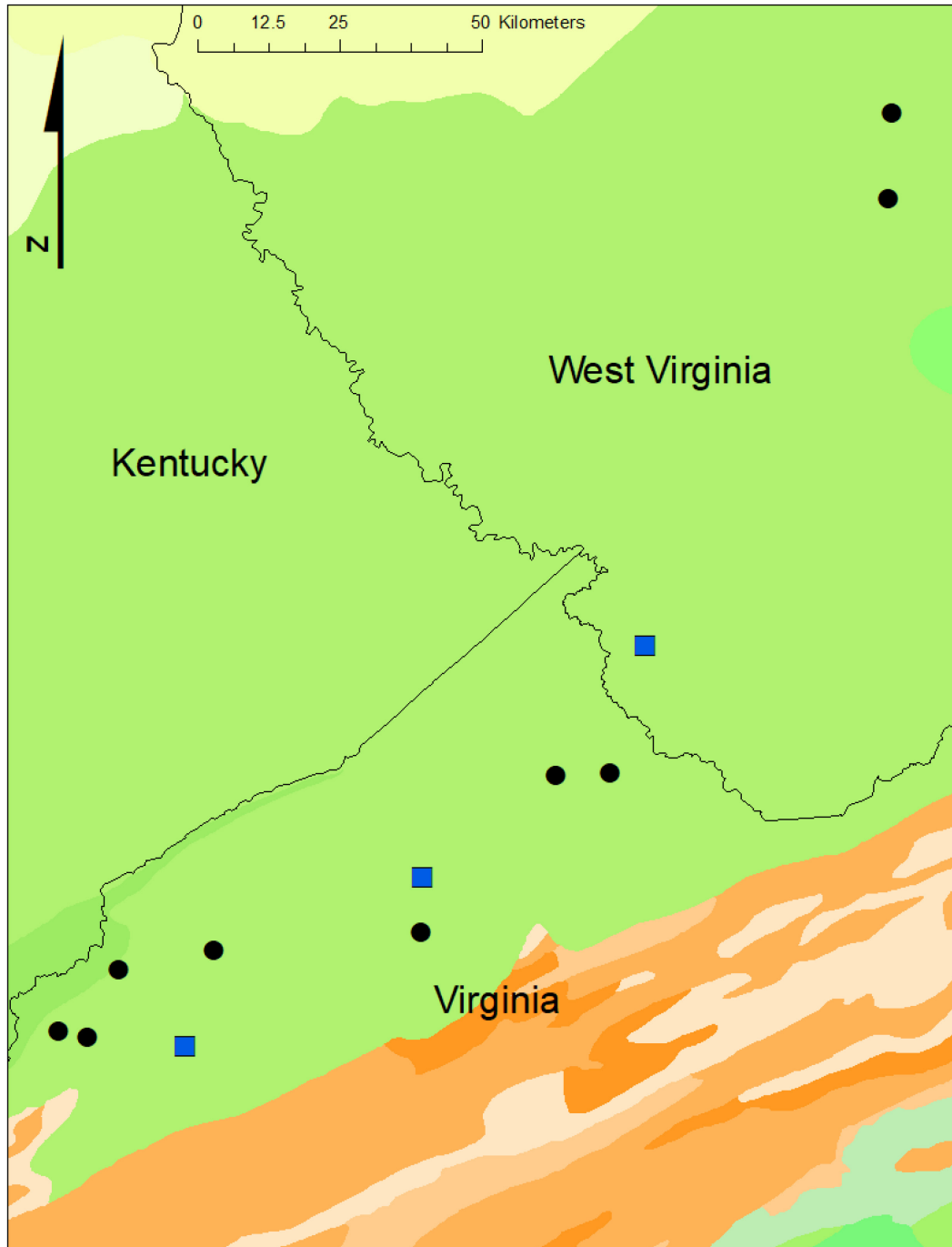


Figure 4.1. Map of study headwater-streams in central Appalachia. Blue squares represent reference streams and black dots represent mining-influenced streams. Map colors represent different level 4 ecoregions. (U.S. EPA)

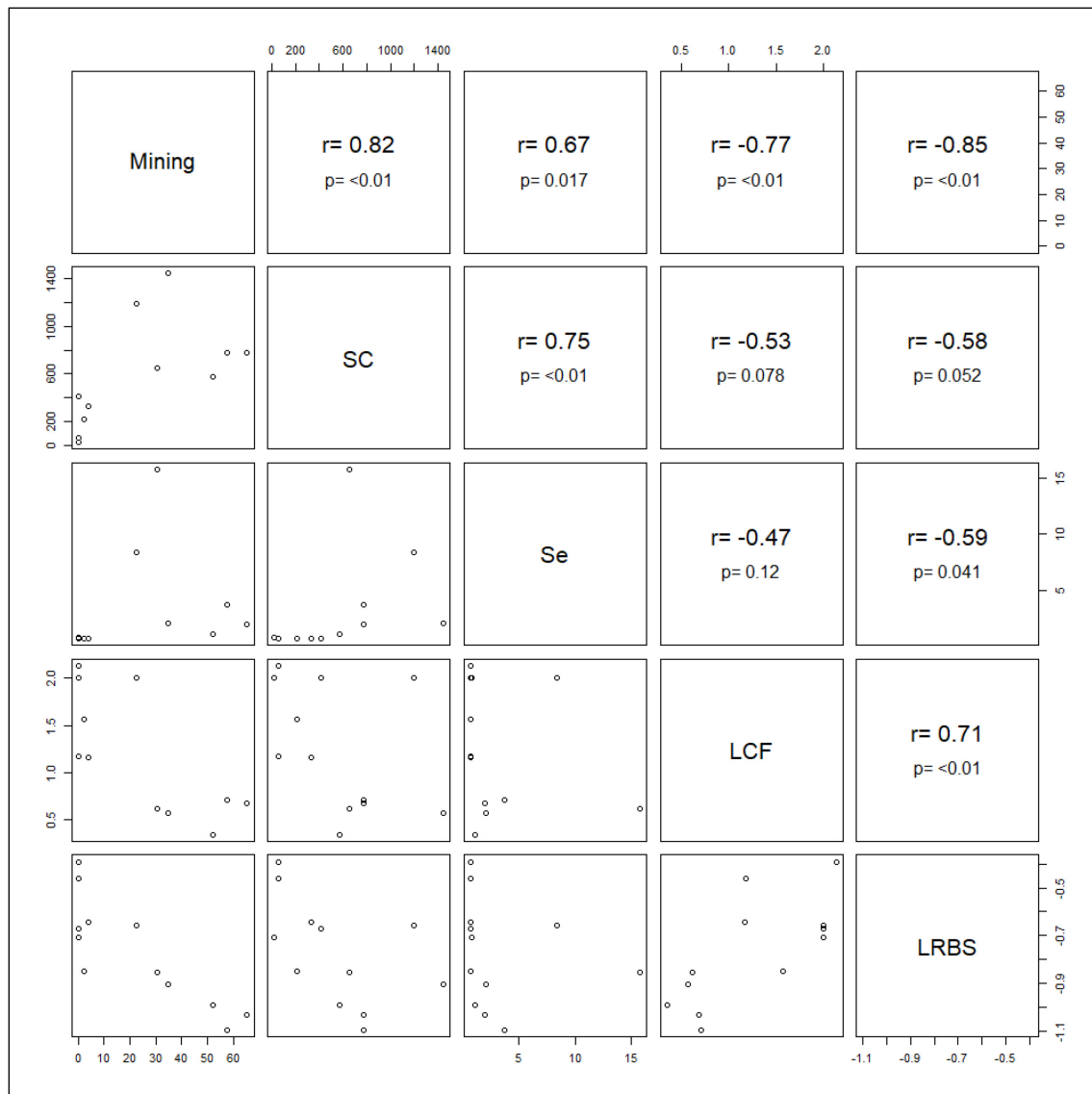


Figure 4.2. Spearman correlation matrix of stressor-indicator variables influencing benthic macroinvertebrate community metrics, with scatterplots in the lower triangle, and Spearman rho coefficients (r) and p-values in the upper triangle. Units for axes are as follows: Mining = %, Specific conductance (SC) = $\mu\text{S cm}^{-1}$, Selenium concentration in water (Se) = $\mu\text{g L}^{-1}$, large cobble-to-fines ratio (LCF) and log of relative bed stability (LRBS) = unitless ratios.

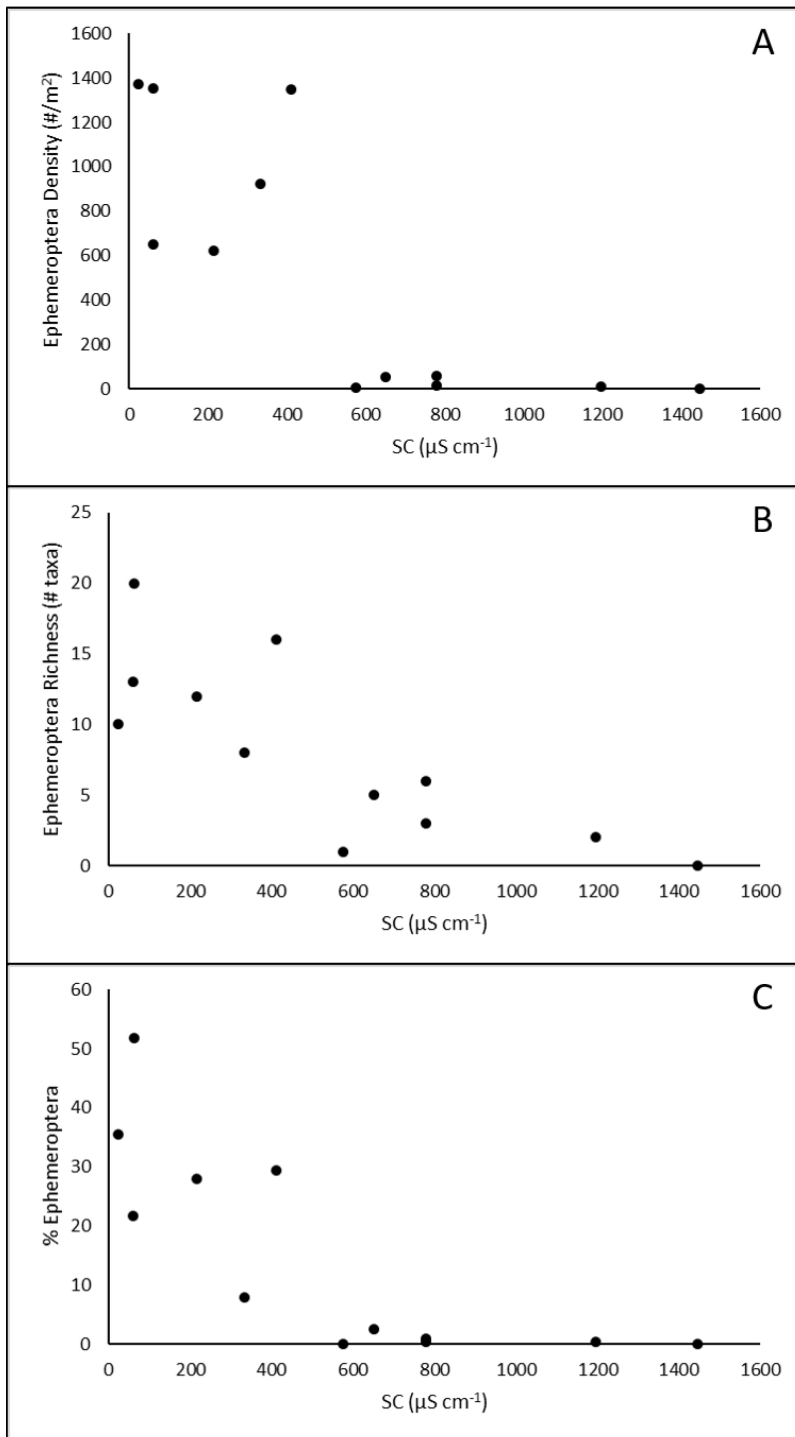


Figure 4.3. Scatterplots of A) Ephemeroptera density vs. specific conductance (SC); B) Ephemeroptera richness vs. SC; C) Ephemeroptera relative abundance vs. SC in headwater streams of central Appalachia.

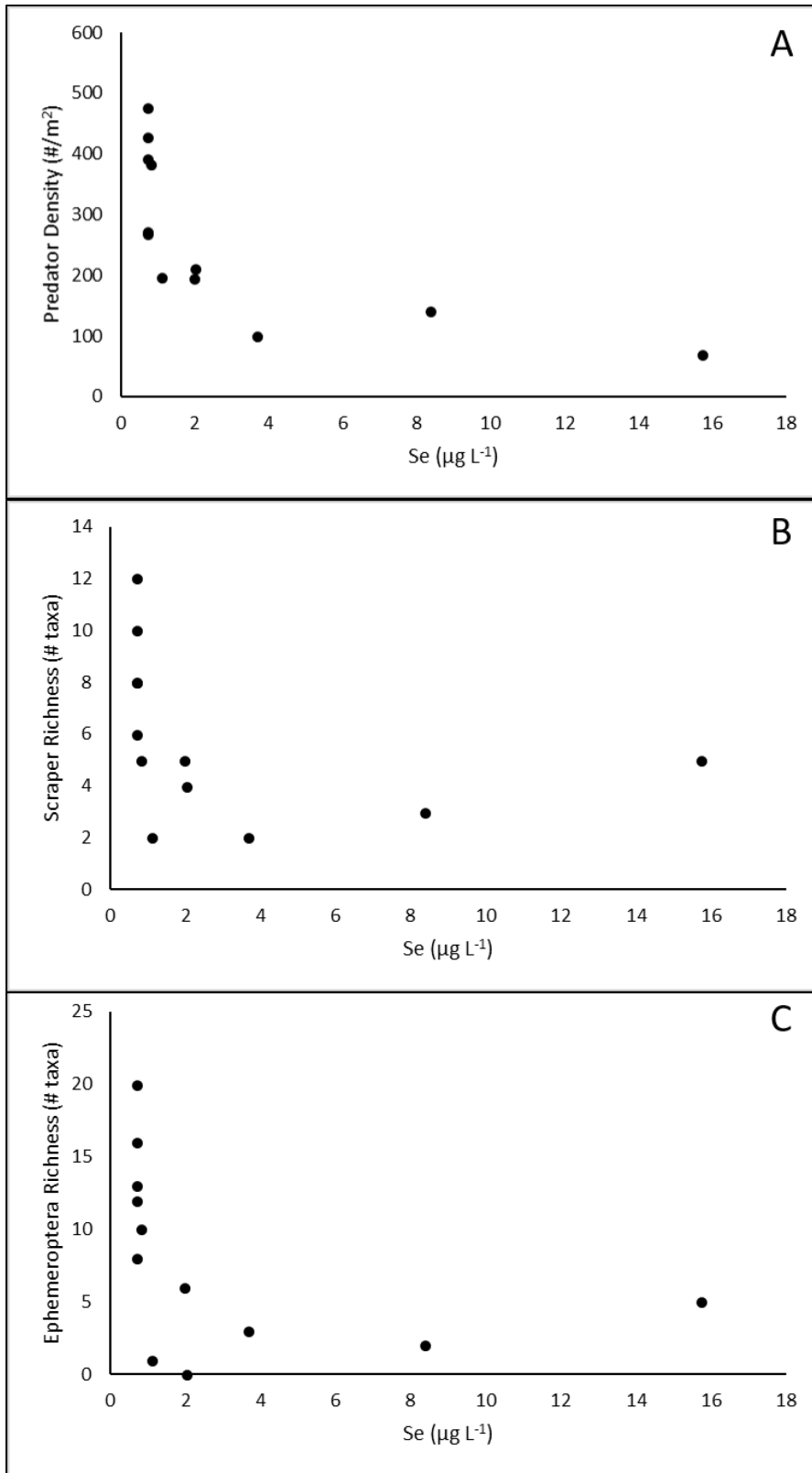


Figure 4.4. Scatterplots of A) Predator density vs. selenium (Se); B) Scraper richness vs. Se; C) Ephemeroptera richness vs. Se in headwater streams of central Appalachia.

