Science of the Total Environment 717 (2020) 137216

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Impacts to water quality and biota persist in mining-influenced Appalachian streams

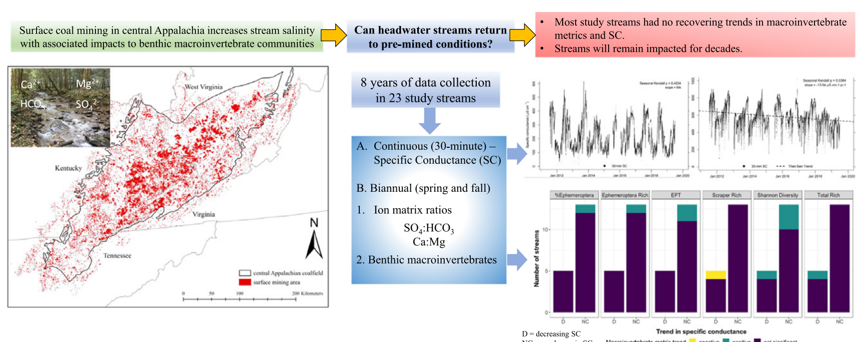
Thomas R. Cianciolo [a,*], Daniel L. McLaughlin [a], Carl E. Zipper [b], Anthony J. Timpano [a], David J. Soucek [c], Stephen H. Schoenholtz [a]

[a] Virginia Polytechnic Institute and State University, Virginia Water Resources Research Center, Virginia Tech, 310 West Campus Dr, RM 210, Blacksburg, VA 24061, USA
[b] Virginia Polytechnic Institute and State University, School of Plant and Environmental Sciences, Virginia Tech, 185 Ag Quad Ln, RM 416, Blacksburg, VA 24061, USA
[c] Illinois Natural History Survey, University of Illinois at Urbana-Champaign, 1816 S. Oak St, Champaign, IL 61820, USA

HIGHLIGHTS

- 23 Appalachian headwater streams where monitored over eight years; 2011 to 2019.
- Surface coal mining influences water chemistry and macroinvertebrate communities.
- Seasonal Kendall analysis was used to identify temporal trends in conductivity.
- Limited recovery of stream health; impacts may remain for decades.
- Long-term monitoring is vital for assessing stream recovery potential.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 December 2019
Received in revised form 6 February 2020
Accepted 7 February 2020
Available online xxxx

Editor: Sergi Sabater

Keywords:
Benthic macroinvertebrates
Conductivity
Salinization
Ion matrix
Temporal trend
Coal mining

ABSTRACT

Elevated dissolved major ions (salinization) from surface coal mining are a common impact to central Appalachian headwater streams. Salinization is associated with alterations of benthic macroinvertebrate communities, as many organisms are adapted to the naturally dilute streams of the region. These geochemical and biological alterations have been observed in streams decades after mining, but it remains unclear whether and at what rate water quality and aquatic biota recover after mining. To address this issue, we analyzed temporal trends in specific conductance (SC), ion matrix ratios, and benthic macroinvertebrate communities over an eight-year period in 23 headwater streams, including 18 salinized by surface coal mining. We found strong, negative correlations between SC and diversity of benthic macroinvertebrate communities. Temporal trend analysis demonstrated limited recovery of water chemistry to natural background conditions. Five of the 18 mining-influenced streams exhibited declining SC; however, annual rates of decline in these streams ranged from 1.9% to 3.7% of mean annual SC, suggesting long time periods will be required to reach established benchmark values (ca. 25 years) or values observed in our five reference study streams (ca. 40 years). Similarly, there was limited evidence for recovery of macroinvertebrate community metrics, even in the few mining-influenced streams with decreasing SC. These findings indicate that salinization and its biological effects persist, likely for decades, in central Appalachian headwater streams. Our work also highlights the value of long-term monitoring data for assessing recovery potential of salinized freshwaters, as well as the need for improved understanding of water quality and biological recovery processes and time frames.

* Corresponding author.
E-mail addresses: thomrc6@vt.edu (T.R. Cianciolo), mclaugd@vt.edu (D.L. McLaughlin), czip@vt.edu (C.E. Zipper), atimpano@vt.edu (A.J. Timpano), soucek@illinois.edu (D.J. Soucek), schoenhs@vt.edu (S.H. Schoenholtz).

https://doi.org/10.1016/j.scitotenv.2020.137216

1. Introduction

Elevated dissolved major ions (i.e., salinization) in freshwaters are a global problem resulting from various forms of land use, including resource extraction, urbanization, and agriculture (Kaushal et al., 2018). Salinization of freshwaters threatens biodiversity and ecosystem function with additional impacts to human health and infrastructure (Cañedo-Argüelles et al., 2016). For example, anthropogenic salinization of freshwater has been linked to altered fish assemblages (Higgins and Wilde, 2005), shifts in macroinvertebrate community structure and trait composition (Szöcs et al., 2014), and decreased carbon storage in wetlands (Herbert et al., 2015). In the central Appalachian coalfield of USA, surface mining activity is a major contributor to increased salinity in headwater streams (Griffith et al., 2012) with resultant benthic macroinvertebrate taxa loss as well as shifts of those communities to more salt-tolerant taxa (Pond et al., 2008; Timpano et al., 2015, 2018a). Moreover, salinity can remain elevated in headwater streams decades after the closure of mining activities (Pond et al., 2014), highlighting the need for long-term study of salinity and associated impacts to biological communities to assess recovery potential of these systems.

In central Appalachia, approximately 3.5% (5900 km^2) of the total land area has been impacted by surface coal mining (Pericak et al., 2018), with documented effects to water chemistry, including increases in major ion concentrations (Pond et al., 2008, 2014; Timpano et al., 2018b). Waste rock, produced when overlaying bedrock layers are removed to expose buried coal seams, is often deposited into adjacent valleys to produce valley fills that bury headwater streams (U.S. EPA, 2011a). Mineral surfaces of this fractured waste rock undergo accelerated weathering, thereby increasing concentrations of dissolved ions in headwater streams receiving discharged waters from mine spoils (Griffith et al., 2012). In central Appalachia, the acids produced by pyrite oxidation are often buffered by neutralizers released from dissolution of carbonates and other associated minerals, resulting in alkaline drainage (Clark et al., 2018a). Resultant alkaline drainage to headwater streams has increased concentrations of major ions including sulfate (SO_4^{2-}), bicarbonate (HCO_3^-), calcium (Ca^{2+}), and magnesium (Mg^{2+}) and has elevated pH, total dissolved solids (TDS), and the reliable salinity surrogate specific conductance (SC) (Pond et al., 2008; Timpano et al., 2015).

Numerous studies have documented that elevated salinity, often measured as SC, impacts benthic macroinvertebrate communities in headwater streams draining mined areas in central Appalachia (e.g., Pond et al., 2008; Boehme et al., 2016; Timpano et al., 2015, 2018a). Streams with elevated salinity have reduced taxon richness and community structure shifted to more salt-tolerant taxa (Pond et al., 2008; Timpano et al., 2015, 2018a). Salt-sensitive taxa including Ephemeroptera (mayflies) can be absent from streams with high salinity (Merricks et al., 2007; Pond, 2010). Moreover, the scraper functional feeding group has been documented to have lower richness and relative abundance in streams with elevated SC compared to reference streams (Pond et al., 2014; Timpano et al., 2015, 2018a). The specific mechanism of scraper decline in streams receiving alkaline mine drainage is uncertain but has been linked to disruption of osmoregulation (Johnson et al., 2015; Clements and Kotalik, 2016) and bioaccumulation of selenium into biofilm food sources (Conley et al., 2011; Whitmore et al., 2018; Drover et al., 2019; Cianciolo et al., 2020). Loss of benthic macroinvertebrate taxa and specific functional feeding groups is of ecological importance because of their significant roles in decomposition and nutrient cycling in headwater streams, and as food sources for higher trophic levels (Wallace and Webster, 1996).

