Long-term and Downstream Patterns in Water Quality and Macroinvertebrate Communities Following Surface Coal Mining in Central Appalachian Headwater Streams

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Annual Research Report 2021-2022
October 3, 2022

Introduction

Surface coal mining in central Appalachia has documented influences to headwater streams, including alterations to water chemistry, particularly increased salinity (Griffith et al. 2012), and benthic macroinvertebrate communities (Pond 2010). Despite these well-recognized impacts, less is known regarding potential stream recovery following mining activities, calling for long-term study of both water chemistry and biological conditions. With continued Powell River Project (PRP) support, our ongoing work focuses on such long-term trends in 23 headwater streams in the coalfield region of southwestern Virginia and southern West Virginia (Figure 1). Since 2011, we have conducted seasonal (fall and spring) sampling of benthic macroinvertebrate communities and water chemistry parameters and have measured specific conductance (SC; surrogate for salinity) at 30-minute intervals. To date, we have observed limited decline in SC and associated recovery of macroinvertebrate communities with time since mining (Cianciolo et al. 2020a). **However, further study is needed to 1) continue tracking potential recovery of both water chemistry and benthic communities, with expanded focus on 2) streamflow influences on water chemistry and 3) headwater contributions (i.e., loading) of water quality constituents to downstream waters.** In 2019-2021, with PRP support, we added to this study by monitoring sub-daily water levels at 14 of these streams and conducting streamflow measurements at six streams; these data are being used to both account for flow-driven variation in water chemistry in long-term trend analysis and to quantify downstream loads (or mass flux) of water chemistry constituents. In the 2021-2022 study reported here, we leveraged deployed instrumentation to continue our long-term monitoring of water chemistry and macroinvertebrate communities and our more recent water level and streamflow measurements. Importantly, these efforts supported larger funding opportunities, including a recently awarded National Science Foundation grant focused on stream carbon processes in our study streams.

Further, in this study, we conducted a new assessment of within-stream spatial variation in water chemistry and macroinvertebrate communities. A 2019 survey on a subset of our streams documented downstream gradients in SC and associated ions, typically exhibiting dilution farther from mined sources. What remains unknown, however, are the effects of such within-stream variability on benthic macroinvertebrate communities. As such, a major element of this 2021-2022 PRP work sampled multiple locations (6-10) within each of six streams to assess downstream patterns in water chemistry, macroinvertebrate communities, and their association. Such information is critical to understand potential downstream recovery and to inform future assessments that better account for spatial variability within headwater streams of central Appalachia.

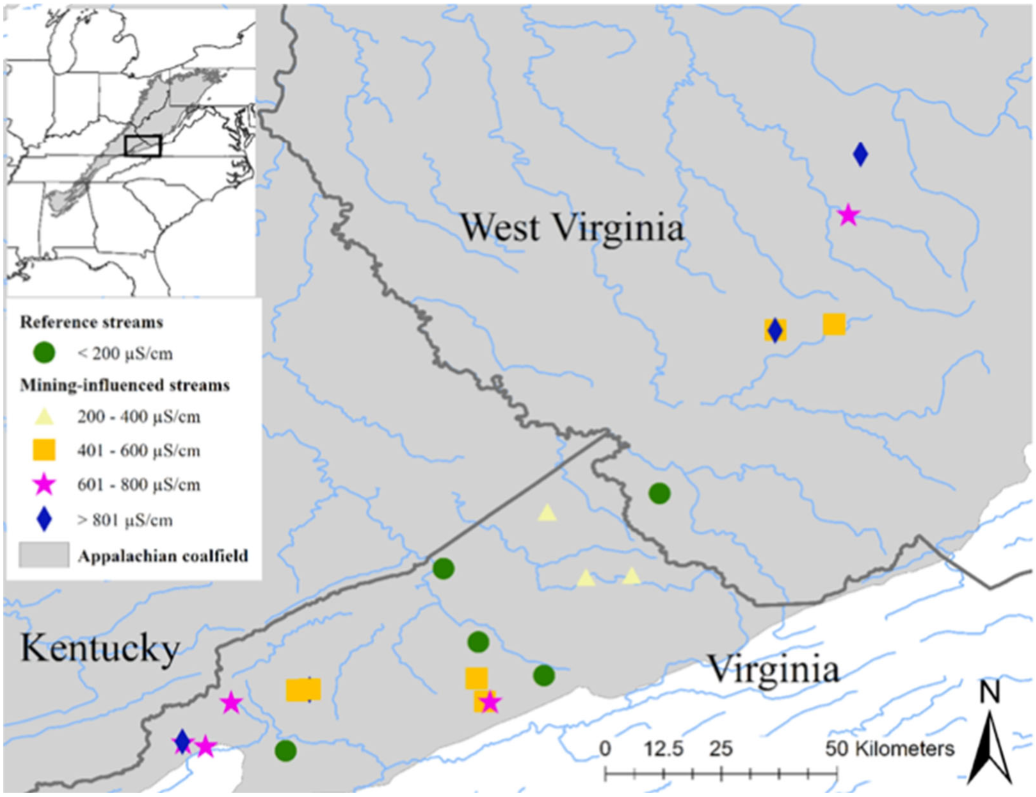

Figure 1. Location of 23 headwater streams used in this study (Cianciolo et al. 2020a).

Objective 1: Expand temporal scope for continuous SC monitoring and seasonal sampling for synoptic water chemistry and benthic macroinvertebrates in 23 central Appalachian headwater streams.

Despite the well-recognized impacts of mining on headwater streams, less is known regarding potential stream recovery, calling for long-term study of both water chemistry and biological conditions. Our ongoing work focuses on such long-term trends and, to date, has found limited declines in SC and associated recovery of macroinvertebrate communities with time since mining (Cianciolo et al. 2020a). However, further study is needed to continue tracking potential recovery of both water chemistry and benthic communities.

In this work, we continued our long-term monitoring of selected water quality parameters in 23 study streams initiated in 2011. In situ SC was recorded every 30 minutes using automated dataloggers already installed within each stream. During this study period, this included downloading, maintenance, and in some cases, replacement of dataloggers during two different site visits. Grab samples of streamwater were collected in spring 2021, fall 2021, and spring 2022 to assess major ion and trace element concentrations. Samples were analyzed for total dissolved solids (TDS), total alkalinity, bicarbonate (HCO_3^-), pH, major cations/trace elements, dissolved organic carbon (DOC), chloride (Cl-) and sulfate (SO_4^{2-}). The SC and grab sample water chemistry data were processed and added to our long-term datasets for continued analysis of water chemistry recovery.

As has been done since 2011, benthic macroinvertebrates were sampled in spring 2021, fall 2021, and spring 2022 in 23 study streams using the semi-quantitative, single habitat (riffle-run) method established by the Virginia Department of Environmental Quality (VDEQ 2008). This method was adapted from U.S. EPA Rapid Bioassessment Protocols (RBP; Barbour et al. 1999) and is comparable to the method used by West Virginia Department of Environmental Protection (WVDEP 2015). Using a 0.3-m D-frame kicknet with 500-μm mesh, a single composite sample (approximately 2 m^2) composed of six approximately 1×0.3-m kicks was collected from separate riffles along a 100-m reach upstream of the SC datalogger. Samples were preserved in 95% ethanol and returned to the lab for sorting and identification. Macroinvertebrate samples were sub-sampled randomly to obtain a 200 (±10%) organism count following VDEQ biomonitoring protocols (VDEQ 2008). Specimens were identified to genus using standard keys (Merritt et al. 2008), except individuals in family Chironomidae and subclass Oligochaeta, which were identified at those levels. These data have also been added to our long-term dataset for recovery trend analysis.

Objective 2: Assess water level- and flow-driven variation in SC to inform analyses of long-term trends in water chemistry.

Dissolved ion element concentrations naturally vary as a result of streamflow variation, often exhibiting either enrichment or dilution with increased flow (Griffith et al. 2012). We have recently found inverse relationships between SC and water level (as an indicator of flow variation) at our study streams, indicating dilution under high flow conditions. Consequently, long-term trend analysis to assess water chemistry recovery is complicated by this additional driver of SC and concentration of associated ions, requiring continued data collection and statistical methods for long-term trend analysis.

During fall 2019, HOBO U20 water level dataloggers were installed across 14 of our 23 study streams to measure stream stage. Dataloggers record stage every 30 minutes. During this project period, these streams were visited on two occasions to download data and maintain dataloggers. Collected stage and SC data were analyzed to assess site-specific distributions of stage and SC, where stage conditions (i.e., stream water levels) were similar across systems but SC, as expected, demonstrated higher values and with more variability at mining-influenced streams (a subset of six streams shown in Figure 2). We also used these data to develop site-specific relationships between SC and stage, thereby indicating flow controls on water chemistry (Figure 3). We observed inverse relationships between SC and water level at all study streams, indicating dilution under high flow conditions. However, the magnitude of this flow-induced dilution was greater in salinized streams (e.g., from ca. 35 to 15 μS/cm in the reference stream EAS vs. from1,000 to 100 μS/cm in the mining-influenced stream RIC).

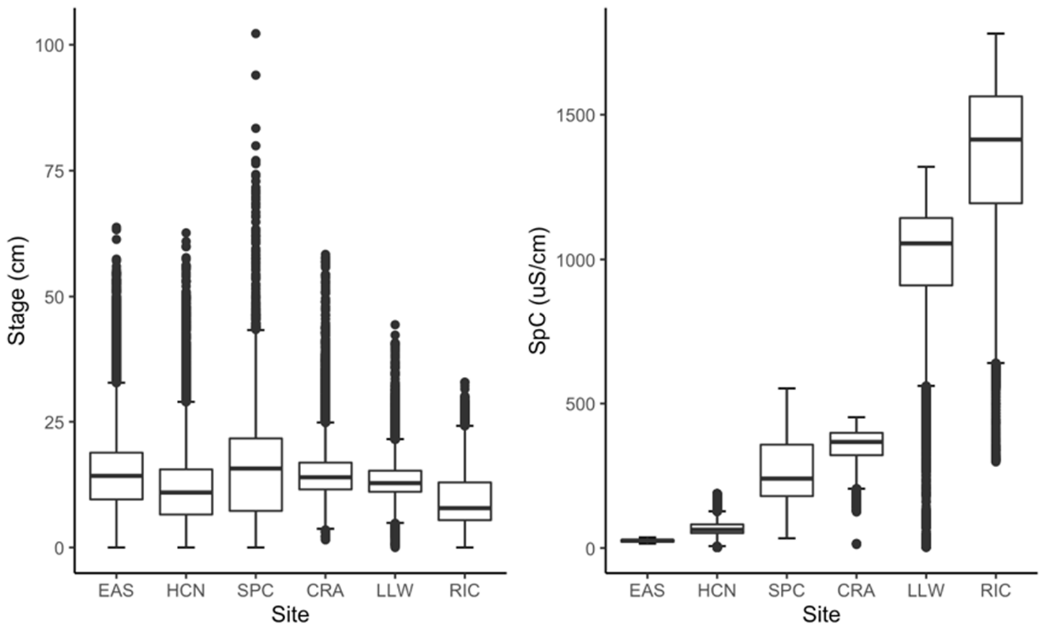

Figure 2. Distributions of 30-minute stage (left) and specific conductance SC (right) between June 2020 and March 2022 at a subset of six of the 14 streams instrumented with water level data loggers. EAS and HCN are reference sites, and SPC, CRA, LLW, and RIC are mining-influenced sites.