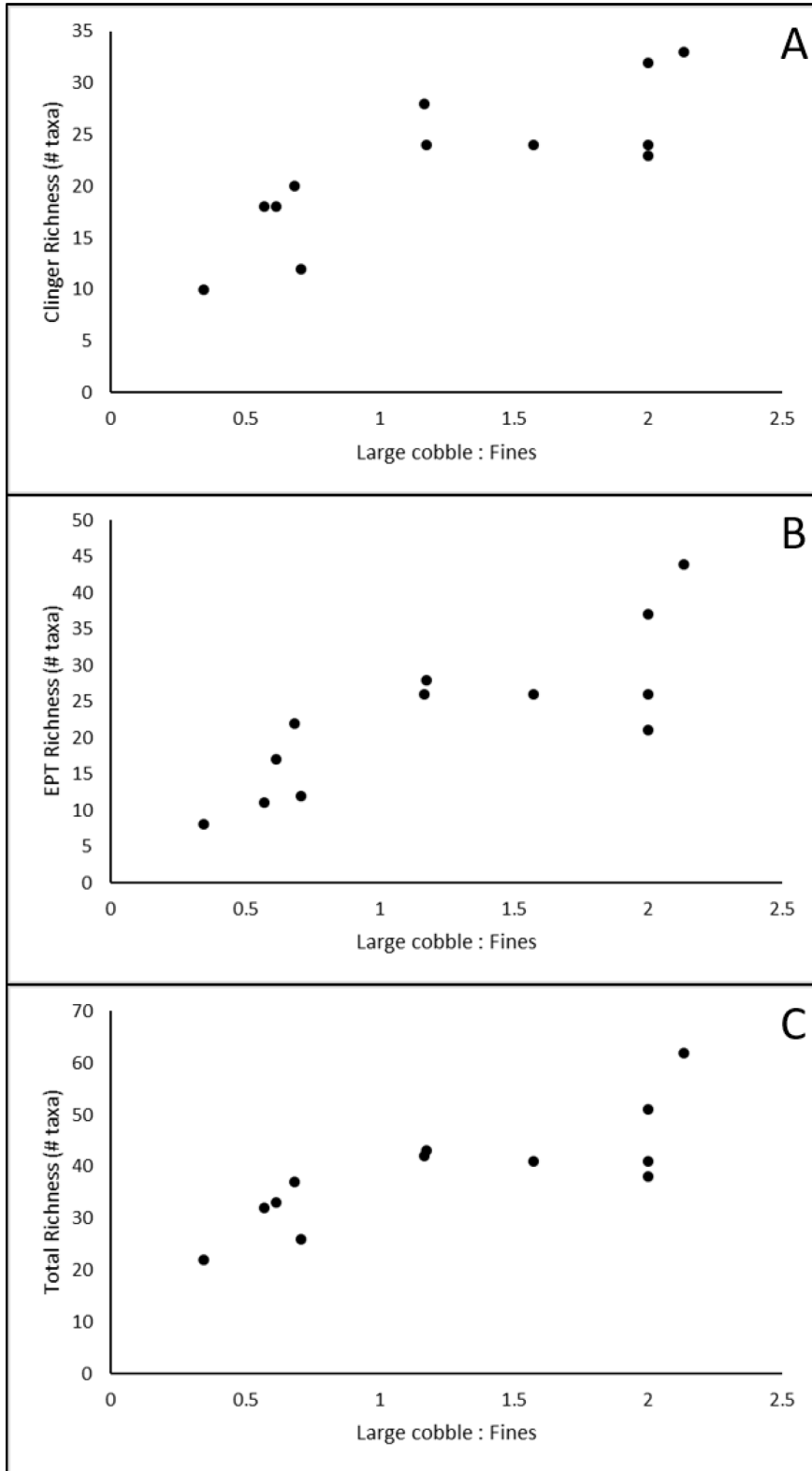


Figure 4.5. Scatterplots of A) Clinger richness vs. Large cobble: Fines; B) EPT richness vs. Large cobble: Fines; C) Total richness vs. Large cobble: Fines in headwater streams of central Appalachia.

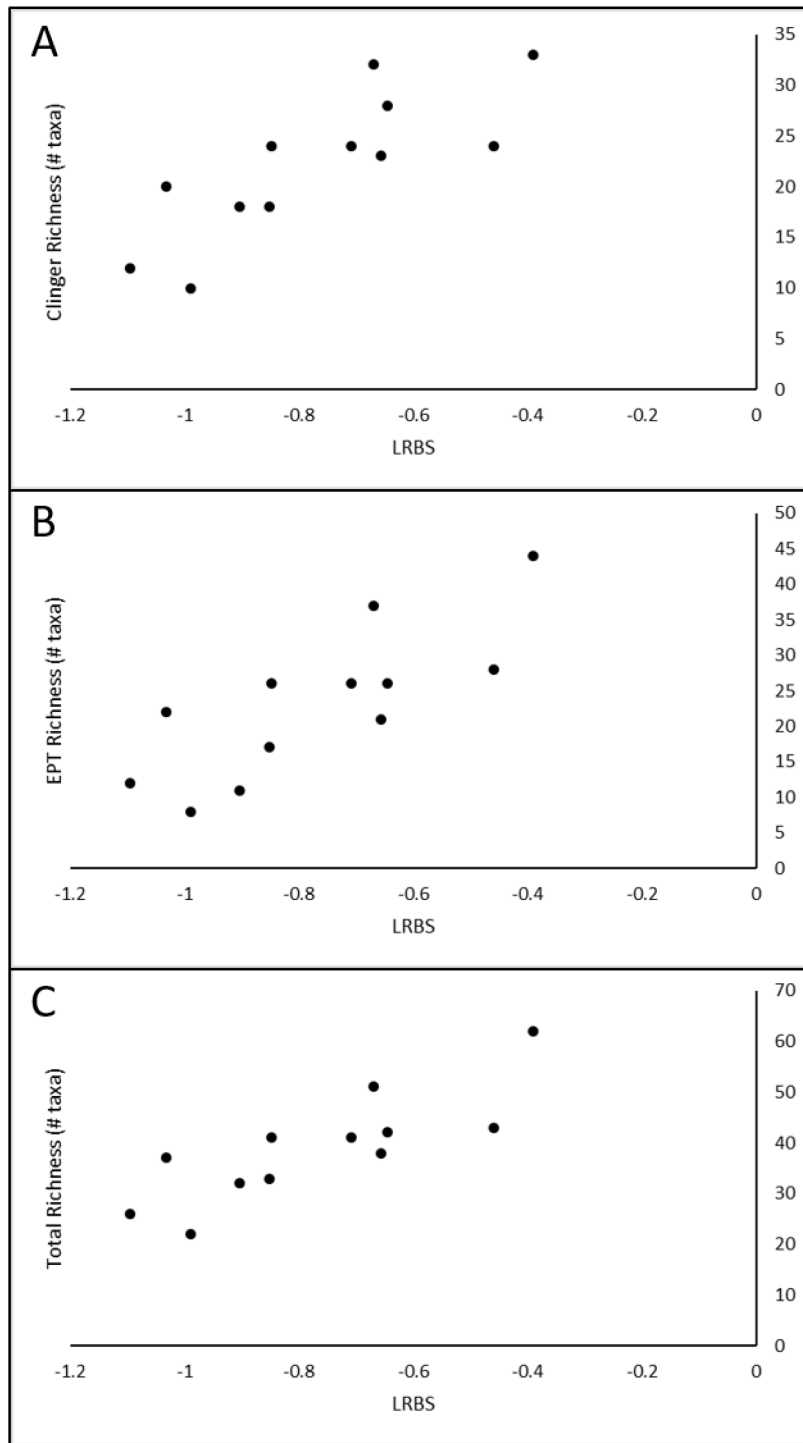


Figure 4.6. Scatterplots of A) Clinger richness vs. log of relative bed stability (LRBS); B) EPT richness vs. LRBS; C) Total richness vs. LRBS in headwater streams of central Appalachia.

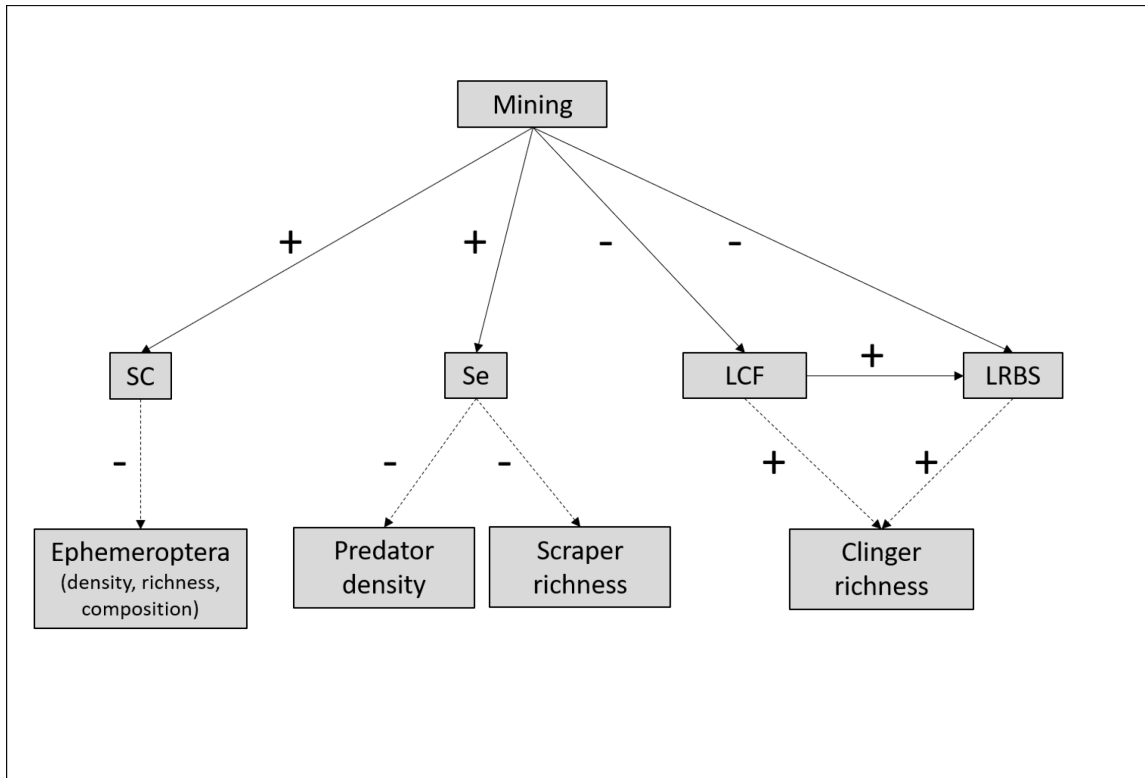


Figure 4.7. Potential causal pathways among selected stressor indicator variables and selected benthic macroinvertebrate metrics in headwater streams in central Appalachia. Signs next to arrows indicate correlation directions (positive or negative). Dotted lines indicate that causal logic, although supported by literature, is more tenuous for biotic pathways than for abiotic pathways. Stressor indicator variables are specific conductance (SC); Selenium concentration in water (Se); large cobble-to-fines ratio (LCF); and log of relative bed stability (LRBS).

Table 4.1. List of candidate stressors and stressor-indicator variables assessed for effects on benthic macroinvertebrate community structure in headwater streams of central Appalachia.

<u>Geography</u>	<u>Substrate</u>	<u>Chemistry</u>
Basin Area	Log Relative Bed Stability (LRBS)	SC
Mining	% fines (< 2 mm)	Se
Latitude	% very fine gravel (2-3 mm)	Mn
Longitude	% fine gravel (4-7 mm)	Al
Elevation	% med gravel (8-15 mm)	Zn
	% coarse gravel (16-31 mm)	Fe
	% very coarse gravel (32-63 mm)	Ni
<u>Geomorphology</u>		
Slope	% gravel (2-63 mm)	
Basin Area : Slope	% small cobble (64-127 mm)	<u>Temperature</u>
Mean bankfull width	% large cobble (128-255 mm)	(Apr 2013-Apr 2014;
Mean bankfull depth	% cobble (64-255 mm)	June - Aug 2013;
	% small boulder (256-511 mm)	Nov 2013 -Feb 2014)
	% med boulder (512-1023 mm)	Min
<u>Hydrology</u>		
Q ₉₅	% bedrock (> 4000 mm)	Max
Q ₅	D10	Median
Q ₅₀	D16	Mean
Q ₉₅ – Q ₅ range	D25	IQR
Q ₅₀ – Q ₅ range	D50	Range
Q ₅₀ – Q ₉₅ range	D75	
Q ₉₅ ua	D84	
Q ₅ ua	D90	
Q ₅₀ ua	IQR	
Q ₉₅ – Q ₅ range ua	Simpson diversity	
Q ₅₀ – Q ₅ range ua	small cobble : fines (SCF)	
Q ₅₀ – Q ₉₅ range ua	large cobble : fines (LCF)	
Flashiness (RBI)	D84 : D50	

Q₅, Q₅₀, and Q₉₅ = 5th, 50th, and 95th quantiles of discharge, respectively; ua = unit-area discharge; D10 = 10th percentile of sediment particle size distribution (with other D-metrics similarly defined); IQR = interquartile range. RBI = Richards-Baker Flashiness Index

Table 4.2. List of candidate benthic macroinvertebrate metrics selected for headwater streams in central Appalachia.

<u>Density (individuals m⁻²)</u>	<u>Richness (No. Taxa)</u>	<u>Evenness</u>	<u>Composition (%)</u>
Total	Total	% Top 2 Dominant	EPT
Total-BHL	Total-BHL	% Top 3 Dominant	EPT-BHL
EPT	EPT	% Top 5 Dominant	E
EPT-BHL	EPT-BHL		E-B
E	E	<u>Diversity</u>	P
E-B	E-B	Simpson	P-L
P	P	Shannon	T
P-L	P-L		T-H
T	T		Baetidae
T-H	T-H		Hydropsychidae
Baetidae	P+T		Leuctridae
Hydropsychidae	Baetidae		Filterer
Leuctridae	Hydropsychidae		Gatherer
Filterer	Leuctridae		Predator
Gatherer	Filterer		Scraper
Predator	Gatherer		Shredder
Scraper	Predator		Burrower
Shredder	Scraper		Clinger
Burrower	Shredder		Sprawler
Clinger	Burrower		Swimmer
Sprawler	Clinger		FRC
Swimmer	Sprawler		Production Rate
	Swimmer		Substrate Stability

E = Ephemeroptera, P = Plecoptera, T = Trichoptera, B = Baetidae, H = Hydropsychidae, L = Leuctridae, FRC = Functional Riparian Corridor. The minus sign indicates exclusion of family from order for analyses.

Table 4.3. Spearman correlations between selected benthic macroinvertebrate metrics and selected stressors in headwater streams influenced by mining in central Appalachia.

Group	Mining	SC	Se	LCF	LRBS
<u>Density</u>					
E-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	0.89***	0.67*	0.62*	-0.61*	-0.65*
Predator	-0.70*	-0.63*	-0.89***	0.45	0.53
P-L	0.87***	0.60*	0.41	-0.71**	-0.78**
<u>Richness</u>					
Gatherer	-0.89***	-0.80**	-0.72**	0.64*	0.67*
E-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	-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***
<u>Diversity</u>					
Simpson	-0.81**	-0.59*	-0.61*	0.89***	0.75**
Shannon	-0.87***	-0.58*	-0.55	0.80**	0.75**
<u>Composition</u>					
% E-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**
FRC	0.77**	0.61*	0.28	-0.56	-0.79**

E = Ephemeroptera, P = Plecoptera, B = Baetidae, L = Leuctridae, FRC = Functional Riparian Corridor. The minus sign in a taxon name indicates exclusion of family from order for analyses.

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

Table 4.4. Stressor ranges across all study streams.

Stressor	Min	Max	Unit
Mining	0	65	%
SC	24.43	1,445	$\mu\text{S cm}^{-1}$
Se	0.7	15.7	$\mu\text{g L}^{-1}$
LCF	0.350	2.13	ratio
LRBS	-1.10	-0.391	ratio

SC = specific conductance; Se = selenium; LCF = large cobble-to-fines ratio; LRBS = log relative bed stability

CHAPTER 5 - Conclusions

Summary

Total density of the benthic macroinvertebrate community in my study streams did not respond to specific conductance (SC) during any of the months when sampled, but densities of several taxonomic and functional groups within the community either increased (e.g. *Leuctra*) or decreased (e.g. Ephemeroptera) with increasing SC, implying that losses of sensitive taxa densities at high SC were being offset by increased densities of tolerant taxa. Total richness also declined with increasing SC, indicating that multiple sensitive taxa were lost in streams with elevated SCs and that communities in those streams were simplified and dominated by a few tolerant taxa.

