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Environmental Toxicology

A Novel Approach to Developing Thresholds for Total Dissolved Solids Using Standardized and Experimental Toxicity Test Methods

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Abstract: The increasing salinization of freshwater streams from anthropogenic land uses and activities is a growing global environmental problem. Increases in individual ions (such as sodium or chloride) and combined measures such as total dissolved solids (TDS) threaten drinking water supplies, agricultural and economic interests, and the ecological health of freshwater streams. Because the toxicity of high ionic strength waters depends on the specific ion composition, few water quality standards exist to protect freshwater streams from salinization. In the present study, we used a novel approach to develop site-specific and ecologically relevant TDS thresholds for the protection of aquatic life. The first step of the approach was to characterize the ion composition of the waterbody or region of interest and prepare artificial samples to match that composition. Using a combination of standardized toxicity test species and more ecologically relevant field-collected species, toxicity tests were then conducted on these artificial samples prepared at a range of TDS concentrations. The advantage of this approach is that water quality criteria can be developed for easy-to-measure generalized parameters such as TDS while ensuring that the criteria are protective of instream aquatic life and account for the complex interactions of the various ions contributing to salinization. We tested this approach in Sand Branch, Loudoun County, Virginia, USA, where salinization from hard rock mining and urban runoff has impaired aquatic life. Acute and chronic TDS thresholds of 938 and 463 mg/L, respectively, were developed in this stream and used for total maximum daily load development in the watershed. The approach provides a potential model for establishing protective thresholds for other waterbodies impacted by salinization. *Environ Toxicol Chem* 2022;41:2782–2796. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Freshwater salinization; freshwater toxicology; total dissolved solids; total maximum daily loads (TMDLs); water quality criteria

INTRODUCTION

The increasing salinization of freshwater streams is a growing global environmental problem (Cañedo-Argüelles et al., 2013) that threatens drinking water supplies (Kaushal et al., 2005), agricultural and economic interests (Williams, 1999), and the ecological health of freshwater streams (Castillo et al., 2018). Kaushal et al. (2018) proposed the concept of "freshwater salinization syndrome," which describes the shifting chemical composition of major ions in fresh water across North America. Freshwater streams naturally export dissolved major ions through the weathering of underlying bedrock and watershed soils, but this process is accelerated by anthropogenic land uses and activities (Kaushal et al., 2017; Liu & Han, 2020). In agricultural landscapes, irrigation and drainage practices, fertilizer and lime use, and tillage practices can contribute to increased concentrations of major ions (Raymond et al., 2008). Roadway deicing salts, water and wastewater discharges, and the weathering of impervious surfaces such as concrete cause salinization effects in urban areas (Corsi et al., 2010; Kaushal et al., 2014; Novotny et al., 2008; Thunqvist, 2004). Mining and resource extraction activities also contribute to increased major ion concentrations in freshwater streams by enhanced weathering of exposed geological material and acidic drainages (Lindberg et al., 2011; Palmer et al., 2010).

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Freshwater salinization sources increase the concentrations of major dissolved ions, such as calcium, magnesium, sodium, potassium, bicarbonate, sulfate, chloride, and nitrate, as well as other trace ions. Increases in these individual dissolved ions are also measured as increases in aggregate parameters such as total dissolved solids (TDS), conductivity, hardness, or alkalinity. Ecological effects from freshwater salinization can result from toxicity of individual ions, toxicity of ion mixtures, or combined effects of osmotic imbalance. Mount et al. (1997) predicted that the relative order of toxicity to freshwater invertebrates and fish was generally $K^+ > HCO_{3-} \sim Mg^{+2} > Cl^- > SO_4^{-2}$, but the degree of toxicity of the individual ions is not fixed and is greatly influenced by background water chemistry (Mount et al., 2016) and the concentrations and ratios of other co-occurring ions (Soucek et al., 2011).

Sensitivity to major ions and TDS also varies greatly across geographic regions, taxa, and functional feeding groups (Castillo et al., 2018), implying that salinization poses a threat to freshwater biodiversity, impacting both the structure and function of freshwater ecosystems. Olson and Hawkins (2017) demonstrated in mesocosm experiments that macroinvertebrate assemblages are influenced by varying species sensitivity to TDS. In field studies, Pond et al. (2008) and Pond (2010) found significant changes in macroinvertebrate assemblages, including a loss of Ephemeroptera species, in streams impacted by surface mining. These changes were correlated with increasing TDS and conductivity across a range of disturbance levels. Although impacts from heavy metals cannot be ruled out, Pond et al. (2008) found that combined measures of salinization (such as TDS and conductivity) were more strongly correlated with benthic macroinvertebrate effects than individual metals concentrations. Lind et al. (2018) even demonstrated that salinization can produce direct ecological community effects as well as top-down and bottom-up indirect effects that alter the abundance of primary producers and consumers.

The impacts of freshwater salinization are also widespread. Kaushal et al. (2018) reported that salinization and associated alkalinization have affected 37% and 90%, respectively, of the drainage area of the United States over the past century. Similarly, the US Environmental Protection Agency's (USEPA's) Assessment, Total Maximum Daily Load (TMDL) Tracking and Implementation System reports over 38 000 miles of river, 850 000 acres of lakes, and 82 000 acres of freshwater wetlands as impaired by salinity related parameters, including specific major ions or cumulative parameters such as TDS and conductivity (USEPA, 2021a). Salinity-related parameters rank as the 14th, eighth, and fourth most prevalent impairment causes in rivers, lakes, and wetlands, respectively (USEPA, 2021a).

Managing and regulating salinization in freshwater ecosystems is a difficult challenge because toxicity may result from effects of individual constituent ions, toxicity of ion mixtures, or combined effects of osmotic imbalance (Goodfellow et al., 2000). For this reason, there are no nationwide criteria to protect aquatic life uses from salinization effects. Many states have a patchwork of criteria that set standards for individual ions such as sodium, chloride, or sulfate. Some have criteria for combined measures such as TDS, but these are typically set to protect drinking water or agricultural uses, rather than aquatic life uses. Researchers have therefore argued for improved regulatory strategies for managing freshwater salinization (Cañedo-Argüelles et al., 2016; Schuler et al., 2019) that include ion-specific monitoring, consideration of regional background ion levels, assessment of site-specific ion composition, better quantification of toxic thresholds and effects, and multi-stakeholder approaches that balance environmental, social, and economic costs and benefits.

Study approach

The objectives of the present study were to propose a novel approach for developing TDS thresholds for the protection of aquatic life in waterbodies impacted by salinization and to implement this approach in a relevant case study. The first step of the approach was to characterize the ion composition of the water body or region of interest and prepare artificial samples to match that composition. This step allows a threshold based on a generalized parameter such as TDS to account for the complex interactions and toxic effects of the various ions that comprise the TDS. It also ensures that the threshold is regionally appropriate and relevant to background water chemistry. The second step of the approach was to conduct toxicity tests on the artificial samples prepared at a range of TDS concentrations and matching the instream ion composition. We propose, and used in the case study example, a combination of standardized toxicity test species and more ecologically relevant field-collected species. This combination provides a connection to the rich toxicological literature through the use of standardized species testing and also provides a more relevant ecological connection to regional salinization impacts through the use of local field-collected species. Standardized toxicity test species are often not particularly sensitive to salinization effects, so the use of sensitive local species such as mayflies provides an overall more protective threshold. The final step of the approach was to use statistical methods consistent with USEPA guidelines for water quality criteria development (USEPA, 1985) to develop acute and chronic thresholds for TDS based on the toxicity test results. This step grounds the approach in traditional water quality criteria development practice. Overall, the approach provides a potential model for establishing TDS thresholds for waterbodies impacted by salinization or for the development of regional freshwater aquatic life criteria for TDS. The advantage of this approach is that water quality criteria can be developed for easy-to-measure generalized parameters such as TDS while ensuring that the criteria are protective of instream aquatic life and account for the complex interactions of the various ions contributing to salinization.