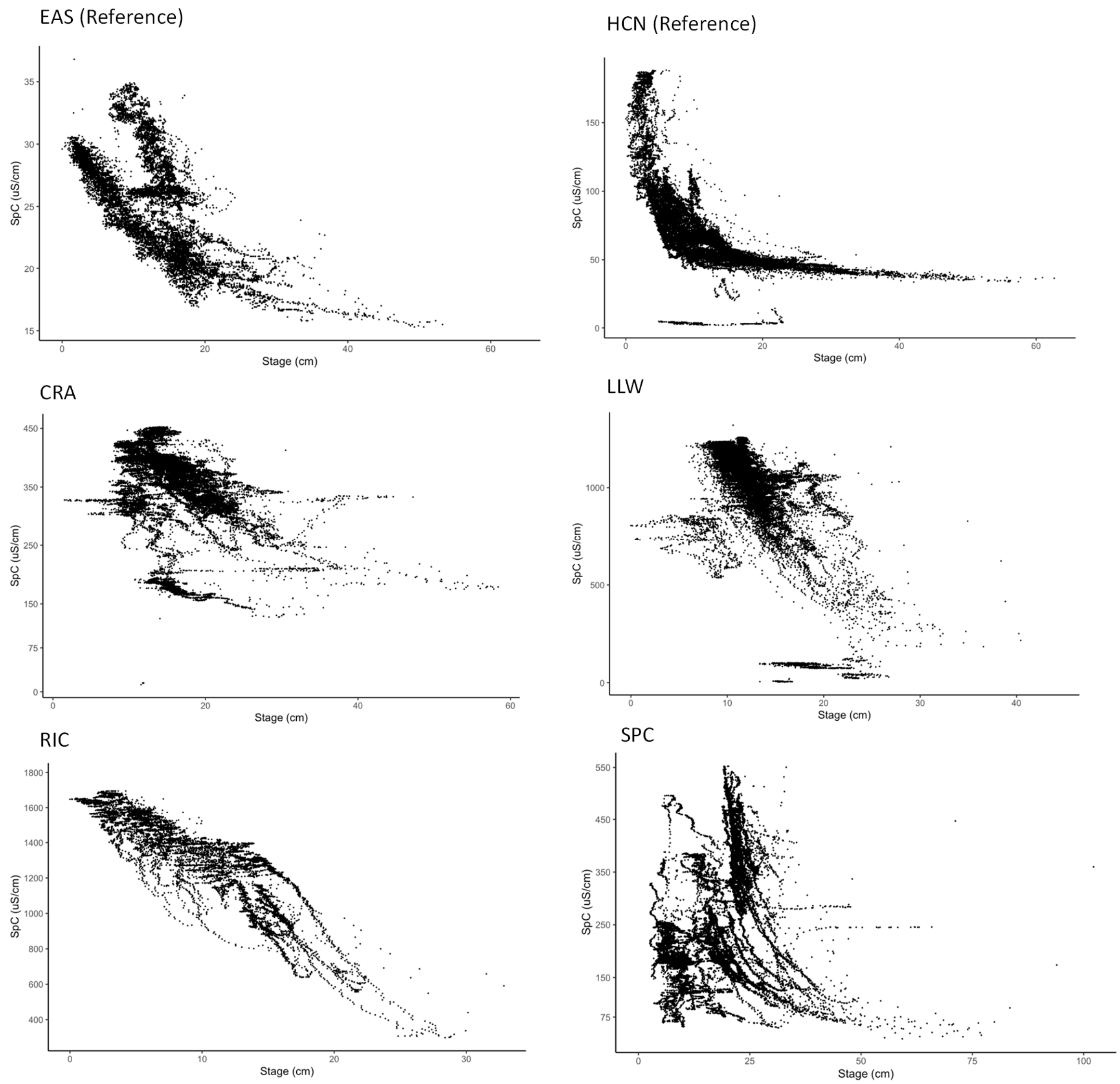

Figure 3. Specific conductance (SC) versus stage relationships between June 2020 and March 2022 at a subset of six of the 14 streams instrumented with water level data loggers. EAS and HCN are reference sites, and SPC, CRA, LLW, and RIC are mining-influenced sites.

Objective 3: Quantify mass fluxes of dissolved major ions and trace elements to downstream waters.

Major ions and trace elements in mining-influenced headwater streams can have important consequences for downstream waters (Johnson et al. 2019). In central Appalachia, however, there has been limited research to understand loads (or mass contributions) of water chemistry constituents (e.g., major ions, trace elements) to downstream waters. Loads can exhibit large variation across sites and with time, driven by both flow and constituent concentrations, requiring estimates of both. As part of PRP-supported work in 2019-2021, we initiated flow measurements at six streams, and continued measurements are needed to develop rating curves for flow time series estimates from our water level data. With PRP support in 2020-2021, we also developed relationships between SC and constituent concentrations. These relationships together with continued data collection for SC, water level, and flow data, can yield downstream loads and inform monitoring approaches relevant to the total maximum daily load (TMDL) framework (CWA §303(d)(1)(C)).

At a subset of streams (2 reference, 4 mining-influenced), we measured flow during 4-5 site visits between April 2021 and May 2022 with the intent to capture a range in flow magnitudes To do so, we used salt tracer additions (Gooseff et al. 2003) and measured response in SC levels. Using the known volume and concentration of the salt solution, and stream background SC, stream discharge was estimated from the following equation:

$$Q = \frac{V}{k\Delta t \ \sum_{i=0}^{n}\left[SC(t) - SC_{bg}\right]} \quad \text{(Eq 1)}$$

where Q is stream discharge (m^3/s), V is the known volume of the injection slug (m^3), k is the calibration constant, SC(t) is the specific conductance at time t, and SC_{bg} is background specific conductance of the stream (Moore, 2005). We continue to collect flow measurements at these streams to further develop rating curves (i.e., relationships between flow and stage) for each stream. Rating curves are being developed to relate measured flow and stream stage, enabling flow estimates from high-frequency (30-min) stage data.

At our six streams with flow measurements, we are working to couple predicted concentrations of water chemistry constituents and flow to yield mass fluxes (or loads) to downstream waters. Ongoing work includes supplementing the 4-to-5 flow measurements at each stream with additional measurements as needed to construct accurate stage-discharge rating curves at each stream. Once those flow-rating curves are completed, we will use 30-minute SC data (Objective #1) and SC-concentration models (2020-2021 PRP Project) to estimate 30-minute concentrations for major ions and trace elements. With these estimated concentrations and 30-minute flow data (via stage- to-flow rating curves), we will determine daily mass loading rates and assess how they vary across sites, seasons, and flow conditions. We are continuing to collect flow data to complete site-specific rating curves necessary for this analysis, but present here an example of the data and approach (Figure 4).

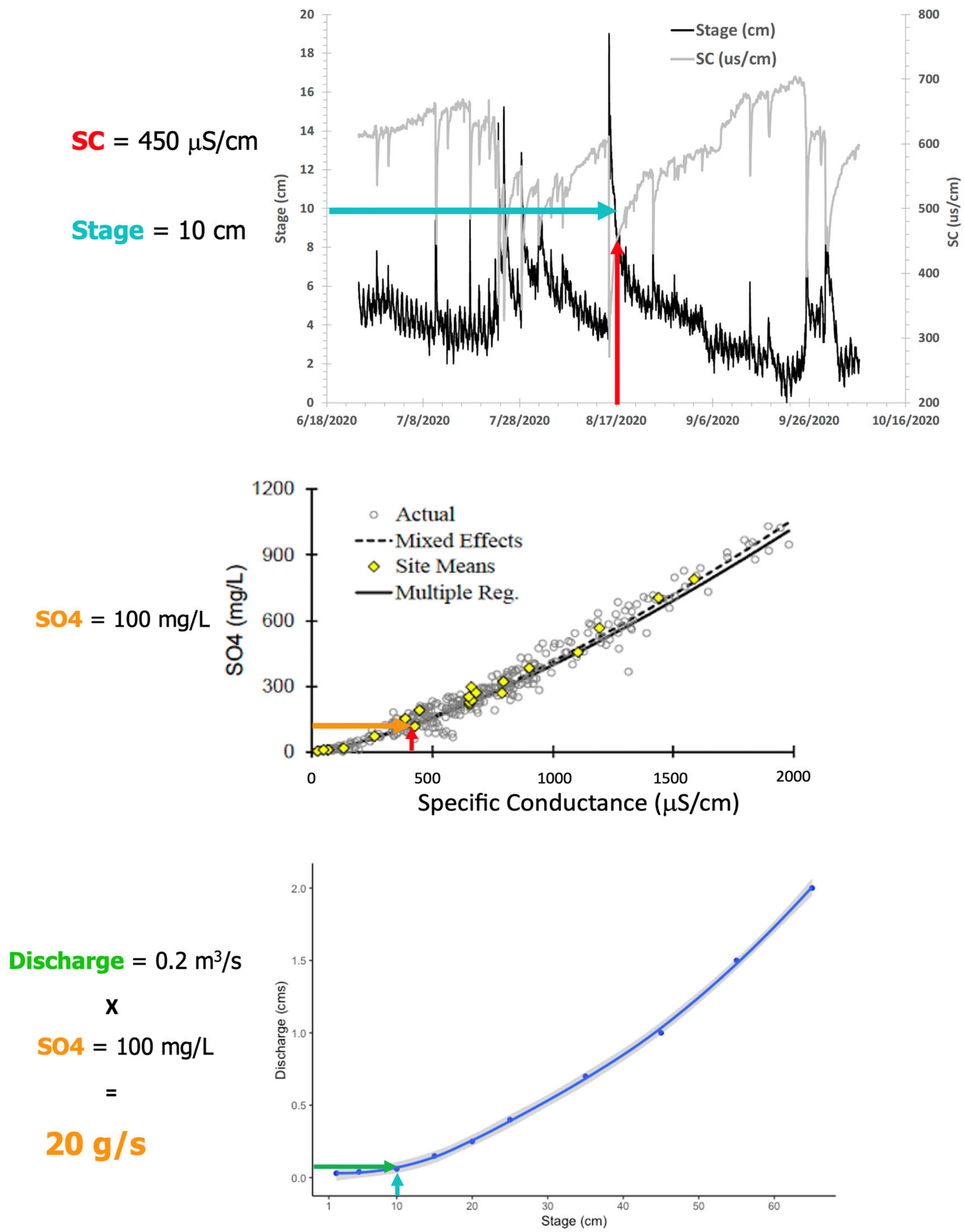

Figure 4. An example for our approach to determine loadings of dissolved major ions and trace elements. <u>Top</u>: stage and specific conductance (SC) data as measured during a ~14-month period (April 2021-May 2022) collected from one of our study streams. <u>Middle</u>: relationship of sulfate (SO4) concentration and specific conductance, as reported in our 2021 PRP report. <u>Bottom</u>: preliminary stage-discharge rating curve, and calculation of SO4 mass loading (mass/time=20 g SO4/second) for the specific date/time represented by the arrows on each of the three charts.