Streams receiving alkaline mine drainage can be influenced by elevated SC long after termination of mining and reclamation, with likely sustained effects to benthic macroinvertebrates (Pond et al., 2014). Daniels et al. (2016) conducted laboratory leaching tests of mine spoils and found slow decline of SC with continued leaching. Evans et al. (2014) documented analogous effects in mine-drainage discharge produced by aging valley fills and estimated that decades would be required for discharge waters to reach SC < 500 μS/cm, a level still greater than the 300 μS/cm U.S. EPA conductivity benchmark (U.S. EPA, 2011b; Cormier et al., 2013). What remains unclear are recovery time periods in headwater streams receiving these discharge waters and the degree to which altered aquatic communities respond to possible salinity declines with time. Persistence of elevated SC and altered benthic macroinvertebrate communities decades after mining have been documented in the region (Pond et al., 2014), but we are unaware of research assessing long-term changes in both salinity and biology within individual streams.

In this work, we evaluated the long-term influence of surface coal mining on salinity and benthic macroinvertebrate communities across 23 central Appalachia headwater streams. Our objectives were to analyze and compare temporal trends in SC, major-ion composition, and benthic macroinvertebrate community structure over an 8-year period (2011 to 2019) and, in doing so, assess the recovery potential of both water chemistry and biology in mining-influenced streams.

2. Methods

2.1. Site selection

We selected 23 first-order headwater streams in the central Appalachian coalfield of Virginia and West Virginia (U.S. EPA Level IV Ecoregion 69d; Omernik and Griffith, 2014) for assessment of long-term trends in water chemistry and benthic macroinvertebrate communities (Fig. 1). Of these, 18 streams are influenced by historical (i.e., before 2011) surface coal mining. Only four of these mining-influenced streams were subject to post-2011 mining activities in their watersheds (Table 1). The other five study streams are reference streams within relatively undisturbed, forested watersheds and with SC values within the lower range observed for streams in the ecoregion (Griffith, 2014). A thorough site selection process was conducted to identify streams that met rigorous abiotic criteria for water chemistry and habitat quality (see methods in Timpano et al., 2015), except for major ions and associated SC in mining-influenced streams. As such, reference and mining-influenced streams have comparable riparian and in-stream habitat conditions and minimal influence from non-salinity stressors (e.g., excessive sedimentation, channelization, extreme pH).

2.2. Data collection

2.2.1. Water chemistry

In situ SC was measured every 30 min between fall 2011 and summer 2019 using automated dataloggers (HOBO Freshwater Conductivity Data Logger, model U24–001, Onset Computer Corp., Bourne, Massachusetts) installed within each stream. Small data gaps are present in the continuous SC time series of some study sites as a result of datalogger malfunction, burial following storm events, and drying of streams during drought conditions. Biannual (i.e., spring and fall) water chemistry monitoring during this same time period included in situ measures of water temperature, SC, and pH via a calibrated handheld multi-probe meter (YSI Professional Plus –YSI, Inc., Yellow Springs, Ohio, USA).

Grab samples were also collected biannually (i.e., spring and fall) from fall 2011 to spring 2019 to assess the ionic composition of stream water under conditions approximating baseflow (i.e., flow not influenced by runoff). Vertically mixed water was collected and immediately filtered through a 0.45-μm pore polyvinylidene fluoride filter into prelabeled sterile polyethylene sample bags. Filtered water was collected in separate 100-ml aliquots for analysis of TDS and alkalinity and in separate 50-ml aliquots for analysis of major cations and anions. The cation sample was preserved to pH < 2 by adding approximately 0.5% (v/v) of a

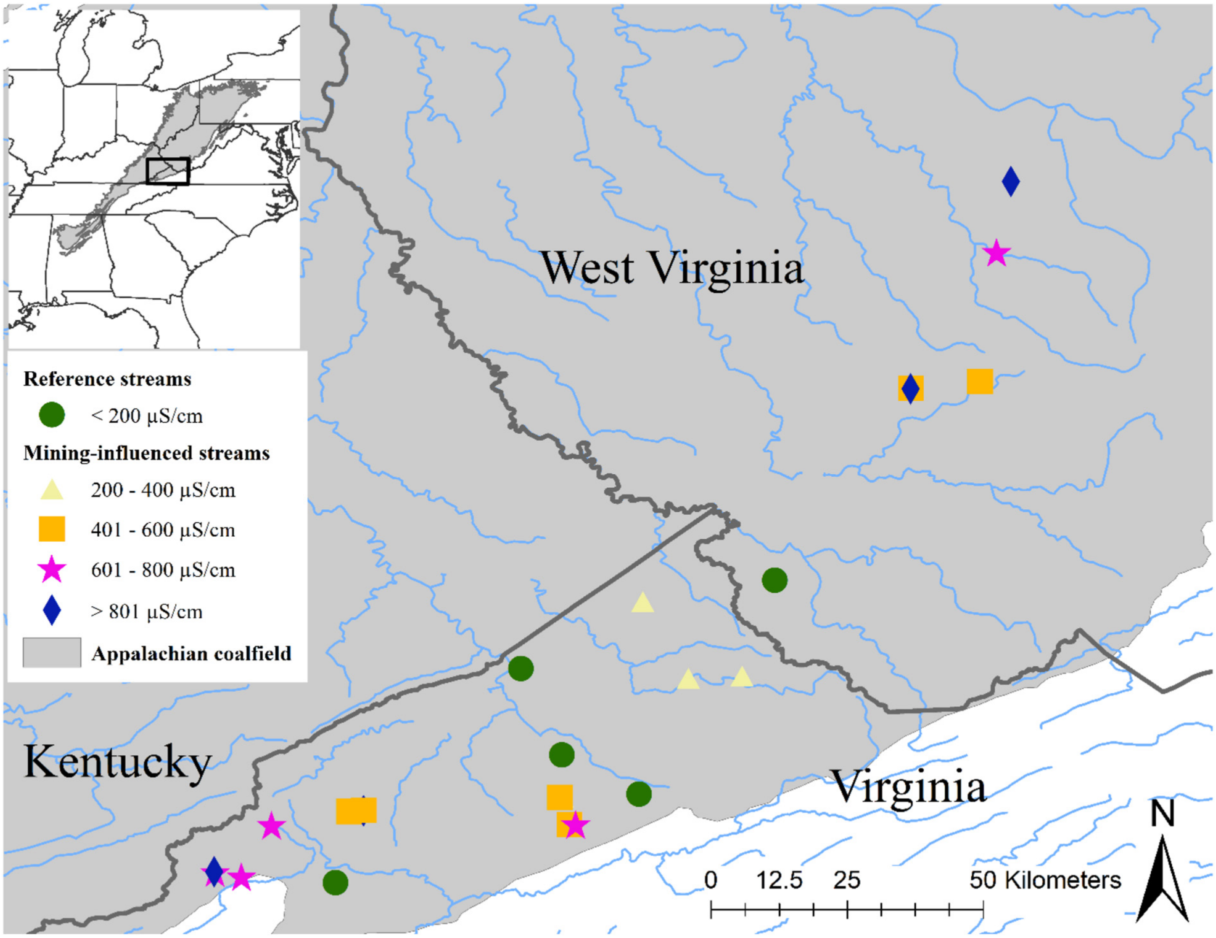


Fig. 1. Map of 23 study headwater streams in the central Appalachia coalfield USA. Circles are reference streams and the remaining shapes are mining-influenced streams. Color and shape of symbol is based on annual mean specific conductance.

solution of 1 + 1 concentrated ultrapure nitric acid and deionized water (UESPA, 1996). Samples were stored at 4 °C until analysis.

Samples were analyzed for TDS by evaporating to constant weight using 50 ml for mining-influenced streams and 100 ml for reference streams in a drying oven at 180 °C (U.S. EPA, 1971). Total Alkalinity was measured by titration of field-filtered water samples with a prepared standard acid (0.02 N HCl) using a potentiometric auto-titrator (TitraLab 865, Radiometer Analytical, Lyon, France) (APHA, 2005). Calculations of HCO_3^- were made from Total Alkalinity and pH measurements (APHA, 2005). Samples were analyzed for major cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) by ICP-MS (Thermo iCAP-RQ) (UESPA, 1996). An ion chromatograph (Dionex ICS 3000) was used to measure concentrations of SO_4^{2-} and Cl^-. Sodium, K^+, and Cl^- are not major contributors to elevated SC in streams influenced by surface coal mining (Pond et al., 2008; Timpano et al., 2015, Supplementary material Table S1), and thus were not included in subsequent analysis.

