ISOLATING EFFECTS OF TOTAL DISSOLVED SOLIDS ON AQUATIC LIFE IN CENTRAL APPALACHIAN COALFIELD STREAMS[1]

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Abstract: Elevated levels of total dissolved solids (TDS) have been identified as stressors to aquatic life in Central Appalachian coalfield streams. At present there are no aquatic life water quality criteria for TDS in the primary coal-producing Central Appalachian states (KY, VA, WV). In all three states, mining-related impacts on aquatic life have been characterized using measures of benthic macroinvertebrate community structure. Impacts of mining on aquatic life have been documented in the literature, but our understanding of impacts from TDS is confounded because elevated TDS rarely occurs independent of other stressors in coalfield streams. Potential TDS covariates in coalfield streams include acidic pH, toxic metals, sedimentation, in-stream and riparian habitat degradation, trophic structure alteration, and hydrologic modification. As a means of isolating TDS effects, we identified 17 headwater streams in Virginia's coalfield region that represent a gradient of TDS concentrations, where influence from non-TDS stressors was minimized (*i.e.*, pH between 6.0 and 9.0, low metal concentrations, reference-quality habitat, primarily forested land-use). Benthic macroinvertebrate communities were sampled from these streams in Spring 2009. Organisms were enumerated and identified to the family/lowest practicable level. These data were then used to calculate common benthic macroinvertebrate community metrics. In addition, TDS and component ions were measured for water collected concurrently with biological samples. Data were analyzed for significant associations between TDS and biological metrics. We identified stream sites with elevated TDS where influence from non-TDS stressors was minimal. Dominant components of TDS were sulfate (46% by weight, mean), bicarbonate (26%), and calcium (13%). Several benthic macroinvertebrate richness measures were correlated negatively with increasing TDS ($p < 0.05$). Relative abundance measures, including Percent Ephemeroptera, were not correlated significantly with TDS ($p > 0.05$) within the range of TDS that we measured (28-792 mg/L). Our results suggest sulfate is a good candidate for single-parameter prediction of biological condition.

Additional keywords: biomonitoring, water quality criteria, conductivity

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Introduction

Background

Elevated levels of total dissolved solids (TDS) have been suggested as stressors to aquatic life in Central Appalachian streams influenced by coal mining (Bodkin et al., 2007, Pond et al., 2008). In coalfield streams, TDS is most often dominated by the dissolved ions SO_4^{2-} and HCO_3^-, with elevated concentrations (relative to reference) of Ca^{2+}, Mg^{2+}, Na^+, K^+, and Cl^- also common (Mount et al., 1997, Pond et al., 2008). At present there are no aquatic life water quality criteria for TDS or dominant ions in the primary coal-producing Central Appalachian states (KY, VA, WV). In all three states, aquatic life conditions are assessed for Clean Water Act compliance using measures of benthic macroinvertebrate community structure.

Dissolved ions, at concentrations above those that occur naturally in Central Appalachian streams, have been shown to cause lethal and sublethal effects to a variety of freshwater invertebrates in laboratory toxicity testing. In mine-influenced streams of the Central Appalachians, in-stream TDS concentration can exceed 2,000 mg/L (Pond et al., 2008). Aquatic bioassays have exposed organisms to a wide range of TDS concentrations. The organisms used in such toxicity testing include common indicator species, such as the cladocerans *Ceriodaphnia dubia* and *Daphnia magna*, the amphipod *Hyalella azteca*, and the midge *Chironomus tentans*, as well as indigenous species such as the mayfly *Isonychia sp*.

Kennedy et al. (2003) exposed *C. dubia* to sulfate-dominated mine effluent and observed significant effects on survival and reproduction at specific conductivities of approximately 6,000 and 3,700 μS/cm (approx. 4,200 & 2,590 mg/L TDS), respectively. Soucek and Kennedy (2005) observed lethal effects of sulfate to *H. azteca* (512 mg/L), *C. dubia* (2,050 mg/L), and *C. tentans* (14,134 mg/L). Chapman et al. (2000) also found reductions in survival and growth of *C. tentans* from simulated mine effluent of approximately 2,000 mg/L TDS. To investigate the effects of TDS on a species representative of populations observed to be impacted at field sites, Kennedy et al. (2004) collected mayflies of the genus *Isonychia* from an unpolluted stream. They then exposed the mayflies to simulated mine effluent for seven days and observed a significant effect on survival at a conductivity of ~1,500 μS/cm (~1,050 mg/L TDS). These laboratory experiments illustrate a clear biological response to elevated TDS, though the results differ among studies, suggesting that TDS tolerance varies widely among different test organisms.

Additional research has shown that TDS toxicity is dependent upon the type and combination of ions in solution. Acute toxicity tests conducted by Mount et al. (1997) exposed *C. dubia* and *D. magna* to 2,453 different solutions of various ion combinations. They found the relative toxicity of individual ions to be: $K^+ > HCO_3^- \approx Mg^{2+} > Cl^- > SO_4^{2-}$. They also found that toxicity of K^+, Cl^-, and $SO_4^{2-}$was reduced in solutions with more than one cation present. Soucek and Kennedy (2005) also found that sulfate toxicity was reduced for *C. dubia* and *H. azteca* when water hardness (Ca^{2+} and Mg^{2+}) was increased. Increased chloride concentration also reduced sulfate toxicity to *H. azteca*. These studies suggest that the biological response to in-stream mineral solutions depends on TDS composition and ion interactions, as well as overall ionic strength or TDS concentration.