Objective 4: Within-Stream Spatial Variation in Water Chemistry and Macroinvertebrate Communities

We have a good understanding of differences in water chemistry and macroinvertebrate communities among our 23 streams, but little is known about their spatial patterns within our streams. Previous research in other stream systems has shown spatial variation in macroinvertebrate communities associated with variable water quality conditions. For example, a study of 14 Wisconsin streams found differing benthic macroinvertebrate assemblies within stream reaches, with differences explained by SC gradients (Shupryt et al. 2021). Similar assessments of macroinvertebrate response to water chemistry patterns in headwater streams of central Appalachia have not been conducted but are needed to inform monitoring protocols and assessment of downstream conditions. Indeed, we have observed clear spatial patterns of water chemistry within our streams. In 2019, preliminary work within a subset of our streams documented strong gradients of SC and associated ions, with downstream dilution likely caused by groundwater and tributary inputs. The River Continuum Concept is a commonly accepted theory that stream biotic communities vary with stream size and distance and associated changes in environmental conditions (Vannote et al. 1980). However, commonly applied stream assessments, included in our ongoing work and in regulatory programs, typically apply bioassessments at singular 100-m sample reaches meant to represent the headwater stream of interest (VDEQ 2008; WVDEP 2015). A spatial macroinvertebrate study within individual streams would help evaluate the ability of a singular reach to represent whole-stream condition. Further, such a spatial study could inform downstream recovery trends for mining-impacted streams compared to reference conditions. We know the impacts of coal mining persist over time (Cianciolo et al. 2020b), but less is known about whether macroinvertebrate communities can recover downstream.

For this within-stream variation study, we identified 14 candidate streams from our 23 long-term sites with accessibility suitable to allow extensive spatial sampling. We then traversed each candidate stream from its confluence with its receiving stream (downstream from our long-term monitoring reach) to the upstream point of origin, measuring SC and assessing multiple habitat characteristics approximately every 200 m. Based on these data, two reference streams and four mining-influenced streams with the highest change in SC-to-distance ratio were selected for further study of spatial patterns in water chemistry and macroinvertebrate communities. Of these six streams, the shortest stream length available to sample was approximately 1,500 m, whereas the longest was approximately 3,000 m.

At each of the six streams, 50-m reaches spaced approximately every 300 m were sampled for both macroinvertebrates and water chemistry, along with additional samples at the long-term monitoring locations and within perennial tributaries. Sampling for both water chemistry and macroinvertebrates occurred in both fall 2021 and spring 2022, following methods described above for long-term monitoring protocols. In each stream, we also installed a SC datalogger (30-min interval) at a new upstream sampling reach, to complement the SC datalogger installed downstream at our long-term monitoring reach. The paired SC data will assess potentially time-varying longitudinal gradients in SC. Last, within each study stream, we used established methods (e.g., USEPA EMAP Physical Habitat protocol) to measure multiple habitat features influential to the benthic macroinvertebrate community, such as: stream gradient, sediment composition, canopy cover, epifaunal substrate, stream depth at thalweg, stream width, stream wetted-width, bank stability, and riparian vegetative zone width.

Comparison of benthic community metrics among streams

The following five aquatic insect metrics are presented below for differences among streams (via multiple samples per stream) as they are the five metrics commonly found to have significant correlation to SC in previous across-stream studies: 1) EPT Richness, 2) E Richness less B, 3) Shannon Diversity, 4) percent composed of top 5 dominant taxa, and 5) Scraper Richness (Figures 5-9). When comparing all community metrics among streams, all were significantly different except for the following six metrics: the top 1, 2, and 5 percent dominant taxa, richness and percentage of scrapers less Ephemeroptera (E), and richness of sprawlers (Table 1). The three aquatic insect metrics with the highest F-statistic and with the largest differences in community composition between streams were: 1) percent Ephemerotera, Plecoptera, and Trichoptera (EPT) less Baetidae (B), Leuctridae (L), and Hydropsychidae (H), 2) percent E less B, and 3) percent Trichoptera (T). EPT are the three most-sensitive orders (commonly referred to as Mayflies, Stoneflies, and Caddisflies, respectively) of aquatic insects and BLH are the three most-tolerant taxa of each respective order. Definitions of metric abbreviations are provided in the Appendix.

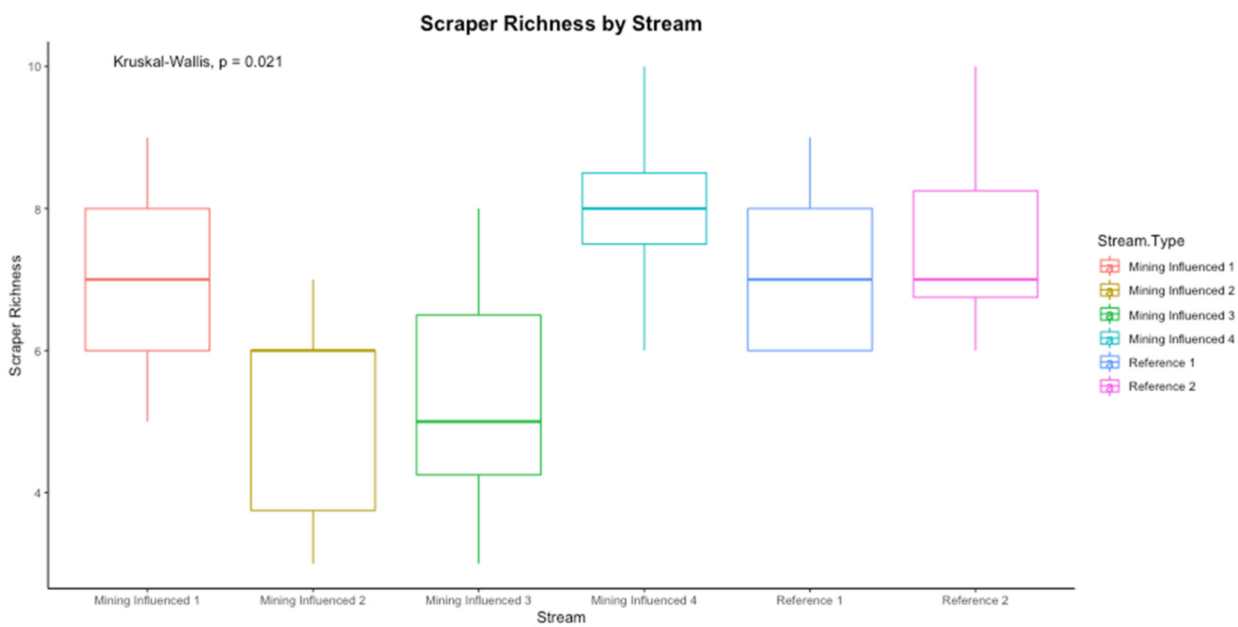

Figure 5. Scraper richness differences among streams using multiple samples per stream.

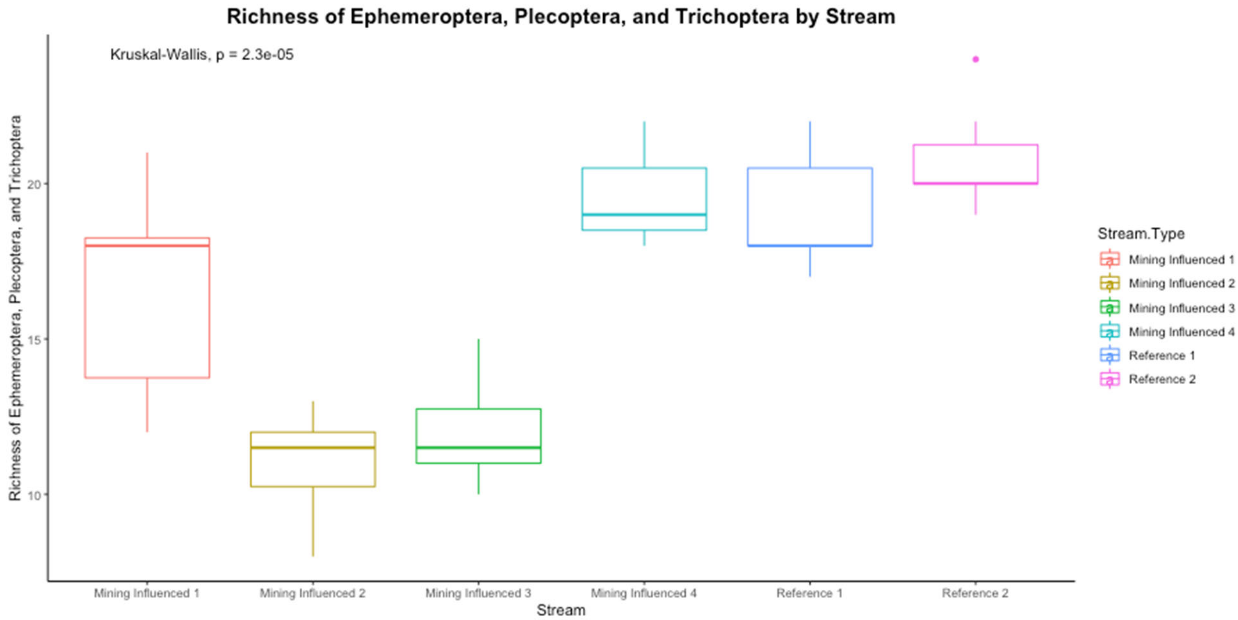

Figure 6. Differences in EPT richness among streams using multiple samples per stream.

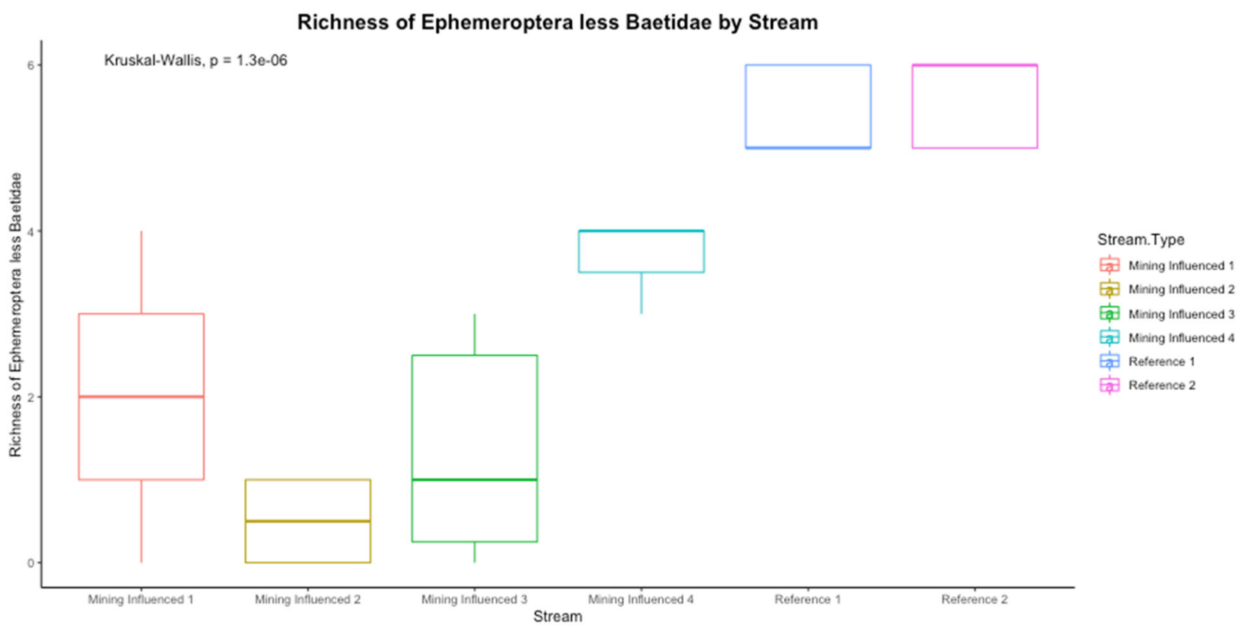

Figure 7. Differences in E less B richness among streams using multiple samples per stream.