We demonstrated this approach as a case study in Sand Branch, Loudoun County, Virginia, USA, where salinization from hard rock mining and urban runoff have impaired aquatic life. Benthic macroinvertebrate community surveys and water quality data were used in a causal analysis framework to identify TDS as a primary stressor in Sand Branch. Water quality analysis of specific ions provided a basis for preparing artificial samples to match Sand Branch ion composition. A range of TDS samples prepared to match the Sand Branch ion composition were then

subjected to toxicity testing using standardized toxicity test species (*Ceriodaphnia dubia*, *Pimephales promelas*, and *Hyalella azteca*) and two field-collected species (a freshwater pleurocerid snail, *Leptoxis carinata*, and a brush legged mayfly, *Isonychia bicolor*). Lastly, acute and chronic TDS thresholds were developed for Sand Branch that protect aquatic life, account for the varying effects of ion composition, and incorporate ecologically relevant and regionally representative species.

MATERIALS AND METHODS

Study site

Sand Branch is a small 2.48-km freshwater stream in Loudoun and Fairfax Counties, Virginia (Figure 1). The stream originates from the discharge of a large hard-rock quarry that was developed in 1958 to provide aggregate for the construction of Dulles International Airport. In addition to the quarry and portions of the airport property, the 356-hectare watershed drains a mixed industrial and commercial center. Land cover in the watershed includes approximately 47% forest/trees, 22% developed impervious, 26% barren (including the quarry), 13% developed pervious, and 2% pasture (Virginia Department of Environmental Quality [VDEQ], 2021a). The Sand Branch watershed lies within the Trap Rock and Conglomerates Uplands and Triassic Lowlands Level IV ecoregions within the Northern Piedmont Level III ecoregion (Woods et al., 1999). Sand Branch is a tributary to Cub Run, which ultimately drains to the Occoquan Reservoir, a major drinking water source for the northern Virginia suburbs of the Washington D.C. metropolitan area.

Sand Branch was listed in Virginia's 2020 303(d)/305(b) Integrated Report as impaired and not supporting the aquatic life designated use based on biological monitoring of the benthic macroinvertebrate community (VDEQ, 2021b). Based on the aquatic life impairment and pursuant to the Clean Water Act, the

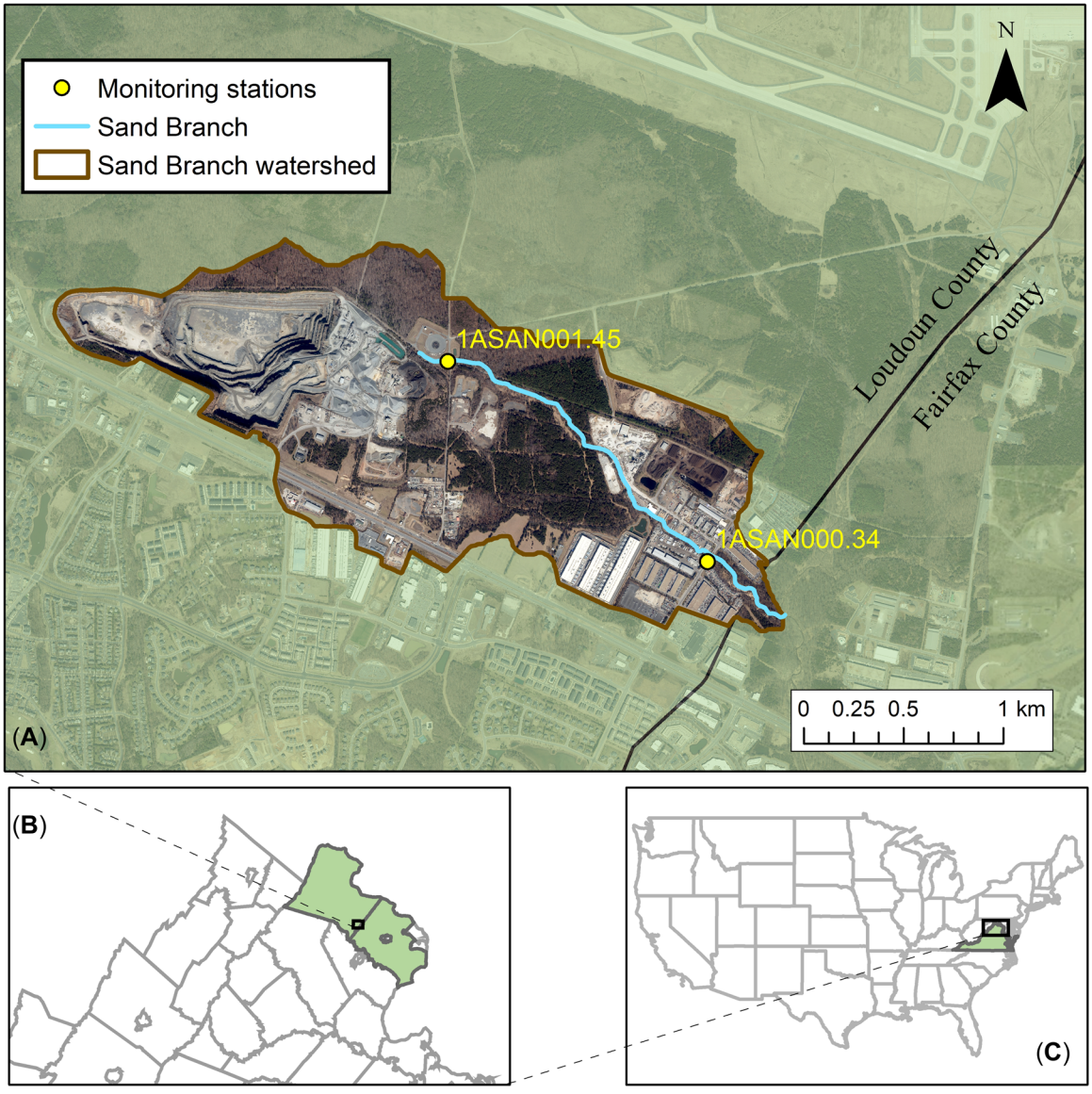

FIGURE 1: Map of Sand Branch watershed in Loudoun and Fairfax County, Virginia. (**A**) Sand Branch watershed with Sand Branch flowing southeast. (**B**) Loudoun and Fairfax Counties in Virginia. (**C**) State of Virginia in the United States.

VDEQ was required to develop a total maximum daily load (TMDL) for pollutants causing or contributing to the impairment. As the first step in this process, VDEQ conducted a benthic stressor analysis to identify the responsible pollutants. The analysis was conducted in accordance with the USEPA Stressor Identification Guidance Document (USEPA, 2000b) using the Causal Analysis/Diagnosis Decision Information System (USEPA, 2018) and found that the probable stressors were excess sediment, phosphorus, and TDS (VDEQ, 2021a). Because Virginia does not have numeric water quality criteria for TDS, an alternative approach was needed for determining the TMDL endpoint that would restore and protect aquatic life in Sand Branch. The present study was developed to meet this need and provide a TDS threshold based on site-specific toxicity testing.

Sand Branch monitoring

The VDEQ has conducted routine monitoring of two stations on Sand Branch (1ASAN001.45 and 1ASAN000.34) since 2015. Among other parameters, the VDEQ has monitored conductivity, TDS, and major ions including calcium, magnesium, sodium, potassium, bicarbonate, sulfate, chloride, and nitrate. Conductivity was measured in the field using a calibrated YSI EXO1 sonde (Xylem). Water quality samples for TDS and major ion analysis were collected in HPDE plastic bottles and transported on ice to the Virginia Division of Consolidated Laboratory Services for analysis using USEPA Method 200.7 for cations (USEPA, 1994), Method 300.1 for anions (USEPA, 1997), and Method 160.1 for TDS (USEPA, 1971). The VDEQ also collected benthic macroinvertebrate samples from Sand Branch in the spring and fall of 2016 and 2020. Benthic macroinvertebrate samples were collected according to the Rapid Bioassessment Protocol (Barbour et al., 1999) using a D-frame kick net to sample a 2-m^2 riffle/run area. Samples were homogenized in a gridded tray, and a randomized 110-organism subsample was sorted (Caton, 1991) and identified to genus. The multi-metric Virginia Stream Condition Index (VSCI) was then calculated for each sample (Burton & Gerritsen, 2003).