Field data have shown that the biotic response to elevated TDS also occurs outside the laboratory with indigenous species. Many recent studies of eastern coal mining-influenced streams have found that benthic macroinvertebrate community structure is altered in mining-influenced streams relative to community structure in streams uninfluenced by mining (Green et al., 2000; Pond, 2004; Pond et al., 2008; and others). In those studies, most mining-influenced streams were observed to be elevated in conductivity/TDS and in all cases, one of those water quality parameters has been significantly and strongly correlated with biotic community structure change.

Although field studies have succeeded in demonstrating the ability of benthic macroinvertebrate monitoring to identify aquatic community structural responses to coal mining activity, much remains unknown about how the benthic macroinvertebrate community responds to specific TDS concentrations and compositions. To date, both non-TDS stressors and elevated TDS likely have had concurrent influences on biota in the streams assessed during field studies. Research reported in this paper sought to better characterize the biotic response to elevated TDS and component ions by isolating the TDS variable through the study of streams where non-TDS stressors were minimized.

Objectives

The goals of this research are to better understand how benthic macroinvertebrate community structure responds to elevated TDS where non-TDS stressors are minimized. To that end, we addressed the following questions:

1) Can TDS be reasonably isolated from other stressors in field studies of this type?
2) What is the ionic composition of TDS in headwater streams of Virginia's Central Appalachian coalfield region?
3) How does benthic macroinvertebrate community structure respond to a gradient of TDS/ion concentration?
4) Which measure of water quality – aggregate measures or individual ions - is most related to biotic response?

Methods

Conceptual Approach

This study was designed to quantify how benthic macroinvertebrate community structure responds to various in-stream concentrations of TDS. Given that many factors can influence the condition of aquatic life, this study sought to create a single-factor analysis by selecting streams that varied primarily by TDS concentration, while minimizing confounding factors that might influence biota. This was accomplished by seeking study streams with attributes such as habitat quality that are as similar as possible to minimally-disturbed reference streams of the region. The design was intended to ensure that TDS, including its component ions, was the primary factor associated with biotic stress in these streams. Factor-effect levels were studied by examining streams spanning a range of TDS levels. The response factor was benthic macroinvertebrate community structure, which was characterized using several common metrics. Correlation analysis was used to reveal associations between biotic metrics and TDS/ion concentration.

Site Selection

This investigation focused on 1st and 2nd order-, or headwater streams of Virginia's Central Appalachian coalfields. Sharing Omernik Level III Ecoregion 69 and coal-bearing geology with much of eastern Kentucky and southern West Virginia, our study region is comparable to neighboring mining regions (Omernik, 1987).

Candidate site selection was conducted by examining a variety of available data using a GIS, augmented by consultation with mine operators and regulators with specific knowledge of site conditions. Virginia Department of Mines, Minerals and Energy provided data on water quality, mine permits, and historical strip-mining site locations, to which were added aerial photography, landuse data, and firsthand stream knowledge of regulators and mine operators. These data were integrated to develop a list of candidate study sites.

Each candidate site was visited to verify conditions. Physicochemical parameters were measured, with particular interest in pH and conductivity. Site reconnaissance prior to sampling also allowed verification of current land uses and ensured minimal catchment disturbance, as per study design. Physical habitat was evaluated using the qualitative visual estimate approach for high-gradient streams as specified in US EPA's Rapid Bioassessment Protocols (RBP) (Barbour et al., 1999).

The 17 suitable sites selected met non-biological reference criteria commonly used for studies of Virginia non-coastal streams (Burton and Gerritsen, 2003), excepting reference criteria concerning conductivity (Table 1). This was done to keep non-TDS abiotic factors as high-quality and as similar as possible among sites. Test sites meeting these criteria were selected within a range of TDS levels of ~200-2000 mg/L, a range commonly associated with mine-influenced streams of the Central Appalachian coalfields (Pond et al., 2008).

Table 1. Abiotic Criteria for Stream Selection

Parameter or Condition (units or range)	Selection Criterion[1]
Dissolved Oxygen (mg/L)	≥ 6.0
pH (std. units)	≥ 6.0 & ≤ 9.0
Epifaunal substrate score (0-20)[2]	≥ 11
Channel alteration score (0-20)[2]	≥ 11
Sediment deposition score (0-20)[2]	≥ 11
Bank disruptive pressure score (0-20)[2]	≥ 11
Riparian vegetation zone width score, per bank (0-10)[2]	≥ 6
Total RBP habitat score (0-200)[2]	≥ 120
Residential land use immediately upstream	None
Property owner or manager permission for access	Obtained

1 – Parameters and numeric selection criteria from Burton and Gerritsen (2003)
2 – RBP habitat, high gradient streams (Barbour et al., 1999)

In addition to meeting the physicochemical and habitat criteria, all test sites also had to be free from obvious influence from residential land use immediately upstream of the monitoring station. This criterion was important to avoid the unpredictable influence of failing septic systems (*e.g.*, dissolved N, P enrichment) or direct stream discharges of household waste (*e.g.*, particulate organic matter, toxics). Finally, accessibility was a practical criterion that had to be met to allow the site to be included in the study. Access permission was obtained for each study site from private landowners and/or mine permittees.