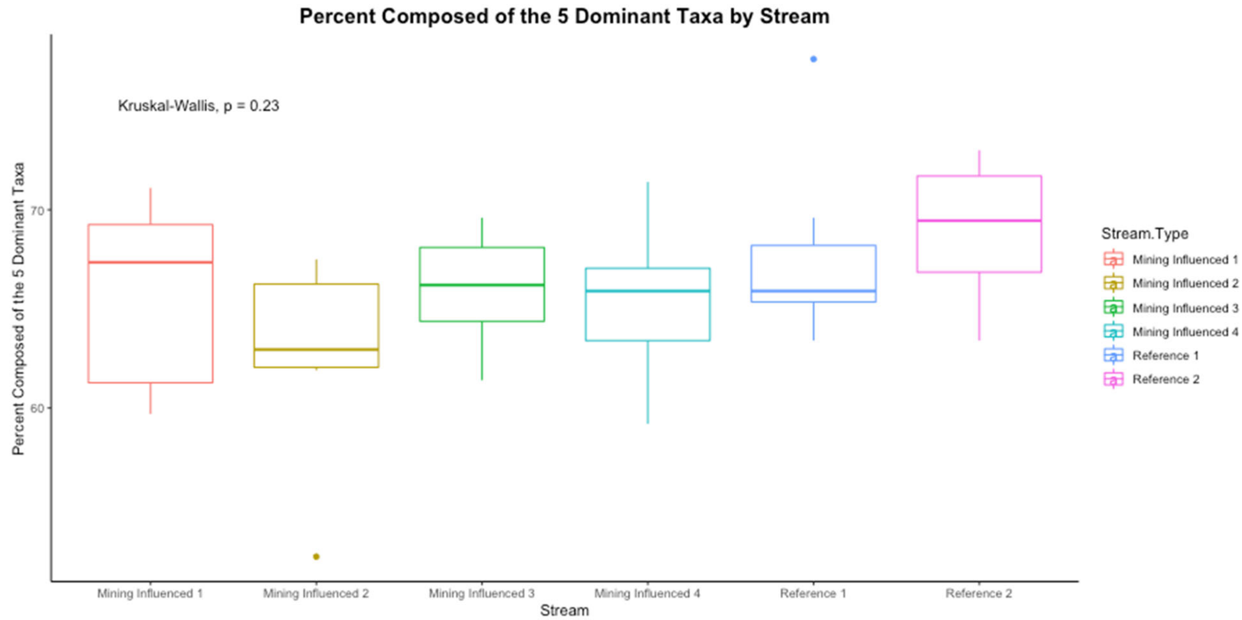

Figure 8. Differences in Percent composed of the 5 dominant taxa among streams using multiple samples per stream.

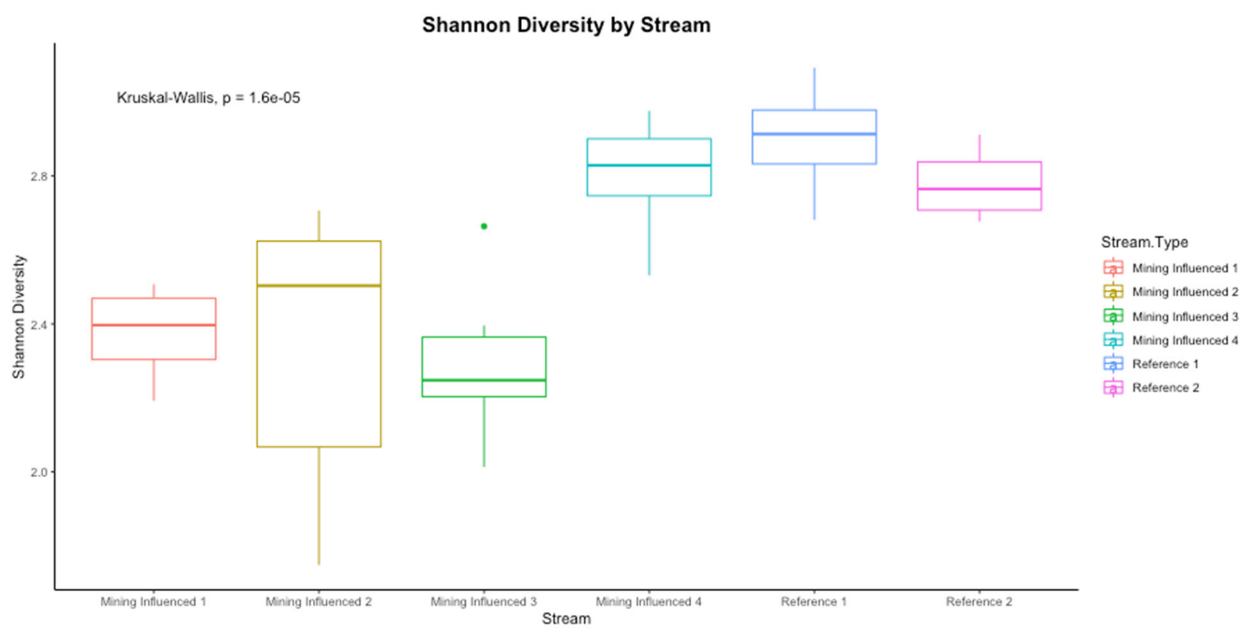

Figure 9. Differences in Shannon Diversity among streams using multiple samples per stream.

Table 1. Mean, standard deviation, Kruskal-Wallis F statistic and p-value for each aquatic insect metric of each study stream in the Fall of 2021. F and p values for metrics presented in Figures 5-9 are bolded. Definitions of metric abbreviations are provided in the Appendix.

	Reference 1		Reference 2		Mining Influenced 1		Mining Influenced 2		Mining Influenced 3		Mining Influenced 4			
Metric	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	F-statistic	p value
Hshannon	2.9	0.14	2.78	0.09	2.38	0.11	2.34	0.4	2.29	0.22	2.8	0.15	**29.76**	**1.65E-05**
Hsimpson	0.92	0.02	0.9	0.01	0.82	0.03	0.83	0.08	0.83	0.05	0.9	0.02	25.51	0.00011103
J	0.83	0.03	0.83	0.02	0.73	0.04	0.74	0.1	0.74	0.05	0.82	0.03	22.48	0.0004243
p1dom	20.06	1.41	20.43	1.05	19.51	1.35	18.52	1.6	19.53	0.91	19.36	1.21	6.97	0.22279858
p2dom	34.63	2.44	35.24	1.81	33.63	2.34	31.93	2.79	33.73	1.56	33.4	2.04	6.88	0.23001683
p5dom	67.71	4.75	68.96	3.56	65.83	4.6	62.5	5.45	65.98	3.07	65.34	4.03	**6.88**	**0.23001683**
pBurrow	25.89	3.6	12.19	5.64	9.14	3.97	20	18.39	27.5	7.7	10.04	2.28	23.85	0.0002322
pCF	11.4	8.52	32.89	2.78	54.49	4.09	39.5	8.72	39	7.14	40.57	5.85	31.81	6.49E-06
pCG	24.51	3.09	23.85	4.78	3.2	1.34	12.87	6.38	17.57	7.59	9.87	4.17	30.9	9.81E-06
pChi	13.47	3.26	10.25	5.5	1.65	0.6	7.15	1.76	7.92	5.15	5.11	1.84	27.34	4.91E-05
pChiO	14.78	3.55	10.49	5.42	2.31	1.09	8.56	2.55	15.48	7.33	5.89	1.85	28.94	2.38E-05
pCling	48.34	8.05	74.14	9.73	84.53	3.69	74.23	17.03	66.88	9.24	83.54	4.68	24.6	0.00016613
pD	23.93	5.17	12.05	5.93	7.74	3.34	21.3	17.98	19.43	5.69	10	2.78	22.24	0.00047033
pE	22.13	6.99	25.03	5.41	4	2.53	4.5	8.73	1.2	1.22	12.93	4.89	31.24	8.41E-06
pE.less.B	21.54	6.83	22.8	5.14	3.7	2.36	0.83	1.37	1.2	1.22	9.86	3.18	35.9	9.93E-07
pEPT	56.12	4.8	77.55	6.83	75.87	8.69	56.28	9.27	59.06	8.1	75.92	4.7	28.37	3.07E-05
pEPT.less.Cheum	54.79	5.7	74.7	7.8	63.75	9.55	51.5	8.51	56.19	8.9	55.9	6.98	21.23	0.00073326
pEPT.less.H	46.28	9.07	46.84	6.3	23.55	6.85	19.69	11.13	21.02	5.04	41.58	4.83	31.56	7.25E-06
pEPT.less.HBL	40.73	4.98	43.28	6.13	22.73	6.98	8.49	3.39	16.18	7.07	37.03	3.46	34.37	2.01E-06
pOligo	1.3	0.89	0.25	0.38	0.66	0.88	1.42	1.07	7.53	5.78	0.77	0.55	14.94	0.01062687
pP	19.74	6.19	10.65	3.15	9.55	4.15	11.08	8.11	12.53	3.82	14.96	6.73	12.3	0.03084033
pP.less.Allo	18.54	5.06	10.65	3.15	9.38	4.1	9.92	7.41	11.62	3.11	4.76	1.37	21.34	0.00069802
pP.less.Amph	19.74	6.19	10.65	3.15	9.55	4.15	11.08	8.11	12.53	3.82	14.96	6.73	12.3	0.03084033
pP.less.LAA	13.57	3.33	9.31	2.57	8.86	4.47	2.38	1.32	6.77	3.86	3.27	1.33	28.8	2.54E-05
pP.less.Leuc	14.77	2.39	9.31	2.57	9.04	4.52	3.53	2.14	7.67	4.9	13.49	6.28	20.92	0.00083953
pPR	13.3	3.41	13.06	1.87	11.73	3.71	5.78	1.81	6.67	3.19	11.99	3.62	19.89	0.00130842
pPT.H	24.14	6.35	21.81	2.71	19.53	8.23	15.2	7.79	19.82	5.97	28.66	4.44	12.8	0.02531255
pSC	29.26	8.58	24.05	4.05	19.09	6.19	20.85	10.89	17.45	4.39	21.16	4.76	11.6	0.04072933
pSC.less.E	14.77	4.01	12.03	3.91	15.44	5.32	19.95	10.62	16.25	3.97	12.06	3.14	9.33	0.0964864
pSH	21.49	7.96	6.09	2.71	11.55	3.18	20.98	11.61	19.3	5.18	16.46	6.25	26.96	5.80E-05
pSprawl	9.86	2.89	2.86	1.63	5.91	3.65	1.7	1.05	5.22	3.66	2.94	1.41	22.96	0.00034315
pSwimm	15.84	6.48	10.83	5.55	0.43	0.32	4.08	7.23	0.4	0.55	3.44	3.42	31.54	7.31E-06
pT	14.24	7.92	41.88	3.47	62.31	6.78	40.7	6.99	45.33	7.38	48.03	5.74	31.27	8.29E-06
pT.less.H	4.39	1.21	11.16	2.23	9.99	5	4.12	2.63	7.3	3.91	13.69	4.32	24.3	0.00018987
pTOL	0.36	0.53	0.06	0.17	0	0	1.74	0.71	0.08	0.2	0.29	0.49	22.87	0.00035717
rich	33.29	2.29	28.25	2.25	26.5	3.16	23.5	4.23	22.33	3.08	31.14	2.48	28.17	3.38E-05
rich.Burrow	7.86	1.57	3.75	1.49	4.13	0.64	5.17	2.04	5.17	0.75	4.14	1.07	21.43	0.0006711
rich.C	4.71	0.95	2.63	0.74	3.75	1.16	3.67	1.75	3.33	1.51	4.29	1.11	11.69	0.03934822
rich.CF	3.86	1.86	5.13	0.83	5.38	1.19	5.33	0.82	3.17	0.75	6.57	0.79	21.47	0.00066011
rich.CG	5.86	1.07	5.38	1.41	2.75	1.16	3.17	1.33	3	0	4.57	0.98	25.33	0.00012051
rich.Cling	19.29	1.98	19.25	1.75	18.88	2.7	14.83	3.54	13.83	2.56	21.43	1.27	23.26	0.00030127
rich.D	6.86	1.46	3.63	1.3	3.88	0.99	7	1.26	4.67	1.75	4.86	1.57	22.98	0.00034081
rich.E	6.14	0.69	7.13	0.83	2.63	1.51	1.17	0.98	1.33	1.37	5.29	0.49	35.42	1.24E-06
rich.E.less.B	5.43	0.53	5.63	0.52	2	1.31	0.5	0.55	1.33	1.37	3.71	0.49	**35.31**	**1.31E-06**
rich.EPT	19.14	2.04	20.75	1.58	16.63	3.2	11	1.79	12	1.79	19.57	1.51	**29.04**	**2.28E-05**
rich.EPT.less.H	16.71	1.6	17.5	1.6	13.25	3.15	7.5	1.38	9.83	1.94	16.29	1.5	29.84	1.59E-05
rich.INT	16.14	2.34	16.75	1.67	14.13	2.53	9.33	2.42	11	2.45	17.57	1.9	26.71	6.48E-05
rich.less.E	27.14	2.27	21.13	2.47	23.88	1.96	22.33	3.56	21	2.68	25.86	2.34	21.45	0.00066654
rich.P	6.57	1.51	6.13	1.25	5.75	1.49	4	1.1	5.83	1.17	5.29	1.38	11	0.05138841
rich.PR	10.29	0.95	6.63	1.6	7	0.93	5.17	1.47	6.67	2.07	7.14	1.95	19.28	0.00170403
rich.PT	13	2.16	13.63	1.51	14	2.14	9.83	1.47	10.67	1.63	14.29	1.38	20.61	0.00095817
rich.SC	7.14	1.21	7.5	1.41	7	1.31	5.17	1.72	5.33	1.86	8	1.29	**13.24**	**0.02126808**
rich.SC.less.E	4	1.41	3.88	0.83	5.13	0.99	4.67	1.75	4	0.63	5.14	1.21	8.71	0.12139335
rich.SH	6	1.53	3.5	0.93	4.38	1.19	4.67	1.21	4.17	1.17	4.86	1.21	11.8	0.0375688
rich.Sprawl	2.86	1.21	1.88	0.64	2.63	0.52	2.5	0.84	2.83	0.98	3.43	1.9	7.08	0.21476243
rich.Swimm	3.14	0.9	3.38	0.74	0.88	0.64	1	0.63	0.5	0.55	2.14	1.07	29.76	1.64E-05
rich.T	6.43	2.15	7.5	0.93	8.25	1.39	5.83	0.75	4.83	1.17	9	0.58	24.79	0.0001528
rich.T.less.H	4	1.15	4.25	0.71	4.88	1.25	2.33	0.82	2.67	1.03	5.71	0.76	26.01	8.87E-05
rich.TOL	0.43	0.53	0.13	0.35	0	0	1.17	0.41	0.17	0.41	0.43	0.79	18.64	0.00224049

