EFFECTS OF WINTER ROAD SALT APPLICATION AND EPISODIC PULSES ON SOUTHERN APPALACHIAN HEADWATER STREAM MACROINVERTEBRATES

A Thesis
by
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Abstract

EFFECTS OF WINTER ROAD SALT APPLICATION AND EPISODIC PULSES ON SOUTHERN APPALACHIAN HEADWATER STREAM MACROINVERTEBRATES

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In many regions of the United States current winter road management operating procedures require that deicing agents be applied to roads before or during each of the winter snow and ice events. Long term water monitoring projects have indicated significant salinization of surface and ground water bodies raising concern from scientists about the impact on aquatic organisms and drinking water quality. Boone, NC is unique in that it has a winter climate comparable to southern New England due to its high elevation in the Southern Appalachian Mountains (982 m), precipitation (132 cm) and quickly developing urban center. Eight headwater streams originating in Boone combine to form the Upper South Fork of the New River. Data collected from these eight streams with sondes from 2010 to 2015 provided water chemistry data (temperature represented by coefficient of variation of temperature, pH, and specific conductivity) and impervious and forested area were determined and correlated to NC DEQ Qual 4 benthic macroinvertebrate collections for which NC biology index (BI), Shannon diversity index, family and genus richness were calculated. Four of the streams have increased urbanization with elevated specific conductivity levels due to road salt in the winter, and four of the streams are less impacted and serve as reference streams. Spearman correlations indicate that the coefficient of variation of temperature, specific conductivity, percent impervious and forested area all have significant moderate correlations with family and genus NCBI values, while specific conductivity, percent impervious and forested area have significant strong correlations with Shannon Diversity. Partial least squares regression indicated that coefficient of variation of temperature, specific conductivity, percent impervious and forested area were significant predictors of observed variation in macroinvertebrate health of the family and genus level. The PLS indicated that specific conductivity, percent impervious and forested area were significant predictors of observed variation in macroinvertebrate diversity. Variations in temperature throughout the seasons and elevated specific conductivity levels have an overall negative impact on macroinvertebrate communities. Additionally the impact of pulse NaCl exposure on aquatic macroinvertebrates survival were examined utilizing the most frequently experienced specific conductivity spikes (2,000 µS cm^{-1}) and the worst case scenario spikes (10,000 µS cm^{-1}) experienced in Boone Creek through the winter months from 2010 to 2015. Exposures to the spikes alone occurred along with exposures that aimed to simulate environmental concentrations. Conductivity levels of 400 µS cm^{-1} and 900 µS cm^{-1} were used to simulate the most frequently experienced levels and the worst case scenario levels, respectively. The 2,000 µS cm^{-1} pulse resting at reference levels (<50 µS cm^{-1}) reduced survivorship up to 83.75% ± 1.8, and 2,000 µS cm^{-1} resting at 400 µS cm^{-1} reduced survivorship up to 73.75% ± 4.6. The 10,000 µS cm^{-1} resting at reference levels (<50 µS cm^{-1}) reduced survivorship up to 10% ± 3.2, and 10,000 µS cm^{-1}

resting at 900 μS cm^{-1} reduced survivorship up to 28.75% ± 6.3. This study suggests that as pulse duration increases for all four scenarios we see a decrease in overall macroinvertebrate survival. An LC_{25} was reached with the 2,000 μS cm^{-1} resting at 400 μS cm^{-1} during the 18 hour pulse duration, and an LC_{50} was reached with the 10,000 μS cm^{-1} resting at 900 μS cm^{-1} during the 6 hr pulse duration. Our research suggests that specific conductivity levels measured in Boone, NC are having a negative impact on overall macroinvertebrate health in the headwater streams of the Upper South Fork of the New River.

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The author would like to express his deepest gratitude to the committee chair, Dr. Shea Tuberty, for providing guidance and displaying patience throughout the graduate program, and for providing the opportunity to work in his laboratory on a unique and meaningful project; Dr. Michael Gangloff, for giving advice on the methodology, data analysis, and aspects regarding presentation of the project; Dr. William Anderson, for providing insight on data analysis and the hydrology side of the project. The author is grateful for his various funding sources including Sigma Xi Grants in Aid of Research, Appalachian State University Office of Student Research and the Cratis D. Williams Graduate School. Without these funds, a large portion of the work would not have been possible. The author is appreciative of contributions made by the following individuals to insure the timely completion of the thesis. Dr. Stephen Klaine (Clemson Univ.) for providing insight on pulse exposures; Dr. Michael Madritch for his instruction in multivariate statistical procedures used in the data analysis; Alex Albers for her guidance with statistical analyses; Richie Hum for aiding in the creation of maps for the study regions and aiding in the use of statistical software; Dr. Derek Martin and Christopher Ely for their help with water parameter data and map creation; Jordan Lollar for aiding in collection of insects and experiments and for creating the macroinvertebrate length dataset; and David Nielsen and Timothy Jones for collecting macroinvertebrates with me on cold and rainy days.