The goal in choosing reference streams was to identify streams within Virginia's Central Appalachian ecoregion that are as close to minimally disturbed as possible. Streams classified as minimally disturbed represent "…the biological condition in places with a minimal amount of human disturbance." (USEPA, 2006). Although the Virginia coalfield region has an extensive history of human settlement, there are still areas in the region where human impacts are minimal (*e.g.*, National Forests, coal-free geology). To maximize comparability to test sites, reference streams were selected, where possible, that drain coal-bearing geology.

Field Methods

At each study site, benthic macroinvertebrate and water quality samples were collected during the Spring 2009 biological index period (March through May) following the single-habitat approach as specified in Virginia Department of Environmental Quality (VDEQ) Biological Monitoring Program Quality Assurance Project Plan for Wadeable Streams and Rivers (VDEQ, 2008). Approximately 2 m^2 of riffle substrate were sampled using a 0.3 m wide D-frame kicknet with 500 μm mesh. A single composite sample was collected at each site, preserved in 95% ethanol and returned to the laboratory for sorting and identification.

Physicochemical parameters of temperature, dissolved oxygen, specific conductance, and pH were measured *in situ* with a calibrated handheld multi-probe meter (Hydrolab Quanta). Single grab samples of water were collected following modified VDEQ Water Quality Monitoring Standard Operating Procedures (SOP) (VDEQ, 2006). Sampling was modified to use vacuum hand pumps and reusable polyethylene filter assemblies rather than peristaltic pumps and single-use capsule filters. All samples were stored in acid-rinsed polypropylene Nalgene bottles. Samples for dissolved metals, TDS, alkalinity, and major ions were filtered in the field immediately following collection using acid-rinsed cellulose ester filters with a nominal pore size of 0.45μm. Samples for metals analysis were preserved to $pH < 2$ with 1+1 concentrated HNO_3 acid. All samples were transported on ice and stored at 4 °C prior to analysis in the laboratory. At each site, all biological and water samples were collected concurrently at base flow to minimize alteration of stream chemistry by direct influx of rainwater. All water quality sampling was conducted upstream of and/or immediately prior to biological sampling.

In-stream and riparian habitat quality were assessed at each site to ensure that habitat quality still met criteria (Table 1). Habitat assessment at all sites was performed using the same RBP methods as used during site selection (Barbour et al., 1999).

Laboratory Methods

Biological sample processing followed modified VDEQ Biomonitoring SOP (VDEQ, 2008). Each sample was sub-sampled to obtain a 200 (±10%) organism count following RBP methods (Barbour et al., 1999). Benthic macroinvertebrates were identified to the family/lowest practicable taxonomic level. Most arthropods were identified to family. The groups of Isopoda and Amphipoda (Order), Collembola (Subclass), and Oligochaeta (Subclass) were common exceptions to the family rule. Calculations of biological metrics for each sample were conducted using tolerance values and functional feeding group designations for families (or higher groups as applicable) for Virginia (Burton and Gerritsen, 2003).

An inductively coupled plasma - optical emission spectrometer (Varian Vista MPX ICP-OES w/ICP Expert software) was used to measure dissolved Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, and all species of Cu, Zn, Mn, Se, Al, Fe ions (APHA, 1998). An ion chromatograph (Dionex DX500) was used to measure Cl^{-} and SO_4^{2-} (APHA, 1998); TDS was measured via filtration of known volumes followed by drying at 180°C (APHA, 1998), with modifications (0.45 µ cellulose ester filter, field filtration); total alkalinity was measured for an aliquot of filtered sample by titration with standard acid (APHA, 1998); and CO_3^{2-}/HCO_3^{-} were calculated from alkalinity and pH measurements (APHA, 1998).

Data Analysis

All statistical analyses were conducted using JMP 8 (SAS Institute, Inc., Cary, North Carolina). Data were analyzed for correlation among measured water quality variables and biological metrics. Non-normal metrics were natural log transformed prior to analysis where appropriate.

Results & Discussion

Site Selection

The site selection process yielded 17 test streams within Virginia's Central Appalachian coalfield region where elevated TDS was the primary stressor to aquatic life (Fig. 1; Timpano et al., 2009). These streams were very similar to reference conditions in all other respects.