Benthic community metrics versus SC within streams

Based on previous studies among streams, the five following aquatic insect metrics are presented below in relation to SC values: 1) EPT Richness, 2) E Richness less B, 3) Shannon Diversity, 4) percent composed of top 5 dominant taxa, and 5) Scraper Richness (Figures 10-14). Of these, significant correlations with SC were found for: Mining-Influenced stream #1 EPT, Mining-influenced stream #2 richness of E less B, and Reference stream #2 scraper richness (although we note the small SC gradient for this stream). When examining all metrics, mining-influenced streams generally had more significant correlations than reference streams, which did exhibit some significant trends albeit versus smaller SC gradients within streams (Table 2). However, most metrics did not exhibit significant trends versus SC within streams, suggesting shifts in community composition may be occurring in each stream based on other environmental factors such as habitat.

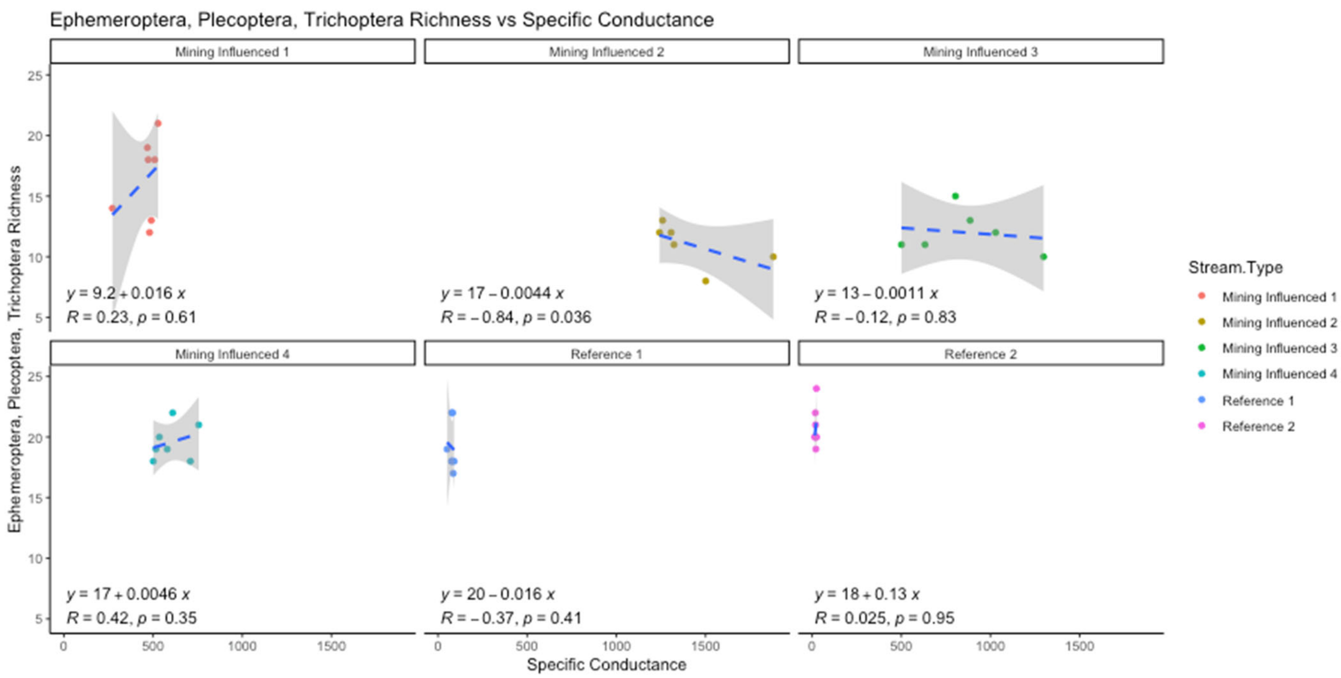

Figure 10. EPT Richness versus SC for within stream samples.

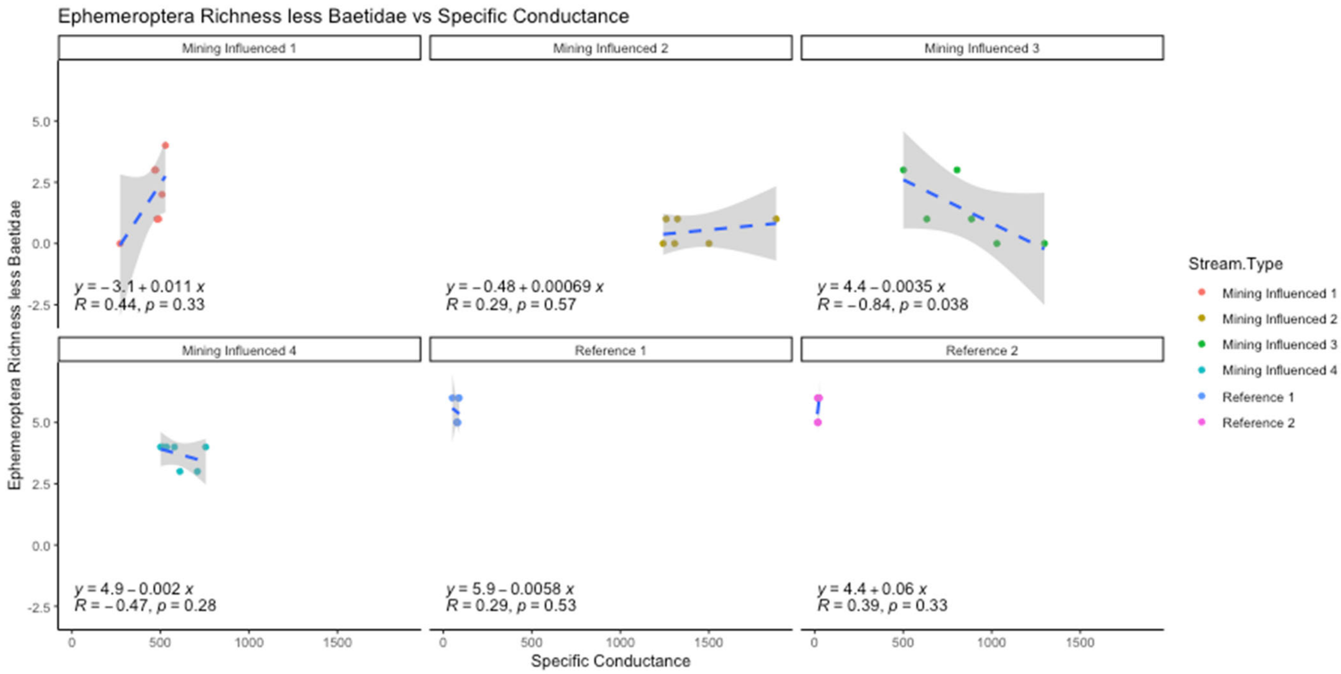

Figure 11. E Richness less B versus SC for within stream samples.