2.2.2. Benthic macroinvertebrates

Benthic macroinvertebrates were sampled biannually (i.e., spring and fall) from fall 2011 to fall 2018 in study streams following the semi-quantitative, single habitat (riffle-run) method specified in Virginia Department of Environmental Quality biomonitoring protocols (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). Samples were collected during fall and spring of each year except fall 2014, fall 2016, spring 2015, and spring 2017. Using a 0.3-m D-frame kicknet with 500-µm mesh, a single composite sample (approximately $2m^2$) 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), which are adapted from RBP methods (Barbour et al., 1999). Specimens were identified to genus level using standard keys (Merritt et al., 2008), except individuals in family Chironomidae and subclass Oligochaeta, which were identified at those levels.

2.3. Data analysis

We assessed recovery potential of stream water quality by investigating temporal trends in salinity. Weekly mean SC values were calculated for all 23 study streams using SC datalogger observations recorded at 30-minute intervals. A modified Seasonal Kendall (SK) analysis (Hirsch and Slack, 1984) using the *kendallSeasonalTrendTest* function from the *Envstats* package in R (Millard, 2013; R Core Team, 2018) was conducted on these weekly mean SC values to assess monotonic temporal trends while accounting for serial dependence and seasonality. Weeks are defined here as "seasons" for the purposes of SK analysis and should not to be confused with meteorological seasons. Our preliminary investigations indicated that definition of statistical seasons as week-long periods did not obscure trends that may have been detected by analysis with longer time periods. The SK test is a

Table 1
Watershed characteristics of 23 study headwater streams in central Appalachia USA.

Stream ID[a]	State	Annual mean SC[b] (μS cm^{-1})	Watershed area (km^2)	% Watershed mined[c] 1985–2010	% Watershed mined 2011–2015	Year of last mining activity[d]
EAS (ref)	VA	25	2.4			
MCB (ref)	VA	51	1.3			
CRO (ref)	VA	64	2.3			
HCN (ref)	WV	68	6.0			
COP (ref)	VA	125	0.8			
GRA	VA	226	4.4	3.2		pre-1985
SPC	VA	349	6.9	2.2		2009
FRY	VA	371	5.7	6.0		2007
HUR	VA	383	1.5	11.4		pre-1985
CRA	WV	416	9.8	N/A[e]		pre-1985
RUT	VA	554	1.9	3.4		pre-1985
LLE	WV	562	0.7	7.8		2005
BIR	VA	571	3.5	3.9		1993
LAB	VA	599	2.8	8.0		2000
MIL	VA	608	2.7	51.2	4.1	2012
ROL	VA	618	1.3	29.7		2010
ROC	VA	690	7.1	34.2	1.6	post-2015
KEL	VA	757	2.6	60.0		2009
POW	VA	761	2.7	58.6	11.4	2014
LLW	WV	1063	2.0	27.2		2006
KUT	VA	1068	1.1	38.8		2009
LLC	WV	1218	4.3	28.2	2.8	post-2015
RIC	VA	1418	4.2	32.1		2005

[a] Reference streams are identified with (ref) after their stream ID. The remaining streams are influenced by surface coal mining.
[b] Annual mean specific conductance (SC) calculated using data collected at 30-minute intervals from fall 2011 to summer 2019.
[c] Mined area of watersheds, 1985–2015, calculated using yearly surface mining layers produced by Pericak et al. (2018).
[d] We define the last year of mining activity as the year when no additional watershed area was converted to surface mining activity.
[e] The mining-influenced stream CRA had no record of land conversion to surface mining from 1985 to 2015. However, aerial photos reveal pre-1977 contour mining areas not captured by Pericak et al. (2018). A direct estimate of mined area was not made in this watershed as a result of the vertical nature of contour mining where coal is removed from the elevation of the coal seam.

non-parametric analysis resistant to data gaps and commonly used for analysis of water-quality time series data to test the significance of temporal trends (e.g., Stoddard et al., 1999; Driscoll et al., 2003; Duan et al., 2018). When significant, we conducted a Theil-Sen slope analysis (Helsel and Hirsch, 2002) using the *TheilSen* function from the *openair* package in R (Carslaw and Ropkins, 2012) to analyze the magnitude of temporal SC trends.

Ionic composition of streamwater was evaluated using both anion and cation molar ratios as matrix indicators (Timpano et al., 2017). Ratio of sulfate to bicarbonate (SO_4:HCO_3) was used as the anion-matrix indicator. Laboratory studies have shown that SO_4^{2-} is the dominant anion early in the leaching process of mine spoils and slowly decreases in concentration whereas HCO_3^- tends to increase with repeated leaching events, suggesting that SO_4:HCO_3 ratio will decline with time since mining (Orndorff et al., 2015; Daniels et al., 2016). Ratio of calcium to magnesium (Ca:Mg) was chosen as a cation-matrix indicator because previous work has observed reduced Ca:Mg ratios in streams influenced by surface coal mining (Timpano et al., 2017) and in mine-spoil leachates (Clark et al., 2018a, 2018b) relative to reference streams. Differences in these ion matrix indicators between reference and mining-influenced streams were analyzed using linear mixed models using the *lmer* function from the *lmerTest* package in R (Kuznetsova et al., 2017) with study stream defined as a random effect and site-type as a fixed effect. Temporal trends of ion ratios for each stream were then analyzed using multiple linear regression with ion ratios as the dependent variable and year and season (spring or fall) as independent variables. We concluded temporal trends in ion ratios were evident when models indicated a statistically significant effect by year.

Biological recovery was assessed by evaluating temporal trends of six metrics describing benthic macroinvertebrate community structure (Barbour et al., 1999). Each of the selected metrics has been shown to respond negatively to increasing SC (Timpano et al., 2015, 2018a); they include measures of taxonomic richness, community composition, and functional feeding groups. Spearman correlation was used to evaluate relationships between salinity and the selected macroinvertebrate metrics. Correlations between SC measured at the time of biological sampling and macroinvertebrate metrics across all 23 study streams were made each year (fall 2011 to fall 2018) for both fall and spring sampling seasons. We then analyzed temporal trends in macroinvertebrate metrics using multiple linear regression with metric as the dependent variable and year (continuous, numeric) and season (spring and fall) as independent variables (Timpano et al., 2017). We concluded temporal trends in benthic macroinvertebrate metrics of community structure were evident when models indicated a statistically significant effect by year. A significance threshold of $p = .05$ was used for all statistical analyses.

3. Results

3.1. Water chemistry

Across all 23 study streams, we found no increasing trends in SC and 17 streams with no significant trend in SC from fall 2011 to summer 2019 (Fig. 2; Supplementary Figs. S1–S23; Table 2). Significant decreasing trends in SC were observed for one reference stream (−1.80 μS/cm yr^{-1}; −2.8% annual mean SC yr^{-1}) and five mining-influenced streams, ranging from −22.74 μS/cm yr^{-1} (−1.9% annual mean SC yr^{-1}) to −10.57 μS/cm yr^{-1} (−3.7% annual mean SC yr^{-1}).

Ratios of SO_4:HCO_3 were higher in mining-influenced streams than reference streams ($p = .026$, Supplementary Fig. S24a), whereas Ca:Mg ratios were higher in reference streams ($p = .028$, Supplementary Fig. S24b). Mixed models revealed 10 of the 18 mining-influenced streams had significant decreasing trends in the SO_4:HCO_3 ratio (Table 2), but there were no significant trends in reference sites. Seven streams (5 mining-influenced, 2 reference) had significant trends in Ca:Mg ratio, all of which were decreasing (Table 2). Supplementary table S2 displays mean values of the ion matrix ratios for each site and magnitude of trend if present.

3.2. Benthic macroinvertebrates

Spring samples exhibited significant ($p < .05$) negative Spearman correlations between SC measured at the time of sampling and all macroinvertebrate metrics for each year (Table 3). Correlations also were predominantly significant and negative for fall, although not as strong for Scraper Richness or Shannon Diversity. Correlations were not significant in fall 2012 for Scraper Richness and Shannon Diversity and in fall 2013 for Scraper Richness.