Dedication

I would like to dedicate this thesis to my parents and brother (Brian, Linda and Tyler Fleetwood), my friends and my significant other. Without your support my time here at Appalachian State University would have been far less enjoyable and exponentially more stressful.

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Foreword

Chapters 1 and 2 of this thesis manuscript will be submitted to the journal, *Environmental Toxicology and Chemistry*. The thesis has been formatted according to the style guide for this journal for rapid acceptance with minimal revisions.

Introduction

Snow and ice-covered roads during the winter months are hazardous to the public, and increase risk of injury along with negatively impacting economic activity [1]. Road salt is one of the favored methods for the deicing of roadways because it is easy to use, effective, and has a relatively low cost [1, 2, 3, 4]. Historically, the application of road salt has been relied on to provide safe passage for people traveling during winter storm events [1, 3]. A study conducted by Marquette University investigated the number of highway incidents that occurred during winter-road conditions and the effectiveness of road salt in reducing collisions. The results supported the conclusion that road-salt reduced the amount of crashes and the cost of injuries and accidents by 88% and 85% respectively [1]. A study conducted by the University of Waterloo investigated the impact on winter-road collisions when solely using road salt for winter-road maintenance, and the combination of road salt with plowing for winter-road maintenance [5]. A 51% reduction in the collision rate occurred when road salt was used alone, and a 65% reduction in the collision rate occurred with road salt was used in conjunction with plowing [5]. The reduction in collisions is lower than the Marquette University study, but there is a definite negative relationship between road salt used for winter-road maintenance and the amount of winter-road collisions [5].

Urban land cover has increased by 186,000 km^2 in 62 years, 1945 to 2007, and this increase will lead to increased road salt application for deicing during snow/ice events which will wash into the nearest body of water after becoming incorporated with the snow/ice [4, 6]. Godwin et al., 2002 and Corsi et al., 2015 have shown that there is a positive relationship between aquatic ecosystem exposure to road salt and increased urban development with time. Elevated salt concentrations have been found to exert a negative impact on the health of aquatic organisms [8, 9, 10]. Application logs starting in 2006 and ending in 2011 show 19.5 million metric tons of road salt were applied each year to Northern US roads [4]. Increase chloride levels have been observed in rivers, groundwater, inland lakes, and even the Laurentian Great Lakes [4].

Salinity is the property of water which results from the shared input of all disassociated mineral salts and the most common contributors are matrix ions that include NaCl [3]. Salinity can be measured in many ways but the most common method is electrical conductivity. Electrical conductivity, on the most basic level, is the ability of a substance to conduct an electrical current [3, 11, 12] and there is a positive correlation between the concentration of dissolved ions in a body of water and the ability to pass an electrical current [3]. As the concentration of dissolved ions in the water increase it will lead to a higher measured conductivity value, and vice versa, which is reported utilizing the unit microSiemens per centimeter ($\mu S\ cm^{-1}$). The standardized method for recording is specific conductivity which is a measurement that is made at or corrected to 25^0C [3, 11].

Salinity exposure, whether it be naturally occurring in a marine ecosystem or occurring in a freshwater ecosystem as a pollutant, impacts aquatic organisms (fish, amphibians, mussels, and invertebrates) via direct contact [3]. Aquatic macroinvertebrates have an especially high risk of exposure due to the fact that their respiratory structures (gills on outside of abdomen, etc.) are in direct contact with any dissolved ions that are in the water. All animals have specific structures that are utilized to transport nutrient ions, and control their individual ionic and osmotic balance [13, 14, 15, 16]. These cell membrane specific structures and functions only work within a specific range of salinities, and the range of salinities will be directly tied to the evolutionary history of the specific organism [13, 17].