During the summer and winter seasons, continuous conductivity monitoring was conducted in Sand Branch. During a 2-week period in August, conductivity was monitored continuously at the downstream station (1ASAN000.34). From August 10, 2020 to August 26, 2020, the VDEQ measured conductivity at 15-min intervals using a YSI EXO1 sonde (Xylem). From December 10, 2020 to February 10, 2021, James Madison University (JMU) measured conductivity at 15-min intervals using a HOBO conductivity logger (Model U24-001; Onset Computer Corp.). During this time period, stream depth was also measured using a HOBO water level logger (Model U20-001-01). Hourly rain and snowfall measurements were obtained from the National Weather Service meteorological station at Washington/Dulles International Airport, DC (KIAD), located within a mile of the Sand Branch watershed. The YSI was cleaned and calibrated every 2 weeks and the more rugged HOBO conductivity logger was cleaned and calibrated monthly. For quality assurance purposes, conductivity measurements were confirmed with a second, newly calibrated instrument at the start and end of each deployment period. The relative percent difference between the continuous monitors and the secondary confirmation reading ranged from only −0.63% to +1.97%, indicating that conductivity measurements were accurate throughout the deployments.

Artificial sample preparation

In the present study, toxicity testing using standardized test species was conducted at Coastal Bioanalysts in Gloucester, VA, and toxicity testing using field-collected species was conducted at James Madison University in Harrisonburg, VA. Samples used for toxicity testing at both laboratories were prepared by researchers at James Madison University. James Madison University researchers prepared artificial samples by adding the appropriate masses of the following salts to match the respective composition of major ions in Sand Branch: sodium bicarbonate, calcium sulfate dihydrate, magnesium sulfate heptahydrate, potassium nitrate, and calcium chloride dihydrate. Researchers at James Madison University used the Generalized Reduced Gradient Nonlinear solver in Microsoft Excel to solve for the mass of the respective salts needed to produce a given TDS and maintain the individual ion composition. The solver was set to minimize the difference between the sample TDS and the target TDS with the constraint that individual ions must be within 7.5% of their respective average contribution in Sand Branch and must be within the range of observed contributions in Sand Branch.

Researchers at James Madison University prepared 5-L batches of the laboratory-prepared sample by adding the respective salts to 5 L of deionized water in a 5-L plastic beaker. The solution was stirred with a magnetic stir bar on a stir plate until the salts were fully dissolved. For James Madison University testing, 5-L preparations were used for each test. For testing at Coastal Bioanalysts, 10, 5-L preparations were composited into 5, 10-L cubitainers for transport to the laboratory. Samples were transported on ice and hand delivered to the laboratory within 24 h of the completion of sample preparation. At the laboratory, the 5, 10-L cubitainers were composited into a 50-L sample for testing. Both laboratories diluted the laboratory-prepared sample with deionized water to prepare a series of five test concentrations using a dilution factor of 0.5. Based on initial range-finding toxicity tests, target TDS values for the highest tested doses were 3500–5300 mg/L TDS.

Nominal TDS values in artificial samples were confirmed by TDS analysis according to Standard Method 2540C: TDS dried at 180 °C (American Public Health Association, American Water Works Association, and World Environment Federation 1998). Quality control samples for TDS analysis showed good accuracy and precision with all blanks below 3 mg/L and relative percent difference of duplicates ranging from 0.43% to 2.3%. Measured TDS values from prepared samples were used in the calculation of toxicity test results.

Toxicity testing using standardized test species

Standardized chronic toxicity tests using *C. dubia*, *P. promelas*, and *H. azteca* were conducted on artificial Sand Branch samples at Coastal Bioanalysts in Gloucester, VA. Fathead

minnow, *P. promelas*, larval survival and growth test (USEPA Method 1000.0) and Daphnid, *C. dubia*, survival and reproduction test (USEPA Method 1002.0) were performed according to USEPA-approved methods for whole effluent toxicity testing (USEPA, 2002b). The *H. azteca* 10-day water only survival and growth test (USEPA Method 100.1) was conducted according to USEPA methods for sediment toxicity testing (USEPA, 2000a) with modifications for use with water only exposure. Ten organisms were placed in each of eight replicate chambers with clean inert sand as a substrate and 250 ml of overlying test solution. *C. dubia* and *P. promelas* tests were performed at 25 °C, and the *H. azteca* test was performed at 23 °C. All tests were conducted with a moderately hard synthetic freshwater control. The laboratory-prepared sample was diluted with deionized water and a 0.5 dilution factor to obtain test concentrations of 4148, 2074, 1037, 518.5, and 259.3 mg/L TDS. Acute toxicity data for fish and daphnid tests were obtained by recording the survival in chronic tests at 96 h of exposure. Survival of *H. azteca* was only recorded at the end of the test, because organisms are buried in the sand substrate until sieving at test completion. All statistics and effect concentrations were calculated with ToxCalc 5.0 (Tidepool Scientific Software) using USEPA-approved statistical methods (USEPA, 2002b).

Toxicity testing using field-collected test species

In addition to the standardized test species, toxicity testing of artificial Sand Branch samples was conducted at James Madison University on two field-collected species: a freshwater pleurocerid snail, *L. carinata*, and a brush legged mayfly, *I. bicolor*. Other mayfly species were considered for testing and included in initial range-finding, but *Isonychia bicolor* was selected for further testing based on field availability, ease of field identification, life cycle, handling success, control survival, and sensitivity. Field-collected organisms were obtained from the North River in Bridgewater, Virginia. The North River has been assessed by the VDEQ as fully supporting the aquatic life use based on benthic community surveys.

Leptoxis carinata test method

James Madison University researchers conducted acute toxicity tests with a field-collected freshwater gastropod snail (*L. carinata*). Tests were conducted in accordance with USEPA acute toxicity test methods (USEPA, 2002a) with modifications for field-collected species. Snails were collected from the North River in Bridgewater, Virginia, by hand picking organisms from the natural cobble substrate. Snails of 7–8 ± 0.25 mm length (longest axis) were targeted for collection. Field staff used a three-dimensional printed size gauge with slots of 6.75 and 8.25 mm to distinguish organisms of the correct size. Organisms were placed in groups of 20 organisms per 500-ml plastic container of river water. Containers were transferred to the laboratory in a cooler with minimal ice. In the laboratory, organisms were transferred to moderately hard synthetic freshwater in 10-gallon glass aquaria. Aquaria were equipped with a pump to circulate water, an air stone to provide aeration, and rocks collected from the field containing naturally colonized periphyton. Snails were held at 20 ± 1 °C for 1 week prior to testing. Taxonomic identification of representative specimens was confirmed by the VDEQ Regional Biologist.

Tests were conducted as 96-h static renewal tests with feeding and renewal at 48 h. For each test treatment, four replicates of five organisms were placed in 500-ml plastic containers with 450 ml of test solution. Moderately hard synthetic freshwater was used as the control, and deionized water was used to dilute laboratory-prepared samples using a dilution factor of 0.5. Tests were conducted in an environmental chamber at 20 ± 1 °C to better match field conditions. Snails were fed by introducing a field-collected rock with naturally colonized periphyton to the test chambers 2 h prior to test renewal at 48 h. Survival and water quality were monitored daily. Mortality was determined as a lack of movement. Snails that were closed inside their shell or not actively moving were placed in the center of the test container for a 2-h observation period. If organisms failed to move during the 2-h observation period and showed no signs of foot or tentacle movement at the end of the period, the organisms were declared dead. All statistics and effect concentrations were calculated with CETIS Ver 1.9.7.10 (Tidepool Scientific Software) using USEPA-approved statistical methods (USEPA, 2002a).

Isonychia bicolor test method

James Madison University researchers conducted acute toxicity tests with the field-collected mayfly species *I. bicolor*. Tests were conducted in accordance with USEPA acute toxicity test methods (USEPA, 2002a) with modifications for field-collected species. Mayflies were collected from the North River in Bridgewater, Virginia, using a 1-m^2 kick net with 1-mm mesh. Organisms were identified in the field and placed in groups of 20 organisms per 500-ml plastic container of river water. Containers were transferred to the laboratory in a cooler with minimal ice to maintain field water temperatures. In the laboratory, organisms were transferred to moderately hard synthetic freshwater in 10-gal glass aquaria. Aquaria were equipped with a pump to circulate water, an air stone to provide aeration, and four 10 × 7 cm low density Matala® filter media pads (Matala USA) to provide substrate and refugia. During holding and acclimation, organisms were fed three whole dried maple leaves at initiation of the holding tank, and ground maple leaves and an algae suspension at approximately 48-h intervals. Ground leaves were prepared by finely grinding 10 dried maple leaves in a blender with 250 ml of deionized water. The algae suspension was prepared by adding 50 mg of dried powdered *Spirulina* algae (Carolina Biological Supply) to 50 ml of deionized water. Naturally colonized periphyton on rocks collected from the stream were also placed in the aquaria so that sloughing of natural periphyton could be utilized as a food source. Mayflies were held at 20 ± 1 °C for 1 week prior to testing. Taxonomic identification of representative specimens was confirmed by the VDEQ Regional Biologist.