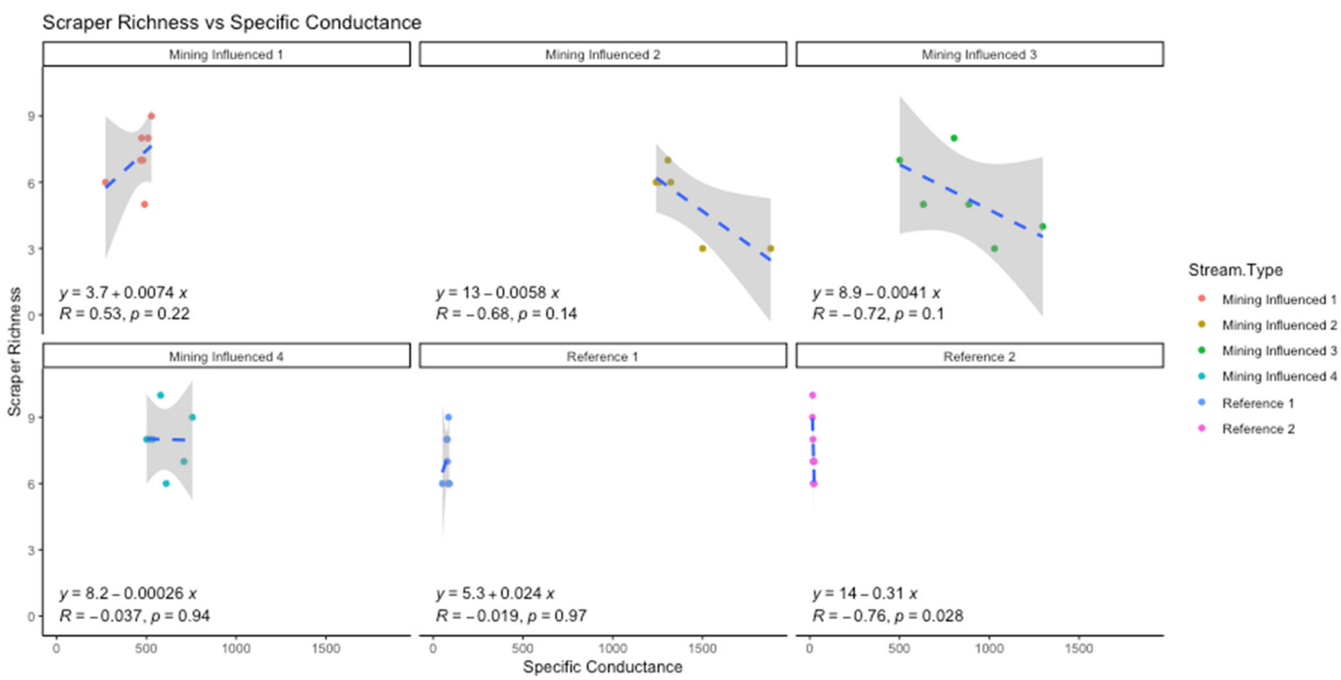

Figure 12. Scraper Richness versus SC for within stream samples.

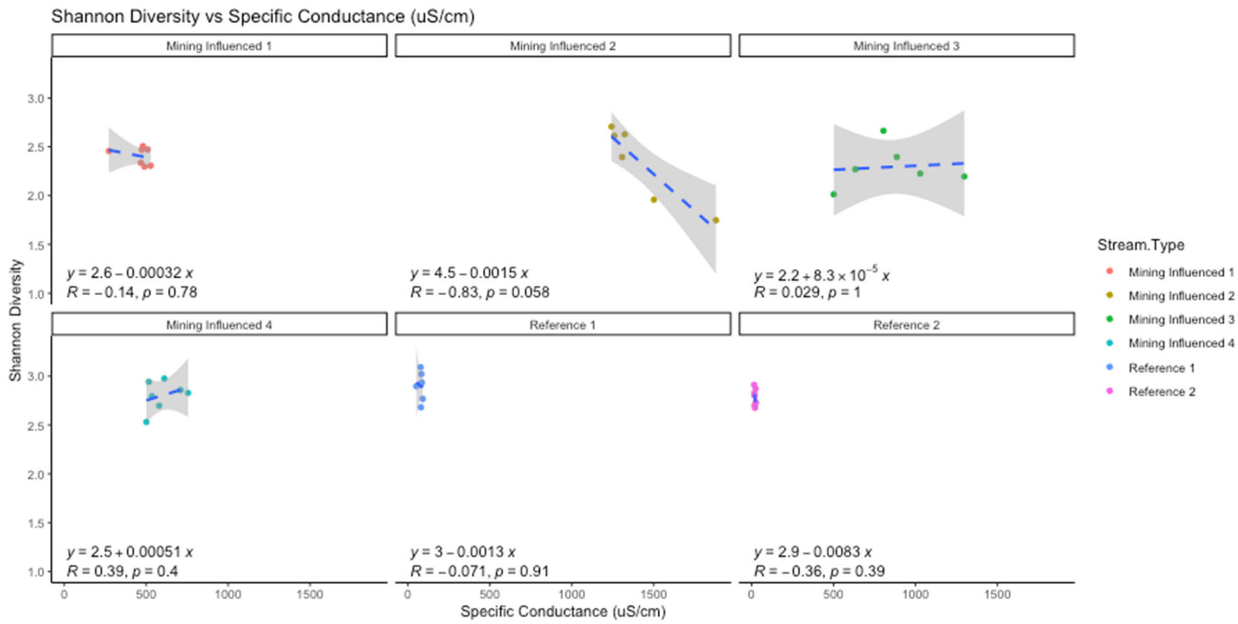

Figure 13. Shannon Diversity versus SC for within stream samples.

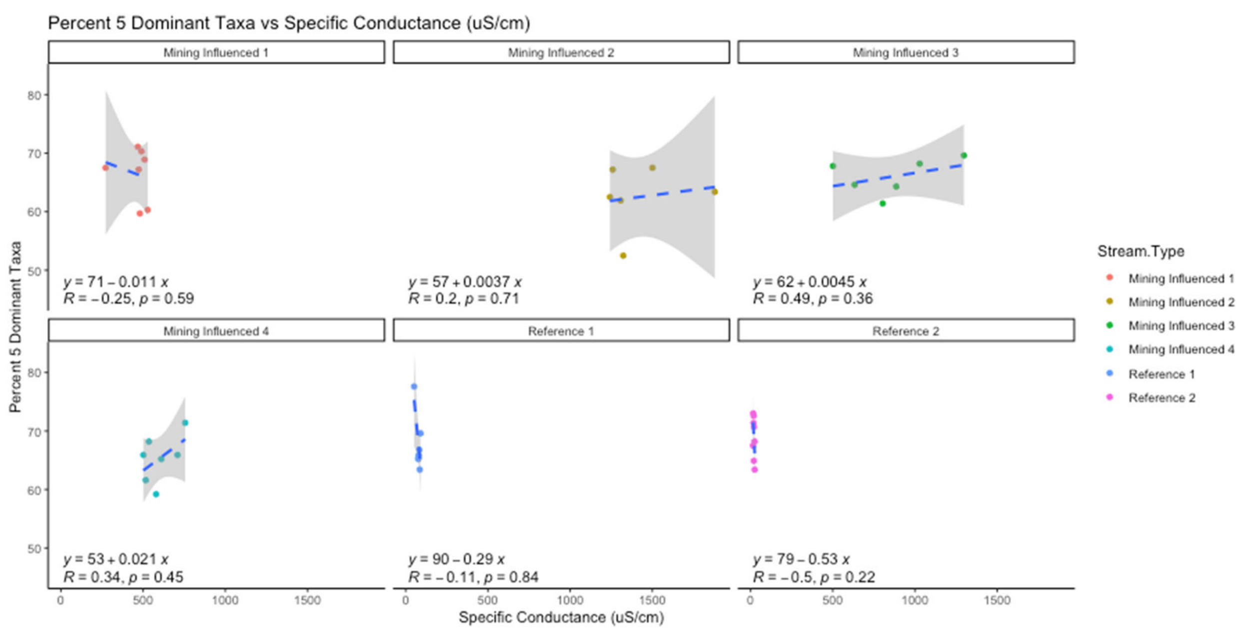

Figure 14. Percent that are the 5 dominant taxa versus SC for within stream samples.

Table 2. Spearman correlations of SC to each aquatic insect metric by stream. P values under 0.05 bolded. Note correlations in reference streams are most likely not causation as SC range is very small. Definitions of metric abbreviations are provided in the Appendix.