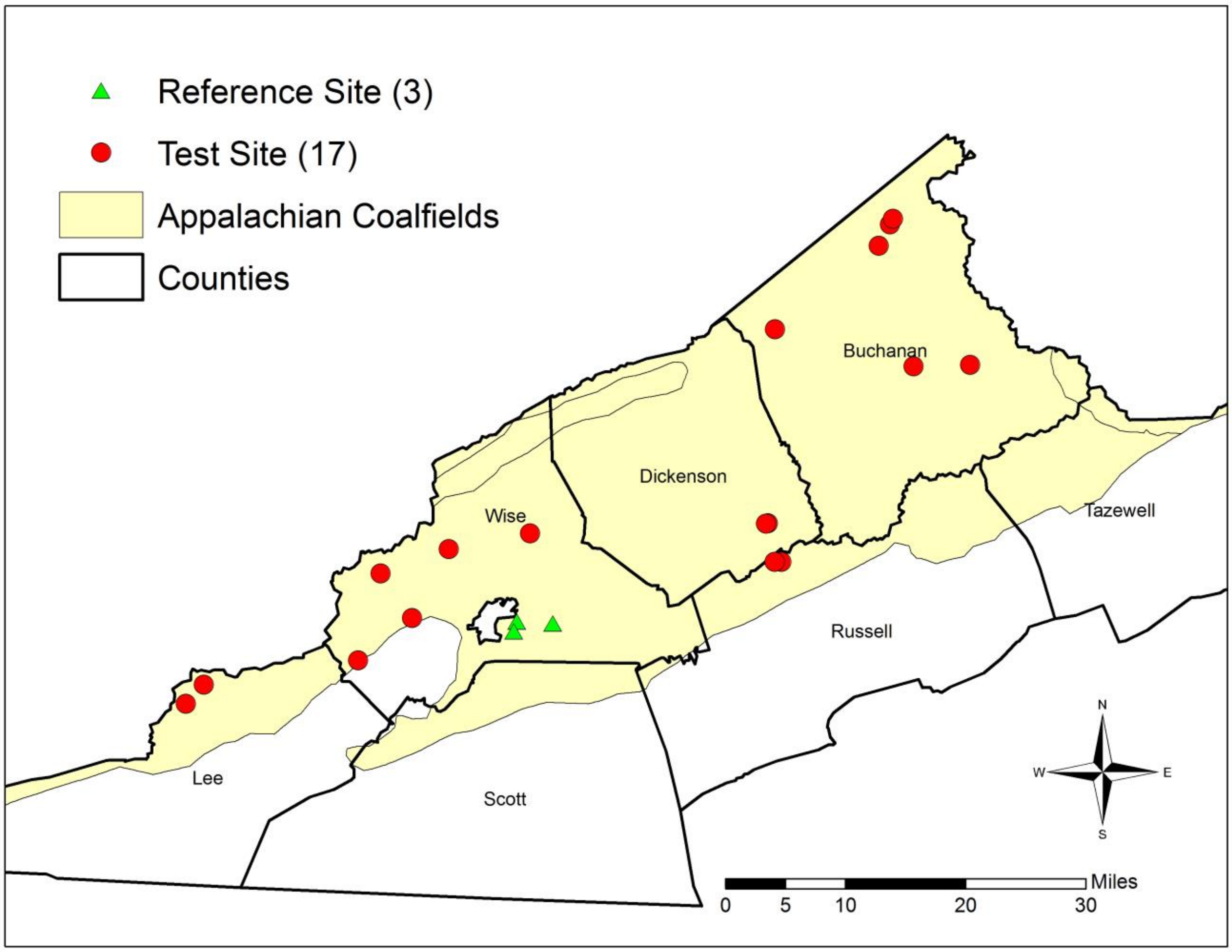

Figure 1. Reference and test sites in the Central Appalachian coalfields of Virginia.

Reference-quality streams draining coal-bearing geology are rare in Virginia's Central Appalachian ecoregion. With an extensive history of mining and associated development, nearly all of the region's coal-geology streams are influenced by a combination of legacy strip-mining, contemporary mining, infrastructure, commercial, industrial, and/or residential development. Three reference streams were located in the Jefferson National Forest in the southern portion of the coalfields based on recommendations from VDEQ biologists, because these streams had served previously as regional references during VDEQ special studies (Fig. 1; Timpano et al., 2009). Data from reference streams were used to establish reference-level habitat quality to ensure that test site habitat was comparable to reference.

Habitat scores for each of the 17 test sites were compared to the mean habitat score of the three reference sites and classified based on comparability to reference. All test sites scored >85% of reference, corresponding to a rating of "Comparable to Reference" (Barbour et al., 1999). Such comparability was deemed acceptable in effectively minimizing the influence of habitat quality on biotic condition, thus allowing the effects of TDS to be isolated and measured.

Physicochemical criteria for pH and dissolved oxygen were within reference limits for all sites. Trace metal analysis indicated water column dissolved metal concentrations below method detection limits for nearly all samples, with no measurements above chronic criteria for Cu, Al, Zn, or Fe.

Water Chemistry

Test sites exhibited a range of TDS/ion concentrations (Table 2). Among test sites, SO_4^{2-} was the most common ion by weight, followed by HCO_3^- and Ca^{2+} (Fig. 2). Ion composition generally conformed to that pattern across test sites (Fig. 3).

Table 2. Distribution statistics for selected water quality parameters (test sites only, n=17). All units mg/L unless noted.