Tests were conducted as 96-h static renewal tests with feeding and renewal at 48 h. For each test treatment, two replicates of 10

organisms were placed in 500-ml plastic containers with 450 ml of test solution. Two 5 × 5 cm pieces of 630 µm Nitex mesh (Genesee Scientific) were placed in each test container to provide a substrate for the mayflies. All test containers were aerated for the duration of the test using a dedicated air pump and air stone. Moderately hard synthetic freshwater was used as the control, and deionized water was used to dilute laboratory-prepared samples using a dilution factor of 0.5. Tests were conducted at 20 ± 1 °C to better match field conditions. Two hours before the 48-h test renewal, each test container was fed 1 ml of a slurry of ground dried maple leaves and 1 ml of an algae suspension. Survival and water quality were monitored daily. Mortality was determined as no movement or response to gentle stimuli. All statistics and effect concentrations were calculated with CETIS Ver 1.9.7.10 (Tidepool Scientific Software) using USEPA-approved statistical methods (USEPA, 2002a).

RESULTS

Benthic data

The health of the benthic macroinvertebrate community was measured in the spring and fall of 2016 and 2020 using the VSCI score (Burton & Gerritsen, 2003). Virginia Stream Condition Index scores were highly variable and ranged from 15.1 to 43.1 at the upstream station and from 9.6 to 53.3 at the downstream station (Figure 2). Spring scores were particularly low, averaging 17.9 and indicating severe ecological impairment. Fall scores were higher, averaging 41.9, but all scores were below the healthy threshold of 60. Species richness was relatively low, ranging from six to 11 species at the upstream station and three to 13 species at the downstream station. No Ephemeroptera or Plecoptera species were found at either site, and only a single non-Hydropsychidae Trichoptera species (*Hydroptila* sp.) was found at each site. The benthic community was dominated by tolerant midge species from the Diptera family Chironomidae and riffle beetles of the Coleopteran genus *Stenelmis*, which together comprised 70% and 68% of the community at the upstream and downstream stations, respectively. Overall, biological surveys showed that Sand Branch was severely impaired with low diversity and a lack of sensitive species (VDEQ, 2021a).

Major ions

Results of routine VDEQ sampling of TDS, conductivity, and major ions are shown in Table 1. Total dissolved solids ranged from 290 to 895 mg/L and averaged 579 mg/L at the downstream station. Conductivity ranged from 361 to 1159 µS/cm and averaged 851 µS/cm. A regression of conductivity to TDS was highly significant ($p < 0.001$ and $r^2 = 0.918$) and proved useful for estimating TDS from the more easily measured conductivity parameter when TDS was not individually measured (Figure 3). On a mass basis, TDS was dominated by sulfate (38%) and bicarbonate (27%), but these contributions were relatively variable over time, with sulfate contributing 11%–59% of TDS and bicarbonate contributing 19%–41%. Chloride was also highly variable, representing 2%–24% of TDS. The remaining ions varied by less than 6% of TDS.

On a milliequivalent basis, the predominant cations were $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$, and the predominant anions were $SO_4^{2-} > HCO_3^{-} > Cl^{-} > NO_3^{-}$ (Figure 4). The sum of cations exceeded anions by 1.4 mEq/L. Some of this departure from charge balance is due to calculating equivalents based on averages for each ion, but this may also reflect additional anions that were not measured. Charge balance on individual samples was much closer, ranging from +0.098 to +0.754, but cations did consistently exceed anions. Overall, unaccounted for anions represented only 6.8% of the total charge, indicating that the eight measured ions contributed the bulk of dissolved solids.

Continuous conductivity

Figure 5 shows continuous monitoring of specific conductivity in Sand Branch during the summer and winter seasons. In August, specific conductivity ranged from 395.2 to 981.3 µS/cm and averaged 852.2 µS/cm. During summer, conductivity gradually increased during dry periods as groundwater and discharge from

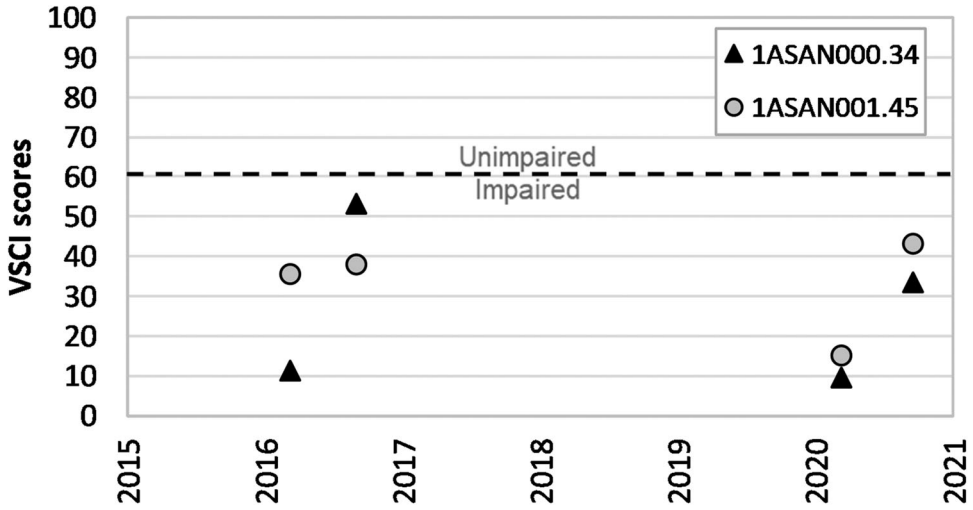

FIGURE 2: Virginia Stream Condition Index (VSCI) scores at Sand Branch stations over time.

TABLE 1: Major ion chemistry of Sand Branch (station 1ASAN000.34)

Parameter	*N*	Date range	Concentration average (and range) of ions (mg/L)	Average (and range) of ions as a percentage of total dissolved solids (%)
Ca^{2+} (mg/L)	7	12/5/17–8/26/20	110 (76.8–138)	16 (14–18)
Mg^{2+} (mg/L)	7	12/5/17–8/26/20	46.1 (30.0–59.3)	6.8 (5.3–7.6)
Na^{+} (mg/L)	13	12/5/17–8/26/20	39.0 (24.8–64.4)	6.6 (4.4–9.7)
K^{+} (mg/L)	13	12/5/17–8/26/20	6.27 (2.14–21.2)	1.2 (0.30–3.4)
HCO_3^{-} (mg/L)	19	1/5/15–8/26/20	156 (118–227)	27 (19–41)
SO_4^{2-} (mg/L)	26	1/5/15–8/26/20	232 (36.3–463)	38 (11–59)
Cl^{-} (mg/L)	27	1/5/15–8/26/20	46.3 (14.4–180)	7.9 (2.0–24)
NO_3^{-} (mg/L)	13	12/5/17–8/26/20	6.87 (0.886–27.7)	1.5 (0.13–6.1)
TDS (mg/L)	27	1/5/15–8/26/20	579 (290–895)	—
Conductivity (µS/cm)	30	1/5/15–8/26/20	851 (361–1159)	—

the quarry and other point sources dominated. Rain events then dramatically decreased conductivity for a short time with the introduction of low-conductivity rainwater and runoff. Over a period of 2–3 days, conductivity gradually rebounded to a baseline level of 900–1200 µS/cm. During winter, conductivity ranged from 308 to 3371 µS/cm and averaged 1034 µS/cm. During winter storm events when deicing salts are added to roadways, dramatic conductivity spikes were observed as stormwater from nonpoint sources introduced additional ions to Sand Branch. Snowfall recorded at Dulles Airport on 12/16/20, 1/25/21, 1/31/21–2/2/21, and 2/7/21 all corresponded to conductivity spikes over 1500 µS/cm. During the largest snowfall of 5.7 inches on 1/31/21–2/2/21, conductivity remained above 1500 µS/cm for more than 3 days and peaked at 3370 µS/cm. These results indicate that Sand Branch maintains a high conductivity baseline from groundwater and point source discharges but experiences additional increases in conductivity during the winter months due to nonpoint source runoff of roadway deicing salts. Although TDS was not measured during wintertime conductivity excursions, conductivity peaks over 1500 µS/cm and a maximum of 3370 µS/cm equated to TDS values of 1124 and 2632 mg/L using the site-specific conductivity to TDS regression (Figure 3).