We observed few significant temporal trends in macroinvertebrate metrics (Table 2). Reference streams had only two significant trends in macroinvertebrate metrics over the study period, including one positive trend in Total Richness ($p = .030$) and one positive trend in Ephemeroptera Richness ($p = .026$). Mining-influenced streams had a total of 9 positive trends and one negative trends among macroinvertebrate metrics. Shannon Diversity had the most trends among streams with four positive trends found at mining-influenced streams. Conversely, Scraper Richness had no positive trends and one negative trend. Supplementary tables S2 and S3 display mean values of the macroinvertebrate metrics for each site and magnitude of trend if present.

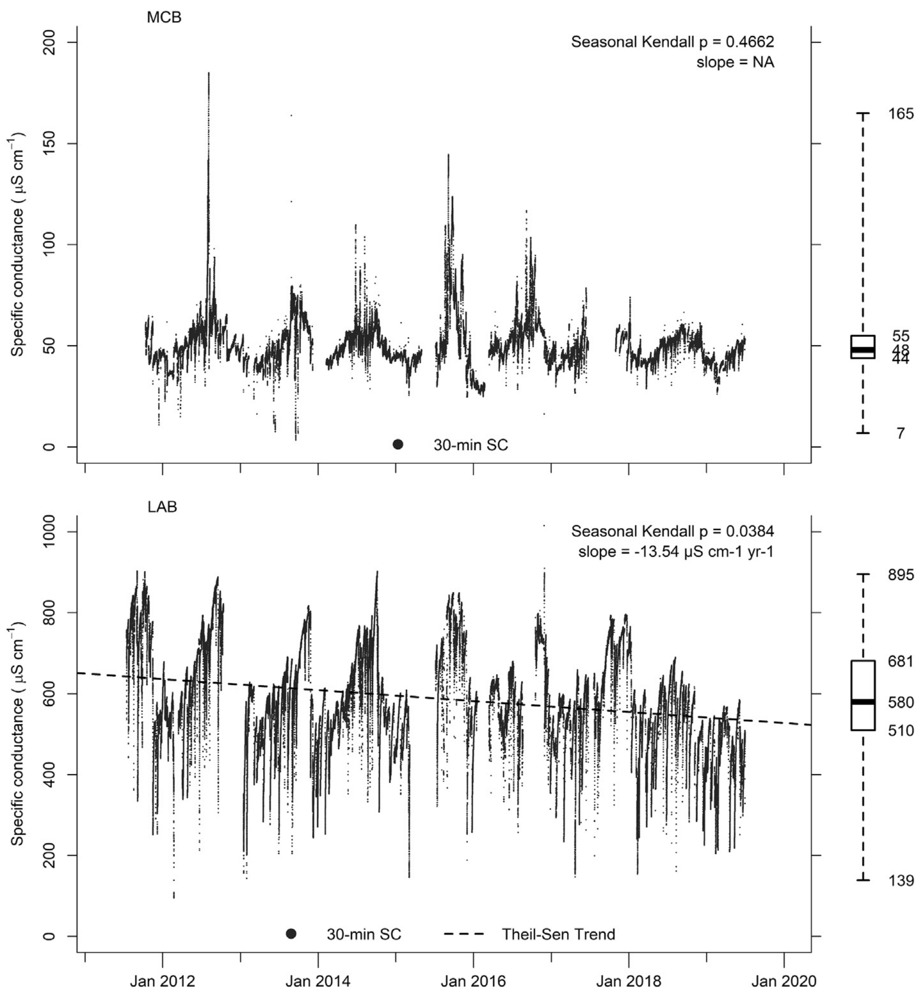


Fig. 2. Examples of temporal trends in weekly mean specific conductance (SC) from fall 2011 to summer 2019 in study streams in central Appalachia USA. Top: reference stream MCB with no SC trend. Bottom: mining-influenced stream LAB, which demonstrated a significant decreasing trend. Seasonal Kendall *p*-value, Theil-Sen slope (dashed line if statistically significant; $p < .05$), and boxplots of 30-minute continuous SC are also shown.

We assessed association between recovery of water quality and recovery of macroinvertebrate communities by comparing temporal trends in SC with those in macroinvertebrate metrics in the 18 mining-influenced streams (Fig. 3, Table 2). Macroinvertebrate-metric trends occurred rarely in streams with SC trends. That is, recovery of water quality did not necessarily equate to recovery of macroinvertebrate communities. We observed only two positive trends ($p < .001$ Shannon Diversity Index; $p = .023$ Shannon Diversity) and one negative trend ($p = .035$ Scraper Richness) in the five mining-influenced streams with decreasing SC. Conversely, we observed nine positive trends and no negative trends in macroinvertebrate metrics in mining-influenced streams with no SC trend.

4. Discussion

4.1. Water chemistry

We evaluated long-term SC trends (fall 2011 to summer 2019) in both mining-influenced and reference streams in central Appalachia to assess the recovery potential of water quality following surface mining while considering natural variation in water chemistry. Salinity in Appalachian headwater streams varies in an annual cyclic pattern in response to flow variation driven by climatic conditions. Timpano et al. (2018b) found salinity deviations within single streams averaged ± 20% of annual mean SC with maximum and minimum SCs occurring in late summer and late winter, respectively. Such seasonal variation is reflected by our high-frequency SC data (Fig. 2, Supplementary Figs. S1–S23), but accounted for in our statistical analysis of longer-term interannual trends. We found only one significant long-term SC trend in our reference streams, albeit with small magnitude of imputed annual change (< 2 µS/cm yr^{-1}), indicating that climatic and related factors did not vary in a manner to cause widespread temporal trends in headwater-stream salinity over our study time frame.

Temporal trend analysis of our 18 mining-influenced streams suggests little recovery of SC over the 8-year study period. Five mining-influenced streams had decreasing SC, but with rates that suggest long recovery times (ca. decades) to return to background levels. The average annual mean SC of the five streams with decreasing SC was 710 µS/cm with an average trend of −16 µS/cm yr^{-1}. Assuming this temporal trend will remain constant over time, it would take approximately 25 years for decline of annual mean SC to reach 300 µS/cm, which is the conductivity-benchmark level identified as protective by U.S. EPA (2011b). Moreover, it would take ca. 40 years to reach the average annual mean SC of the reference streams in this study (67 µS/cm).

Constant rates of SC decline are not expected in streams receiving alkaline mine drainage. Rather, laboratory and field mine spoil leaching experiments have shown that SC generally follows a decay pattern where rates of decline are highest immediately after overburden is deposited and become more gradual as time increases (Sena et al., 2014; Daniels et al., 2016). Data from one Pennsylvania stream over 45 years

Table 2
Summary of temporal trends in water chemistry and benthic macroinvertebrate community metrics in 23 study headwater streams in central Appalachia USA.

Stream ID[a]	Annual Mean SC[b] (μS cm^{-1})	SC slope (μS cm^{-1} yr^{-1})[b]	SO_4:HCO_3	Ca:Mg	Richness	EPT Richness[c]	Ephemeroptera Richness	% Ephemeroptera	Scraper Richness	Shannon Diversity
EAS (ref)	25			Neg[d]						
MCB (ref)	51			Neg						
CRO (ref)	64	−1.80			Pos					
HCN (ref)	68						Pos			
COP (ref)	125									
GRA	226									Pos
SPC	349					Pos		Pos		Pos
FRY	371									
HUR	383		Neg							Pos
CRA	416									
RUT	554		Neg	Neg						
LLE	562		Neg							
BIR	571	−10.57								
LAB	599	−13.54	Neg		Pos					Pos
MIL	608	−22.74	Neg	Neg						
ROL	618									
ROC	690	−11.53	Neg	Neg					Neg	
KEL	757		Neg	Neg						
POW	761		Neg				Pos			
LLW	1063					Pos				
KUT	1068	−19.56	Neg							
LLC	1218									
RIC	1418		Neg	Neg						

[a] Reference streams are identified with (ref) after their stream ID. The remaining streams are influenced by surface coal mining.
[b] SC: Specific Conductance.
[c] Combined number of taxa from orders Ephemeroptera, Plecoptera, and Trichoptera.
[d] A significance threshold of 0.05 was used for all analysis.

revealed mining-origin SO_4^{2-}, a substantial contributor to and strong correlate with SC, declined after mining in a non-linear manner, with rates of SO_4^{2-} decline decreasing steadily over time (Sams and Beer, 2000). Therefore, it is possible that in our historically mining-influenced streams, strong initial declines in SC following geologic disturbance may have already occurred and were not measured during this study; and that SC of waters emerging from weathered waste rock have reached more stable patterns in response to long-term, continued leaching (Daniels et al., 2016). Continued long-term study may be necessary to fully assess trends, albeit at very slow rates, that may occur over longer periods.