	Temp (°C)	pH (SU)	DO	Cond. (µS/cm)	TDS	SO_4^{2-}	HCO_3^-	Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-
Minimum	10.9	6.6	7.8	25	27.8	4.2	5.1	2.2	1.5	1.0	1.4	0.9
10th percentile	12.1	7.1	8.2	216	126.2	56.6	19.6	22.5	10.9	4.3	1.8	1.0
Median	13.4	7.9	9.3	490	298.0	155.0	89.9	45.2	24.0	18.5	2.9	1.9
90th percentile	15.0	8.3	10.0	856	593.1	420.5	173.2	95.7	54.8	52.2	4.4	8.6
Maximum	17.5	8.5	10.2	970	791.6	531.4	301.7	119.9	75.4	135.9	5.0	9.8

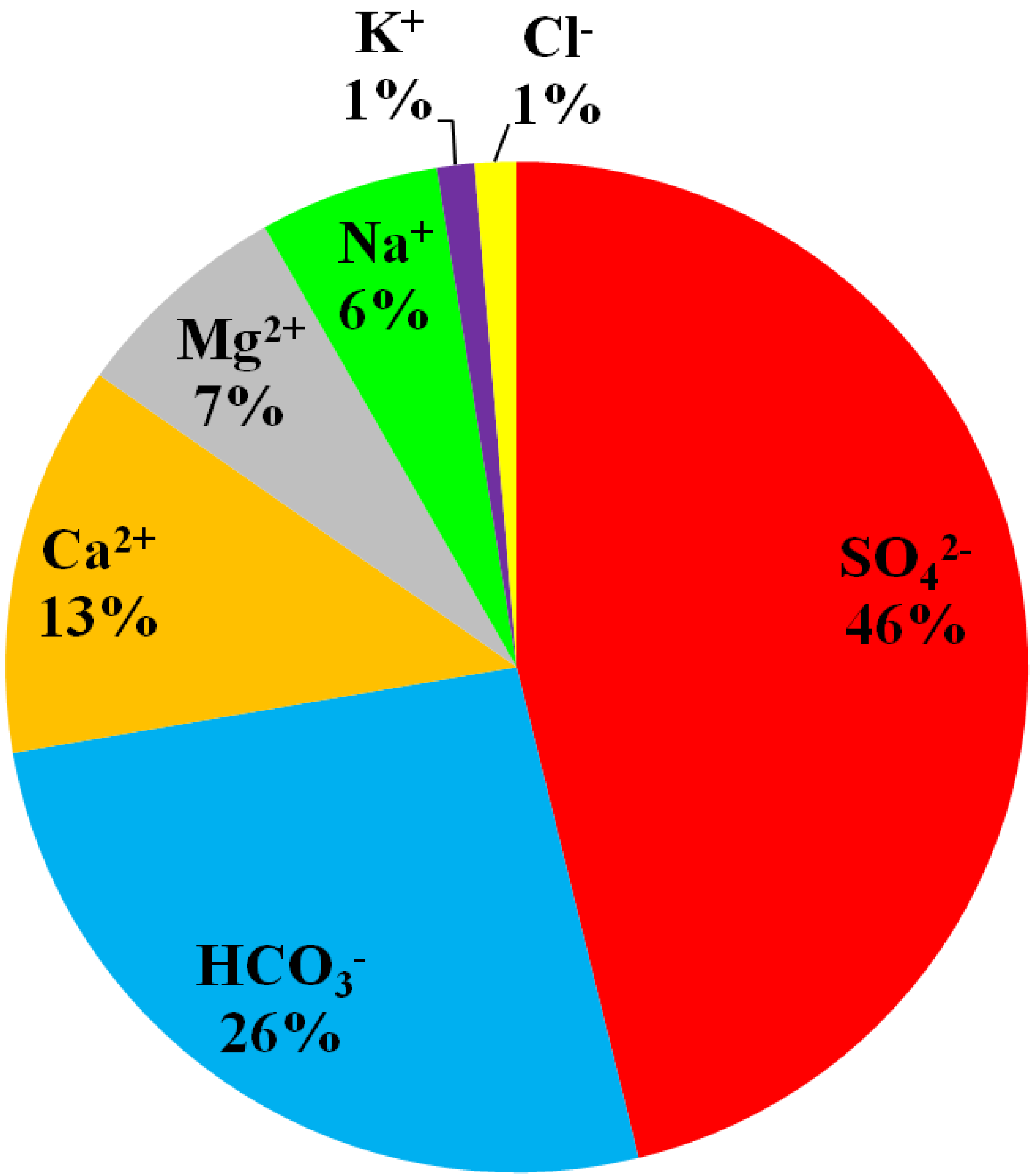

Figure 2. Mean relative proportions, by weight, of major ions (test sites only, n=17).