Agreement between artificial samples and instream ion composition

James Madison University researchers prepared artificial samples to achieve a range of TDS levels while maintaining the ion composition of Sand Branch as detailed in Table 1. Figure 6 compares the ion composition of Sand Branch samples to artificial samples. For each ion, the resulting artificial samples were within the range of field-observed TDS contributions and within 7.5% of the average TDS contributions. With the given set of reagents (sodium bicarbonate, calcium

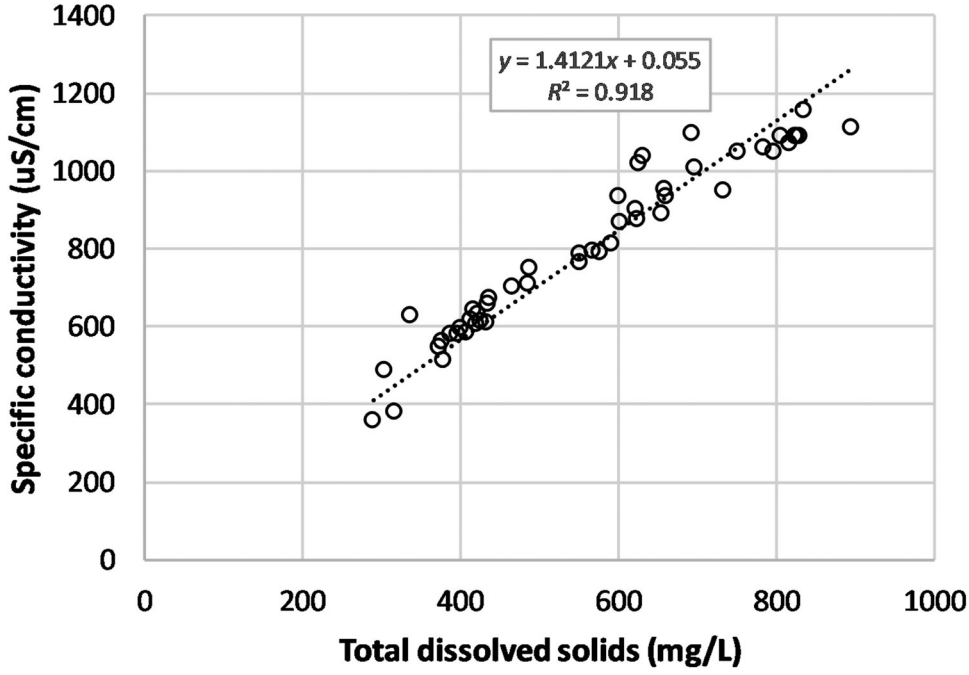

FIGURE 3: Regression of total dissolved solids (TDS) to specific conductivity in Sand Branch. Data points represent paired TDS and specific conductivity measurements from individual Sand Branch samples.

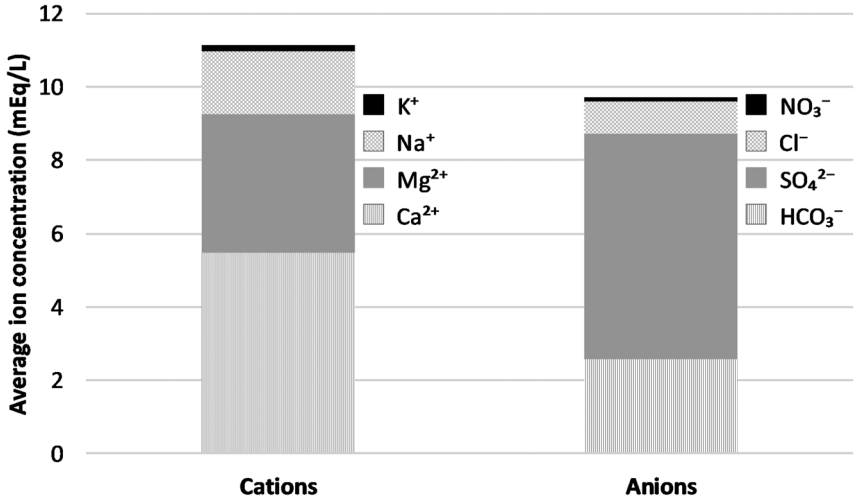

FIGURE 4: Average milliequivalent contributions of ions in Sand Branch water chemistry.

sulfate dihydrate, magnesium sulfate heptahydrate, potassium nitrate, and calcium chloride dihydrate), preparations could not get closer to the average than 7.5% for chloride and bicarbonate. Other ions were within 0.6%–4.3% of average values observed in the field.

Toxicity tests with standardized species

Coastal Bioanalysts successfully performed *C. dubia*, *P. promelas*, and *H. azteca* acute and chronic tests on the artificial Sand Branch sample. The tests met all acute and chronic test acceptability criteria. Control survival was 100% in the *C. dubia* and *P. promelas* tests and 97.5% in the *H. azteca* test. Control reproduction in the *C. dubia* test was 30.8 neonates per female, control biomass in the *P. promelas* test was 0.69 mg per surviving organism, and control growth in the *H. azteca* test was 0.08 mg per surviving organism. Reference toxicant tests for all three species were within control limits for the laboratory.

Toxicity results from standardized test species are shown in Table 2. Acute toxicity tests resulted in 96-h median lethal concentrations (LC50s) of 3195 mg/L TDS for *C. dubia*, 1511 mg/L TDS for *P. promelas*, and 10-d LC50 of >4148 mg/L TDS for

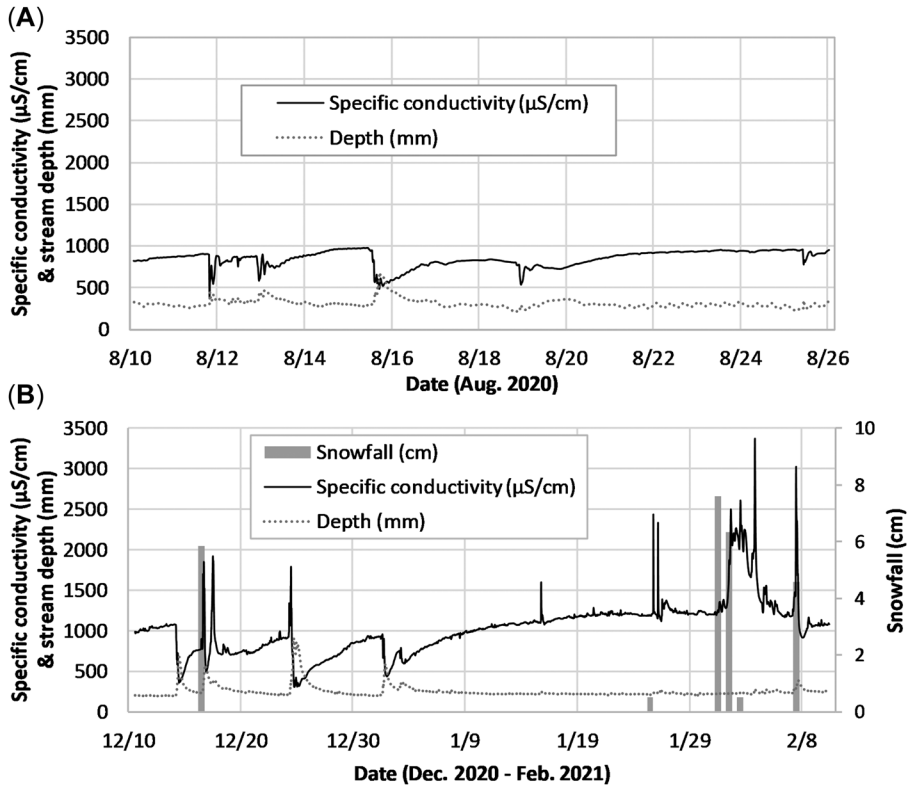

FIGURE 5: Continuous conductivity monitoring in Sand Branch during summer (**A**) and winter (**B**) seasons.