Appalachian landscapes are formed from the same geologic strata that are disturbed by mining, although with near-surface materials that have been intensively weathered. Because these landscapes give rise to streams with SC typically <200 μS/cm, and often <100 μS/cm (Supplementary Table S1), the same could be eventually expected for streams receiving alkaline mine discharge. Such results, however, have not been observed. A study of 137 Virginia valley fills found that 15–25 years on average were required for SC values in mining effluent (or leachate) to decline to <500 μS/cm (Evans et al., 2014) but declines to natural background levels were not noted. Pond et al. (2014) found West Virginia valley fills continued to discharge dissolved major ions, resulting in stream water with high SC (mean = 692 μS/cm, and ranging from 318 to 1409 μS/cm) 11 to 33 years following mining completion. Further, a field lysimeter study in Kentucky found SC values of leachate >1000 μS cm^{-1} initially after spoil placement, but those levels declined and stabilized at ~ 400 μS cm^{-1} within nine years (Agouridis et al., 2012; Sena et al., 2014). Our work supports these studies, which together demonstrate that SC does not recover to natural conditions even decades after mining activities cease and that the required time periods to do so are largely unknown.

Changes in ion matrix can also occur in mining-influenced streams with continued weathering of waste-rock material. We expected that SO_4:HCO_3 ratios would decline over time based on previous laboratory

Table 3
Spearman correlations between specific conductance and selected benthic macroinvertebrate metrics across all 23 study headwater streams in central Appalachia USA by season and year of sampling.

	Fall[a]						Spring[b]				
Metric	2011	2012	2013	2015	2017	2018	2012	2013	2014	2016	2018
Richness	−0.75*	−0.51*	−0.78*	−0.56*	−0.77*	−0.74*	−0.75*	−0.76*	−0.72*	−0.66*	−0.74*
EPT[c] Richness	−0.80*	−0.62*	−0.71*	−0.59*	−0.71*	−0.76*	−0.81*	−0.81*	−0.81*	−0.82*	−0.76*
Ephemeroptera Richness	−0.80*	−0.76*	−0.79*	−0.82*	−0.80*	−0.80*	−0.87*	−0.88*	−0.83*	−0.93*	−0.80*
Scraper Richness	−0.61*	−0.39	−0.33	−0.53*	−0.69*	−0.73*	−0.71*	−0.59*	−0.74*	−0.74*	−0.73*
Shannon Diversity	−0.61*	−0.41	−0.55*	−0.55*	−0.60*	−0.67*	−0.61*	−0.67*	−0.76*	−0.71*	−0.67*
% Ephemeroptera	−0.73*	−0.79*	−0.76*	−0.84*	−0.78*	−0.84*	−0.69*	−0.87*	−0.86*	−0.83*	−0.84*

* $p < .05$.
[a] Fall benthic macroinvertebrate sampling index period lasts from September through November (VDEQ, 2008).
[b] Spring benthic macroinvertebrate sampling index period lasts March through May (VDEQ, 2008).
[c] Combined number of taxa from orders Ephemeroptera, Plecoptera, and Trichoptera.

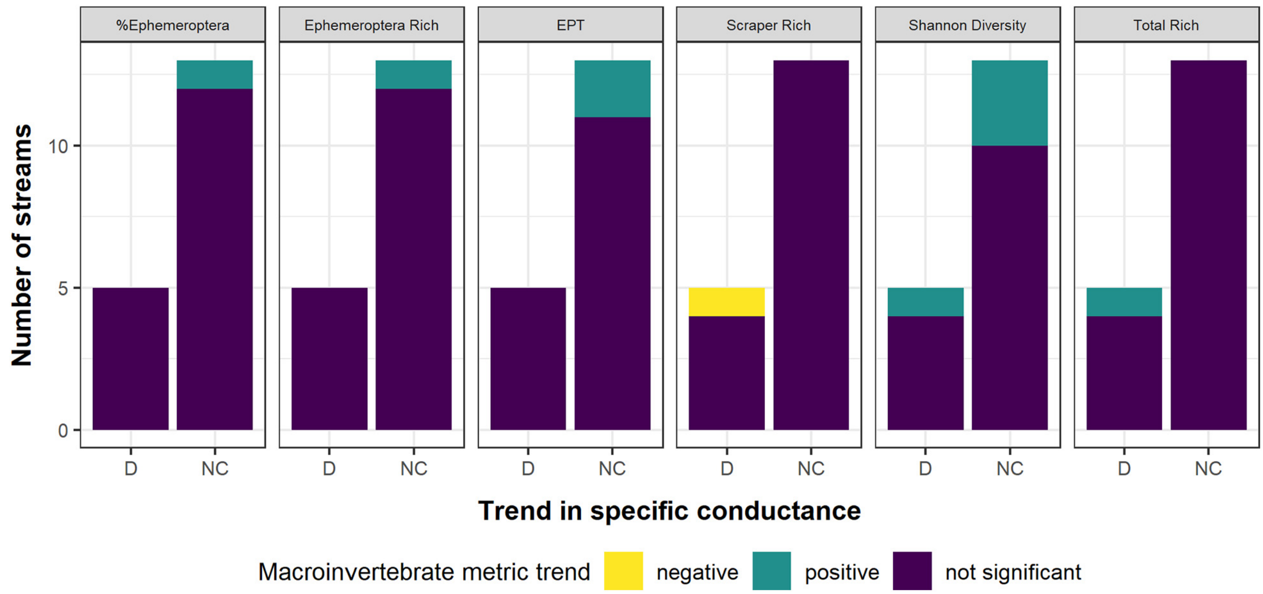


Fig. 3. Temporal trends in macroinvertebrate metrics for 18 mining-influenced streams in central Appalachia USA with decreasing (D) trends in specific conductance ($n = 5$) and those with no change (NC) in specific conductance ($n = 13$) from fall 2011 to summer 2019.

studies of mine spoil leaching (Orndorff et al., 2015; Daniels et al., 2016). This change in water chemistry occurs as continued leaching causes pyrite depletion and declining pyrite-oxidation influence on water-discharge chemistry while dissolution of carbonates, feldspars, and other pH-increasing minerals proceeds as a dominant water-chemistry influence (Clark et al., 2018a). Negative trends in SO_4:HCO_3 ratio at 10 of 15 mining-influenced streams with the highest SCs (annual mean > 380 μS cm^{-1}), suggests weathering of mine spoil in a manner that is consistent with known geochemical processes. This finding demonstrates water-chemistry change toward a condition more similar to the natural-background state. Further, most (4 of 5) streams exhibiting declining SC also exhibited declining SO_4:HCO_3 ratios; but reasons for the lack of more-frequent observation of correspondence between temporal patterns of SC and SO_4:HCO_3 ratios are not known.

In addition, we expected that the Ca:Mg ratio would increase over time, reasoning that aging of mine spoils would result in a gradual return to background conditions. However, we found no increasing trends for Ca:Mg in mining-influenced streams. Bulk Mg concentrations in Appalachian mine spoils often exceed Ca concentrations, and Ca:Mg molar ratios of approximately 1:1 occur commonly in leachates from mine spoils (Clark et al., 2018a) as we observed here. Geochemical sources and processes responsible for increased release of Mg from Appalachian mine spoils are poorly understood (Clark et al., 2018b).