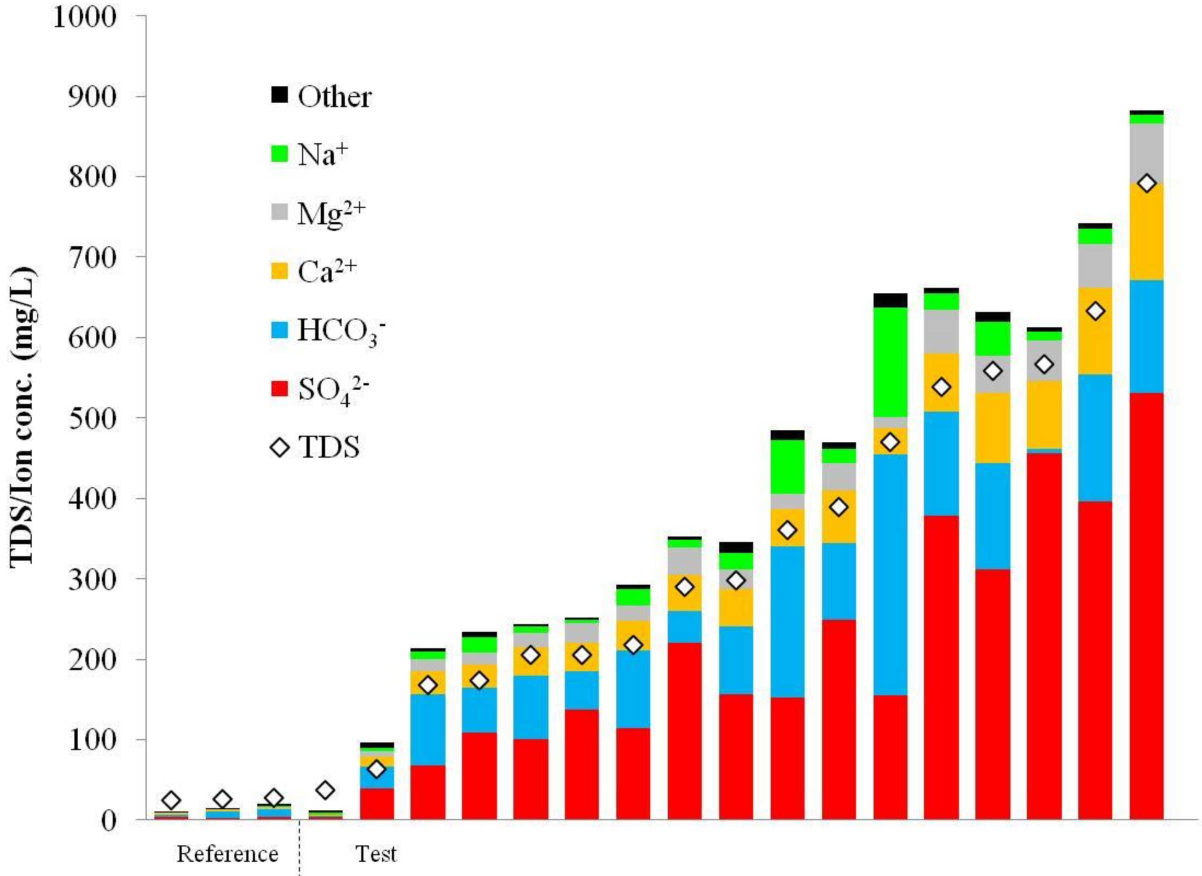

Figure 3. TDS and major component ion concentrations for all study sites.

Water quality parameters were highly correlated (Table 3), suggesting that a single parameter such as conductivity, TDS, or sulfate could represent water quality for a site.

Table 3. Pearson product-moment correlations for major ions and related measures (test sites only, n=17). All correlations shown are significant ($p < 0.05$).

	Ca^{2+}	SO_4^{2-}	TDS	Cond.	Mg^{2+}	K^+
SO_4^{2-}	0.96					
TDS	0.94	0.94				
Cond.	0.92	0.90	0.98			
Mg^{2+}	0.96	0.98	0.92	0.87		
K^+	0.76	0.70	0.85	0.87	0.69	
HCO_3^-			0.50	0.56		0.68

Associations Between Biology and Water Quality

Many metrics of benthic macroinvertebrate community structure and function were considered (Table 4).

Table 4. Candidate biological metrics by category, with expected response to anthropogenic disturbances such as water quality degradation; and metric definition; superscripts designate transformation used for statistical analysis (if applicable). Adapted from Burton and Gerritsen (2003).

Metric by Category	Response	Definition
Taxonomic Richness		*Number of different taxa in each specified group*
Number of Taxa	decrease	Total number of different taxa in sample
Number of EPT Taxa	decrease	Number of different taxa in the generally sensitive orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)
Number of E Taxa	decrease	Number of different Ephemeroptera taxa (mayflies)
Number of P Taxa	decrease	Number of different Plecoptera taxa (stoneflies)
Number of T Taxa	decrease	Number of different Trichoptera taxa (caddisflies)
Number of Diptera Taxa	decrease	Number of different Diptera taxa ("true" flies, such as midges and blackflies)
Composition		*Percent of individuals within total sample of…*
Percent EPT	decrease	…the generally sensitive orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)
Percent E	decrease	…the order Ephemeroptera (mayflies)
Percent Plecoptera	decrease	…the order Plecoptera (stoneflies)
Percent Trichoptera[b]	decrease	…the order Trichoptera (caddisflies)
Percent EPT less Hydropsychidae	decrease	…the generally sensitive orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), less the generally tolerant Trichoptera family Hydropsychidae
Percent PT Less Hyd.	decrease	…the generally sensitive orders Plecoptera (stoneflies) and Trichoptera (caddisflies), less the generally tolerant Trichoptera family Hydropsychidae
Percent T less Hydropsychidae [b]	decrease	…the generally sensitive order Trichoptera (caddisflies), less the generally tolerant Trichoptera family Hydropsychidae
Percent Diptera[a]	increase	…the order Diptera ("true" flies, such as midges and blackflies)
Percent Chironomidae[a]	increase	…the Diptera family Chironomidae (midges)
Percent Baetidae	variable	…the generally tolerant Ephemeroptera family Baetidae
Percent Nemouridae	variable	…the generally tolerant Plecoptera family Nemouridae
Percent Hydropsychidae [b]	variable	…the generally tolerant Trichoptera family Hydropsychidae

Table 4, continued. Candidate biological metrics by category, with expected response to perturbation, definition, and transformation used (if applicable). Adapted from Burton and Gerritsen (2003).