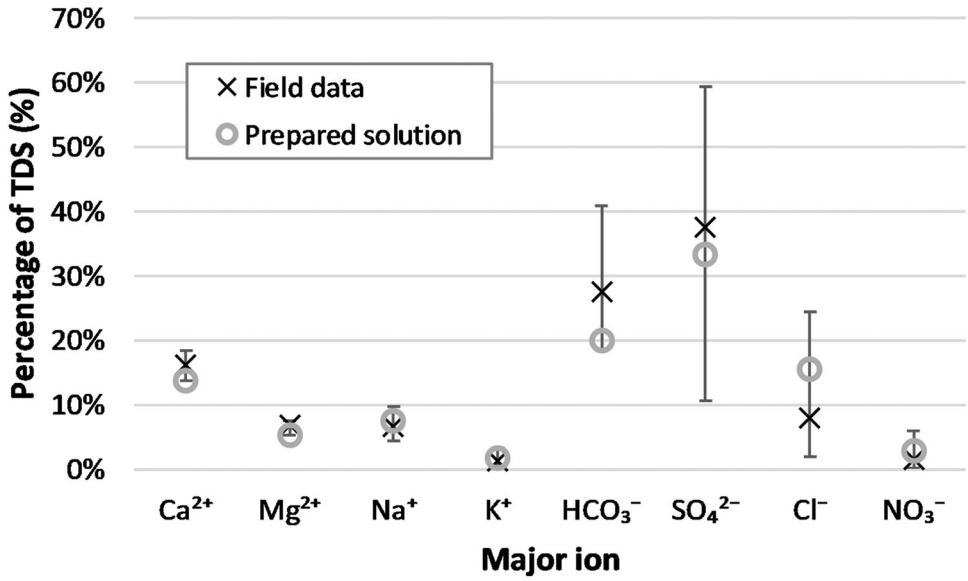

FIGURE 6: Ion composition measured in Sand Branch (average with range) compared to ion composition of artificial samples prepared for use in toxicity testing. TDS = total dissolved solids.

H. azteca. The chronic 25% inhibition concentrations (IC25s) were 1440, 1233, and 3669 mg/L TDS for the respective species. These results indicate that *P. promelas* is the most sensitive to elevated TDS, whereas *H. azteca* is the least sensitive.

Toxicity tests with field-collected species

James Madison University researchers successfully performed two *L. carinata* acute tests and two *I. bicolor* acute tests on artificial Sand Branch samples. Control survival was 90% in both *I. bicolor* acute tests and ranged from 95% to 100% in *L. carinata* tests. In *I. bicolor* tests, a small number of organisms emerged into adults during the test. In the 9/6/21 test, three organisms emerged, and in the 9/20/21 test, two organisms emerged. This is a small percentage of the 120 test organisms (<2.5%) exposed in each test, so the impact on test results is minimal. For LC50 calculation, emerging organisms were considered to have survived the exposure and were not considered dead.

Toxicity results from field-collected species are shown in Table 3. *Leptoxis carinata* results were extremely consistent, with 96-h LC50s of 3327 and 3349 mg/L TDS. The mayfly, *I. bicolor*, was more sensitive to TDS, but results were also more variable. *Isonychia bicolor* 96-h LC50s were 2527 and 1339 mg/L TDS. This is somewhat expected because *L. carinata* were relatively consistent in size (6.75–8.25 mm) and therefore presumably also in age. Field-collected *I. bicolor* represented more variable sizes and ages based on the pattern of growth and molting in comparison to the season and timing of collection.

TDS threshold calculation

Acute and chronic TDS thresholds for Sand Branch were calculated using Equation 1 in accordance with USEPA's guidelines for water quality criteria development (USEPA, 1985). To protect against acute effects, a final acute value was calculated based on 96-h LC50s from toxicity tests with standardized and field-collected species exposed to artificial Sand Branch samples. Acute LC50s ranged from 1511 mg/L TDS for *P. promelas* (96-h LC50) to >4148 mg/L TDS for *H. azteca* (10-d LC50). Acute sensitivity was in the order of *P. promelas* > *I. bicolor* > *C. dubia* > *L. carinata* > *H. azteca* from most to least sensitive. Based on this rank ordering of sensitivity, *H. azteca* data were excluded from

TABLE 2: Toxicity test results of artificial Sand Branch samples using standardized test species

Test	Test period	Endpoint	NOEC (mg/L TDS)	LOEC (mg/L TDS)	IC25 (mg/L TDS)	LC50 (mg/L TDS)[a]	Control performance	QA flags
Ceriodaphnia dubia	6/23/21–	Survival	2074	4148	—	3195	100% survival	None
chronic test	6/30/21	Reproduction	1037	2074	1440	—	30.8 neonates	None
Pimephales promelas	6/23/21–	Survival	1037	2074	—	1511	100% survival	None
chronic test	6/30/21	Biomass	1037	2074	1233	—	0.6853 mg	None
Hyalella azteca	6/23/21–	Survival	4148	>4148	—	>4148	97.5% survival	None
chronic test	7/3/21	Growth	4148	>4148	3669	—	0.0838 mg	None

[a]96-h LC50 for *C. dubia* and *P. promelas* and 10-day LC50 for *H. zteca*.
TDS = total dissolved solids; NOEC = no-observable-effect concentration; LOEC = lowes-observable-effect concentration; IC25 = 25% inhibition concentration; LC50 = median lethal concentration; QA = quality assurance.

TABLE 3: Toxicity test results of artificial Sand Branch samples using field-collected test species

Test	Test period	Endpoint	NOEC (mg/L TDS)	LOEC (mg/L TDS)	96-h LC50 (mg/L TDS)	Control performance	QA flags
Leptoxis carinata acute test	7/5/21–7/9/21	Survival	2678	5236	3327	100% survival	None
L. carinata acute test	8/30/21–9/3/21	Survival	1370	2786	3349	95% survival	None
Geometric mean					3338		
Isonychia bicolor acute test	9/6/21–9/10/21	Survival	2216	4336	2527	90% survival	3 organisms emerged
I. bicolor acute test	9/20/21–9/24/21	Survival	535	1046	1339	90% survival	2 organisms emerged
Geometric mean					1839		

TDS = total dissolved solids; NOEC = no-observable-effect concentration; LOEC = lowest-observable-effect concentration; LC50 = median lethal concentration; QA = quality assurance.

the calculation, whereas data from the four most sensitive species were used to calculate a final acute value of 938 mg/L TDS for Sand Branch (Table 4).

$$S^2 = \frac{\Sigma((\ln \text{GMAV})^2) - \frac{((\Sigma \ln \text{GMAV}))^2}{4}}{\Sigma(F) - \frac{(\Sigma(\sqrt{P}))^2}{4}}$$
$$L = \left(\sum(\ln \text{GMAV}) - \frac{S(\Sigma(\sqrt{P}))}{4}\right) \quad (1)$$
$$A = S(\sqrt{0.05}) + L$$
$$\text{FV} = e^A$$

where GMV is the genus mean (acute or chronic) value, F is the sum of the respective probabilities, P, P is the respective probability for each GMV, calculated as $R/(N+1)$, where R is the respective rank of each *GMV* value and N is the highest rank, and FV is the final (acute or chronic) value.

To protect against chronic effects, a final chronic value was calculated from Equation 1 based on IC25 data from toxicity tests on artificial Sand Branch samples. For the standardized toxicity test species, IC25s were used directly, but for the field-collected species, acute LC50s were translated to chronic IC25s using literature-derived acute-to-chronic ratios.