4.2. Benthic macroinvertebrates

Despite some mining-influenced streams showing slow recovery of water quality, we found limited evidence of coincident recovery by benthic macroinvertebrate communities over the study time frame. Our work and others (e.g., Pond et al., 2008, 2014; Timpano et al., 2015, 2018a) document strong negative relationships between SC and benthic macroinvertebrates in central Appalachian headwater streams, suggesting that decreasing SC trends should correspond with associated recovery of macroinvertebrate communities. However, for the five mining-influenced streams with significant negative SC trends, the SC trends were small (-10.6 μS/cm yr^{-1} to -22.7 μS/cm yr^{-1}) and with annual mean SC values ≥570 μS/cm, a value well above the EPA conductivity benchmark (300 μS/cm) and previously estimated critical SC thresholds (see Figs. 3, 4 in Timpano et al., 2018a). As such, it is possible that SC has not declined sufficiently to enable recovery of macroinvertebrate communities. It is also possible that our use of rapid bioassessment methods (i.e., identification of only 200 ± 10% individuals from semi-quantitative samples), while commonly used in other studies assessing macroinvertebrate communities in central Appalachia and sufficient for detecting salinization effects (e.g., Merricks et al., 2007; Pond et al., 2008, 2014; Cormier et al., 2013; Boehme et al., 2016; Timpano et al., 2015, 2018b), was not sufficiently sensitive to enable detection of temporal trends. A study conducted in a subset of our study streams showed that metrics derived from high-enumeration quantitative sampling (i.e., identification of all individuals collected in replicate Surber samples) were more sensitive to SC compared to metrics derived from semi-quantitative sampling (Pence, 2019). Further, rapid bioassessment sampling may underestimate taxa richness by as much as 50% in our study streams, particularly in low-SC streams where richness was higher (Pence, 2019). Thus, given that any declining SC trends we observed were slight, and sensitivity of our biological metrics to SC changes was limited, it is possible that SC has not declined sufficiently to enable detection of macroinvertebrate community recovery as commonly measured using rapid bioassessment methods. We also note the distinction between measures of temporal trends in 30-minute, continuous SC data compared to estimated trends in biological metrics using only seasonal sampling. Further, biological effects of saline waters occur as a function of ion matrix as well as concentration of ions (Mount et al., 1997). Four of six streams with positive biological-metric trends, all with mean SCs >380 μS cm^{-1}, were also characterized by declining SO_4:HCO_3 ratios (Table 2), highlighting the importance of ion matrix and future research needs to better understand its influence on macroinvertebrate communities.

Further, although we and others have observed strong relationships between SC and macroinvertebrate taxa loss (e.g., Pond et al., 2008; Timpano et al., 2015, 2018a), salinity may not be the only stressor to benthic macroinvertebrates in these headwater streams. Drover et al. (2019) documented significant, negative relationships between Ephemeroptera and Scraper Richness metrics and water column selenium (Se) concentrations in a subset of 15 of our study streams. Selenium is a naturally occurring trace element but can be elevated to potentially harmful levels in streams influenced by surface mining (U.S. EPA, 2016) and has been shown to bioaccumulate in and cause toxicity to benthic macroinvertebrates (DeBruyn and Chapman, 2007; Conley et al., 2011; Whitmore et al., 2018; Cianciolo et al., 2020). In particular, Se can bioaccumulate into stream biofilm (Arnold et al., 2017; Whitmore et al., 2018; Cianciolo et al., 2020), which is consumed by macroinvertebrates, including scrapers and several salt-sensitive Ephemeroptera taxa. Therefore, it is possible that Se is an additional stressor to these groups, potentially explaining some of the discrepancy between gradual recovery in water chemistry (i.e., decreasing SC) and absence of positive trends in macroinvertebrate metrics, particularly in Ephemeroptera and scrapers. Selenium in mine-spoil leachates typically declines with continued leaching in a manner similar to, but more rapidly than, major ions and SC (Ziemkiewicz et al., 2011; Clark et al., 2018b); however, Se can remain in aquatic food webs years after inputs have ceased (Lemly, 1997; Swift, 2002). Other trace elements are also released at low concentrations from mine spoils (Clark et al., 2018b) and bioaccumulate in these systems, the effects of which have not been studied.

Biological recovery of Appalachian headwaters will be influenced by more than just water quality. Even if water quality returns to natural condition, restoration of a diverse macroinvertebrate community in those streams will depend on the integrity of nearby source communities available to recolonize each stream. Because surface coal mining can have extensive and multiple impacts to stream networks and the terrestrial ecosystems that support them (Palmer et al., 2010), it is possible that such widescale disturbance could impede biological recovery of impacted streams by extirpating source populations of taxa in nearby waters that might otherwise recolonize those streams. It is unclear whether biological recovery in our streams has been limited by disturbances other than altered local water chemistry, as large proportions of most catchments remain relatively undisturbed. However, elsewhere in Appalachia, there is evidence that extensive degradation of nearby waters (within 5 km) can isolate intact streams from diverse taxa pools, thus limiting recolonization potential and community diversity despite adequate water quality (Merriam and Petty, 2016). Similarly, the presence of unsalinized tributaries with intact communities can increase diversity of salinized receiving streams (Pond et al., 2014). Hence, recovery of water quality alone may be insufficient to realize biological recovery. Therefore, maintaining biological diversity of nearby waters may be critical to successful biological recovery of once-salinized streams.

Surface coal mining affects >3.5% of the total land area in central Appalachia (Pericak et al., 2018). Mining effects on water resources, however, are more extensive, because mining disturbance in a small fraction of a watershed can influence downstream water chemistry and biology (Bernhardt et al., 2012). In southern WV, for example, >20% of the river network may be biologically altered as a result of surface coal mining (Bernhardt et al., 2012). Our results suggest this extensive biological impact may continue for many years after mining activities cease even if water quality improves slowly over time. Further, three streams in our study have had no active mining since 1985 and still contain annual mean SC levels greater than the 300 $\mu S\ cm^{-1}$ conductivity benchmark, with no declining SC trends. It remains unclear what the ecological impact of altered macroinvertebrate communities will be over such long periods, highlighting the need for long-term study of salinization impacts to ecosystem structure and functions in headwater streams influenced by surface coal mining.

5. Conclusion

Our findings demonstrate that salinization and its biological effects are likely to persist for decades in central Appalachian headwater streams influenced by surface coal mining. Multiple mining-influenced streams exhibited reduced sulfate dominance of the ion matrix, suggesting eventual water-chemistry recovery from mining influence; but declining salinity trends were rare and of low magnitude, suggesting recovery times on the order of decades or longer. Moreover, those slowly declining trends in salinity did not equate to concurrent benthic-macroinvertebrate recovery during our eight-year study period. These findings also highlight the value of long-term monitoring data for assessing recovery potential of salinized freshwaters, as well as the need for improved understanding of water quality and biological recovery processes and time frames.

Funding

Funding: This work was supported by the U.S. Department of Interior Office of Surface Mining Reclamation and Enforcement (grant numbers S11AC20004, S12AC20023, and S15AC20028); and the Powell River Project at Virginia Tech.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to express our gratitude to the many people who made this 8-year research project possible, including, M. Underwood for benthic macroinvertebrate identification, K. Peeler, J. Parks, Z. Orndorff, and M. Council-Troche for water chemistry analysis, P. Donovan for spatial analysis, and the many field helpers who worked long hours collecting samples. In addition, the rigorous site selection process would not have been possible without collaboration with mining personnel, state agencies, and private landowners.

Appendix A. Supplementary data

A summary table of measured water chemistry parameters, high-frequency specific conductance figures for all study streams, and a table with mean values and temporal trend slopes for ion ratios and biological metrics are provided alongside this article in supplementary material. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.137216.

References

Agouridis, C.T., Angel, P.N., Taylor, T.J., Barton, C.D., Warner, R.C., Yu, X., Wood, C., 2012. Water quality characteristics of discharge from reforested loose-dumped mine spoil in eastern Kentucky. J. Environ. Qual. 41 (2), 454–468. https://doi.org/10.2134/jeq2011.0158.