Ephemeroptera (mayflies) evolved in low-salt environments. They have adapted to freshwater conditions via their epithelium, which is selectively permeable to the uptake of certain ions and less permeable to larger ions and water [3]. The relationship of this group and environments with varying conductivity levels (background = ≤200 µS cm-1; high = > 1,500 µS cm-1; these values were set by the U. S. EPA) have been extensively studied [3]. The data show that environments near or below background conductivity levels had 99.2% (852 out of 859) of Ephemeroptera (relative abundance) were present and only 0.8% (7 out of 859) that were absent [3]. The data also shows that environments with high conductivity had 45% (50 out of 111) of Ephemeroptera (relative abundance) were present and 55% (61 out of 111) were absent [3]. Ephemeroptera seem to be the model order of aquatic macroinvertebrates used to show the impact of increased levels of conductivity but other groups of macroinvertebrates also are impacted by conductivity in similar ways [18]. Pond, 2010, show that environments near or below background conductivity levels (<150 µS cm^{-1}) had 100% (163 genera) of total West Virginia benthic invertebrate genera, and 100% (104 genera) of total Kentucky benthic invertebrate genera. High conductivity environments (≥1500 µS cm^{-1}) had a 24.5% (123 genera) reduction in West Virginia benthic invertebrate genera, and a 44.2% (58 genera) reduction in Kentucky benthic invertebrate genera [19].

Increased aqueous salts are dissolved ions that are readily available for uptake in freshwater environments, and will lead to ion imbalances within freshwater organisms that cause a disruption of normal neural activity [13, 14]. Too many Cl^- ions will excessively inhibit nerve activity, and this inhibition will result in paralysis and consequently death [3, 20, 21]. Increased salt levels in freshwater systems can be extremely problematic, because studies have shown that this phenomenon can occur in all animals with a nervous system [15].

Numerous studies have investigated the impact of increased conductivity levels and salinity levels on aquatic organisms. Increased salinity and conductivity is caused by the total concentration of dissolved ions, and these major ions are the primary culprits: Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, SO_4^{2-}, CO_3^{2-}, and HCO_3^- [22]. Investigators have examined the impact of the major ions on aquatic life and utilizing varying concentrations have tried to identify various toxicity levels such as lethal concentration to 50% of test organisms (LC_{50}), LC_{25}, and effect concentration to 25% of test organisms (EC_{25}) [23]. Investigators also have used non-lethal endpoints when studying the impact of major ions such as amount of drift by macroinvertebrates, total community metabolism, community composition, growth and reproduction of individuals, and monitoring of biotic indices of an impacted site. Investigators conducting these experiments also utilize experimental concentrations that are derived from field sampling. The standard water quality measurements (dissolved oxygen, pH, temperature, specific conductivity, etc.) of local streams typically allow experimenters to justify the experimental levels that will be utilized, and these measurements will typically be taken from data sonde multi-year water monitoring projects or from grab samples taken from set locations over a certain amount of time.

Salinity toxicity experimentation has led to the establishment of freshwater aquatic life standards by the Environmental Protection Agency (EPA). These standards help ensure that aquatic communities will persist when exposed to these levels in their environment. Chronic exposure to invertebrates is defined as a four day average, and the water quality standard established for the southern Appalachian Mountains for specific conductivity is 300 $\mu S\ cm^{-1}$ and a chloride concentration of 230 $mg\ L^{-1}$ [3, 4, 10]. The acute exposure water quality standard for invertebrates, a one hour average, is approximately 1,720 $\mu S\ cm^{-1}$ and 860 $mg\ L^{-1}$ respectively [3, 4, 10]. The standards set for chloride concentration are meant to be exceeded only once every three years [24]. It is important to note that these water quality standards were established for the region of the country that experience mountain mining, hydraulic fracturing, and de-icing road salt practices. Studies have shown that the chronic toxicity benchmark provides a reasonable level of protection for most aquatic insect groups. However, near the chronic benchmark certain taxonomic groups are impacted greater than others. Mayflies are very sensitive to elevated conductivity levels, and near the chronic toxicity benchmark there was an increase in mayfly drift, a reduction in community metabolism, and a reduction in the abundance of certain mayfly groups (e.g. Baetidae and Heptageniidae) [25]. Increased conductivity values ($> 1{,}200\ \mu S\ cm^{-1}$, which is less than the acute toxicity benchmark) were shown to have major restructuring effects on stream benthic communities [25]. Investigators have shown that standards derived from exclusive laboratory experiments may not be protective of natural benthic communities. However, Clements and Kotalik [25] established that the chronic toxicity benchmark set by the EPA is reasonably protective of aquatic insect communities in streams with naturally low conductivity.