Researchers at James Madison University searched the USEPA's ECOTOXicology Knowledgebase (USEPA, 2021b) of over a million toxicology results for acute and chronic data for *I. bicolor* and *L. carinata* exposure to major ions. There were two studies that exposed *I. bicolor* to sodium chloride and calculated acute (Echols et al., 2010) and chronic (Echols et al., 2013) effect concentrations. The 96-h LC50 was 3.1 g/L NaCl, and the geometric mean of IC25 values for growth measured as number of exuviae (or molts) over 7 days was 1.1 g/L NaCl. These results equate to an acute-to-chronic ratio of 2.82 for *I. bicolor* exposure to major ions. Applying the same acute-to-chronic ratio to acute results from our study would equate to a chronic IC25 of 652 mg/L TDS. This value was used for *I. bicolor* in the calculation of the final chronic value for TDS.

A search of the USEPA's ECOTOXicology knowledgebase returned no data for *L. carinata* exposure to major ions. For this reason, the acute-to-chronic ratio used for *L. carinata* was an average of the acute-to-chronic ratios for other species used in the present study. Acute-to-chronic ratios were relatively low and ranged from 1.22 for *P. promelas* to 2.82 for *I. bicolor*, with an average acute-to-chronic ratio of 2.09. When this value was applied to *L. carinata* acute data, it resulted in a chronic IC25 of 1597 mg/L TDS. This value was used for *L. carinata* in the calculation of the final chronic value for TDS.

Based on measured IC25s for standardized species and acute-to-chronic ratio-derived IC25s for field-collected species, chronic values ranged from 652 mg/L TDS for *I. bicolor* to 3669 mg/L TDS for *H. azteca*. Chronic sensitivity was in the order of *I. bicolor* >

TABLE 4: Calculation of acute threshold for total dissolved solids in Sand Branch

Species	GMAV (96-h LC50)	*R*	ln(GMAV)	ln(GMAV)^2	*P*	$\sqrt{P}$
Pimephales promelas	1511	1	7.320527	53.590115	0.166667	0.408248
Isonychia bicolor	1839	2	7.516977	56.504947	0.333333	0.57735
Ceriodaphnia dubia	3195	3	8.069342	65.114286	0.5	0.707107
Leptoxis carinata	3338	4	8.113127	65.822831	0.666667	0.816497
Sum	—	—	28.24581	199.94074	1.666667	2.509202
S^2	5.100087	—	—	—	—	—
S	2.258337	—	—	—	—	—
L	6.338337	—	—	—	—	—
A	6.843317	—	—	—	—	—
					TDS (mg/L)	Conductivity (µS/cm)
				FAV	93	1324

FAV = final acute value; TDS = total dissolved solids; GMAV = genus mean acute value; LC50 = median lethal concentration.

TABLE 5: Calculation of chronic threshold for total dissolved solids in Sand Branch

Species	GMCV (IC25)	*R*	ln(GMCV)	ln(GMCV)^2	*P*	$\sqrt{P}$
Isonychia bicolor	652	1	6.48024	41.993515	0.166667	0.408248
Pimephales promelas	1233	2	7.117206	50.654614	0.333333	0.57735
Ceriodaphnia dubia	1440	3	7.272398	52.887778	0.5	0.707107
Leptoxis carinata	1597	4	7.375963	54.404831	0.666667	0.816497
Sum	—	—	28.24581	199.94074	1.666667	2.509202
S^2	5.227924	—	—	—	—	—
S	2.286465	—	—	—	—	—
L	5.627151	—	—	—	—	—
A	6.13842	—	—	—	—	—
					TDS (mg/L)	Conductivity (µS/cm)
				FCV	463	654

TDS = total dissolved solids; FCV = final acute value; GMAV = genus mean chronic value; IC25 = 25% inhibition concentration.

P. promelas > *C. dubia* > *L. carinata* > *H. azteca* from most to least sensitive. Based on this rank ordering of sensitivity, *H. azteca* data were excluded from the calculation, whereas data from the four most sensitive species were used to calculate a final chronic value of 463 mg/L TDS for Sand Branch (Table 5).

Based on the USEPA's methodology for water quality criteria development (USEPA, 1985), an acute TDS threshold of 938 mg/L and a chronic TDS threshold of 463 mg/L were developed for Sand Branch and used as targets for TMDL development. Using the Sand Branch TDS to conductivity regression, these thresholds translate to protective conductivity values of 1324 µS/cm for acute effects and 654 µS/cm for chronic effects.

DISCUSSION AND CONCLUSION

Use of field-collected toxicity test species

The use of field-collected species for toxicity testing is enticing because the selected test species can represent more ecologically relevant and potentially more sensitive members of the aquatic community than currently represented by standardized laboratory-cultured species. In the present study, we utilized a field-collected mayfly (*I. bicolor*) and a snail species (*L. carinata*) in addition to standardized toxicity test species. This increased the ecological relevance of testing, because both species are common members of the benthic macroinvertebrate community in free-flowing Eastern US streams. The addition of the mayfly also increased the sensitivity of testing, with *I. bicolor* being the most sensitive of the five tested species. This is consistent with the findings of others who have identified mayflies as more sensitive to TDS than common test species (Clements & Kotalik, 2016; Dunlop et al., 2008; Kefford et al., 2012; Kunz et al., 2013; Pond, 2010). In comparison with 12 other macroinvertebrate taxonomic orders, Dunlop et al. (2008) found mayflies to be the most sensitive to salinity in Eastern Australian streams.

Despite the advantages of improved ecological relevance and increased sensitivity, toxicity testing with field-collected species continues to provide challenges, particularly with sensitive mayfly species. In the present study, preliminary method development testing and evaluation of four different field-collected species were conducted prior to selecting *I. bicolor* and *L. carinata*. Two other mayfly species, *Ephemerella hispida* and *Maccaffertium* sp., were also evaluated for use based on availability at the field site, conduciveness of their life cycle to routine use, ease of field identification, success in handling and acclimation to laboratory conditions, acceptable control survival, and sensitivity to TDS. In general, the snail species was by far the most conducive to laboratory toxicity testing. It was abundant throughout the field season, easy to collect and identify, responded well to laboratory acclimation, and exhibited high control survival in initial testing. Snails were less sensitive to TDS than mayfly species, but much more conducive to laboratory toxicity testing.

The snail species also produced greater precision in results. In duplicated tests, *L. carinata* LC50s differed by less than 1% in our study. By comparison, duplicated *I. bicolor* tests produced LC50s that differed by 61% (relative percent difference calculated for LC50s of 2527 and 1339 mg/L TDS). These results are consistent with the findings of Echols et al. (2010), who found similar variability in *I. bicolor* exposure to NaCl, where 96-h LC50s ranged from 2250 to 3780 mg/L (relative percent difference of 51%). Echols et al. (2013) concluded that variability in the size and age of field-collected mayflies contributed to this variability in toxicity test sensitivity.

Among the mayfly species, life cycle patterns periodically disrupted field availability and testing. *E. hispida* were abundant in the field in late spring, but mass emergence of the species in May limited the availability of nymphs large enough to easily capture for the remainder of the summer field season. *I. bicolor* emerged predominantly in June, but nymphs of various instars remained abundant throughout the field season. *Maccaffertium* sp. emerged at a high rate throughout the field season and frequently disrupted testing. Due to their instinct to cling to surfaces, *Maccaffertium* sp. were the most difficult to handle, but all three mayfly species acclimated reasonably well to laboratory conditions and moderately hard synthetic freshwater. Control

survival during initial testing was highly variable for *I. bicolor* (averaging 78% and ranging from 55% to 95%) and suboptimal for *Maccaffertium* sp. (70%–75%). Throughout method development, however, control survival of *I. bicolor* was increased by reduced handling and initiating aeration of test containers. Of the mayfly species, *I. bicolor* was selected for continued use in the present study based on relative field abundance and availability, minimal interference from emergence (outside of the June timeframe), easy identification and handling, and acceptable control survival with the use of aeration.

Echols et al. (2013) experienced similar challenges with *I. bicolor* and *Maccaffertium* sp. Seasonal patterns of emergence greatly altered the availability of organisms throughout the year, and *Maccaffertium* sp. were unavailable during certain seasons. This makes the use of field-collected organisms difficult for consistent testing regimes. Control survival for *I. bicolor* was also highly variable, ranging from 20% to 100% at 7 days (Echols et al., 2013). Echols et al. (2013) concluded that control survival was particularly problematic when test temperatures were above 20 °C. These challenges of year-round availability and necessary techniques and conditions to optimize control survival continue to make the use of field-collected mayfly species for routine toxicity testing difficult to implement.