American Public Health Association (APHA), 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed. American Public Health Assoc, Washington, DC.

Arnold, M.C., Bier, R.L., Lindberg, T.T., Bernhardt, E.S., Di Giulio, R.T., 2017. Biofilm mediated uptake of selenium in streams with mountaintop coal mine drainage. Limnologica 65, 10–13. https://doi.org/10.1016/j.limno.2017.05.002.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish (Washington, DC).

Bernhardt, E.S., Lutz, B.D., King, R.S., Fay, J.P., Carter, C.E., Helton, A.M., Campagna, D., Amos, J., 2012. How many mountains can we mine? Assessing the regional degradation of central Appalachian rivers by surface coal mining. Environmental Science & Technology 46 (15), 8115–8122. https://doi.org/10.1021/es301144q.

Boehme, E.A., Zipper, C.E., Schoenholtz, S.H., Soucek, D.J., Timpano, A.J., 2016. Temporal dynamics of benthic macroinvertebrate communities and their response to elevated specific conductance in Appalachian coalfield headwater streams. Ecol. Indic. 64, 171–180. https://doi.org/10.1016/j.ecolind.2015.12.020.

Cañedo-Argüelles, M., Hawkins, C.P., Kefford, B.J., Schäfer, R.B., Dyack, B.J., Brucet, S., Buchwalter, D., Dunlop, J., Frör, O., Lazorchak, J., Coring, E., 2016. Saving freshwater from salts. Science 351 (6276), 914–916. https://doi.org/10.1126/science.aad3488.

Carslaw, D.C., Ropkins, K., 2012. Openair — an R package for air quality data analysis. Environ. Model Softw. 27–28, 52–61.

Cianciolo, T.R., McLaughlin, D.L., Zipper, C.E., Timpano, A.J., Soucek, D.J., Whitmore, K.M., Schoenholtz, S.H., 2020. Selenium bioaccumulation across trophic levels and along a longitudinal gradient in headwater streams. Environ. Toxicol. Chem. https://doi.org/10.1002/etc.4660.

Clark, E.V., Daniels, W.L., Zipper, C.E., Eriksson, K., 2018a. Mineralogical influences on water quality from weathering of surface coal mine spoils. Appl. Geochem. 91, 97–106. https://doi.org/10.1016/j.apgeochem.2018.02.001.

Clark, E.V., Zipper, C.E., Daniels, W.L., Keefe, M.J., 2018b. Appalachian coal mine spoil elemental release patterns and depletion. Appl. Geochem. 98, 109–120. https://doi.org/10.1016/j.apgeochem.2018.09.016.

Clements, W.H., Kotalik, C., 2016. Effects of major ions on natural benthic communities: an experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity. Freshwater Science 35 (1), 126–138. https://doi.org/10.1086/685085.

Conley, J.M., Funk, D.H., Cariello, N.J., Buchwalter, D.B., 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly Centroptilum triangulifer. Ecotoxicology 20 (8), 1840–1851. https://doi.org/10.1007/s10646-011-0722-1.

Cormier, S.M., Suter, G.W., Zheng, L., 2013. Derivation of a benchmark for freshwater ionic strength. Environ. Toxicol. Chem. 32 (2), 263–271. https://doi.org/10.1002/etc.2064.

Daniels, W.L., Zipper, C.E., Orndorff, Z.W., Skousen, J., Barton, C.D., McDonald, L.M., Beck, M.A., 2016. Predicting total dissolved solids release from central Appalachian coal mine spoils. Environ. Pollut. 216, 371–379. https://doi.org/10.1016/j.envpol.2016.05.044.

DeBruyn, A.M., Chapman, P.M., 2007. Selenium toxicity to invertebrates: will proposed thresholds for toxicity to fish and birds also protect their prey? Environmental Science & Technology 41 (5), 1766–1770. https://doi.org/10.1021/es062253j.

Driscoll, C.T., Driscoll, K.M., Roy, K.M., Mitchell, M.J., 2003. Chemical response of lakes in the Adirondack region of New York to declines in acidic deposition. Environmental Science & Technology 37 (10), 2036–2042. https://doi.org/10.1021/es020924h.

Drover, D.R., Schoenholtz, S.H., Soucek, D.J., Zipper, C.E., 2019. Multiple stressors influence benthic macroinvertebrate communities in central Appalachian coalfield streams. Hydrobiologia https://doi.org/10.1007/s10750-019-04081-4.

Duan, W., He, B., Chen, Y., Zou, S., Wang, Y., Nover, D., Chen, W., Yang, G., 2018. Identification of long-term trends and seasonality in high-frequency water quality data from the Yangtze River basin, China. PLoS One 13 (2). https://doi.org/10.1371/journal.pone.0188889.

Evans, D.M., Zipper, C.E., Donovan, P.F., Daniels, W.L., 2014. Long-term trends of specific conductance in waters discharged by coal-mine valley fills in central Appalachia, USA. J. Am. Water Resour. Assoc. 50 (6), 1449–1460. https://doi.org/10.1111/jawr.12198.

Griffith, M.B., 2014. Natural variation and current reference for specific conductivity and major ions in wadeable streams of the conterminous USA. Freshwater Science 33 (1), 1–17. https://doi.org/10.1086/674704.

Griffith, M.B., Norton, S.B., Alexander, L.C., Pollard, A.I., LeDuc, S.D., 2012. The effects of mountaintop mines and valley fills on the physicochemical quality of stream ecosystems in the Central Appalachians: a review. Sci. Total Environ. (4), 17, 1–12 https://doi.org/10.1016/j.scitotenv.2011.12.042.

Helsel, D.R., Hirsch, R.M., 2002. Statistical Methods in Water Resources. 323. US Geological Survey, Reston, VA.

Herbert, E.R., Boon, P., Burgin, A.J., Neubauer, S.C., Franklin, R.B., Ardón, M., Hopfensperger, K.N., Lamers, L.P., Gell, P., 2015. A global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands. Ecosphere 6 (10), 1–43. https://doi.org/10.1890/ES14-00534.1.

Higgins, C.L., Wilde, G.R., 2005. The role of salinity in structuring fish assemblages in a prairie stream system. Hydrobiologia 549 (1), 197–203. https://doi.org/10.1007/s10750-005-0844-7.

Hirsch, R.M., Slack, J.R., 1984. A nonparametric trend test for seasonal data with serial dependence. Water Resour. Res. 20 (6), 727–732. https://doi.org/10.1029/WR020i006p00727.

Johnson, B.R., Weaver, P.C., Nietch, C.T., Lazorchak, J.M., Struewing, K.A., Funk, D.H., 2015. Elevated major ion concentrations inhibit larval mayfly growth and development. Environ. Toxicol. Chem. 34 (1), 167–172. https://doi.org/10.1002/etc.2777.

Kaushal, S.S., Likens, G.E., Pace, M.L., Utz, R.M., Haq, S., Gorman, J., Grese, M., 2018. Freshwater salinization syndrome on a continental scale. Proc. Natl. Acad. Sci. 115 (4), E574–E583. https://doi.org/10.1073/pnas.1711234115.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82 (13), 1–26.

Lemly, A.D., 1997. Ecosystem recovery following selenium contamination in a freshwater reservoir. Ecotoxicol. Environ. Saf. 36 (3), 275–281. https://doi.org/10.1006/eesa.1996.1515.

Merriam, E.R., Petty, J.T., 2016. Under siege: isolated tributaries are threatened by regionally impaired metacommunities. Sci. Total Environ. 560–561, 170–178. https://doi.org/10.1016/j.scitotenv.2016.04.053.

Merricks, T.C., Cherry, D.S., Zipper, C.E., Currie, R.J., Valenti, T.W., 2007. Coal-mine hollow fill and settling pond influences on headwater streams in southern West Virginia, USA. Environ. Monit. Assess. 129 (1–3), 359–378. https://doi.org/10.1007/s10661-006-9369-4.

Merritt, R.W., Cummins, K.W., Berg, M.B., Holzenthal, R.W., Prather, A.L., Marshall, S.A., 2008. Introduction to the Aquatic Insects of North America. Fourth edition. Kendall Hunt Publishing, Dubuque, Iowa.