Taxonomic replacement is a method that has seen use in ecological studies, but no study to my knowledge has used it to assess stream communities influenced by central Appalachian coal mining. In Chapter 3, I used taxonomic replacement analysis to determine if any macroinvertebrate groups responded to SC by replacing sensitive taxa with tolerant taxa. What I found was that in some cases, neither the density nor richness of the groups responded to SC, but taxonomic replacement was occurring along the SC gradient. Results from this investigation revealed which taxa were replacing and which taxa were being replaced along the SC gradient. For example, in May 2013 SC-sensitive Trichoptera (*Cyrnellus*, *Dolophilodes*, *Wormaldia*) and Ephemeroptera (*Isonychia*) filterers found in samples from low-SC streams were replaced by more SC-tolerant Diptera (*Simulium* and *Prosimulium*) filterers in high-SC streams.

I found three stressor-indicator variables (SIVs) in addition to SC that were associated with changes in community structure: selenium, large cobble-to-fines ratio, and relative bed stability. These additional SIVs could be connected in causal pathways both to mining and to specific benthic macroinvertebrate metrics (Figure 5.1). This research confirms findings by other studies that elevated major-ion concentrations appear to be a significant factor in the alteration of benthic macroinvertebrate communities. However, this research reveals that other mechanisms such as elevated selenium and fine sediment may contribute to community alterations observed in high-SC streams.

Implications

Chapter 3 illustrated the importance of accounting for dominant taxa when using metrics such as Ephemeroptera-Plecoptera-Trichoptera (EPT) density or relative abundance to determine the biological status of the sampled benthic macroinvertebrate community in a stream. For example, community-structure alteration can be overlooked if EPT density is a metric used to detect community changes associated with SC influence when a dominant member of EPT that responds positively to increasing SC, such as the stonefly *Leuctra*, is present. *Leuctra* was found in this study to dominate numerically some samples, and its positive association with SC changed the direction of correlation from

negative to non-significant (e.g. EPT density vs. SC) and sometimes from negative to positive (e.g. Plecoptera density vs. SC and shredder density vs. SC).

Tests for comparisons between quantitative and semi-quantitative sampling were not performed for this study, but use of quantitative sampling to provide capabilities that are not possible with semi-quantitative sampling, such as the ability to detect where and when population densities are extremely low is supported by this study. This is evident from data analyses in chapter 3, particularly with Ephemeroptera (Figure 3.3). Estimates of density in addition to relative abundance can help to determine if high relative abundance of a particular taxon is caused by high density of the taxon of interest, low density of another taxon, or a combination.

Taxonomic replacement detected alteration of sampled benthic macroinvertebrate community structure in groups and months when neither density nor richness analyses detected alteration (e.g. Total, EPT, and filterer in May). Consequences of replacement are particularly important within functional feeding groups (FFGs). Differing taxa process food differently so replacement of salt-sensitive taxa present in low-SC streams by tolerant taxa in streams with elevated SC does not necessarily equate to redundancy of function even within FFGs.

Seasonality of sample composition is important to consider for both timing and frequency of sampling. Because sample composition changes seasonally, the responses of taxonomic and functional groups to various stressors, in this case SC, are expected to change seasonally.

Knowledge of how community structure responds to various stressors and SIVs in addition to SC, and at what SIV levels those responses occur, can be informative for stream restoration and management. In chapter 4, I identified elements of stream structure (e.g. substrate) that could be manipulated to improve habitat for target benthic macroinvertebrate groups. Integration of correct habitat design into stream restoration and water resource management is critical for restoring and maintaining benthic community structure and function of headwater streams.

Recommendations

Future research

One of the shortcomings of this project was the small sample sizes. A larger sample size would make it possible to conduct complex modeling of associations between specific taxonomic and functional groups and specific SIVs to further inform our knowledge of the ecology of these headwater streams.

Development of long-term datasets that include both multiple SIVs and quantitative samples of benthic macroinvertebrates that initiate tracking during the mining phase and continue periodic sampling for several years or decades into the recovery phase could possibly confirm patterns seen in this study. Perhaps more importantly, such monitoring could determine if patterns emerge showing that macroinvertebrate communities change

from those characteristic of mining streams to those characteristic of regional reference streams (i.e. recovery). A long-term dataset with a sufficient number of streams would make it possible to determine the time frame required for stream characteristics to return to reference conditions (if they do), and how that change impacts specific components of benthic macroinvertebrate communities.

Another recommendation is to perform functional assessments in conjunction with structural assessments to understand how community structure begets stream ecosystem functions in central Appalachian mining systems along gradients of various SIVs. This is the next logical step towards determining whether loss of diversity and certain functional groups translates into a loss of functionality and ecosystem services in these headwater stream ecosystems.

Policy and management

The results of this study generally support findings using current headwater stream assessment practices such as semi-quantitative sampling and multimetric indices. The methods used in this study could also supplement those practices, perhaps in a selected subset of streams. Excepting assessments of relative bed stability, the full suite of sampling, downloading loggers, and other measurements done for this study were performed in less than three hours by one person per stream visit; the relative bed stability measurements, however, required at least two people and additional time.

The streams in this study were selected with intent to represent reference conditions for all stream habitat features except for SC. Despite this attempt using a semi-quantitative rapid habitat assessment method, quantitative measurements of stream attributes revealed gradients of several SIVs from low-mining-impacted to high-mining-impacted streams. Those gradients were also correlated to select benthic macroinvertebrate community structure metrics, suggesting that quantitative habitat measurements increase the ability to detect changes in biotic condition when habitat differences among streams are not visually detectable. Hence, it is that quantitative habitat measurements are useful supplements to the visual assessments that are used commonly.

Perhaps the most important message to take away from this work concerns the emphasis on SC as a benthic macroinvertebrate community stressor in mining-influenced streams in central Appalachia. Results of this study indicate SC is not the only mining-related stressor affecting macroinvertebrate community structure. I recommend that a multi-stressor approach be considered for future research and management practices. Supplemental sampling and measurement procedures, including using density and taxonomic replacement as tools to detect community structure alterations, are potentially useful for determining protection and mitigation priorities for headwater streams.

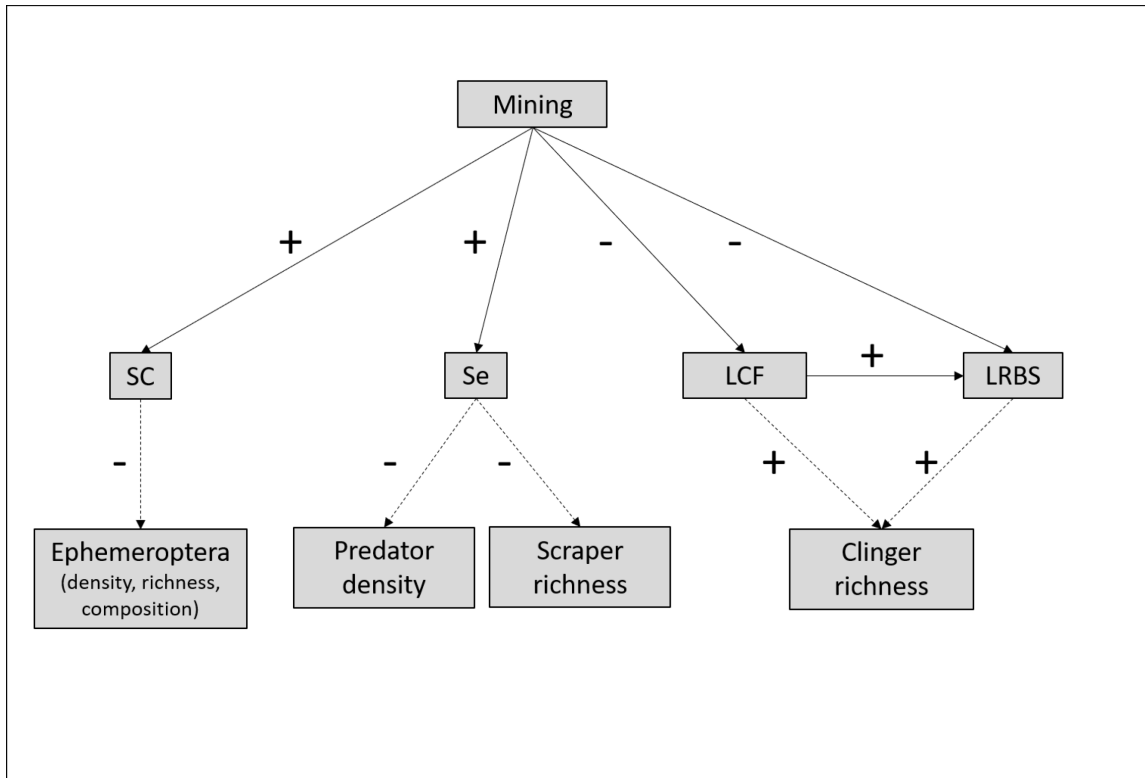


Figure 5.1. Potential causal pathways among selected stressor-indicator variables and selected benthic macroinvertebrate metrics. Signs next to arrows indicate correlation directions (positive or negative). Dotted lines indicate that causal logic, although supported by literature, is more tenuous for biotic pathways than for abiotic pathways.

APPENDIX A – Data summaries for stressor-indicator variables

The following data summaries are stressor-indicator variables used for analyses in chapter 4.

Table A.1. Geography data summaries. Basin area, geographic coordinates, and elevation were all estimated using ArcMap v.10 (ESRI Inc., Redlands, CA, USA) software with digital elevation rasters downloaded from the National Map (United States Geological Survey (USGS); <http://nationalmap.gov/>) in 2014. Mining as a percent of the basin area was obtained from Timpano *et al.* (2017; see Literature Cited in Chapter 4).

Stream ID	Basin area (km ²)	Mining (%)	Latitude	Longitude	Elevation (m)
CRO	2.31	0	37.13019	82.21793	449
EAS	2.39	0	36.91766	82.59197	820
GRA	4.38	2.1	37.25777	82.00913	397
HCN	5.91	0	37.42064	81.86633	360
KEL	2.63	57.7	36.93574	82.79259	564
LLC	4.34	22.6	38.08411	81.47602	297
MIL	2.75	52.1	36.92759	82.74711	559
POW	2.69	65.3	37.01295	82.69753	771
RFF	4.61	0.2	37.05943	82.22127	498
RIC	4.24	34.7	37.03730	82.54575	560
ROC	7.21	30.7	37.96590	81.50045	286
SPC	6.73	3.8	37.26115	81.92038	495

Table A.2. Geomorphology data summaries. Measurements were taken in June 2014 and represent mean values of multiple lateral transects approximately 30 m apart in a 150 m reach.

Stream ID	Slope	Basin area: Slope	Mean bankfull width (m)	Mean bankfull depth (m)
CRO	0.033	70.0	6.09	0.64
EAS	0.063	37.9	4.47	0.53
GRA	0.052	77.3	7.70	0.60
HCN	0.032	184.7	7.20	0.83
KEL	0.064	39.1	5.82	0.61
LLC	0.026	159.6	5.60	0.79
MIL	0.033	79.1	4.31	0.41
POW	0.049	54.9	4.64	0.54
RFF	0.044	104.8	5.05	0.46
RIC	0.026	163.1	6.46	0.62
ROC	0.027	267.0	6.10	0.99
SPC	0.032	210.3	7.26	0.60

Slope is expressed as rise/run; Basin area is expressed as km²

Table A.3. Streamflow summaries were obtained from continuous Levellogger data converted into discharge using rating curves. The raw stage data were obtained between February and April 2014. The rating curves were constructed from six stage and discharge measurement dates.

Stream ID	Q ₉₅	Q ₅₀	Q ₅	Q ₉₅ – Q ₅ range	Q ₅₀ – Q ₅ range	Q ₅₀ – Q ₉₅ range
CRO	0.112	0.054	0.030	0.082	0.024	0.059
EAS	0.125	0.048	0.020	0.105	0.028	0.077
GRA	0.161	0.102	0.069	0.093	0.033	0.060
HCN	0.223	0.095	0.048	0.175	0.047	0.128
KEL	0.050	0.031	0.022	0.028	0.010	0.019
LLC	0.400	0.150	0.069	0.331	0.081	0.250
MIL	0.040	0.024	0.019	0.022	0.005	0.017
POW	0.085	0.046	0.036	0.048	0.010	0.038
RFF	0.304	0.144	0.093	0.211	0.051	0.160
RIC	0.235	0.138	0.092	0.143	0.046	0.097
ROC	0.345	0.104	0.070	0.274	0.034	0.241
SPC	0.362	0.120	0.065	0.297	0.055	0.242

All streamflow summaries are in units of m³ s⁻¹;
Q₉₅, Q₅₀, and Q₅ are 95th, 50th, and 5th percentiles discharge

Table A.4. Unit-area streamflow summaries were obtained from continuous Levellogger data converted into discharge using rating curves. The raw stage data were obtained between February and April 2014. The rating curves were constructed from six stage and discharge measurement dates.

Stream ID	Q ₉₅	Q ₅₀	Q ₅	Q ₉₅ – Q ₅ range	Q ₅₀ – Q ₅ range	Q ₅₀ – Q ₉₅ range	Flashiness
CRO	0.049	0.023	0.013	0.036	0.010	0.025	0.155
EAS	0.052	0.020	0.008	0.044	0.012	0.032	0.176
GRA	0.040	0.025	0.017	0.023	0.008	0.015	0.101
HCN	0.038	0.016	0.008	0.030	0.008	0.022	0.189
KEL	0.020	0.013	0.009	0.011	0.004	0.007	0.094
LLC	0.096	0.036	0.017	0.080	0.020	0.060	0.291
MIL	0.015	0.009	0.007	0.008	0.002	0.006	0.108
POW	0.031	0.017	0.014	0.018	0.004	0.014	0.068
RFF	0.066	0.031	0.020	0.046	0.011	0.035	0.119
RIC	0.055	0.033	0.022	0.034	0.011	0.023	0.141
ROC	0.048	0.014	0.010	0.038	0.005	0.033	0.285
SPC	0.054	0.018	0.010	0.044	0.008	0.036	0.198

Flashiness is unitless and is given by Baker *et al.* 2004;

All other units are m/s and represent unit-area discharge;

Q₉₅, Q₅₀, and Q₅ are 95th, 50th, and 5th percentiles discharge

Table A.5. Substrate data summaries calculated from pebble counts of 200 pebbles/ stream collected in March 2014.

Stream ID	LRBS	Fines	Very fine gravel	Fine gravel	Med gravel	Coarse gravel	Very coarse gravel	Gravel	Small cobble	Large cobble	Cobble	Small boulder	Med boulder	Bedrock
CRO	-0.39	7.5	0.0	5.5	6.0	8.0	27.0	46.5	22.5	16.0	38.5	2.5	0.5	4.5
EAS	-0.71	5.5	0.0	4.0	9.5	19.5	15.0	48.0	20.0	11.0	31.0	8.5	2.0	5.0
GRA	-0.85	7.0	0.0	3.5	11.5	13.5	24.0	52.5	25.0	11.0	36.0	2.0	0.5	2.0
HCN	-0.46	11.5	0.0	1.5	8.5	11.5	23.5	45.0	23.0	13.5	36.5	4.0	0.0	3.0
KEL	-1.10	15.5	0.0	3.5	9.5	9.0	17.0	39.0	22.0	11.0	33.0	4.0	1.5	7.0
LLC	-0.66	4.5	2.0	1.5	1.5	11.0	37.0	53.0	30.0	9.0	39.0	3.0	0.5	0.0
MIL	-0.99	13.0	0.0	4.0	12.5	23.5	23.5	63.5	13.0	4.5	17.5	3.0	1.0	2.0
POW	-1.03	11.0	1.0	7.5	11.5	16.0	21.5	57.5	18.5	7.5	26.0	4.5	0.5	0.5
RFF	-0.67	7.0	1.0	4.0	12.5	18.5	19.0	55.0	20.0	14.0	34.0	1.5	0.5	2.0
RIC	-0.90	14.0	0.5	4.0	14.0	15.5	16.0	50.0	15.0	8.0	23.0	2.5	1.5	9.0
ROC	-0.85	13.0	0.0	1.5	6.0	20.0	26.5	54.0	19.5	8.0	27.5	4.0	1.5	0.0
SPC	-0.65	12.0	0.5	2.5	9.0	15.5	17.0	44.5	21.0	14.0	35.0	3.5	2.0	3.0

All units are % of total pebble count, except LRBS which is a unitless ratio

Table A.6. Substrate data summaries calculated from pebble counts of 200 pebbles/ stream collected in March 2014. All numbers with a “D” prefix are percentiles.