	Reference 1		Reference 2		Mining Influenced 1		Mining Influenced 2		Mining Influenced 3		Mining Influenced 4	
Metric	cor	p	cor	p	cor	p	cor	p	cor	p	cor	p
Hshannon	-0.071	0.879	-0.357	0.385	-0.143	0.760	**-0.829**	**0.042**	0.029	0.957	0.393	0.383
Hsimpson	-0.500	0.253	-0.357	0.385	-0.107	0.819	**-0.829**	**0.042**	0.314	0.544	0.250	0.589
J	-0.321	0.482	-0.667	0.071	-0.321	0.482	**-0.829**	**0.042**	0.086	0.872	0.250	0.589
p1dom	-0.107	0.819	-0.500	0.207	-0.250	0.589	0.200	0.704	0.486	0.329	0.342	0.452
p2dom	-0.107	0.819	-0.500	0.207	-0.250	0.589	0.200	0.704	0.486	0.329	0.342	0.452
p5dom	-0.107	0.819	-0.500	0.207	-0.250	0.589	0.200	0.704	0.486	0.329	0.342	0.452
pBurrow	-0.143	0.760	0.429	0.289	-0.036	0.939	0.486	0.329	0.543	0.266	0.714	0.071
pCF	-0.286	0.535	-0.476	0.233	0.500	0.253	0.086	0.872	-0.486	0.329	-0.143	0.760
pCG	-0.357	0.432	0.190	0.651	**-0.857**	**0.014**	0.714	0.111	0.600	0.208	0.536	0.215
pChi	-0.321	0.482	0.262	0.531	-0.270	0.558	0.486	0.329	0.771	0.072	0.107	0.819
pChiO	-0.179	0.702	0.262	0.531	**-0.786**	**0.036**	0.486	0.329	0.771	0.072	0.393	0.383
pCling	-0.536	0.215	-0.690	0.058	0.342	0.452	-0.771	0.072	-0.771	0.072	-0.321	0.482
pD	0.107	0.819	0.429	0.289	0.000	1.000	0.600	0.208	0.543	0.266	0.429	0.337
pE	0.393	0.383	0.048	0.911	-0.107	0.819	0.029	0.957	**-0.986**	**0.00031**	-0.306	0.504
pE.less.B	0.393	0.383	-0.071	0.867	-0.107	0.819	0.152	0.774	**-0.986**	**0.00031**	-0.607	0.148
pEPT	0.500	0.253	-0.071	0.867	0.071	0.879	-0.257	0.623	0.086	0.872	-0.286	0.535
pEPT.less.Cheum	0.500	0.253	0.024	0.955	0.036	0.939	-0.257	0.623	0.086	0.872	-0.071	0.879
pEPT.less.H	0.571	0.180	-0.071	0.867	0.143	0.760	-0.657	0.156	0.771	0.072	-0.107	0.819
pEPT.less.HBL	0.321	0.482	0.000	1.000	0.143	0.760	0.029	0.957	**0.943**	**0.005**	-0.143	0.760
pOligo	0.214	0.645	-0.289	0.488	-0.637	0.124	0.600	0.208	0.143	0.787	0.296	0.518
pP	0.321	0.482	-0.381	0.352	0.143	0.760	**-0.943**	**0.005**	0.257	0.623	0.357	0.432
pP.less.Allo	0.250	0.589	-0.381	0.352	0.143	0.760	**-0.943**	**0.005**	0.257	0.623	0.071	0.879
pP.less.Amph	0.321	0.482	-0.381	0.352	0.143	0.760	**-0.943**	**0.005**	0.257	0.623	0.357	0.432
pP.less.LAA	-0.357	0.432	-0.381	0.352	0.250	0.589	-0.257	0.623	0.771	0.072	0.250	0.589
pP.less.Leuc	-0.179	0.702	-0.381	0.352	0.250	0.589	-0.486	0.329	**0.886**	**0.019**	0.107	0.819
pPR	-0.357	0.432	0.108	0.799	0.357	0.432	-0.600	0.208	0.486	0.329	-0.321	0.482
pPT.H	**0.786**	**0.036**	-0.241	0.565	0.143	0.760	-1.000	0	0.771	0.072	0.286	0.535
pSC	0.071	0.879	-0.060	0.888	-0.321	0.482	-0.771	0.072	-0.543	0.266	0.143	0.760
pSC.less.E	-0.714	0.071	-0.238	0.570	-0.607	0.148	**-0.829**	**0.042**	-0.029	0.957	0.179	0.702
pSH	0.714	0.071	-0.310	0.456	0.036	0.939	-0.029	0.957	0.314	0.544	0.107	0.819
pSprawl	0.429	0.337	**0.905**	**0.002**	0.107	0.819	-0.696	0.125	**0.943**	**0.005**	-0.143	0.760
pSwimm	0.607	0.148	**0.810**	**0.015**	-0.075	0.873	0.406	0.425	-0.339	0.510	0.054	0.908
pT	-0.286	0.535	-0.287	0.490	0.321	0.482	0.371	0.468	0.116	0.827	-0.429	0.337
pT.less.H	0.500	0.253	0.167	0.693	0.179	0.702	0.086	0.872	**0.943**	**0.005**	-0.286	0.535
pTOL	0.236	0.610	0.577	0.134	NA	NA	0.200	0.704	-0.393	0.441	0.668	0.101
rich	0.342	0.452	0.180	0.670	0.360	0.427	-0.600	0.208	-0.143	0.787	**0.954**	**0.001**
rich.Burrow	0.243	0.599	0.200	0.634	-0.219	0.637	-0.030	0.954	0.648	0.164	0.543	0.208
rich.C	0.020	0.967	-0.456	0.256	-0.075	0.873	-0.638	0.173	-0.647	0.165	0.147	0.753
rich.CF	-0.259	0.574	0.088	0.835	0.275	0.550	-0.216	0.681	-0.309	0.552	**0.777**	**0.040**
rich.CG	0.599	0.155	0.509	0.197	-0.430	0.335	0.334	0.518	NA	NA	**0.767**	**0.044**
rich.Cling	-0.018	0.969	-0.331	0.423	0.429	0.337	-0.580	0.228	-0.334	0.518	0.692	0.085
rich.D	0.487	0.268	0.420	0.300	0.418	0.350	-0.395	0.439	0.455	0.364	0.741	0.057
rich.E	0.000	1.000	0.441	0.274	0.259	0.574	-0.093	0.862	**-0.837**	**0.038**	0.316	0.490
rich.E.less.B	0.289	0.530	0.394	0.334	0.436	0.328	0.293	0.573	**-0.837**	**0.038**	-0.474	0.282
rich.EPT	-0.374	0.408	0.025	0.952	0.234	0.613	**-0.841**	**0.036**	-0.116	0.827	0.418	0.350
rich.EPT.less.H	-0.018	0.969	0.185	0.660	0.270	0.558	-0.759	0.080	-0.029	0.957	0.364	0.423
rich.INT	-0.090	0.848	0.277	0.506	0.234	0.613	-0.696	0.125	-0.235	0.653	0.418	0.350
rich.less.E	0.327	0.474	0.133	0.753	0.281	0.542	-0.771	0.072	0.353	0.492	**0.927**	**0.003**
rich.P	-0.200	0.667	0.110	0.795	0.291	0.527	**-0.878**	**0.021**	0.441	0.381	0.056	0.905
rich.PR	-0.315	0.491	0.683	0.062	**0.791**	**0.034**	**-0.928**	**0.008**	0.334	0.518	0.703	0.078
rich.PT	-0.367	0.418	-0.123	0.772	0.327	0.474	**-0.899**	**0.015**	0.463	0.355	0.430	0.335
rich.SC	-0.019	0.968	**-0.761**	**0.028**	0.527	0.224	-0.679	0.138	-0.725	0.103	-0.037	0.937
rich.SC.less.E	0.164	0.726	**-0.718**	**0.045**	-0.139	0.766	**-0.941**	**0.005**	-0.338	0.512	0.197	0.672
rich.SH	0.571	0.180	-0.426	0.293	0.367	0.418	-0.441	0.381	0.618	0.191	-0.449	0.312
rich.Sprawl	-0.150	0.749	0.206	0.624	0.474	0.282	-0.676	0.140	0.507	0.305	-0.164	0.726
rich.Swimm	0.132	0.777	0.404	0.321	-0.100	0.832	**0.845**	**0.034**	-0.488	0.326	0.599	0.155
rich.T	0.000	1.000	-0.350	0.395	-0.055	0.908	-0.463	0.355	0.147	0.781	0.668	0.101
rich.T.less.H	0.112	0.811	-0.143	0.735	-0.236	0.610	0.123	0.816	0.334	0.518	0.116	0.805
rich.TOL	0.289	0.530	0.577	0.134	NA	NA	-0.393	0.441	-0.393	0.441	0.579	0.173

Drivers of stream community composition

There is a clear separation of reference from mining-influenced streams based on individual counts (n= 200 per sample) in nonmetric multidimensional scaling (NMDS) analyses. The 11 water chemistry variables and 10 habitat metrics that differed the most among streams, according to Kruskal-Wallis analysis, were chosen to be overlaid as vectors. The top 11 water chemistry variables after redundant parameters were removed included: na.mgl, sc.uScm, cl.mgl, alkalinity.mgl, li.ugl, sr.ugl, ni.ugl, k.mgl, u.ugl, ca:mg, and so4:hco3, with SC and alkalinity largely explaining separation among stream groups (Figure 15). The top 10 habitat metrics included: average slope, average riparian width right bank (R), average riparian width left bank (L), average vegetative protection R, mean pebble size D50, average wetted width, percent fines, percent small cobble, percent pebbles, and average embeddedness, with some of these weighing more in axis two (i.e., vertical ordination space) than water quality variables (Figure 16). Definitions of water quality and habitat metric abbreviations are provided in the Appendix.

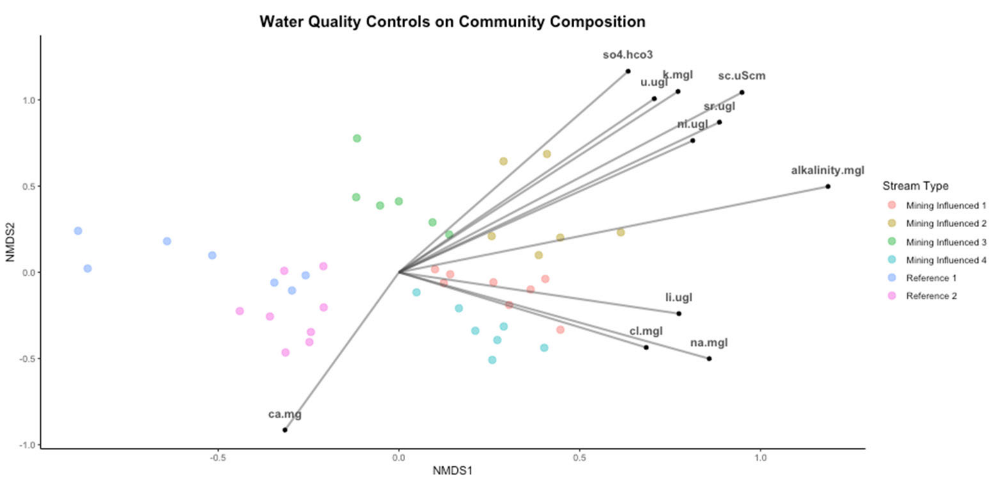

Figure 15. NMDS of raw taxa counts for each site colored by stream with vectors of selected water quality overlaid.

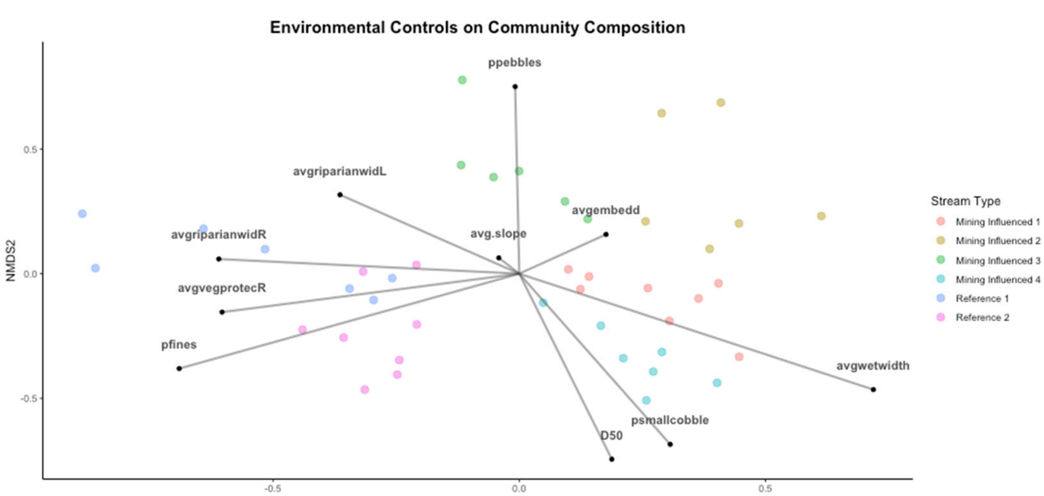

Figure 16. NMDS of raw taxa counts for each site colored by stream with vectors of substrate metrics overlaid.

Table 3. Kruskal-Wallis F statistic and p-value for each water chemistry metric (left) and habitat metric (right) of each study stream in the Fall of 2021. Definitions of water quality and habitat metric abbreviations are provided in the Appendix.

Metric	F-statistic	p.value
ac.uScm	38.69	2.74E-07
ag.ugl	5.00	0.41588019
al.ugl	22.48	0.00042455
alkalinity.mgl	38.12	3.57E-07
as.ugl	26.95	5.83E-05
ba.ugl	33.65	2.79E-06
ca.mg	34.86	1.60E-06
ca.mgl	37.54	4.66E-07
cd.ugl	28.05	3.56E-05
cl.mgl	38.47	3.03E-07
co.ugl	28.16	3.38E-05
cr.ugl	18.61	0.00226822
cu.ugl	30.64	1.10E-05
do.mgl	21.99	0.00052497
fe.ugl	24.40	0.00018184
hardness.mgl	37.65	4.43E-07
hco3.mgl	38.12	3.57E-07
k.mgl	36.80	6.56E-07
li.ugl	37.92	3.92E-07
mg.mgl	37.52	4.71E-07
mn.ugl	29.93	1.52E-05
mo.ugl	5.00	0.41588019
na.mgl	39.35	2.02E-07
nh3.n.mgl	7.91	0.16129871
ni.ugl	37.05	5.84E-07
no2no3.n.mgl	29.83	1.60E-05
npoc.mgl	28.29	3.20E-05
ortho.po4.p.mgl	15.05	0.01015905
pb.ugl	6.00	0.30621892
ph	17.67	0.00339508
sc.uScm	39.22	2.14E-07
se.ugl	30.90	9.79E-06
so4.hco3	34.12	2.25E-06
so4.mgl	38.53	2.95E-07
sr.ugl	37.56	4.63E-07
temp.C	18.83	0.0020671
ti.ugl	29.56	1.80E-05
tn.mgl	12.90	0.02428991
tp.mgl	12.27	0.03129623
u.ugl	35.32	1.30E-06
v.ugl	29.75	1.65E-05
zn.ugl	26.68	6.58E-05

Metric	F-statistic	p value
avg.slope	26.95	5.84E-05
avgbankstabL	5.73	0.33300
avgbankstaR	2.28	0.80972
avgcancov	13.25	0.02113
avgdepth	10.05	0.07377
avgembedd	15.84	0.00732
avgriparianwidL	21.16	0.00075
avgriparianwidR	21.61	0.00062
avgvegprotecL	14.81	0.01119
avgvegprotecR	21.00	0.00081
avgwetwidth	18.87	0.00204
D10	14.30	0.0138
D16	14.48	0.0128
D25	11.57	0.0412
D50	19.53	0.0015
D75	10.22	0.0691
D84	7.37	0.1944
D84to50	15.82	0.0074
D90	6.70	0.2443
Hsimpson	12.40	0.0297
IQR	9.43	0.0932
LCF	9.21	0.1009
LWD.m2	2.55	0.76868
pfines	18.32	0.0026
plargecobble	6.12	0.2943
ppebbles	16.49	0.0056
psmallcobble	17.64	0.0034
SCF	12.33	0.0305

Abundance data were also used to determine similarity among samples and their correlation with distance downstream for each stream. Both Reference streams had significant correlations, suggesting their aquatic macroinvertebrate communities become more dissimilar with distance downstream. The Mining-Influenced #3 stream displayed similar trends to reference conditions. This stream is also the stream with the highest gradient of SC over the shortest distance from upstream to downstream, suggesting that extreme dilution gradients of SC in mining influenced streams may also create shifts in community.