The general consensus within the research concludes that as conductivity of freshwater streams increase there is a corresponding decrease in stream health in the form of reduced biodiversity/richness, increased biotic indices of streams, increased tolerant organisms and decreased sensitive organisms. Contributing new research from the highest elevations of the southern Appalachians has been the goal of a five year water monitoring project in the headwater streams of the USFNR.

To our knowledge, no studies have investigated the impact of pulse NaCl exposure on aquatic macroinvertebrates. Studies have examined the impact of various salts on macroinvertebrate communities, and the salts used in these studies include Na^+, Ca^{2+}, Mg^{2+}, K^+,

Cl^-, SO_4^{2-}, CO_3^{2-}, and HCO_3^- [22]. Contribution of these major ions comes from multiple sources outside of de-icing road salt such as: irrigation practices, mining activities, and hydraulic fracturing [19, 26, 27, 28]. As stated earlier it is important to note that EPA conductivity benchmarks were set utilizing levels from regions of the country that experience year round impacts from mountain mining and hydraulic fracturing. This could cause major discrepancies with studies focusing on watersheds that are impacted solely by de-icing road salt, and have no current impact of mountaintop mining or hydraulic fracturing practices. When investigating specific conductivity increases in natural systems no experiments have focused on the patterns of increase that occur. Based on data from our sondes, specific conductivity increases from road salt in storm water occur very rapidly and peak quickly. These spikes are following by long periods at background conductivity levels or elevated levels for months at a time, which will be referred to as freshening events. The specific conductivity peaks and valleys are important sources of information and need to be analyzed to truly understand how increases in salt concentrations impact the macroinvertebrate community of a watershed.

Methods

Study Area

Boone, NC has an elevation of 982 meters which consequently leads to enhanced precipitation, and a winter climate that compares more closely to southern New England [29]. Boone on average receives approximately 88.9 centimeters of snowfall annually, and this leads to a unique microclimate when compared to the rest of North Carolina or the southeastern US region [29]. Headwater streams originating in Boone combine to form the Upper South Fork of the New River (USFNR) which flows north to meet the North Fork of the New River near the NC/VA state line.

The headwater streams of the USFNR were chosen for this study due to their proximity to Boone and the range of measured conductivities, rates of development and impervious surfaces. They include: Boone Creek (BC), East Fork of the New River (EFNR), Flannery Fork Creek (FF), Goshen Creek (GC), Hodges Creek (HC), Middle Fork of the New River (MFNR), Winklers Creek (WC), and the South Fork of the New River proper (SFNR; represented as State Farm). At each site the Appalachian Aquatic Science Research team (AppAqua) manages In-Situ Inc. Multi-Parameter Water Quality TROLL 9500s (data sondes) that are located on the headwater streams of the USFNR.

Benthic Macroinvertebrate Collections

Macroinvertebrate collections (n=40) were taken from the streams around Boone by groups at Appalachian State University (ASU) (n=34) and the North Carolina Division of Water Quality (n=6). Collections from ASU utilized the Qual-4 Method which includes one riffle-kick, one under bank D-net sweep, one leaf-pack, and visual collections from submerged rocks and woody debris [30]. This method was designed to be conducted only in small streams, which are defined as having a drainage area ≤ 3.0 square miles [31]. All macroinvertebrates are included in calculations to represent the community [30]. All macroinvertebrate collections from ASU were identified down to family and genus and species (if possible with dichotomous key) [17]. Collections from the North Carolina Division of Water Quality were identified to species. North Carolina biotic index (NCBI), represent the relative tolerance of the benthic community to the presence of general stressors on a scale from 0.0 to 10.0, with the lower values indicating pristine conditions and higher values indicating stress [30]. North Carolina biotic index values were calculated for each collection utilizing tolerance values on both the Family (mean of all included genera) and Genus level. Shannon Diversity Index (H), Simpson (D) Diversity Index, richness (S), and evenness (J) were calculated for each collection.

Correlation with USFNR Water Physiochemical Data

This study utilized water physiochemical data through August 22, 2010 to January 31, 2017. Parameters were recorded every fifteen minutes by the data sondes and these include temperature (°C), specific conductivity ($\mu S\ cm^{-1}$), and pH. Additional parameters calculated include coefficient of variation of temperature, percent impervious and forested area (percent impervious and forested were calculated utilizing 1 meter resolution in the Feature Analyst software in ArcMap v. 10.3) for each watershed within the USFNR. Each macroinvertebrate collection was paired with its corresponding daily mean water parameter data; except for the percent impervious and forested area because each watershed was given a single representative value.