Stream ID	D10	D16	D25	D50	D75	D84	D90	IQR	Simpson	SCF	LCF	D84:D50
CRO	6.0	12.8	27.8	60.0	119.0	164.8	194.2	91.3	0.85	3.0	2.1	2.8
EAS	9.0	14.8	20.8	53.0	139.3	242.1	421.0	118.5	0.88	3.6	2.0	4.6
GRA	6.9	12.8	20.5	51.5	95.0	126.2	159.1	74.5	0.85	3.6	1.6	2.5
HCN	1.0	10.0	19.0	55.0	108.5	149.0	218.1	89.5	0.86	2.0	1.2	2.7
KEL	1.0	4.0	12.0	59.0	125.3	177.1	309.3	113.3	0.88	1.4	0.7	3.0
LLC	16.9	27.8	36.0	63.0	96.0	112.2	140.9	60.0	0.77	6.7	2.0	1.8
MIL	1.0	6.8	13.0	29.5	57.3	81.8	128.3	44.3	0.86	1.0	0.3	2.8
POW	1.0	6.0	9.8	37.5	87.5	104.3	151.8	77.8	0.87	1.7	0.7	2.8
RFF	6.0	10.8	16.0	44.5	97.5	131.5	170.7	81.5	0.86	2.9	2.0	3.0
RIC	1.0	6.0	10.0	35.5	101.0	174.9	577.7	91.0	0.89	1.1	0.6	5.2
ROC	1.0	10.0	18.8	42.0	85.3	110.5	146.2	66.5	0.85	1.5	0.6	2.6
SPC	1.0	8.0	16.0	47.0	116.0	159.0	232.2	100.0	0.88	1.8	1.2	3.4

All units are mm, except for Simpson, SCF, LCF, and D84:D50 which are unitless

Table A.7. Water chemistry data summaries – April 2014

Stream ID	SC ($\mu\text{S cm}^{-1}$)	Se	Mn	Al	Zn	Fe	Ni
MRL		5.0	1.0	1.0	10.0	10.0	0.1
MDL		0.7	0.1	0.1	0.4	0.8	0.0
CRO	61	0.7	1.6	6.6	7.5	36.0	0.1
EAS	24	0.8	0.7	5.2	6.7	12.2	0.0
GRA	216	0.7	3.6	14.1	31.0	342.4	0.5
HCN	61	0.7	2.4	8.7	9.5	54.4	1.0
KEL	778	3.7	5.4	2.6	9.9	23.7	0.9
LLC	1196	8.4	28.6	13.5	7.7	21.6	3.0
MIL	576	1.1	6.9	3.3	4.7	28.2	0.7
POW	778	2.0	5.0	3.5	6.5	178.8	0.9
RFF	412	0.7	1.2	4.3	4.7	11.4	0.3
RIC	1445	2.0	8.7	4.6	5.4	17.5	1.7
ROC	651	15.7	8.4	11.7	74.8	249.6	2.1
SPC	332	0.7	22.1	16.3	10.8	222.6	1.1

All variable units are $\mu\text{g L}^{-1}$, unless otherwise stated;

MRL is minimum reporting level;

MDL is minimum detectable level

Table A.8. Annual water temperature data summaries (Apr 2013 – Apr 2014).

Stream ID	Min	Max	Median	Mean	IQR	Range
CRO	0.5	22.5	12.0	11.9	11.6	22.0
EAS	1.1	19.7	11.2	11.2	9.3	18.6
GRA	1.2	22.6	13.0	12.8	11.1	21.4
HCN	0.7	21.7	11.6	11.6	11.0	21.0
KEL	0.9	22.1	12.4	12.0	7.5	21.1
LLC	-0.2	23.4	13.9	12.8	11.5	23.6
MIL	0.7	22.8	12.1	12.0	11.3	22.1
POW	1.0	20.5	11.2	11.3	9.3	19.5
RFF	0.9	24.0	11.8	12.1	9.2	23.1
RIC	1.0	22.4	12.3	12.0	11.1	21.4
ROC	-0.2	21.4	8.2	9.0	9.3	21.6
SPC	0.9	21.7	13.6	12.5	11.0	20.9

All units are in degrees Celsius;

IQR = Interquartile range

Table A.9. Summer water temperature data summaries (June – Aug 2013)

Stream ID	Min	Max	Median	Mean	IQR	Range
CRO	15.2	22.4	18.6	18.7	2.2	7.2
EAS	13.5	18.9	16.6	16.5	1.9	5.4
GRA	16.5	22.6	19.6	19.6	1.8	6.1
HCN	15.6	21.4	18.6	18.6	1.6	5.8
KEL	14.2	22.1	16.6	16.5	1.2	7.8
LLC	15.6	23.4	19.8	19.8	2.2	7.8
MIL	15.3	22.8	18.9	18.9	1.8	7.6
POW	14.5	20.0	16.7	17.0	1.8	5.5
RFF	14.4	21.1	17.0	17.0	1.7	6.6
RIC	15.1	21.5	18.8	18.7	1.7	6.4
ROC	15.0	21.6	18.2	18.2	1.8	6.7
SPC	14.8	21.7	18.6	18.6	1.6	6.9

All units are in degrees Celsius;

IQR = Interquartile range

Table A.10. Winter water temperature data summaries (November 2013 – February 2014).

Stream ID	Min	Max	Median	Mean	IQR	Range
CRO	0.9	11.3	4.3	4.4	4.8	10.5
EAS	1.1	10.3	5.5	5.5	2.8	9.2
GRA	1.2	12.1	5.8	5.7	4.0	10.9
HCN	0.8	11.0	4.7	4.7	4.2	10.2
KEL	0.9	11.1	7.4	7.1	2.1	10.2
LLC	-0.2	8.3	3.3	3.4	2.9	8.5
MIL	0.7	11.9	4.9	4.8	3.5	11.2
POW	1.0	12.5	5.8	5.8	3.3	11.5
RFF	0.9	11.5	6.5	6.4	2.7	10.6
RIC	1.0	11.3	4.6	4.7	3.7	10.3
ROC	-0.2	12.1	3.9	3.9	3.7	12.3
SPC	0.9	11.7	5.4	5.3	3.6	10.9

All units are in degrees Celsius;

IQR = Interquartile range

APPENDIX B – Benthic macroinvertebrate density taxon x stream matrices

Table B.1. May 2013 specific conductance (SC) and benthic macroinvertebrate density (# individuals m⁻²). Taxa are in first column, stream data are in remainder of columns. Taxa data are based on first three Hess subsamples. Mean SC is the mean of SC values since previous benthic macroinvertebrate sampling. All data correspond to analyses from chapter 3.

Taxon/Stream ID	COP	EAS	FRY	GRA	KEL	KUT	MIL	POW	RFF	SPC
Mean SC (µS cm ⁻¹)	96	23	349	180	784	1096	612	735	426	281
Acentrella	0	0	194	0	0	0	0	0	233	287
Acroneuria	0	4	0	4	0	0	0	0	0	4
Aeshna	0	0	0	0	0	4	8	0	0	0
Agapetus	19	0	0	4	0	0	0	0	23	0
Agnetina	0	0	31	0	0	0	0	0	0	8
Alloperla	0	12	0	0	0	0	0	0	0	0
Ameletus	16	8	0	0	0	0	0	8	0	0
Amphinemura	4	0	4	0	31	8	581	143	0	19
Antocha	0	4	23	0	0	4	4	0	0	4
Arigomphus	0	0	0	0	0	0	0	0	8	0
Attenella	0	4	0	0	0	0	0	0	0	0
Baetis	93	426	0	4	4	349	267	27	16	407
Beloneuria	0	0	8	0	0	0	0	0	0	0
Boyeria	0	0	0	0	4	0	4	0	0	0
Ceratopogonidae	0	16	0	0	0	4	4	0	0	4
Ceratopsyche	0	0	4	0	0	0	8	0	16	0
Cernotina	0	0	4	0	0	0	0	0	0	0
Chauliodes	0	0	4	0	0	0	0	0	0	4
Chelifera	4	4	81	4	16	66	132	112	217	97
Cheumatopsyche	0	0	12	4	4	0	31	0	4	12
Chironomidae	50	182	411	132	333	151	500	62	186	581
Cinygmula	31	116	8	27	8	0	0	0	4	0
Clinocera	0	0	0	0	0	0	0	0	8	0
Collembola	0	12	0	0	8	0	0	0	0	0
Cynellus	4	0	0	0	0	0	0	0	8	8
Dicranota	4	16	0	0	0	0	0	4	0	0
Diplectrona	62	16	54	12	0	66	66	174	62	47
Dolophilodes	8	0	12	12	0	0	4	8	105	89
Dromogomphus	0	0	0	0	0	0	0	0	0	4
Drumella	19	19	81	0	0	0	0	0	225	39
Ectopria	16	12	0	35	8	4	12	31	23	62
Epeorus	47	58	4	0	0	0	0	0	0	8
Ephemera	4	0	0	0	0	0	0	0	0	0
Ephemerella	81	12	0	0	0	0	0	0	0	31
Glossosoma	0	0	0	0	0	0	0	0	0	12

Taxon/Stream ID	COP	EAS	FRY	GRA	KEL	KUT	MIL	POW	RFF	SPC
Gomphidae	0	0	0	0	0	0	4	0	0	0
Gomphus	0	0	0	0	0	0	19	0	0	0
Haploperla	0	8	0	0	0	0	0	0	23	0
Hebrus	0	0	0	0	0	0	0	0	0	12
Hemerodromia	0	0	0	0	0	0	35	4	4	8
Heptagenia	0	0	0	0	0	0	0	0	8	0
Heterocloeon	0	19	31	0	12	39	128	16	0	512
Hexatoma	0	16	0	0	8	0	0	0	4	0
Hydropsyche	0	0	0	0	0	0	4	0	0	4
Hydroptila	0	0	0	0	0	8	4	0	0	0
Isogenoides	0	0	0	0	0	0	0	0	12	0
Isonychia	4	0	0	0	0	0	0	0	0	0
Isoperla	31	8	0	0	0	0	0	16	0	0
Lanthus	0	0	4	4	0	0	4	8	0	0
Lepidostoma	0	4	0	4	0	0	0	0	0	0
Leptophlebia	0	0	12	0	0	0	0	0	0	0
Leuctra	140	360	1101	2019	1271	2275	5523	4132	1205	2314
Limnophila	0	0	0	0	0	4	0	0	0	0
Maccaffertium	0	0	8	43	0	0	0	0	0	0
Macronychus	0	0	43	0	0	0	0	0	0	0
Malirekus	23	4	0	0	0	0	0	0	0	0
Neoplasta	0	0	0	0	0	4	0	4	23	35
Nigronia	0	0	4	0	0	0	8	0	23	4
Oligochaeta	0	16	27	8	314	19	35	16	376	140
Optioservus	0	0	31	0	0	0	12	47	159	23
Oulimnius	85	143	205	0	0	0	0	357	85	50
Paraleptophlebia	27	112	0	0	0	0	0	0	0	0
Peltoperla	39	0	0	0	0	0	0	0	0	0
Perlesta	0	0	0	4	0	0	8	0	27	39
Perlidae	0	0	16	0	8	4	0	0	12	0
Perlodidae	0	0	0	0	0	0	4	0	4	0
Polycentropus	0	12	12	35	0	0	4	0	0	0
Potamyia	0	0	0	0	0	0	0	4	0	0
Prosimulium	0	0	0	0	16	35	27	4	4	0
Psephenus	0	0	109	8	0	0	19	0	50	16
Pteronarcys	0	0	12	0	0	0	0	0	19	0
Rhyacophila	0	4	8	0	0	23	31	12	35	16
Simuliidae	0	0	0	0	70	19	27	0	4	0
Simulium	0	0	0	0	4	8	12	0	0	0
Stenelmis	0	0	0	0	0	0	0	0	0	4
Stenonema	0	0	4	0	0	0	0	0	0	0
Stylogomphus	0	0	0	4	0	0	0	0	0	0

Taxon/Stream ID	COP	EAS	FRY	GRA	KEL	KUT	MIL	POW	RFF	SPC
Stylurus	0	0	0	4	0	0	0	0	0	0
Sweltsa	0	0	4	0	0	0	0	0	0	0
Tallaperla	0	23	0	0	0	0	0	4	0	4
Tipula	0	0	0	0	0	4	12	0	0	0
Utaperla	0	0	0	0	0	0	0	0	8	0
Wormaldia	0	0	0	4	0	0	0	4	101	23

Table B.2. July 2013 specific conductance (SC) and benthic macroinvertebrate density (# individuals m⁻²). Taxa are in first column, stream data are in remainder of columns. Taxa data are based on first three Hess subsamples. Mean SC is the mean of SC values since previous benthic macroinvertebrate sampling. All data correspond to analyses from chapter 3.

Taxon/Stream ID	COP	CRO	EAS	FRY	GRA	KEL	KUT	MIL	POW	RFF	RIC	SPC
Mean SC ($\mu\text{S cm}^{-1}$)	130	67	27	394	246	848	1309	656	886	456	1583	382
Acentrella	0	0	50	8	8	0	12	0	0	16	4	0
Acerpenna	0	8	0	0	0	0	0	0	0	0	0	0
Acroneuria	4	19	50	0	43	0	0	0	0	8	0	39
Aeshnidae	0	0	0	0	0	0	0	0	0	4	0	0
Agapetus	0	8	0	0	0	0	0	0	0	0	0	0
Agnetina	0	0	0	0	0	0	0	0	0	0	0	16
Allocapnia	0	0	0	0	0	0	0	0	0	35	0	0
Alloperla	8	0	16	4	0	0	0	0	0	43	4	0
Ameletus	0	4	0	0	0	0	0	0	0	0	0	0
Antocha	0	4	8	16	0	0	0	4	0	16	0	4
Baetis	112	66	4	0	4	0	4	4	12	0	0	0
Beloneuria	0	0	47	0	0	0	0	0	0	0	0	0
Boyeria	0	0	0	0	0	0	0	0	0	0	4	0
Calopteryx	0	0	0	0	0	0	0	0	0	0	4	0
Capniidae	0	0	0	0	0	0	0	0	0	12	0	0
Ceratopogonidae	16	4	8	0	0	4	4	12	0	0	12	0
Ceratopsyche	66	70	205	81	58	23	171	171	43	116	105	16
Chelifera	8	12	16	8	0	35	12	4	23	8	16	19
Cheumatopsyche	0	8	4	8	0	19	74	74	0	23	12	0
Chironomidae	275	109	744	112	89	58	31	74	43	314	54	89
Cinygmula	0	0	19	0	0	0	0	0	0	0	0	0
Collembola	0	0	0	4	0	0	8	0	0	0	0	0
Dicranota	0	0	8	0	0	0	0	0	0	0	0	0

Taxon/Stream ID	COP	CRO	EAS	FRY	GRA	KEL	KUT	MIL	POW	RFF	RIC	SPC
Diphettor	50	0	4	0	0	0	0	0	0	0	0	0
Diplectrona	31	19	47	0	8	0	16	35	39	12	4	16
Diura	35	0	0	0	0	0	0	0	0	0	0	0
Dixa	8	0	0	0	0	0	0	4	0	0	0	0
Drunella	0	4	0	0	0	0	0	0	0	0	0	0
Ectopria	140	4	19	4	0	16	23	8	23	12	8	8
Ephemera	4	12	0	0	0	0	0	0	0	12	0	0
Ephemerella	0	4	0	0	0	0	0	0	0	0	0	8
Glossosoma	8	0	23	4	0	0	0	0	0	0	0	0
Goera	0	0	0	0	0	0	0	0	0	12	0	0
Gomphidae	0	0	0	4	0	0	0	0	0	4	0	0
Hansonoperla	35	58	23	0	0	0	0	0	0	0	8	0
Haploperla	16	0	0	0	0	0	0	0	0	0	0	0
Hemerodromia	8	0	0	4	0	0	0	0	0	8	4	0
Heptagenia	12	93	81	12	0	0	0	0	0	8	0	19
Heptageniidae	0	0	0	0	0	0	0	0	0	8	0	0
Heterocloeon	4	0	35	0	0	0	0	12	0	0	0	0
Hexatoma	8	19	16	0	0	0	0	0	0	0	8	0
Isoperla	4	0	0	0	0	0	0	0	0	0	0	0
Lanthus	4	0	4	0	0	54	8	27	19	0	0	4
Leptotarsus	0	4	0	0	0	0	0	0	0	0	0	0
Leucrocuta	12	4	16	0	0	0	0	0	0	0	0	0
Leuctra	426	27	667	806	484	930	1225	2465	1012	1364	1868	1729
Limonia	0	0	0	0	0	0	0	0	0	0	8	0
Maccaffertium	0	4	0	19	39	0	0	0	0	12	0	109
Macrostemum	0	0	0	4	0	0	0	0	0	0	0	0
Malirekus	0	0	4	0	0	0	0	0	0	0	0	0