Millard, S.P., 2013. EnvStats: An R Package for Environmental Statistics. Springer, New York 978-1-4614-8455-4.

Mount, D.R., Gulley, D.D., Hockett, J.R., Garrison, T.D., Evans, J.M., 1997. Statistical models to predict the toxicity of major ions to Ceriodaphnia dubia, Daphnia magna and Pimephales promelas (fathead minnows). Environmental Toxicology and Chemistry: An International Journal 16 (10), 2009–2019. https://doi.org/10.1002/etc.5620161005.

Omernik, J.M., Griffith, G.E., 2014. Ecoregions of the conterminous United States: evolution of a hierarchical spatial framework. Environ. Manag. 54 (6), 1249–1266. https://doi.org/10.1007/s00267-014-0364-1.

Orndorff, Z.W., Daniels, W.L., Zipper, C.E., Eick, M., Beck, M., 2015. A column evaluation of Appalachian coal mine spoils' temporal leaching behavior. Environ. Pollut. 204, 39–47. https://doi.org/10.1016/j.envpol.2015.03.049.

Palmer, M.A., Bernhardt, E.S., Schlesinger, W.H., Eshleman, K.N., Foufoula-Georgiou, E., Hendryx, M.S., Lemly, A.D., Likens, G.E., Loucks, O.L., Power, M.E., White, P.S., 2010. Mountaintop mining consequences. Science 327 (5962), 148–149. https://doi.org/10.1126/science.1180543.

Pence, R.A., 2019. Comparison of Quantitative and Semi-Quantitative Assessments of Benthic Macroinvertebrate Community Response to Elevated Salinity in Central Appalachian Coalfield Streams. M.S. Thesis. Virginia Tech, Blacksburg, VA.

Pericak, A.A., Thomas, C.J., Kroodsma, D.A., Wasson, M.F., Ross, M.R., Clinton, N.E., Campagna, D.J., Franklin, Y., Bernhardt, E.S., Amos, J.F., 2018. Mapping the yearly extent of surface coal mining in central Appalachia using Landsat and Google earth engine. PLoS One 13 (7). https://doi.org/10.1371/journal.pone.0197758.

Pond, G.J., 2010. Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA). Hydrobiologia 641 (1), 185–201. https://doi.org/10.1007/s10750-009-0081-6.

Pond, G.J., Passmore, M.E., Borsuk, F.A., Reynolds, L., Rose, C.J., 2008. Downstream effects of mountaintop coal mining: comparing biological conditions using family-and genus-level macroinvertebrate bioassessment tools. J. N. Am. Benthol. Soc. 27 (3), 717–737. https://doi.org/10.1899/08-015.1.

Pond, G.J., Passmore, M.E., Pointon, N.D., Felbinger, J.K., Walker, C.A., Krock, K.J., Fulton, J.B., Nash, W.L., 2014. Long-term impacts on macroinvertebrates downstream of reclaimed mountaintop mining valley fills in central Appalachia. Environ. Manag. 54 (4), 919–933. https://doi.org/10.1007/s00267-014-0319-6.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL. https://www.R-project.org/.

Sams, I.I.I.J.I., Beer, K.M., 2000. Effects of Coal-Mine Drainage on Stream Water Quality in the Allegheny and Monongahela River Basins—Sulfate Transport and Trends. Water-Res Invest Rep 99–4208U.S. Geological Survey, NWQA Program, Lemoyne, PA, p. 2000.

Sena, K., Barton, C., Angel, P., Agouridis, C., Warner, R., 2014. Influence of spoil type on chemistry and hydrology of interflow on a surface coal mine in the eastern US coalfield. Water Air Soil Pollut. 225 (11), 2171. https://doi.org/10.1007/s11270-014-2171-y.

Stoddard, J.L., Jeffries, D.S., Lükewille, A., Clair, T.A., Dillon, P.J., Driscoll, C.T., Forsius, M., Johannessen, M., Kahl, J.S., Kellogg, J.H., Kemp, A., 1999. Regional trends in aquatic recovery from acidification in North America and Europe. Nature 401 (6753), 575.

Swift, M.C., 2002. Stream ecosystem response to, and recovery from, experimental exposure to selenium. J. Aquat. Ecosyst. Stress. Recover. 9 (3), 159–184. https://doi.org/10.1023/A:1021299003516.

Szöcs, E., Coring, E., Bäthe, J., Schäfer, R.B., 2014. Effects of anthropogenic salinization on biological traits and community composition of stream macroinvertebrates. Sci. Total Environ. 468, 943–949. https://doi.org/10.1016/j.scitotenv.2013.08.058.

Timpano, A.J., Schoenholtz, S.H., Soucek, D.J., Zipper, C.E., 2015. Salinity as a limiting factor for biological condition in mining-influenced central Appalachian headwater streams. J. Am. Water Resour. Assoc. 51 (1), 240–250. https://doi.org/10.1111/jawr.12247.

Timpano, A.J., Vander Vorste, R., Soucek, D.J., Whitmore, K., Zipper, C.E., Schoenholtz, S.H., 2017. Stream Ecosystem Response to Mining-Induced Salinization in Central Appalachia. Final Report to the Office of Surface Mining Reclamation and Enforcement (OSMRE Cooperative Agreement S15AC20028). Virginia Water Resources Research Institute.

Timpano, A.J., Schoenholtz, S.H., Soucek, D.J., Zipper, C.E., 2018a. Benthic macroinvertebrate community response to salinization in headwater streams in Appalachia USA over multiple years. Ecol. Indic. 91, 645–656. https://doi.org/10.1016/j.ecolind.2018.04.031.

Timpano, A.J., Zipper, C.E., Soucek, D.J., Schoenholtz, S.H., 2018b. Seasonal pattern of anthropogenic salinization in temperate forested headwater streams. Water Res. 133, 8–18. https://doi.org/10.1016/j.watres.2018.01.012.

U.S. EPA (Environmental Protection Agency), 1971. Method 160.1: Residue, filterable (gravimetric, dried at 180 °C). Methods for the Chemical Analysis of Water and Wastes (MCAWW) (EPA/600/4-79/020).

U.S. EPA (Environmental Protection Agency), 1996. Method1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels. EPA-821-R-96-011.

U.S. EPA (Environmental Protection Agency), 2011a. The Effects of Mountaintop Mines and Valley Fills on Aquatic Ecosystems of the Central Appalachian Coalfields. Office of Research and Development, National Center for Environmental Assessment, Washington, DC (EPA/600/R-09/138F).

U.S. EPA (Environmental Protection Agency), 2011b. A Field-Based Aquatic Life Benchmark for Conductivity in Central Appalachian Streams. 2011. U.S. Environmental Protection Agency, NCEA, Washington, DC (EPA/600/R-10/023A).

U.S. EPA (Environmental Protection Agency), 2016. Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016. EPA 822-R-16-006 (Washington, D.C., 2016).

VDEQ, 2008. Biological Monitoring Program Quality Assurance Project Plan for Wadeable Streams and Rivers. Virginia Department of Environmental Quality Water Quality Monitoring and Assessment Programs, Richmond, Virginia.

Wallace, J.B., Webster, J.R., 1996. The role of macroinvertebrates in stream ecosystem function. Annu. Rev. Entomol. 41 (1), 115–139. https://doi.org/10.1146/annurev.en.41.010196.000555.

Whitmore, K.M., Schoenholtz, S.H., Soucek, D.J., Hopkins, W.A., Zipper, C.E., 2018. Selenium dynamics in headwater streams of the central Appalachian coalfield. Environ. Toxicol. Chem. 37 (10), 2714–2726. https://doi.org/10.1002/etc.4245.

WVDEP, 2015. Watershed Assessment Branch 2015 Field Sampling Standard Operating Procedures. West Virginia Department of Environmental Protection Division of Water and Waste Management, Watershed Assessment Branch, Charleston, WV.

Ziemkiewicz, P.F., O'Neal, M., Lovett, R.J., 2011. Selenium leaching kinetics and in situ control. Mine Water Environ. 30 (2), 141–150. https://doi.org/10.1007/s10230-011-0154-4.