Metric by Category	Response	Definition
Diversity		*Percent of individuals in sample that comprise…*
Percent 1 Dominant Taxon	increase	…the most abundant taxon
Percent 2 Dominant Taxa	increase	…the two most abundant taxa
Percent 5 Dominant Taxa	increase	…the five most abundant taxa
Trophic Groups		*Percent of individuals in sample (or number of different taxa), that obtain food by…*
Percent Collectors[a]	decrease	…collecting/gathering depositional organic matter
Percent Filterers[b]	variable	…filtering suspended organic matter
Percent Predators	variable	…preying on other organisms
Percent Scrapers	decrease	…scraping algae and associated material from substrate surfaces
Percent Shredders	decrease	…shredding/chewing coarse organic matter such as leaves and detritus
Number of Collector Taxa	decrease	(number of taxa classified primarily as Collectors)
Number of Filterer Taxa	variable	(number of taxa classified primarily as Filterers)
Number of Predator Taxa	variable	(number of taxa classified primarily as Predators)
Number of Scraper Taxa	decrease	(number of taxa classified primarily as Scrapers)
Number of Shredder Taxa	decrease	(number of taxa classified primarily as Shredders)
Tolerance		
Hilsenhoff Biotic Index	increase	abundance-weighted average of organic pollution tolerance for assemblage

[a] transformed to normal distribution for analysis as Ln(X)
[b] transformed to normal distribution for analysis as Ln(1+X)

Correlation analysis was conducted on the variables, either as measured or as transformed to normal distributions. Significant correlations were noted (Table 5). All relationships between water quality parameters and biotic metrics were negative or not significant (Fig. 4).

Table 5. Pearson product-moment correlations for biological metrics and water quality parameters (test sites only, n=17). Metrics are as defined in Burton and Gerritsen (2003). All correlations are significant ($p < 0.05$).

Metric	Ca^{2+}	SO_4^{2-}	Mg^{2+}	TDS	Cond.	K^+
Number of EPT Taxa	-0.81	-0.81	-0.79	-0.76	-0.76	-0.64
Number of E Taxa	-0.75	-0.79	-0.77	-0.71	-0.71	-0.59
Number of P Taxa	-0.78	-0.75	-0.73	-0.72	-0.72	-0.60
Percent 5 Dominant Taxa	0.75	0.71	0.71	0.64	0.62	0.52
Number of Collector Taxa	-0.66	-0.71	-0.71	-0.61	-0.58	-0.55
Number of Taxa	-0.63	-0.56	-0.57	-0.50	-0.49	
Number of T Taxa	-0.52					

No single water quality parameter stood out as a lone predictor of biological condition, but SO_4^{2-} may be the best choice among the water quality parameters we measured if use of a single parameter is desired. Correlation analysis revealed that Ca^{2+}, SO_4^{2-}, and Mg^{2+} are significantly correlated to the greatest number of biological metrics, each more so than TDS or conductivity, whereas HCO_3^- and Na^+ were not significantly correlated with any biological metrics. Sulfate was the dominant ion in nearly all samples, whereas Ca^{2+} and Mg^{2+} contribute less to TDS (Fig. 2 and 3). In addition, observed maximum SO_4^{2-} concentration (531 mg/L) was much higher than maximum concentrations of Ca^{2+} (120 mg/L) or Mg^{2+} (75 mg/L) (Table 2). Furthermore, the three ions are all strongly correlated to each other (Table 3). Sulfate has been used as a reliable indicator of mining activity (Pond et al., 2008). These data suggest that SO_4^{2-} may be a suitable candidate for prediction of aquatic life response in mining-influenced streams, although that preliminary finding will be subject to additional investigation by further study.

The strongest correlations to TDS and related measures were with the generally sensitive groups of aquatic insects (the orders Ephemeroptera, Plecoptera, and Trichoptera, or EPT). In this regard, our results echo the findings of other studies of mining-influenced streams that also observed correlations of elevated TDS with reduced richness of EPT taxa (*e.g.*, Pond et al., 2008; Pond, 2004; Green et al., 2000).