Taxon/Stream ID	COP	CRO	EAS	FRY	GRA	KEL	KUT	MIL	POW	RFF	RIC	SPC
Nemoura	0	0	0	0	0	0	8	19	0	0	0	0
Neoplasta	0	0	0	4	0	0	0	0	0	4	0	0
Nigronia	12	0	0	12	0	0	0	8	0	50	0	35
Oligochaeta	47	8	4	8	43	671	0	0	4	19	31	16
Optioservus	105	47	225	47	4	4	0	8	31	112	23	39
Oulimnius	0	0	0	16	0	0	0	0	0	0	0	0
Paragnetina	0	0	8	0	0	0	0	0	0	0	0	0
Paraleptophlebia	43	8	16	0	16	0	0	0	0	0	0	0
Pedicia	0	0	0	0	4	0	0	0	0	0	0	0
Peltoperla	27	0	16	0	0	0	0	0	0	0	0	0
Perlidae	0	0	0	12	0	0	0	0	0	35	0	0
Pilaria	0	0	0	0	0	0	0	0	0	0	4	0
Platyhelminthes	0	0	0	0	0	0	0	0	0	4	0	0
Plauditus	0	0	0	0	0	0	4	0	0	0	0	0
Polycentropus	0	0	16	0	0	4	4	0	0	0	0	4
Psephenus	0	35	0	47	8	0	4	16	0	47	8	27
Pycnopsyche	0	0	0	0	0	0	0	0	0	4	0	0
Rhagovelia	0	12	0	0	4	0	0	0	8	0	8	0
Rhyacophila	12	0	43	0	4	0	8	23	0	0	0	8
Sialis	0	0	0	0	0	4	0	0	0	0	4	8
Simuliidae	0	0	8	0	0	0	0	267	0	0	12	0
Simulium	0	0	0	0	0	0	8	0	0	0	0	0
Stenacron	0	16	0	0	0	0	0	0	0	0	0	0
Stenelmis	12	8	0	16	0	0	0	0	0	39	0	0
Stenonema	27	27	4	0	70	0	0	0	0	43	0	182
Stylogomphus	0	0	0	0	39	0	0	0	0	0	4	8
Tallaperla	0	0	39	0	0	0	0	0	0	0	0	0

Taxon/Stream ID	COP	CRO	EAS	FRY	GRA	KEL	KUT	MIL	POW	RFF	RIC	SPC
Tipula	4	0	0	0	0	4	0	0	0	0	0	0
Twinnia	0	0	0	0	0	0	4	0	0	0	0	0
Wormaldia	12	0	4	0	0	0	0	0	0	0	0	0

Table B.3. September 2013 specific conductance (SC) and benthic macroinvertebrate density (# individuals m⁻²). Taxa are in the first column; stream data are in remainder of columns. Taxa data are based on first three Hess subsamples. Mean SC is the mean of SC values since previous benthic macroinvertebrate sampling. All data correspond to analyses from chapter 3.

Taxon/Stream ID	CRO	FRY	GRA	KEL	KUT	MIL	POW	RFF	RIC	SPC
Mean SC ($\mu\text{S cm}^{-1}$)	73	414	284	778	1199	637	951	454	1650	429
Acentrella	0	0	0	0	0	0	0	16	0	0
Acroneuria	16	8	47	4	0	0	0	12	12	8
Allocapnia	0	0	0	0	0	0	0	0	0	4
Alloperla	0	19	0	0	0	0	4	39	0	4
Antocha	0	4	0	0	0	0	0	0	19	0
Atrichopogon	4	0	0	0	0	0	0	0	0	0
Baetis	12	4	0	0	0	0	0	58	0	0
Beloneuria	12	0	0	0	0	0	0	0	0	27
Boyeria	0	0	0	0	4	8	0	0	0	0
Ceratopogonidae	0	23	4	23	0	0	0	0	4	0
Ceratopsyche	47	78	16	27	112	93	43	500	380	62
Cernotina	0	0	0	4	0	0	0	0	0	0
Chelifera	0	0	0	0	0	0	0	0	8	0
Cheumatopsyche	8	16	19	19	0	112	8	62	287	39
Chironomidae	112	78	27	23	58	43	50	271	302	47
Chloroperlidae	0	0	0	0	0	0	4	0	0	0
Cinygmula	12	0	0	0	0	0	0	0	0	0
Collembola	4	4	0	12	8	4	0	8	4	0
Dicranota	0	0	0	0	0	0	0	12	0	0
Diphetor	19	0	0	0	0	0	0	0	0	0
Diplectrona	50	12	54	81	194	174	54	221	4	112
Dolophilodes	0	0	0	0	0	0	0	39	0	0
Ectopria	8	4	27	4	4	12	0	12	23	16
Elmidae	12	0	0	0	0	0	0	0	0	0
Ephemera	16	39	0	8	0	0	0	12	0	0
Ephemerellidae	0	12	0	0	0	4	0	0	0	19
Eurylophella	8	0	0	4	0	0	0	0	0	0
Glossosoma	0	12	0	0	0	0	0	16	0	0
Goera	0	0	0	0	0	0	0	4	0	0
Gomphidae	4	16	4	4	0	35	16	12	16	12
Heptageniidae	0	8	4	0	0	0	0	0	0	27
Hexatoma	4	0	0	4	0	0	0	0	16	0
Lanthus	12	0	4	27	16	16	0	0	4	0
Leuctra	12	74	85	128	492	376	570	35	481	147
Limnephilidae	4	0	0	0	0	0	0	0	0	0
Limnophila	0	0	0	0	0	0	0	0	0	4

Taxon/Stream ID	CRO	FRY	GRA	KEL	KUT	MIL	POW	RFF	RIC	SPC
Maccaffertium	12	43	19	0	0	0	0	0	0	74
Neoplasta	0	4	0	0	0	0	0	0	4	0
Nigronia	0	4	4	0	0	0	0	31	0	16
Oligochaeta	16	47	0	81	4	0	0	39	8	16
Optioservus	19	244	12	0	0	16	23	252	372	50
Ormosia	4	0	0	0	0	0	0	0	0	0
Oulimnius	12	47	0	0	0	0	58	47	19	8
Paracapnia	74	12	120	8	0	0	0	12	0	47
Paraleptophlebia	85	0	0	0	0	0	0	0	0	0
Plauditus	0	0	0	0	4	0	0	19	0	0
Pleidae	4	0	0	4	0	8	4	0	8	0
Polycentropus	0	0	0	0	0	0	4	0	0	8
Psephenus	16	357	66	0	0	19	0	78	8	62
Pteronarcys	0	0	0	0	0	0	0	8	0	0
Rhagovelia	0	0	0	0	0	0	4	0	0	0
Rhyacophila	16	39	0	12	12	12	4	16	0	16
Sialis	0	0	0	0	4	0	0	0	0	0
Simuliidae	0	0	0	0	0	12	0	4	0	0
Soyedina	0	4	0	0	0	0	0	0	0	0
Stenonema	4	23	19	0	0	0	0	0	0	19
Stylogomphus	8	0	8	0	0	0	0	0	0	0
Tipula	0	4	0	0	0	4	4	0	0	8
Wormaldia	0	0	0	0	0	0	0	8	0	0

Table B.4. November 2013 specific conductance (SC) and benthic macroinvertebrate density (# individuals m⁻²). Taxa are in the first column, stream data are in remainder of columns. Taxa data are based on first three Hess subsamples. Mean SC is the mean of SC values since previous benthic macroinvertebrate sampling. All data correspond to analyses from chapter 3.

Taxon/Stream ID	CRO	FRY	GRA	HCN	KEL	KUT	MIL	POW	RFF	RIC	ROC	SPC
Mean SC ($\mu\text{S cm}^{-1}$)	82	436	402	84	801	1249	765	944	542	1856	588	523
Acentrella	0	0	0	0	0	0	0	0	58	0	0	0
Acerpenna	12	0	0	0	0	0	0	0	0	0	0	0
Acroneuria	23	4	31	0	0	0	0	0	8	4	4	23
Allocapnia	62	961	93	194	167	89	287	601	570	1341	376	39
Alloperla	27	8	66	23	0	4	0	16	182	0	0	23
Ameletus	159	23	16	4	0	0	35	345	12	4	0	12
Anax	0	0	0	0	0	0	0	0	0	4	0	0
Antocha	0	12	0	35	0	0	0	0	27	12	0	8
Baetidae	0	0	0	0	0	0	0	0	16	0	0	0
Baetis	81	0	4	0	0	4	0	0	252	0	0	0
Baetisca	0	0	0	4	0	0	0	0	0	0	0	0
Centropilum	4	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	19	8	0	62	0	0	12	0	27	12	8	12
Ceratopsyche	0	295	0	0	39	12	4	8	434	1186	43	43
Cernotina	0	0	23	8	0	0	0	16	0	0	4	19
Chelifera	0	0	0	8	0	0	0	0	0	0	0	0
Cheumatopsyche	0	171	0	16	27	12	54	0	136	539	39	35
Chimarra	0	0	0	4	0	0	0	0	0	0	0	4
Chironomidae	357	709	306	1116	116	337	1504	229	430	659	54	93
Cinygmula	70	0	0	23	0	0	0	0	0	0	0	0
Collembola	1229	85	4	43	209	4	4	0	0	0	19	23
Dicranota	0	0	0	0	0	0	0	0	8	0	0	0
Diphetor	31	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream ID	CRO	FRY	GRA	HCN	KEL	KUT	MIL	POW	RFF	RIC	ROC	SPC
Diplectrona	12	112	12	4	97	453	58	39	81	159	39	23
Dixa	4	0	0	0	0	0	4	0	0	0	0	0
Ectopria	12	4	0	4	0	23	4	47	19	35	0	0
Elmidae	0	0	0	0	0	0	0	0	66	0	0	0
Ephemera	47	16	4	89	0	0	0	0	8	0	0	0
Ephemerellidae	93	39	31	349	0	0	0	0	50	0	0	47
Eurylophella	78	4	4	221	0	0	0	0	0	0	0	8
Glossosoma	0	8	0	0	0	0	0	0	4	0	0	0
Goera	16	47	23	0	0	0	0	0	8	0	0	0
Goeridae	0	8	0	0	0	0	0	0	0	0	0	0
Gomphidae	0	0	4	4	0	4	19	4	0	39	16	31
Hemerodromia	0	4	0	0	0	0	0	0	0	0	0	4
Heptageniidae	23	8	12	0	0	0	0	0	12	0	0	0
Heterocloeon	0	4	0	0	0	4	0	0	0	0	0	0
Hexatoma	23	0	0	8	0	0	0	8	0	16	0	4
Isogenoides	0	0	0	0	0	0	0	0	23	0	0	0
Isoperla	0	0	0	0	0	0	0	4	35	0	0	0
Lanthus	4	4	8	16	12	23	4	23	0	0	0	0
Leptophlebia	0	0	0	23	0	0	0	0	0	0	0	0
Leptophlebiidae	0	0	0	8	0	0	0	0	4	0	0	0
Leuctra	8	62	4	35	62	163	81	248	35	74	16	31
Limnophila	8	0	0	0	0	0	0	0	0	0	0	0
Limonia	0	0	0	0	0	0	0	0	0	4	0	0
Maccaffertium	19	4	19	132	0	0	0	0	50	0	4	70
Nemouridae	0	16	0	12	0	0	0	0	4	8	0	0
Neophylax	50	143	101	81	0	12	58	16	47	8	12	23
Neoplasta	0	4	0	12	0	0	0	0	4	0	0	0

Taxon/Stream ID	CRO	FRY	GRA	HCN	KEL	KUT	MIL	POW	RFF	RIC	ROC	SPC
Nigronia	0	0	0	0	0	0	8	0	27	8	0	19
Oligochaeta	8	147	209	302	101	31	47	12	19	0	31	16
Optioservus	19	422	4	97	0	0	43	109	888	198	31	74
Oulimnius	39	492	0	198	4	4	0	143	171	12	8	58
Paracapnia	167	337	163	50	23	4	151	0	322	128	0	112
Paraleptophlebia	136	0	4	8	0	0	0	0	0	0	0	0
Pedicia	0	0	0	0	0	4	0	0	0	0	0	0
Peltoperla	0	0	4	0	0	0	0	0	0	0	0	0
Peltoperlidae	0	0	0	0	0	0	0	0	8	0	0	0
Perlodidae	19	23	0	4	0	0	0	0	0	0	4	0
Pilaria	4	43	0	8	0	0	0	0	0	0	0	0
Platyhelminthes	0	0	0	0	0	0	0	4	0	0	0	0
Plauditus	0	0	0	0	0	0	0	0	190	0	8	0
Pleidae	0	0	0	0	4	0	4	0	0	0	0	0
Polycentropus	0	4	0	35	0	0	0	0	0	0	0	47
Psephenus	12	128	43	8	0	0	0	0	62	0	0	54
Pteronarcys	8	4	0	0	0	0	0	0	4	0	0	0
Pycnopsyche	8	97	0	0	0	0	19	0	4	0	0	0
Rhyacophila	0	16	0	23	19	35	16	12	50	54	4	19
Serratella	0	0	0	0	4	0	8	0	0	0	0	0
Shipsa	0	0	0	0	0	0	0	0	0	0	8	0
Sialis	0	4	0	0	4	0	0	0	4	4	4	4
Simuliidae	0	0	0	4	4	0	0	0	0	4	0	0
Stenacron	23	0	0	12	0	0	0	0	0	0	0	0
Stenelmis	0	8	0	0	0	0	0	0	0	0	0	0
Stenonema	0	16	12	50	0	0	0	0	16	0	0	35
Stylogomphus	12	8	35	12	0	0	8	0	0	4	0	0

Taxon/Stream ID	CRO	FRY	GRA	HCN	KEL	KUT	MIL	POW	RFF	RIC	ROC	SPC
Taeniopteryx	0	0	0	23	0	4	4	0	4	4	0	8
Tipula	0	0	0	0	0	4	19	0	4	4	0	0
Veliidae	0	0	0	0	0	0	0	4	0	0	0	0

Table B.5. January 2014 specific conductance (SC) and benthic macroinvertebrate density (# individuals m⁻²). Taxa are in the first column, stream data are in the remainder of columns. Taxa data are based on first three Hess subsamples. Mean SC is the mean of SC values since previous benthic macroinvertebrate sampling. All data correspond to analyses from chapter 3.