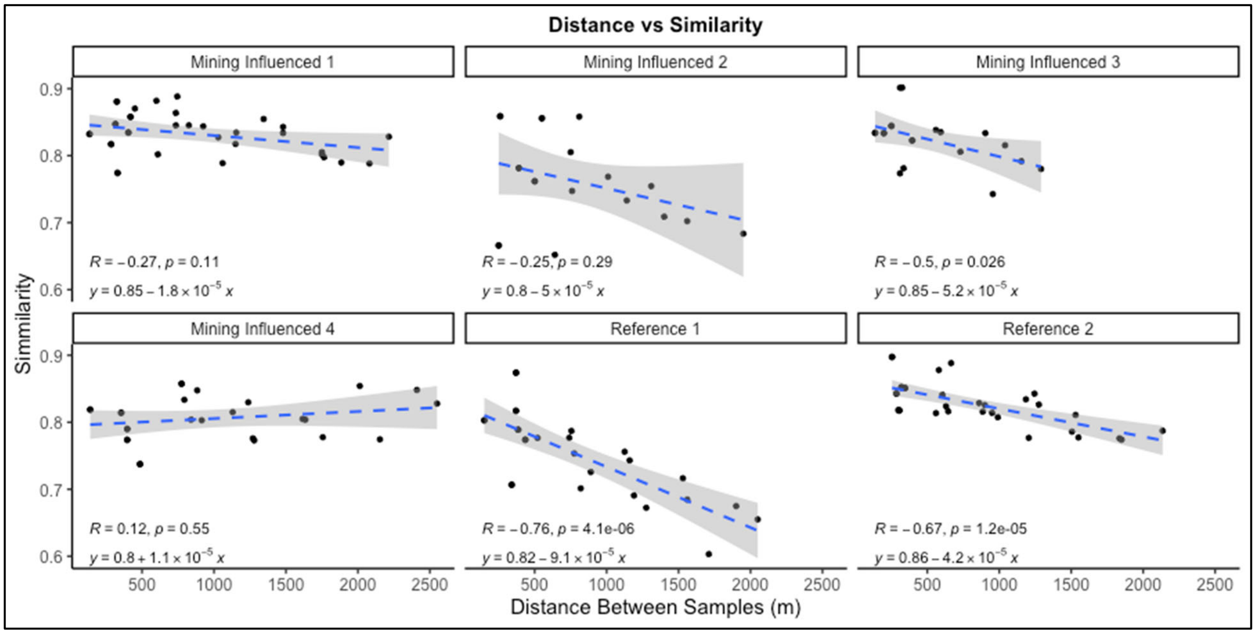

Figure 17. Spearman correlation of Bray-Curtis dissimilarity values versus distance between samples for each stream.

Summary

With this PRP funding support, we i) continued monitoring of SC, stage, and flow; ii) conducted seasonal macroinvertebrate and water quality sampling to add to our long-term dataset and enable future long-term recovery assessments; and iii) conducted within-in stream assessment of water chemistry and macroinvertebrate communities. Contributions of this work include:

- Using deployed instrumentation and three synoptic sampling events, we added to our long-term dataset for continued analysis of potential stream recovery following mining.
- Combining SC and stage data demonstrated general dilution of SC with higher stage (and thus higher flow) and will allow us to account for this flow control in future assessment of stream water chemistry recovery.
- We conducted 4-5 rounds of streamflow measurements at our six selected streams, and thus more flow measurements are needed to develop accurate rating curves between flow and stage. We emphasize, however, that PRP funding support has allowed us to maintain stage recorders, to initiate flow measurement methods, and to develop SC-concentration relationships; these resources will be leveraged with future efforts to continue data collection and flow measurements. Once rating curves are completed, we will use data and developed models for novel estimates of downstream mass export of measured water chemistry constituents.
- We sampled 6-8 locations within six streams, both in fall and spring, for water chemistry and macroinvertebrate communities. Spring samples are still being processed, but fall samples preliminarily indicate: i) both within- and across-stream variation in specific community composition metrics; ii) most metrics do not exhibit significant correlations versus SC gradients within streams; and iii) analysis of drivers indicate other variables, including habitat, may help to explain within-stream variation.

Appendix

Table A1. Benthic macroinvertebrate metrics.

Metric	Abbreviation
% Chironomidae	pChi
% Chironomidae and Oligochaeta	pChiO
% Diptera	pD
% Ephemeroptera	pE
%Ephemeroptera less Baetidae	pE.less.B
% Ephemeropter, Plecoptera, and Trichoptera	pEPT
% Ephemeropter, Plecoptera, and Trichoptera less Cheumatopsyche	pEPT.less.Cheum
% Ephemeropter, Plecoptera, and Trichoptera less Hydropsychidae, Baetidae, and Leuctridae	pEPT.less.H
% Ephemeropter, Plecoptera, and Trichoptera less Hydropsychidae	pEPT.less.HBL
% Oligochaeta	pOligo
% Plecoptera	pP
% Plecoptera less Allocapnia	pP.less.Allo
% Plecoptera less Amphinemura	pP.less.Amph
% Plecoptera less Leuctra, Allocapnia, and Amphinemura	pP.less.LAA
% Plecoptera less Leuctra	pP.less.Leuc
% Plecoptera and Trichoptera less Hydropsychidae	pPT.H
% Trichoptera	pT
% Trichoptera less Hydropsychidae	pT.less.H
Diptera Taxa	rich.D
Ephemeroptera Taxa	rich.E
Ephemeroptera Taxa less Baetidae	rich.E.less.B
Ephemeropter, Plecoptera, and Trichoptera Taxa	rich.EPT
Ephemeropter, Plecoptera, and Trichoptera Taxa less Hydropsychidae	rich.EPT.less.H
All Taxa Except Ephemeroptera	rich.less.E
Plecoptera Taxa	rich.P
Plecoptera and Trichoptera Taxa	rich.PT
Trichoptera Taxa	rich.T
Trichoptera Taxa less Hydropsychidae	rich.T.less.H
% Collector-Filterers	pCF
% Collector-Gatherers	pCG
% Predators	pPR
% Scrapers	pSC
% Scrapers less Ephemeroptera	pSC.less.E
% Shredders	pSH
Collector Taxa	rich.C
Collector-Filterer Taxa	rich.CF
Collector-Gatherer Taxa	rich.CG
Predator Taxa	rich.PR
Scraper Taxa	rich.SC
Scraper Taxa less Ephemeroptera	rich.SC.less.E
Shredder Taxa	rich.SH
% Burrowers	pBurrow
% Clingers	pCling
% Scrapers	pSC
% Scrapers less Ephemeroptera	pSC.less.E
% Sprawlers	pSprawl
% Swimmers	pSwimm
Burrower Taxa	rich.Burrow
Clinger Taxa	rich.Cling
Sprawler Taxa	rich.Sprawl
Swimmer Taxa	rich.Swimm
Pielou's Evenness	J
% 1 Dominant Taxon	p1dom
% 2 Dominant Taxa	p2dom
% 5 Dominant Taxa	p5dom
% Tolerant Taxa	pTOL
Intolerant Taxa	rich.INT
Tolerant Taxa	rich.TOL
Shannon Diversity	Hshannon
Simpson Diversity	Hsimpson

Table A2. Habit metrics.

Category	Metric	Abbreviation
EPA Rapid Assessment Sampling Reach Averages	Slope	avg.slope
	Bank Stability Left Bank	avgbankstabL
	Bank Stability Right Bank	avgbankstaR
	Canopy Cover	avgcancov
	Wetted Depth	avgdepth
	Embeddedness	avgembedd
	Riparian Width Left Bank	avgriparianwidL
	Riparian Width Right Bank	avgriparianwidR
	Vegetative Protection Left Bank	avgvegprotecL
	Vegetative Protection Right Bank	avgvegprotecR
	Wetted Width	avgwetwidth
	Large Woody Debris (square meters)	LWD.m2
Sediment Size Distribution	Size at Which 10% of all Particles are Found	D10
	Size at Which 16% of all Particles are Found	D16
	Size at Which 25% of all Particles are Found	D25
	Size at Which 50% of all Particles are Found	D50
	Size at Which 75% of all Particles are Found	D75
	Size at Which 84% of all Particles are Found	D84
	Size at Which 50-84% of all Particles are Found	D84to50
	Size at Which 90% of all Particles are Found	D90
	Sediment Diversity	Hsimpson
	Inner Quartile Range of Sediment Sizes	IQR
	Large Cobbles to Fine Ratio	LCF
	% Fines	pfines
	% Large Cobbles	plargecobble
	% Pebbles	ppebbles
	% Small Cobbles	psmallcobble
	Small Cobbles to Fine Ratio	SCF

Table A3. Water quality metrics.

Category	Metric	Abbreviaton
Metals	Sodium	na.mgl
	Chloride	cl.mgl
	Lithium	li.ugl
	Strontium	sr.ugl
	Calcium	ca.mgl
	Magnesium	mg.mgl
	Nickel	ni.ugl
	Potassium	k.mgl
	Uranium	u.ugl
	Calcium to Magnesium Ratio	ca.mg
	Barium	ba.ugl
	Selenium	se.ugl
	Copper	cu.ugl
	Manganese	mn.ugl
	Vanadium	v.ugl
	Titanium	ti.ugl
	Cobalt	co.ugl
	Cadmium	cd.ugl
	Arsenic	as.ugl
	Zinc	zn.ugl
	Iron	fe.ugl
	Aluminum	al.ugl
	Chromium	cr.ugl
	Lead	pb.ugl
	Silver	ag.ugl
	Molybdenum	mo.ugl
Nutrients	Sulfate	so4.mgl
	Bicarbonate	hco3.mgl
	Ratio of Sulfate to Bicarbonate	so4.hco3
	nitrate + nitrite	no2no3.n.mgl
	Non-Purgeable Organic Carbon	npoc.mgl
	Ortho-Phosphate	ortho.po4.p.mgl
	Total Nitrogen	tn.mgl
	Total Phosphorus	tp.mgl
	Nitrate	nh3.n.mgl
Field Measurements	Specific Conductance	sc.uScm
	Actual Conductance	ac.uScm
	Alkalinity	alkalinity.mgl
	Hardness	hardness
	Dissolved Oxygen	do.mgl
	Temperature Celcius	temp.C
	Potential Hydrogen (pH)	ph

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