In a literature review and workshop discussion, Sibley et al. (2020) identified a number of research needs in this area. For field-collected species, the workshop recommended evaluating and optimizing holding, feeding, and testing conditions, understanding dietary requirements, and developing methods to better quantify and standardize organism age. The workshop also recommended steps for continuing efforts to develop laboratory culturing methods for sensitive mayfly species. These steps included further work to define physiological limits, enhance reference testing and control charting, and implement interlaboratory testing. Laboratory culture and testing methods have recently been developed for the mayfly, *Neocloeon triangulifer* (Soucek & Dickinson, 2015; Soucek et al., 2018, 2020), but this method has yet to be standardized and approved for national use.

In light of ongoing work to better refine and optimize field-collected mayfly tests or develop interlaboratory validated culture-based mayfly tests, such tests may not be appropriate for stand-alone compliance testing use at this time. However, such tests can fill the current void for sensitive benthic macroinvertebrate species if used within a broader testing program. In our study, field-collected mayfly and snail species were used in conjunction with other standardized toxicity test species and as part of broader field bioassay and chemical monitoring approaches.

Site-specific TDS threshold development approach

The present study used a novel approach to develop a site-specific and ecologically-relevant TDS threshold. Statistical calculations from the USEPA guidelines for water quality criteria development (USEPA, 1985) were used to develop TDS thresholds from toxicity tests of standardized and field-collected species on artificial samples prepared to match the ion composition of the impaired stream. This approach can be used to establish regional freshwater aquatic life criteria for generalized parameters such as TDS. It can also be used to developed site-specific thresholds for waterbodies impacted by salinization. In the present case study, the approach was used in TMDL development to set acute thresholds of 938 mg/L TDS or 1324 µS/cm conductivity and chronic thresholds of 463 mg/L TDS or 654 µS/cm conductivity to restore aquatic life.

The chronic threshold developed in our study is slightly higher than those developed in other TDS TMDLs in Virginia to date. Among eight other TMDLs, thresholds ranged from 334 mg/L TDS (VDEQ, 2006) to 373 mg/L TDS (Virginia Department of Mines, Minerals and Energy & VDEQ, 2007). These thresholds, however, were developed based on monitoring from unimpaired reference watersheds and not from toxicity test data. As such, they represent conditions that would be supportive of healthy aquatic life, but do not represent true thresholds. Thresholds of impairment would have to be higher than these reference watershed-based thresholds, so the toxicity-derived threshold of 463 mg/L TDS in our study appears to be at least internally consistent with other Virginia TMDLs.

Throughout the toxicology literature, protective threshold values developed for TDS or conductivity have been highly variable, from conductivity thresholds as low as 300 µS/cm (Cormier et al., 2018; USEPA, 2011) to TDS thresholds over 2000 mg/L (Chapman et al., 2000). A wide range in threshold values for TDS is understandable given the varying sensitivity of species used for testing (Kefford et al., 2012; Soucek et al., 2011) and the varying toxicity of TDS mixtures based on the ion composition (Goodfellow et al., 2000; Mount et al., 1997). To account for differences in ion composition, the present study prepared samples with site-specific ion composition as other researchers have done in Canadian lakes (Chapman & McPherson, 2016) and for mining effluents in Appalachia (Kennedy et al., 2004, 2005) and Alaska (Chapman et al., 2000).

To account for the differences in species sensitivity, our study used five species including a sensitive mayfly species, because previous studies have demonstrated that mayflies are among the most sensitive to TDS effects (Clements & Kotalik, 2016; Dunlop et al., 2008; Kefford et al., 2012; Kunz et al., 2013; Pond, 2010). Total dissolved solid thresholds based on standardized toxicity testing species have generally been higher than for approaches that have utilized more sensitive mayfly species, such as the present study. For instance, Kennedy et al. (2005) determined a chronic TDS threshold of 2331 mg/L for *C. dubia*, Chapman et al. (2000) determined TDS thresholds of 1100 mg/L TDS for *Chironomus tentans* and >2000 mg/L for *Oncorhynchus mykiss*, and Chapman and McPherson (2016) determined TDS thresholds of 1100, 1390, 1470, and 2200 mg/L TDS for *Daphnia magna*, *Chironomus dilutus*, *Pseudokirchneriella subcapitata*, and *P. promelas*, respectively. In our study, *I. bicolor* was the most sensitive species, followed by *P. promelas*, *C. dubia*, and *L. carinata*. *Hyalella azteca* was the least sensitive to TDS, which is unsurprising, considering that *H. azteca* can be successfully used in the testing of saline estuarine sediments (Nebeker & Miller, 1988).

Studies that used mayfly species determined much lower TDS thresholds that are consistent with those developed in the present study. In simulated coal mine effluent, Kennedy et al. (2004) determined a no-observable-effect concentration of 619 µS/cm conductivity for 7-day *I. bicolor* survival and growth (measured as molted exuviae per replicate), consistent with the 654 µS/cm conductivity chronic threshold developed in our study. Similarly, Pond (2004) showed that on surface mined lands, mayfly taxa decreased significantly at conductivity levels above 500 µS/cm. USEPA (2011) and Cormier et al. (2018) developed a much lower protective benchmark of approximately 300 µS/cm conductivity for the Appalachian region, but some have been critical of the field-based approach employed (Roark et al., 2013). Clements and Kotalik (2016) used mesocosm studies to confirm that this benchmark was protective of aquatic insect communities in naturally low-conductivity streams, but they also found that thresholds increased for communities with naturally higher background conductivity. Effect concentration 20% values were 42% lower for communities with low background conductivity (60–70 µS/cm) than for communities with higher background conductivity (200–250 µS/cm). Sand Branch is in the Trap Rock Conglomerate Uplands and Triassic Lowlands ecoregion, where background conductivity is naturally higher. Among 43 VDEQ monitoring stations in this ecoregion, the median conductivity is 310 µS/cm, so it is reasonable that a site-specific threshold in this ecoregion would be considerably higher than the 300 µS/cm proposed by USEPA (2011) and Cormier et al. (2018). An analysis of the 43 VDEQ monitoring stations in the Trap Rock Conglomerate Uplands and Triassic Lowlands ecoregion also provides reasonable assurance that the 654 µS/cm threshold developed from our study can be achieved. The chronic threshold of 654 µS/cm conductivity is at the 90th percentile of stations within the ecoregion, and only four stations exceed this value: the two stations on Sand Branch and two stations on Cub Run near the confluence with Sand Branch.

In conclusion, the present study presented a novel approach to developing aquatic life thresholds for TDS, a generalized measure of salinization. This approach accounts for site-specific and ion-specific toxicity, considers the range of sensitivity among ecologically relevant aquatic species, and is consistent with the literature on TDS toxicity. Although more research is needed to improve and standardize toxicity test methods for sensitive mayfly species, our study provides a potential model for establishing TDS thresholds for waterbodies impacted by salinization and for the development of regional freshwater aquatic life criteria for TDS.

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Author Contributions Statement—**Robert N. Brent:** Conceptualization; Formal analysis; Methodology; Project administration; Writing—original draft. **Jared Kunkel:** Investigation; Validation; Writing—review & editing. **Zachary Tomek:** Investigation; Validation; Writing—review & editing. **Dalton Buchardt:** Investigation; Validation; Writing—review & editing. **Peter F. DeLisle:** Investigation; Formal analysis; Validation; Supervision; Writing—review & editing. **Sarah Silvers:** Funding acquisition; Project administration; Resources; Writing—review & editing.

Data Availability Statement—Most data are available with the article. The authors wish to provide additional supplemental data to the public on request. The project involves data collected by a state agency (Virginia Department of Environmental Quality), an academic institution (James Madison University), and a corporation (Coastal Bioanalysts). As such, the authors prefer to not post a combined dataset with agency and non-agency data on a third-party site when the totality of state agency data is more generally available and routinely updated through the National Water Quality Database. Rather, the authors prefer to respond to specific requests (brentrn@jmu.edu) to ensure that the data provided meets the needs of the request and accurately reflects the individual sources of data.

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