Taxon/Stream ID	COP	CRO	EAS	FRY	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
Mean SC ($\mu\text{S cm}^{-1}$)	111	57	20	291	742	983	736	562	743	260	1258	568	256
Acerpenna	16	54	12	0	0	0	0	0	0	0	0	0	0
Acroneuria	19	8	58	31	0	0	31	0	4	43	23	0	19
Allocapnia	16	8	0	558	0	0	8	66	27	27	124	23	19
Alloperla	58	39	0	4	0	0	0	0	0	16	0	8	8
Ameletus	19	27	43	58	4	0	0	78	8	0	4	0	8
Amphinemura	31	0	4	163	806	353	70	205	163	388	198	4	236
Antocha	4	0	0	58	0	0	0	0	0	23	0	0	0
Baetidae	0	0	0	4	0	0	0	4	0	0	0	0	0
Baetis	0	0	0	31	23	70	0	4	58	275	0	4	31
Beloneuria	0	4	0	0	0	0	0	0	0	0	0	0	4
Boyeria	0	0	0	0	4	0	0	4	0	0	0	0	0
Ceratopogonidae	23	0	19	0	8	4	4	0	4	27	4	0	0
Ceratopsyche	198	70	4	306	39	85	353	43	70	686	1159	62	101
Cernotina	8	0	4	0	0	0	0	0	0	8	23	4	8
Chelifera	0	0	4	0	8	12	0	4	8	0	31	0	4
Cheumatopsyche	8	0	4	109	132	8	74	50	0	54	399	12	101
Chimarra	0	0	0	0	0	0	0	0	0	0	4	0	4
Chironomidae	442	391	535	318	399	787	388	267	101	864	1271	50	578
Chloroperlidae	0	8	0	0	0	0	0	0	0	0	0	0	0
Cinygmula	4	12	655	0	0	0	0	0	0	4	0	0	0
Clinocera	0	0	0	0	0	0	4	0	0	0	0	0	0
Collembola	16	8	0	4	4	0	4	0	0	4	4	0	0
Cordulegaster	4	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream ID	COP	CRO	EAS	FRY	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
Cultus	0	0	0	4	0	0	0	0	0	4	0	0	0
Dicranota	0	0	0	0	0	0	8	0	0	0	0	0	0
Diphetor	0	31	4	0	0	0	0	0	0	0	0	0	0
Diplectrona	345	132	47	271	492	322	178	112	225	271	209	74	236
Diploperla	0	16	4	12	0	0	0	0	0	0	0	0	0
Diura	101	12	8	4	0	0	4	0	43	4	0	0	0
Dixa	0	0	0	4	0	0	0	0	0	0	0	0	0
Dolophilodes	4	0	0	12	0	0	12	0	0	19	4	0	4
Ectopria	58	16	8	8	16	4	0	4	16	23	27	4	8
Epeorus	89	264	593	23	0	0	0	0	4	39	0	0	16
Ephemera	4	4	8	8	0	0	0	0	0	4	0	0	12
Ephemerella	880	0	97	43	0	0	0	8	0	31	0	0	74
Ephemerellidae	0	0	0	0	0	0	0	0	4	0	0	8	0
Eurylophella	256	43	47	81	0	0	0	0	0	12	0	0	43
Glossosoma	8	8	0	4	0	0	8	0	0	0	0	4	0
Goera	0	4	23	23	0	4	19	4	0	4	8	0	31
Gomphidae	4	0	4	0	0	8	12	4	12	0	8	0	8
Hemerodromia	0	0	0	0	12	0	0	4	0	4	0	0	0
Heptageniidae	0	4	0	0	0	0	0	0	8	0	0	0	0
Hexatoma	27	16	23	0	0	0	0	0	0	0	19	0	0
Hydroperla	4	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsyche	0	0	0	0	0	0	16	0	0	12	0	0	0
Isogenoides	0	4	0	0	0	0	0	0	0	0	0	0	0
Isoperla	39	4	0	27	0	0	19	0	23	16	0	0	4
Lanthus	4	0	4	0	35	8	0	12	0	8	47	0	0
Leptophlebiidae	0	31	0	0	0	0	0	4	4	0	0	0	0
Leucrocuta	0	0	31	0	0	0	0	0	0	0	0	0	0

Taxon/Stream ID	COP	CRO	EAS	FRY	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
Leuctra	58	16	93	240	2465	1368	4	291	682	267	543	0	500
Maccaffertium	8	0	0	4	0	0	0	0	0	8	0	0	70
Macronychus	4	0	0	0	0	0	0	0	0	0	0	0	0
Malirekus	12	0	0	0	0	0	0	0	0	0	0	0	0
Metachela	0	0	0	0	0	0	0	16	0	0	0	0	0
Nemoura	0	0	0	136	0	0	186	128	35	50	4	236	151
Nemouridae	0	0	0	0	0	0	0	0	0	0	4	0	0
Neophylax	23	93	23	205	4	4	43	19	0	112	31	0	66
Neoplasta	0	19	31	4	35	62	8	27	16	12	78	0	19
Neuriclepsis	0	0	0	0	0	0	0	0	0	4	8	0	0
Nigronia	0	0	0	16	0	0	0	0	0	27	0	0	27
Oemopteryx	0	0	0	124	0	0	132	0	0	124	0	0	8
Oligochaeta	8	0	8	0	516	43	8	16	8	39	31	4	16
Optioservus	0	8	12	93	0	0	43	4	0	89	116	12	23
Ormosia	12	4	0	0	0	0	8	0	0	0	0	4	0
Ostrocerca	0	0	0	0	0	0	0	19	23	0	35	0	31
Oulimnius	136	27	35	43	0	0	0	0	19	105	19	8	8
Paracapnia	89	221	27	178	0	0	4	0	0	62	0	0	159
Paraleptophlebia	23	23	124	0	0	0	0	0	0	0	0	0	0
Pedicia	12	0	4	0	0	0	0	0	0	0	4	0	0
Peltoperla	0	4	4	0	0	0	0	0	0	0	0	0	0
Perlodidae	4	0	0	0	0	0	4	0	0	4	0	0	8
Pilaria	4	0	4	0	0	0	0	0	0	0	0	0	8
Plauditus	0	0	0	47	39	31	0	0	12	190	4	4	12
Polycentropus	12	0	12	0	0	0	0	0	0	0	0	0	8
Psephenus	0	8	0	78	0	0	0	0	0	19	0	4	66
Pseudocleon	0	0	0	12	0	0	0	0	0	0	0	0	0

Taxon/Stream ID	COP	CRO	EAS	FRY	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
Pteronarcys	43	8	50	16	0	0	8	0	0	39	0	0	12
Pycnopsyche	0	16	0	0	0	0	0	0	0	0	16	0	4
Remenus	0	0	0	0	0	0	0	0	0	4	0	0	0
Rhyacophila	81	16	50	58	47	54	43	23	70	112	93	8	66
Serratella	50	271	27	19	0	0	0	0	0	182	0	0	391
Shipsa	0	0	0	12	4	0	58	0	16	0	0	0	81
Sialis	0	0	0	0	0	0	0	0	0	0	4	0	0
Simuliidae	66	287	23	388	23	4	8	0	0	244	23	0	0
Soyedina	0	0	0	16	0	0	23	0	0	0	0	89	19
Stenonema	4	27	0	0	0	0	0	0	0	0	0	0	0
Stylogomphus	0	4	0	4	0	0	0	0	0	0	8	0	8
Sweltsa	4	0	0	0	0	0	0	0	0	0	0	0	0
Taeniopteryx	0	0	0	0	0	0	16	8	0	0	4	4	35
Tallaperla	4	0	0	0	0	0	0	0	16	0	0	0	0
Tipula	4	0	0	8	8	4	4	43	16	19	8	8	0
Tipulidae	0	0	0	0	0	0	0	0	0	4	0	0	0
Ustrocerca	0	0	0	19	0	0	0	0	0	0	0	0	0
Wormaldia	0	8	0	0	4	0	8	0	0	4	0	0	4
Yugus	16	0	4	0	0	0	0	0	8	4	0	0	0

Table B.6. April 2014 specific conductance (SC) and benthic macroinvertebrate density (# individuals m⁻²). Taxa are in the first column, stream data are in the remainder of columns. Taxa data are based on first three Hess subsamples. Mean SC is the mean of SC values since previous benthic macroinvertebrate sampling. All data correspond to analyses from chapter 3.

Taxon/Stream ID	CRO	EAS	GRA	HCN	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
Mean SC ($\mu\text{S cm}^{-1}$)	61	24	216	61	778	1061	1196	576	778	412	1445	651	332
Acentrella	19	0	345	23	0	0	0	0	0	1159	0	31	252
Acerpenna	12	0	0	0	0	0	0	0	0	0	0	0	0
Acroneuria	62	31	39	0	0	0	43	4	35	16	8	0	31
Alloperla	0	0	0	4	0	0	0	0	0	0	0	0	0
Ameletus	128	4	12	0	0	0	0	0	19	8	0	0	0
Amphinemura	27	31	16	31	616	19	275	1039	1442	248	186	481	403
Antocha	0	4	27	50	4	8	4	0	0	23	8	12	4
Arigomphus	0	0	0	0	0	0	0	0	0	0	0	4	0
Atherix	0	0	0	0	0	0	0	0	0	0	4	0	0
Baetis	0	12	0	0	12	19	0	0	43	27	0	4	0
Baetisca	0	0	0	4	0	0	0	0	0	0	0	0	0
Beloneuria	4	0	0	0	0	0	0	0	0	0	0	0	39
Brachycentrus	50	0	0	8	0	0	0	0	0	8	0	0	0
Ceratopogonidae	27	27	0	16	0	0	43	0	4	8	16	0	23
Ceratopsyche	4	4	0	4	4	0	101	0	0	174	16	27	16
Cernotina	0	0	23	0	0	0	0	0	0	0	0	0	4
Chelifera	12	54	16	97	124	50	27	116	248	16	89	12	291
Cheumatopsyche	8	4	8	47	23	4	124	43	0	47	140	23	78
Chironomidae	659	771	422	853	481	547	589	500	833	469	857	1174	4756
Cinygmula	43	415	19	8	0	0	0	0	12	0	0	8	12
Clinocera	0	0	0	0	0	0	4	0	4	0	0	4	16
Clioperla	0	0	0	19	0	0	0	0	0	0	0	0	0
Cultus	0	0	0	0	0	0	0	0	0	0	8	0	0

Taxon/Stream ID	CRO	EAS	GRA	HCN	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
Cynellus	23	0	23	0	0	0	16	0	8	31	0	0	23
Dicranota	0	8	0	0	0	0	0	0	0	0	0	0	4
Diphetor	70	0	0	0	0	0	0	0	0	4	0	0	0
Diplectrona	70	70	19	0	54	43	85	43	465	136	0	35	124
Dolophilodes	4	0	0	0	0	0	0	0	0	0	0	0	0
Drunella	116	229	50	58	4	0	8	0	0	213	0	8	78
Ectopria	12	27	12	27	0	12	12	4	12	4	27	4	31
Empididae	0	0	4	0	0	0	0	0	0	0	0	0	0
Epeorus	124	578	12	0	0	0	0	0	0	43	0	0	16
Ephemera	19	0	4	12	0	0	0	0	0	8	0	0	0
Ephemerella	143	147	39	426	0	0	0	4	8	35	0	0	477
Eurylophella	47	0	0	27	0	0	0	0	0	0	0	0	62
Glossosoma	0	0	0	0	0	0	0	0	0	0	0	4	0
Gomphidae	0	0	4	0	0	0	0	0	0	0	4	0	0
Habrophlebia	31	0	0	0	0	0	0	0	0	0	0	0	0
Habrophlebiodes	136	0	0	27	0	0	0	0	0	16	0	0	0
Haploperla	27	31	194	12	0	0	19	0	0	8	0	4	27
Hemerodromia	0	0	23	85	8	0	12	31	16	4	19	19	19
Heptagenia	0	0	0	0	0	0	0	0	0	8	0	0	0
Heterocloeon	19	0	0	4	0	0	0	0	0	0	0	0	0
Hexatoma	12	31	0	0	0	0	4	0	8	0	8	0	0
Hydropsyche	0	0	0	0	0	0	0	0	0	0	0	8	0
Isogenoides	8	0	0	0	0	0	0	0	0	0	0	0	19
Isonychia	78	0	0	0	0	0	0	0	0	35	0	0	0
Isoperla	4	0	0	12	0	0	4	0	0	16	0	0	0
Lanthus	8	0	31	16	39	0	27	0	0	16	27	0	16
Lepidostoma	35	27	0	0	0	4	8	0	4	0	0	0	0

Taxon/Stream ID	CRO	EAS	GRA	HCN	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
Leptophlebiidae	78	0	0	0	0	0	0	0	0	0	0	0	0
Leuctra	151	787	783	864	4101	4047	442	3236	3740	984	1016	171	4674
Limnophila	0	0	0	0	0	0	0	0	4	0	0	0	0
Maccaffertium	0	0	12	43	0	0	0	0	0	4	0	0	19
Microcylloepus	0	0	0	0	0	0	0	0	0	0	19	0	0
Nemouridae	0	0	4	0	0	0	0	0	0	0	0	0	0
Neophylax	12	0	0	0	0	0	0	0	0	0	0	0	0
Neoplasta	0	0	0	0	0	0	0	0	0	0	0	4	12
Nigronia	0	0	0	4	0	0	0	4	0	19	4	0	35
Ochrotrichia	0	0	0	0	0	27	0	0	58	0	0	0	0
Oligochaeta	58	39	132	31	601	147	12	109	54	244	47	78	1926
Optioservus	66	0	0	136	0	0	155	0	0	132	66	58	101
Ormosia	4	4	0	0	0	0	0	0	0	0	0	4	0
Oulimnius	0	457	0	0	0	0	0	0	535	0	47	0	0
Paraleptophlebia	43	43	8	12	0	0	0	0	0	8	0	4	0
Perlesta	0	16	0	0	0	0	0	27	8	0	167	0	0
Perlodidae	0	0	8	0	0	0	0	0	0	0	0	4	109
Polycentropus	12	0	8	23	0	0	8	0	78	0	0	4	101
Prosimulium	0	0	0	0	43	0	0	8	0	0	0	0	0
Psephenus	12	0	19	31	0	0	4	0	0	16	4	0	50
Pteronarcys	19	4	0	0	0	0	0	0	0	8	0	0	0
Pycnopsyche	8	0	0	4	0	0	4	0	0	0	8	0	0
Remenus	0	0	0	0	0	0	0	0	0	4	0	0	0
Rhyacophila	0	16	0	0	8	8	4	4	19	16	16	4	0
Sialis	0	0	4	0	0	0	0	0	0	0	0	0	0
Simuliidae	8	0	4	8	4	0	16	0	0	0	0	4	0
Simulium	0	4	0	0	0	0	0	0	12	0	0	0	0

Taxon/Stream ID	CRO	EAS	GRA	HCN	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
Stactobiella	0	0	0	0	0	0	0	0	93	0	0	0	0
Stenacron	8	167	0	0	0	0	0	0	8	12	0	0	0
Stenelmis	0	0	0	0	0	0	0	0	0	54	0	0	0
Stenonema	16	0	8	12	0	0	0	0	0	0	0	0	0
Stylogomphus	0	8	0	0	47	12	0	128	58	0	12	0	0
Suwallia	12	0	0	4	0	0	0	0	0	0	0	0	0
Sweltsa	0	19	0	0	0	0	0	0	0	8	0	0	0
Taeniopterygidae	0	0	4	0	0	0	0	0	0	0	0	0	0
Tallaperla	0	0	0	0	0	0	0	0	4	0	0	0	0
Tipula	4	0	0	0	8	12	0	8	8	4	0	16	4
Yugus	12	8	12	0	4	0	31	0	54	54	0	0	0

Table B.7. April 2014 benthic macroinvertebrate density (# individuals m⁻²). Taxa are in the first column; stream data are in the remainder of columns. Taxa data are based on six Hess subsamples. All data correspond to analyses from chapter 4.

