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Original Articles

Benthic macroinvertebrate community response to salinization in headwater streams in Appalachia USA over multiple years

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ABSTRACT

Salinization of freshwaters threatens aquatic ecosystems globally via effects that include reductions in benthic macroinvertebrate diversity. Enhanced understanding of salinity effects in freshwater ecosystems could aid mitigation efforts. Salinization effects on benthic macroinvertebrate community structure were quantified in a 4.5-year study of 25 headwater streams spanning a gradient of salinity in the central Appalachian mountains of USA. Community structure as sampled was strongly seasonal, justifying treatment of Spring and Fall data separately. Stream communities became increasingly different from reference condition as salinity increased, with stronger relationships between biota and salinity in Spring than in Fall. Intra-seasonal variation in community structure was also revealed across sampling dates. Genera of the order Ephemeroptera appeared as most sensitive to salinity, as indicated by rapid declines in richness and relative abundance as salinity increased. Plecoptera and Trichoptera richness and relative abundance metrics appeared as less sensitive to salinity, and some Plecoptera genera exhibited increased relative abundance at elevated salinity. Other community metrics were weakly associated with salinity and exhibited greater variability than Ephemeroptera metrics, suggesting that Ephemeroptera richness and relative abundance are sensitive indicators with which to gauge onset of salinity effects. Declines in richness and relative abundance of non-Baetidae Ephemeroptera were associated strongly with increasing salinity, with effects observed at specific conductance levels as low as 200 µS/cm in Spring based on seasonal discrete conductivity sampling. Chronic salinization has persistent effects on community structure over multiple years, but those effects are not uniform among taxa or consistent across seasons. Our findings suggest that effective detection of the onset of community change in salinized streams is best accomplished using Spring data and focusing on community metrics that incorporate salt-sensitive Ephemeroptera taxa.

1. Introduction

Salinization of freshwaters threatens aquatic ecosystems globally, with increased dissolved concentrations of major ions (i.e., Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, $SO_4{}^{2-}$, $HCO_3{}^{-}$) from a variety of sources associated with alterations of benthic macroinvertebrate community structure (Cañedo-Argüelles et al., 2013). There is increasing scientific recognition of the need for water management policies to mitigate salinization impacts to freshwater biota (Cañedo-Argüelles et al., 2016). However, much remains unknown about how salinity affects aquatic communities.

In the USA, central Appalachian headwater streams are subject to long-term salinization from surface coal mining as natural waters can leach major ions from mine spoils, raising in-stream salinity in excess of background levels for decades, at minimum (Pond et al., 2014; Evans et al., 2014). Assessment of mining-induced salinization effects has been largely conducted using field-based observational surveys, which have documented substantial alterations of the benthic macroinvertebrate community as specific conductance (SC – a salinity surrogate) increases (e.g., Paybins et al., 2000; Hartman et al., 2005; Pond et al., 2008; Gerritsen et al., 2010; Bernhardt et al., 2012). Investigations of such alterations have used differing methodologies for measuring and relating salinity stressors and biological effects, limiting comparability among studies. Biotic effect levels of SC ranging from < 200 to > 900 µS/cm have been reported in other studies, but Timpano et al. (2015) noted that those studies varied in their choice of salinity measure (predictor), biological endpoint (response), and modeling framework.

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Freshwater salinization has been associated with aquatic life impacts globally, including in South Africa (Goetsch and Palmer, 1997), Australia (Kefford, 1998; Wright et al., 2017), France (Piscart et al., 2005), Spain (García-Criado et al., 1999; Cañedo-Argüelles et al., 2012), and Germany (Arle and Wagner, 2013). In those studies, impacts were characterized as declines in one or more measures of benthic macroinvertebrate community structure, such as richness, diversity, abundance, or evenness. In addition to loss of salt-sensitive taxa, invasion of salt-tolerant non-native species was observed (Piscart et al., 2005).

Laboratory toxicity testing has shown that dissolved salts, when elevated above natural background concentrations, can cause lethal and sublethal effects to a variety of freshwater invertebrates (Chapman et al., 2000; Kennedy et al., 2003; Soucek and Kennedy, 2005). Several recent laboratory experiments have focused on measuring salt toxicity to mayflies (Insecta:Ephemeroptera), a group particularly sensitive to salinity in mining-influenced streams (Johnson et al., 2015). Chronic experiments with the mayfly *Neocloeon triangulifer* (Ephemeroptera:Baetidae) have demonstrated lethal and sublethal effects from individual salts (e.g., Johnson et al., 2015; Soucek and Dickinson, 2015), as well as from ion mixtures typical of surface waters receiving alkaline mine drainage (e.g., Kunz et al., 2013). Ongoing experimental research into ecotoxicology of salts and salt mixtures is illuminating mechanisms and responses of indicator species, but field-based data form the basis of current efforts to establish salinity "benchmarks"- minimum SC levels at which biotic community alterations from a reference condition are observed (Cormier et al., 2013). Therefore, quantifying field-based salinity sensitivity is an important complement to laboratory experimentation as a means for gaining improved understanding of salinity effects on benthic macroinvertebrates.