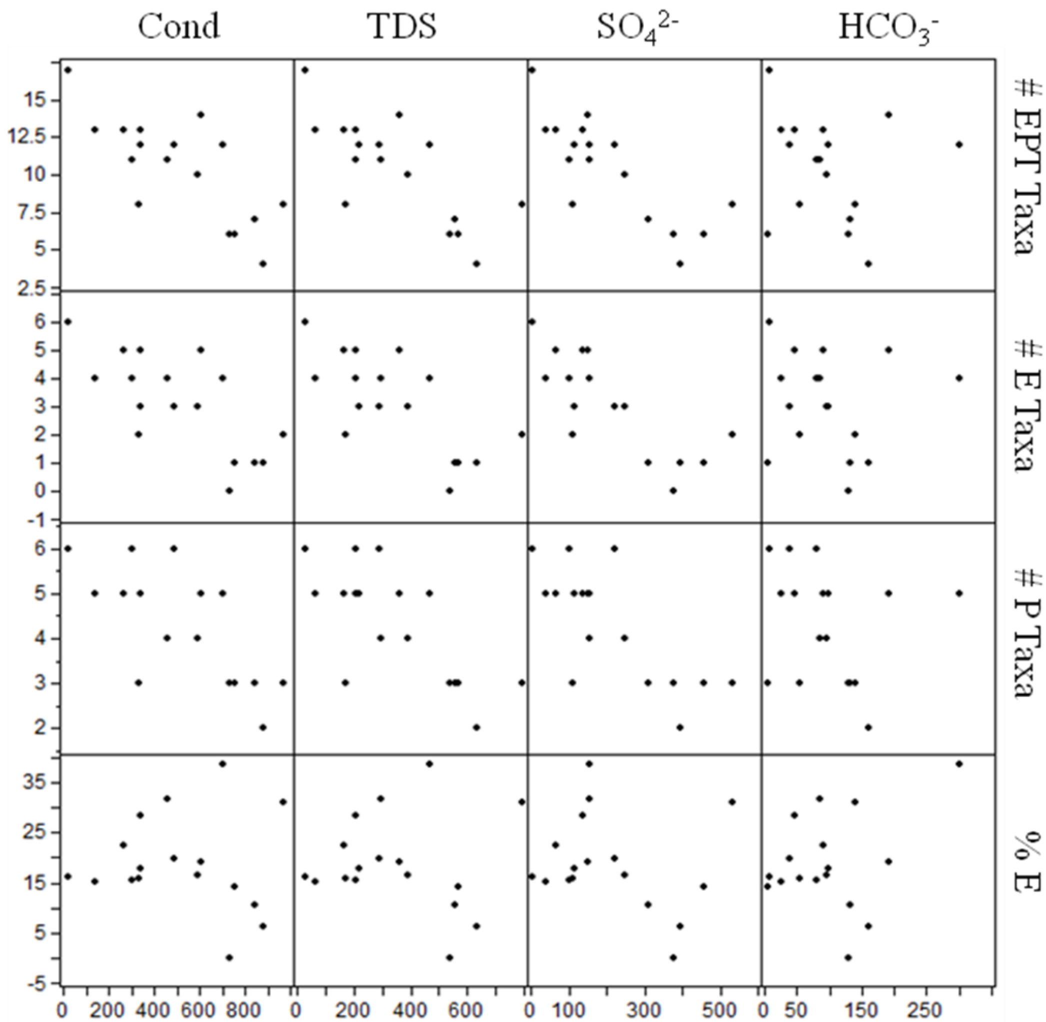

Figure 4. Scatter plot matrix of select chemical and biological parameters (test sites only, n=17). Units are mg/L except conductivity (μS/cm). All correlations are significant ($p < 0.05$) except those involving bicarbonate (HCO_3^-) and Percent Ephemeroptera (% E).

We did not observe the Percent Ephemeroptera (mayflies) metric to be correlated with any chemical variables, including TDS (Fig. 4). Other studies of mining-influenced streams have observed significant negative correlations between mayfly relative abundance and TDS or conductivity (*e.g.*, Pond et al., 2008; Pond, 2004; Green et al., 2000). However, while mayfly relative abundance was not correlated with dissolved ion measures in our data, mayfly richness (Number of E Taxa) was negatively correlated with TDS and related measures (Table 5). This result demonstrates that at our study sites for the Spring 2009 sampling period, as TDS increased across sites, the benthic macroinvertebrate community structure responded by shifting to fewer mayfly taxa, but overall mayfly relative abundance did not respond predictably to increasing TDS (Fig. 4).

The metrics most strongly correlated with TDS and related parameters were measures of community richness, rather than measures of relative abundance. This suggests that although family-level community richness (the number of taxa present) may decline with increasing TDS, abundance of individuals within the remaining families, and perhaps overall order abundance, may remain less affected, at least within the range of TDS and component ion concentrations that we assessed.

Conclusions

We found that TDS can be reasonably isolated from other stressors in the Virginia Central Appalachian coalfields through strategic site selection, as we were successful in locating sites with elevated TDS that exhibited minimal influence from non-TDS stressors. This was critical to understanding the influence of dissolved ions on benthic macroinvertebrate community structure with minimal confounding from common TDS covariates.

The TDS in waters we sampled tended to be dominated by SO_4^{2-} and HCO_3^-, with Ca^{2+} being the third most prevalent ion by mass. Bicarbonate was not significantly correlated with any benthic community metrics. Sulfate comprised nearly half of the dissolved ion mass on average, and it was the dominant water quality parameter most highly correlated with biotic metrics. For these reasons, we think SO_4^{2-} concentration (w/v) is a candidate for use as a single-parameter predictor of biological condition.

Biological metrics that exhibited a significant negative correlation with TDS/ions were those of family-level community richness, especially those measuring richness of the generally sensitive insect orders of Ephemeroptera, Plecoptera, and Trichoptera. Order-level relative abundance metrics, including Percent Ephemeroptera, were not correlated with conductivity, TDS, or component ion concentration. This suggests that while family-level community richness (the number of taxa present) may decline with increasing TDS, abundance of individuals within the remaining families, and perhaps overall order abundance, may remain less affected, at least within the range of TDS and component ion concentrations that we assessed.

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