Taxon/Stream ID	CRO	EAS	GRA	HCN	KEL	LLC	MIL	POW	RFF	RIC	ROC	SPC
Acentrella	23	0	428	19	0	2	0	0	924	0	29	415
Acerpenna	25	0	0	0	0	0	0	0	0	0	0	0
Acroneuria	39	27	37	0	0	21	0	17	8	6	0	17
Agnetina	0	0	0	0	0	0	0	0	12	0	0	0
Alloperla	0	0	0	2	0	2	0	0	0	0	0	4
Ameletus	136	31	6	2	0	0	0	21	4	0	0	0
Amphinemura	16	16	31	35	752	248	672	899	188	234	463	275
Antocha	0	2	17	50	2	2	2	0	29	6	12	4
Atherix	0	0	0	0	0	0	0	0	0	2	0	0
Baetidae	0	0	2	0	0	0	0	0	0	0	0	0
Baetis	35	19	0	2	12	0	0	21	16	0	2	0
Baetisca	0	0	0	2	0	0	0	0	0	0	0	0
Beloneuria	2	0	0	2	0	0	0	0	25	0	0	87
Brachycentrus	41	0	0	4	0	2	0	0	6	0	0	0
Calopteryx	0	0	0	0	0	0	6	0	0	0	0	0
Ceratopogonidae	17	39	0	39	14	23	29	6	19	10	4	16
Ceratopsyche	2	2	0	2	2	85	0	0	120	122	47	8
Cernotina	0	0	14	0	0	0	0	0	2	0	0	2
Chelifera	6	31	8	136	91	23	93	233	47	60	19	223
Cheumatopsyche	6	2	8	56	17	91	23	0	41	128	31	60
Chironomidae	661	1031	351	1010	486	566	1517	783	578	1405	942	3816
Cinygmula	70	473	17	10	0	0	0	6	0	0	6	10
Clinocera	0	6	2	0	2	2	0	2	0	0	2	17
Cordulegaster	0	0	0	0	0	0	0	2	0	0	0	0
Cultus	0	0	0	0	0	0	0	0	0	4	0	0

Taxon/Stream ID	CRO	EAS	GRA	HCN	KEL	LLC	MIL	POW	RFF	RIC	ROC	SPC
Cynellus	16	2	14	0	0	14	0	4	16	8	0	19
Dicranota	0	14	0	8	0	2	0	0	0	0	2	4
Diphetor	231	0	0	0	0	0	0	0	2	0	0	0
Diplectrona	45	39	35	2	43	87	23	287	101	16	68	93
Diploperla	0	0	0	0	0	2	0	0	0	0	0	0
Dolichopodidae	4	0	0	0	0	0	0	0	0	0	0	0
Dolophilodes	4	0	2	0	0	6	0	0	0	0	0	0
Drunella	99	182	70	37	2	6	0	0	194	0	17	70
Dubiraphia	0	0	0	0	0	0	0	0	0	16	0	0
Ectopria	16	21	14	45	2	12	4	17	23	29	4	27
Empididae	0	0	2	0	0	0	0	0	0	0	0	0
Epeorus	91	405	14	0	2	0	0	0	23	0	0	8
Ephemera	17	6	2	27	0	0	0	0	105	0	0	2
Ephemerella	192	107	58	360	0	0	4	4	19	0	0	339
Eurylophella	62	4	6	126	0	0	0	0	6	0	0	56
Glossosoma	0	0	0	0	0	0	0	0	0	0	2	0
Gomphidae	0	0	2	0	0	0	0	0	0	2	0	2
Habrophlebia	35	0	0	0	0	0	0	0	0	0	0	0
Habrophlebiodes	171	0	0	17	0	0	0	0	10	0	0	0
Haploperla	29	21	219	14	0	10	0	14	12	0	4	19
Hemerodromia	2	0	19	99	4	17	33	8	6	12	16	17
Heptagenia	35	0	0	0	0	0	0	0	4	0	0	0
Heptageniidae	0	0	0	0	0	0	0	0	6	0	0	0
Heterocloeon	14	0	0	0	0	0	0	0	0	0	0	0
Hexatoma	10	35	0	4	0	4	0	4	0	8	0	0
Hydropsyche	0	0	0	0	0	2	0	0	0	0	8	2
Isogenoides	4	0	0	0	0	0	0	0	0	0	0	10

Taxon/Stream ID	CRO	EAS	GRA	HCN	KEL	LLC	MIL	POW	RFF	RIC	ROC	SPC
Isonychia	45	0	0	0	0	0	0	0	17	0	0	0
Isoperla	19	0	0	16	0	4	0	0	12	0	2	0
Lanthus	10	0	25	27	31	19	10	0	21	21	6	16
Lepidostoma	27	21	0	2	19	4	37	19	0	0	0	0
Leptophlebiidae	23	0	0	0	0	0	0	0	0	0	0	0
Leuctra	91	653	645	552	3176	347	2337	2789	1370	1037	188	4194
Limnophila	0	6	0	0	0	0	0	6	0	0	0	0
Maccaffertium	0	0	8	37	0	0	0	0	6	0	0	21
Microcylloepus	0	0	0	0	0	0	0	0	0	10	0	0
Nemouridae	0	0	2	0	0	0	0	0	0	0	0	0
Neophylax	10	0	0	0	0	0	0	0	0	0	0	0
Neoplasta	0	0	2	0	0	2	0	0	6	6	2	14
Nigronia	0	0	0	2	0	2	4	0	17	2	0	21
Ochrotrichia	0	2	0	0	0	0	0	39	0	0	0	0
Oligochaeta	41	35	91	43	576	33	105	62	194	76	58	1457
Optioservus	52	0	4	134	0	141	4	0	244	60	83	81
Ormosia	2	2	0	0	0	0	0	0	0	0	2	0
Oulimnius	0	432	0	0	0	0	0	516	0	64	0	0
Paraleptophlebia	41	52	6	6	0	0	0	0	8	0	2	0
Perlesta	0	8	0	0	0	0	16	12	0	103	0	0
Perlodidae	2	0	10	4	0	19	0	0	10	0	6	54
Pilaria	2	2	0	0	0	0	0	0	0	0	0	0
Polycentropus	8	2	12	12	0	4	0	39	6	0	4	74
Prosimulium	0	0	0	0	23	0	4	2	0	2	0	0
Psephenus	10	0	19	16	0	2	0	0	19	2	2	47
Pseudocloeon	0	0	0	0	0	0	0	2	0	0	0	0
Pteronarcys	10	4	0	0	0	0	0	0	6	0	0	0

Taxon/Stream ID	CRO	EAS	GRA	HCN	KEL	LLC	MIL	POW	RFF	RIC	ROC	SPC
Pycnopsyche	14	0	0	2	0	4	0	2	4	16	0	0
Remenus	2	0	0	0	0	0	0	0	2	0	0	0
Rhyacophila	2	17	0	0	6	2	2	17	8	17	4	4
Shipsa	0	0	0	0	43	0	0	0	0	0	0	0
Sialis	0	0	2	0	4	0	0	0	0	0	0	0
Simuliidae	4	0	2	4	2	12	0	0	0	2	6	0
Simulium	0	2	0	0	0	0	0	6	0	0	0	0
Stactobiella	0	0	0	0	0	0	0	52	0	0	0	0
Stenacron	4	93	0	0	0	0	0	4	6	0	0	0
Stenelmis	4	0	0	14	0	0	0	0	52	0	0	0
Stenonema	8	0	4	6	0	0	0	0	0	0	0	0
Stylogomphus	2	6	0	0	35	0	97	37	0	19	0	0
Suwallia	12	0	0	0	0	0	0	2	0	0	0	10
Sweltsa	4	10	0	2	0	0	0	0	4	0	0	0
Taeniopterygidae	0	0	2	0	0	0	0	0	0	0	0	0
Tallaperla	0	0	0	0	0	0	0	2	0	0	0	0
Tipula	2	0	0	0	4	2	6	4	2	0	12	2
Tipulidae	2	0	0	0	0	0	0	0	0	0	0	0
Yugus	4	8	12	0	2	0	0	29	29	0	0	19

Table B.8. Benthic macroinvertebrate sampling dates for each stream.

Stream ID	May 2013	Jul 2013	Sep 2013	Nov 2013	Jan 2014	Apr 2014
COP	22	19	ns	ns	18	ns
CRO	ns	20	22	27	19	27
EAS	19	18	ns	ns	20	27
FRY	22	19	22	27	19	ns
GRA	23	20	23	26	ns	26
HCN	ns	ns	ns	25	ns	25
KEL	20	17	21	28	20	28
KUT	20	17	21	28	20	28
LLC	ns	ns	ns	ns	17	25
MIL	20	17	21	28	20	28
POW	21	18	20	28	21	28
RFF	22	19	22	27	19	27
RIC	ns	18	20	29	21	29
ROC	ns	ns	ns	25	17	25
SPC	23	20	23	26	18	26

ns = no sample collected or insufficient number of sub-samples available

APPENDIX C – Master taxa list

Table C.1. List of taxa in alphabetical order with primary FFG and habit, and taxonomic order and family listed.

Taxon	FFG	Habit	Order	Family
Acentrella	Gatherer	Swimmer	Ephemeroptera	Baetidae
Acerpenna	Gatherer	Swimmer	Ephemeroptera	Baetidae
Acroneuria	Predator	Clinger	Plecoptera	Perlidae
Aeshna	Predator	Climber	Anisoptera	Aeshnidae
Aeshnidae	Predator	Climber	Anisoptera	Aeshnidae
Agapetus	Scraper	Clinger	Trichoptera	Glossosomatidae
Agnetina	Predator	Clinger	Plecoptera	Perlidae
Allocaupnia	Shredder	Clinger	Plecoptera	Capniidae
Alloperla	Predator	Clinger	Plecoptera	Chloroperlidae
Ameletus	Scraper	Swimmer	Ephemeroptera	Ameletidae
Amphinemura	Shredder	Sprawler	Plecoptera	Nemouridae
Anax	Predator	Climber	Anisoptera	Aeshnidae
Antocha	Gatherer	Clinger	Diptera	Tipulidae
Arctoconopa	Shredder	Burrower	Diptera	Tipulidae
Arigomphus	Predator	Burrower	Anisoptera	Gomphidae
Atherix	Predator	Sprawler	Diptera	Athericidae
Atrichopogon	Predator	Sprawler	Diptera	Ceratopogonidae
Attenella	Gatherer	Clinger	Ephemeroptera	Ephemerellidae
Baetidae	Gatherer	Swimmer	Ephemeroptera	Baetidae
Baetis	Gatherer	Swimmer	Ephemeroptera	Baetidae
Baetisca	Gatherer	Sprawler	Ephemeroptera	Baetiscidae
Beloneuria	Predator	Clinger	Plecoptera	Perlidae
Boyeria	Predator	Climber	Anisoptera	Aeshnidae
Brachycentrus	Filterer	Clinger	Trichoptera	Brachycentridae
Caenis	Gatherer	Sprawler	Ephemeroptera	Caenidae
Calopteryx	Predator	Climber	Zygoptera	Calopterygidae
Capniidae	Shredder	Sprawler	Plecoptera	Capniidae
Centropilum	Gatherer	Swimmer	Ephemeroptera	Baetidae
Ceratopogonidae	Predator	Sprawler	Diptera	Ceratopogonidae
Ceratopsyche	Filterer	Clinger	Trichoptera	Hydropsychidae
Cernotina	Predator	Clinger	Trichoptera	Polycentropodidae
Chauliodes	Predator	Clinger	Megaloptera	Corydalidae
Chelifera	Gatherer	Sprawler	Diptera	Empididae
Cheumatopsyche	Filterer	Clinger	Trichoptera	Hydropsychidae
Chimarra	Filterer	Clinger	Trichoptera	Philopotamidae
Chironomidae	Gatherer	Burrower	Diptera	Chironomidae
Chloroperlidae	Predator	Clinger	Plecoptera	Chloroperlidae
Cinygmula	Scraper	Clinger	Ephemeroptera	Heptageniidae
Clinocera	Predator	Clinger	Diptera	Empididae

Taxon	FFG	Habit	Order	Family
Clioperla	Predator	Clinger	Plecoptera	Perlodidae
Cordulegaster	Predator	Burrower	Anisoptera	Cordulegastridae
Cultus	Predator	Clinger	Plecoptera	Perlodidae
Cynellus	Filterer	Clinger	Trichoptera	Polycentropodidae
Dicranota	Predator	Sprawler	Diptera	Tipulidae
Dipheter	Gatherer	Swimmer	Ephemeroptera	Baetidae
Diplectrona	Filterer	Clinger	Trichoptera	Hydropsychidae
Diploperla	Predator	Clinger	Plecoptera	Perlodidae
Diura	Predator	Clinger	Plecoptera	Perlodidae
Dixa	Gatherer	Swimmer	Diptera	Dixidae
Dolichopodidae	Predator	Sprawler	Diptera	Dolichopodidae
Dolophilodes	Filterer	Clinger	Trichoptera	Philopotamidae
Dromogomphus	Predator	Burrower	Anisoptera	Gomphidae
Drunella	Scraper	Clinger	Ephemeroptera	Ephemerellidae
Dubiraphia	Gatherer	Clinger	Coleoptera	Elmidae
Ectopria	Scraper	Clinger	Coleoptera	Psephenidae
Elmidae	Scraper	Clinger	Coleoptera	Elmidae
Empididae	Predator	Sprawler	Diptera	Empididae
Epeorus	Gatherer	Clinger	Ephemeroptera	Heptageniidae
Ephemera	Gatherer	Burrower	Ephemeroptera	Ephemeridae
Ephemerella	Gatherer	Clinger	Ephemeroptera	Ephemerellidae
Ephemerellidae	Gatherer	Clinger	Ephemeroptera	Ephemerellidae
Eurylophella	Gatherer	Clinger	Ephemeroptera	Ephemerellidae
Glossosoma	Scraper	Clinger	Trichoptera	Glossosomatidae
Goera	Scraper	Clinger	Trichoptera	Goeridae
Goeridae	Scraper	Clinger	Trichoptera	Goeridae
Gomphidae	Predator	Burrower	Anisoptera	Gomphidae
Gomphus	Predator	Burrower	Anisoptera	Gomphidae
Habrophlebia	Gatherer	Swimmer	Ephemeroptera	Leptophlebiidae
Habrophlebiodes	Scraper	Swimmer	Ephemeroptera	Leptophlebiidae
Hansonoperla	Predator	Clinger	Plecoptera	Perlidae
Haploperla	Predator	Clinger	Plecoptera	Chloroperlidae
Hebrus	Predator	Climber	Hemiptera	Hebridae
Hemerodromia	Predator	Sprawler	Diptera	Empididae
Heptagenia	Scraper	Clinger	Ephemeroptera	Heptageniidae
Heptageniidae	Scraper	Clinger	Ephemeroptera	Heptageniidae
Heterocloeon	Scraper	Swimmer	Ephemeroptera	Baetidae
Hexatoma	Predator	Burrower	Diptera	Tipulidae
Hydroperla	Predator	Clinger	Plecoptera	Perlodidae
Hydropsyche	Filterer	Clinger	Trichoptera	Hydropsychidae
Hydroptila	Scraper	Clinger	Trichoptera	Hydroptilidae
Isogenoides	Predator	Clinger	Plecoptera	Perlodidae

Taxon	FFG	Habit	Order	Family
Isonychia	Filterer	Swimmer	Ephemeroptera	Isonychiidae
Isoperla	Predator	Clinger	Plecoptera	Perlodidae
Lanthus	Predator	Burrower	Anisoptera	Gomphidae
Lepidostoma	Shredder	Climber	Trichoptera	Lepidostomatidae
Leptophlebia	Gatherer	Swimmer	Ephemeroptera	Leptophlebiidae
Leptophlebiidae	Gatherer	Swimmer	Ephemeroptera	Leptophlebiidae
Leptotarsus	Gatherer	Burrower	Diptera	Tipulidae
Leucrocuta	Scraper	Clinger	Ephemeroptera	Heptageniidae
Leuctra	Shredder	Sprawler	Plecoptera	Leuctridae
Limnephilidae	Shredder	Climber	Trichoptera	Limnephilidae
Limnophila	Predator	Burrower	Diptera	Tipulidae
Limonia	Shredder	Burrower	Diptera	Tipulidae
Maccaffertium	Scraper	Clinger	Ephemeroptera	Heptageniidae
Macronychus	Gatherer	Clinger	Coleoptera	Elmidae
Macrostemum	Filterer	Clinger	Trichoptera	Hydropsychidae
Malirekus	Predator	Clinger	Plecoptera	Perlodidae
Metachela	Predator	Sprawler	Diptera	Empididae
Microcylloepus	Gatherer	Clinger	Coleoptera	Elmidae
Muscidae	Predator	Burrower	Diptera	Empididae
Nemoura	Shredder	Sprawler	Plecoptera	Nemouridae
Nemouridae	Shredder	Sprawler	Plecoptera	Nemouridae
Neophylax	Scraper	Clinger	Trichoptera	Uenoidae
Neoplasta	Predator	Sprawler	Diptera	Empididae
Neureclipsis	Filterer	Clinger	Trichoptera	Polycentropodidae
Nigronia	Predator	Clinger	Megaloptera	Corydalidae
Ochrotrichia	Gatherer	Clinger	Trichoptera	Hydroptilidae
Oconoperla	Predator	Clinger	Plecoptera	Perlodidae
Oemopteryx	Shredder	Sprawler	Plecoptera	Taeniopterygidae
Oligochaeta	Gatherer	Burrower	Oligochaeta	Oligochaeta
Optioservus	Scraper	Clinger	Coleoptera	Elmidae
Ormosia	Gatherer	Burrower	Diptera	Tipulidae
Ostrocerca	Shredder	Sprawler	Plecoptera	Nemouridae
Oulimnius	Scraper	Clinger	Coleoptera	Elmidae
Paracapnia	Shredder	Sprawler	Plecoptera	Capniidae
Paragnetina	Predator	Clinger	Plecoptera	Perlidae
Paraleptophlebia	Gatherer	Swimmer	Ephemeroptera	Leptophlebiidae
Pedicia	Predator	Burrower	Diptera	Tipulidae
Peltoperla	Shredder	Clinger	Plecoptera	Peltoperlidae
Peltoperlidae	Shredder	Clinger	Plecoptera	Peltoperlidae
Perlesta	Predator	Clinger	Plecoptera	Perlidae
Perlidae	Predator	Clinger	Plecoptera	Perlidae
Perlodidae	Predator	Clinger	Plecoptera	Perlodidae