Biological assessments commonly use benthic macroinvertebrates as indicators; results of such assessments can vary based on taxonomic composition of samples, response metrics derived therefrom, and choice of metric endpoint used to define effects (Cao and Hawkins, 2011). In addition, choice of SC predictor and modeling framework can influence conclusions drawn regarding salinity levels associated with specific biological effects. Therefore, we sought to assess salinity-biota relationships while accounting for sources of variation in water quality of headwater streams subjected to mining influence. Our objectives were to characterize response to salinity of benthic macroinvertebrates at the community level, identify relative salt-sensitivity of individual genera and taxonomic groups, model salinity response of the most salt-sensitive community metrics, and determine minimum SC levels associated with community change.

2. Methods

2.1. Stream selection

Twenty-five forested first-order streams were selected for assessment of SC and benthic macroinvertebrates in the central Appalachian coalfield of Virginia and West Virginia. Streams were selected to minimize influence by non-salinity stressors on benthic macroinvertebrate communities (Timpano et al., 2015). Twenty streams with mining-induced salinity elevated above background were considered "test" streams, and five streams with minimal disturbance and no mining were considered "reference" streams. See Timpano et al. (2015, 2018) for details regarding stream selection criteria, selection methods, and stream attributes.

2.2. Benthic macroinvertebrates

We surveyed the benthic macroinvertebrate community in each stream over a 4.5-year period during Fall (2011, 2012, 2013, 2015) and Spring (2012, 2013, 2014, 2016) seasons. Timing of sample collection was consistent during each season across years, with most samples collected during April (median date = 16 April) and October (median date = 20 October). Samples from all streams were generally collected within approximately nine days of one another during a season. Exact timing of biological sampling within a season was influenced strongly by stream flow, with no sampling occurring during flow extremes. We took care to collect all samples during baseflow (i.e., stream flow not influenced by storm flow) and at least seven days after extreme flows that were estimated to have caused scouring. Baseflow sampling helped to ensure adequate time for recolonization of substrate after high flow and allowed for concurrent collection of baseflow water samples and measurement of SC.

Samples were collected using the single-habitat method for high-gradient streams as per U.S. EPA Rapid Bioassessment Protocols (Barbour et al., 1999). Using a 0.3-m D-frame kicknet with 500-μm mesh, a single composite sample (approximately 2 m^2) composed of six 1 × 0.3-m kicks was collected along a 100-m reach at each stream. Because of presence of Endangered Species Act-listed crustaceans and mollusks in the region, all specimens from those groups were returned to the streams unharmed. Samples were preserved in 95% ethanol and returned to the laboratory for sorting and identification.

Biological samples were sub-sampled randomly to obtain a 200 (± 10%) organism count following Virginia Department of Environmental Quality methods (VDEQ, 2008), which are adapted from RBP methods (Barbour et al., 1999) and are comparable to methods used by West Virginia Department of Environmental Protection (WVDEP, 2015). Specimens were identified to genus/lowest practicable level using standard keys (Stewart et al., 1993; Wiggins, 1996; Smith, 2001; Merritt et al., 2008), except individuals in family Chironomidae and subclass Oligochaeta, which were identified at those levels.

2.3. Stream water chemistry

Water temperature, dissolved oxygen, SC, and pH were measured *in situ* at baseflow in Spring and Fall at the time of biological sample collection with a calibrated handheld multi-probe meter (Hanna HI-9828 - Hanna Instruments, Inc., Woonsocket, Rhode Island, USA; or YSI Professional Plus – YSI, Inc., Yellow Springs, Ohio, USA). To assess dissolved ion concentrations, grab-samples of water were collected approximately monthly (≤19 times) at each stream during baseflow (i.e., stream flow not influenced by storm flow) from May 2011 through April 2013, as well as concurrently with biological sampling. Stream water samples were filtered immediately using polyvinylidene difluoride syringe filters with a nominal pore size of 0.45 μm and stored in sterile polyethylene sample bags. Aliquots for analysis of cations were preserved to pH < 2 with 1 + 1 concentrated ultrapure nitric acid. All samples were transported to the laboratory on ice and stored at 4 °C until analysis.

In the laboratory, water samples were analyzed for major cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), and dissolved trace elements (Al, Cu, Fe, Mn, Se, Zn) using either an inductively coupled plasma-optical emission spectrometer (Varian Vista MPX ICP-OES w/ICP Expert software, Varian Instruments, Walnut Creek, California USA) or an inductively coupled plasma-mass spectrometer (Thermo Electron X-Series ICP-MS, Thermo Fisher Scientific, Waltham, Massachusetts USA) (APHA, 2005). An ion chromatograph (Dionex DX500, Dionex Corp., Sunnyvale, California USA) was used to measure Cl^- and SO_4^{2-} (APHA, 2005). Total alkalinity was measured by titration with standard acid (APHA, 2005) using a potentiometric auto-titrator (TitraLab 865, Radiometer Analytical, Lyon, France). Concentrations of the anions CO_3^{2-} and HCO_3^- were calculated from alkalinity and pH measurements (APHA, 2005). Water chemistry data were examined to determine if trace elements were present at chronically toxic levels (exceeding criteria continuous concentrations [CCC]) (USEPA, 2012, 2016; ILEPA, 2001). This was done to determine if there was potential for trace element toxicity to confound observed salinity-biota associations.