Taxon	FFG	Habit	Order	Family
Pilaria	Predator	Burrower	Diptera	Tipulidae
Plauditus	Scraper	Swimmer	Ephemeroptera	Baetidae
Pleidae	Predator	Swimmer	Hemiptera	Pleidae
Polycentropodidae	Filterer	Clinger	Trichoptera	Polycentropodidae
Polycentropus	Predator	Clinger	Trichoptera	Polycentropodidae
Potamyia	Filterer	Clinger	Trichoptera	Hydropsychidae
Prosimulium	Filterer	Clinger	Diptera	Simuliidae
Psephenus	Scraper	Clinger	Coleoptera	Psephenidae
Pseudocloeon	Scraper	Swimmer	Ephemeroptera	Baetidae
Pteronarcys	Shredder	Clinger	Plecoptera	Pteronarcyidae
Pycnopsyche	Shredder	Sprawler	Trichoptera	Limnephilidae
Rasvena	Predator	Clinger	Plecoptera	Chloroperlidae
Remenus	Predator	Clinger	Plecoptera	Perlodidae
Rhagovelia	Predator	Skater	Hemiptera	Veliidae
Rhyacophila	Predator	Clinger	Trichoptera	Rhyacophilidae
Serratella	Gatherer	Clinger	Ephemeroptera	Ephemerellidae
Shipsa	Shredder	Sprawler	Plecoptera	Nemouridae
Sialis	Predator	Burrower	Megaloptera	Sialidae
Simuliidae	Filterer	Clinger	Diptera	Simuliidae
Simulium	Filterer	Clinger	Diptera	Simuliidae
Soyedina	Shredder	Sprawler	Plecoptera	Nemouridae
Stactobiella	Shredder	Clinger	Trichoptera	Hydroptilidae
Stenacron	Gatherer	Clinger	Ephemeroptera	Heptageniidae
Stenelmis	Scraper	Clinger	Coleoptera	Elmidae
Stenonema	Scraper	Clinger	Ephemeroptera	Heptageniidae
Stratiomyidae	Gatherer	Sprawler	Diptera	Stratiomyidae
Stylogomphus	Predator	Burrower	Anisoptera	Gomphidae
Stylurus	Predator	Burrower	Anisoptera	Gomphidae
Suwallia	Predator	Clinger	Plecoptera	Chloroperlidae
Sweltsa	Predator	Clinger	Plecoptera	Chloroperlidae
Tabanidae	Predator	Sprawler	Diptera	Tabanidae
Taenionema	Shredder	Sprawler	Plecoptera	Taeniopterygidae
Taeniopterygidae	Shredder	Sprawler	Plecoptera	Taeniopterygidae
Taeniopteryx	Shredder	Sprawler	Plecoptera	Taeniopterygidae
Tallaperla	Shredder	Clinger	Plecoptera	Peltoperlidae
Tipula	Shredder	Burrower	Diptera	Tipulidae
Tipulidae	Shredder	Burrower	Diptera	Tipulidae
Twinnia	Filterer	Clinger	Diptera	Simuliidae
Utaerla	Predator	Clinger	Plecoptera	Chloroperlidae
Veliidae	Predator	Skater	Hemiptera	Veliidae
Wormaldia	Filterer	Clinger	Trichoptera	Philopotamidae
Yugus	Predator	Clinger	Plecoptera	Perlodidae

APPENDIX D – Supporting figures

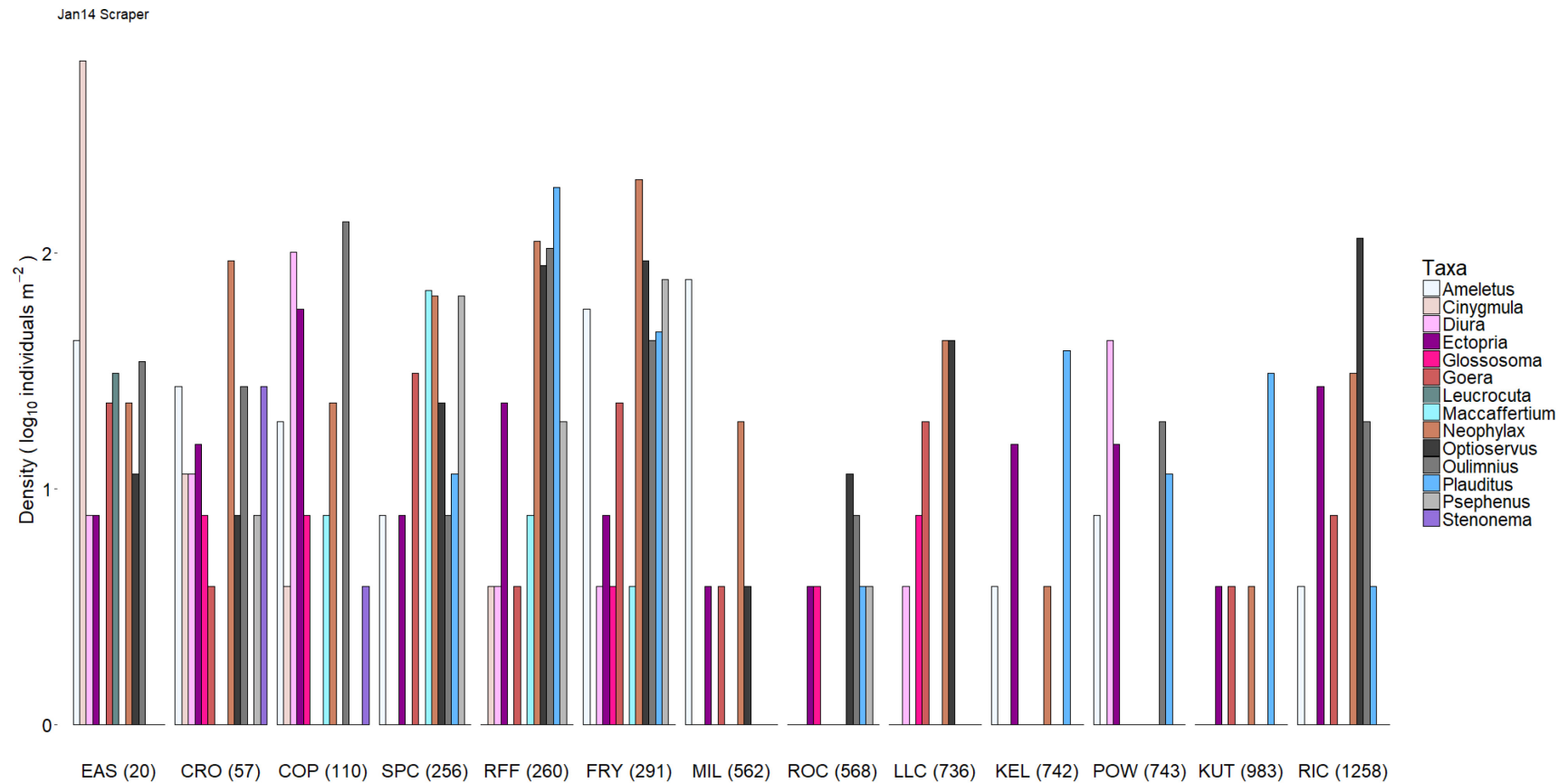


Figure D.1. January 2014 scraper densities. X-axis represents stream IDs with stream SC ($\mu\text{S cm}^{-1}$) in parentheses. Ameletus, Cinygmula, Leucrocuta, Maccaffertium, Plauditus, and Stenonema are Ephemeroptera genera. Ephemeroptera scraper density and richness is depleted in high-SC streams.

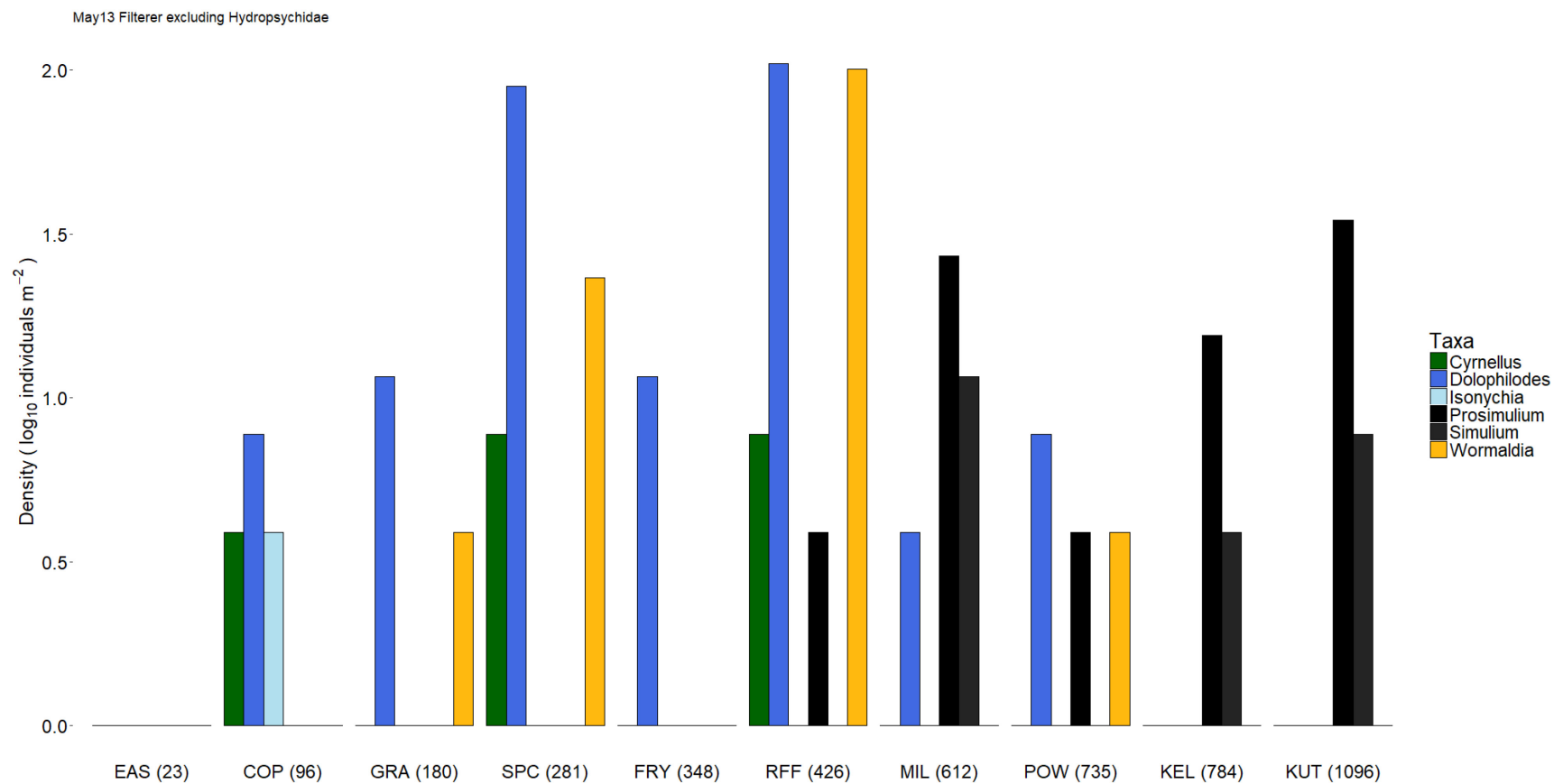


Figure D.2. May 2013 filterer-minus-Hydropsychidae densities. X-axis represents stream IDs with stream SC ($\mu\text{S cm}^{-1}$) in parentheses. This figure illustrates the concept of taxonomic replacement as presented in chapter 3, with two genera of blackflies replacing mayflies and caddisflies.

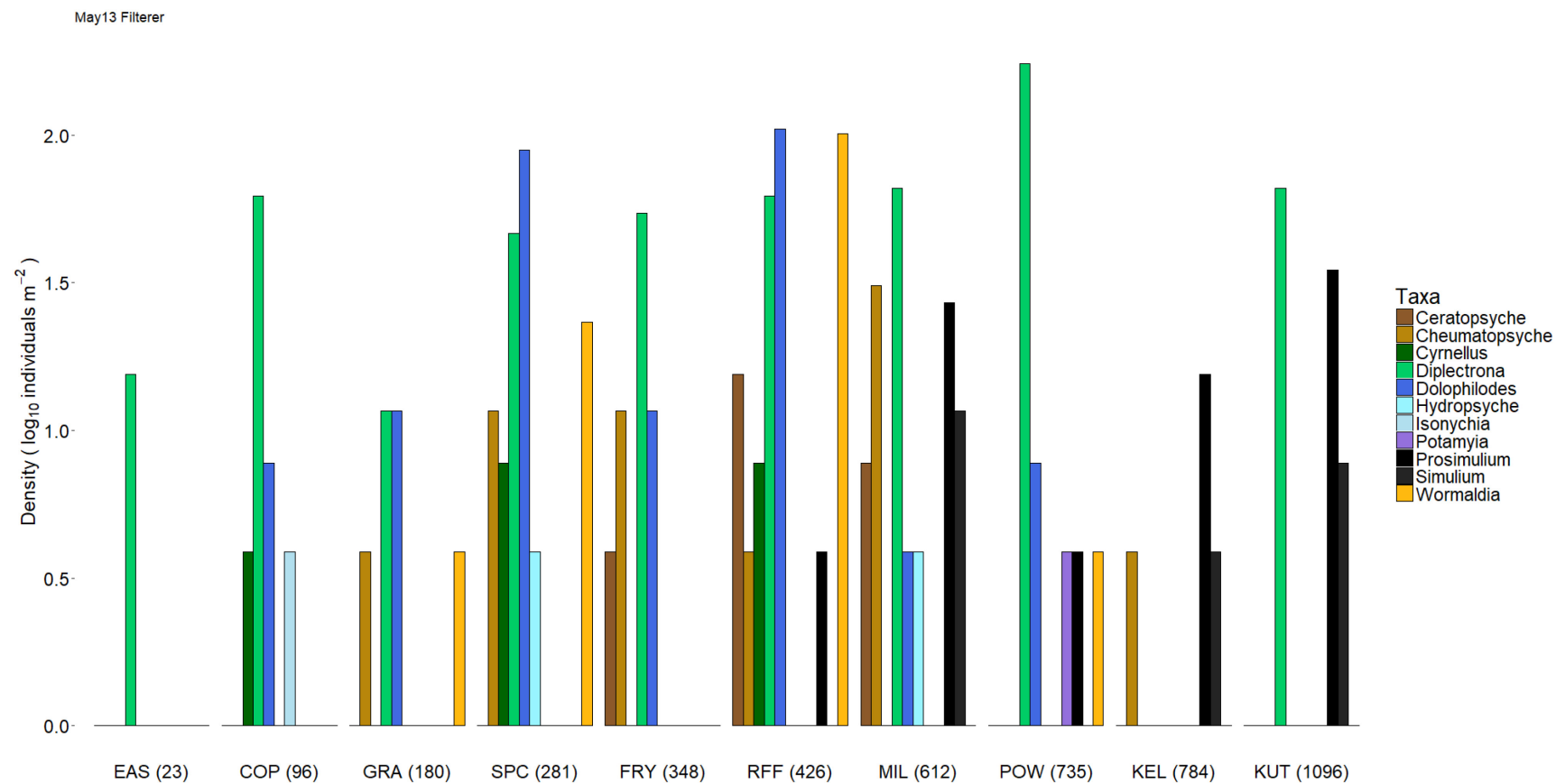


Figure D.3. May 2013 filterer densities. X-axis represents stream IDs with stream SC ($\mu\text{S cm}^{-1}$) in parentheses.