Flathead Mayfly (*Heptageniidae*) collection

The project utilized the insect order Ephemeroptera (Mayfly) with a primary focus on the Family Heptageniidae (mean North Carolina tolerance value = 2.44, derived from 28 taxa). Two primary genera were collected from Heptageniidae – *Maccaffertium* and *Epeorus*. Prior studies examining the impact of major ion levels, point and non-point pollution have utilized Heptageniidae [25, 32]. Individuals (n = 1,800) were taken from Flannery Fork at Rocky Creek road [36.189302, -81.676557]. The site was chosen because it is a reference stream and one of the few places that the desired family (Heptageniidae) can be found throughout the year in large quantities while having little to no impact from the surrounding environment. The insects were collected by a standard kick seine procedure or by washing off rocks from riffle habitat, transferred into a shallow pan, and carefully removed from the water using a small piece of Nitex mesh in order to prevent damage to individuals, which could lead to mortality. Once collected, the organisms were transported back to the lab and placed in exposure vessels in the experimental system. Experiments occurred in a natural flow through/recycle system with sufficient aeration and held at a constant temperature of 12^0C. The components of the system include: two Frigid Units Custom Rearing Water Baths 0.9144 m Length X 63.5 cm Width X 12.7 cm Depth, 124 watt Apex Titanium Chiller, Pan World 50PX-X Magnetic Water Pump, Aquatic Eco-Systems Sweetwater Linear Air Compressor, SMART UV Lite Sterilizers, Aquatic Eco-Systems VB 25 Filter Bag, Utilitech Outdoor 2 Outlet Timer, and eight Sylvania Fluorescent Tubes 2.44 m T-12. The exposure vessels were constructed from VWR Glass Petri dishes 100x20 mm, and 20 x15 cm 600 µM Nitex screening.

Specific Conductivity Cumulative Distribution Analysis

In order to analyze the data gathered by the data sondes placed in the outflows of the eight headwater streams in Boone, NC some definitions need to be established. For our study, a "specific conductivity storm event" is any increase in conductivity that is ≥ 15% within an hour, and the duration of the storm were determined by how long it takes for the conductivity readings to return to background levels. This rate of change was selected, because once this characteristic occurred the storm event would surpass either the EPA chronic toxicity 4-day average concentration (300 µS cm^{-1}) or the acute toxicity 1-hour average concentration (1720 µS cm^{-1}) of ambient water quality criteria. A ‘pulse’ exposure is when the conductivity of a system spikes drastically in comparison to the background levels. The term ‘pulse exposure’ will be important when detailing the exposure part of the experiment.

Boone Creek (BC) and the South Fork of the New River (SFNR) were selected in order to construct cumulative distributions based on the conductivity levels in the winter weather months (November – April) between 2010 and 2015. Boone Creek represents a heavily developed stream with 33.44% impervious surface, and the SFNR represents a moderately developed stream with 16.59% impervious surface. South Fork of the New River represents the average of all the monitored streams in the watershed. Both experience exposure to winter road management. The sonde located on Boone Creek [36.208486, -81.653429] is 4,000 meters upstream of the SFNR sonde [36.208486, -81.653429].

Water quality data were scanned for BC and SFNR to look for any conductivity storm events that match the characteristic stated above. Once an increase of ≥15% was found the data from the beginning to the end of the storm event (returns to background levels) was extracted. This process will continue until every storm event has been identified during the monitored years (2010 – 2015) for each stream. After each storm event has been isolated the data will then be broken down into 1-hour increments, and then the mean of each hour was calculated. The cumulative distribution was determined from this new dataset. It displayed different specific conductivity levels and how many hours each stream spent at the respective concentration range. The cumulative distribution data helped determine the different specific conductivity levels that were utilized for the pulse exposure trials [33, 34]. Two levels were utilized for experimentation, the most frequently experienced specific conductivity peak and the worst-case scenario peak.

Two exposure methods were utilized during the pulse experiments: 1) baseline exposure – macroinvertebrates were exposed to conductivity levels derived from the cumulative distribution but after the duration of the exposure has ended the macroinvertebrates were returned to water in the system that is held at reference stream levels (< 50 µS cm^{-1}); 2) background storm event exposure – the procedure was performed as stated in method one, but instead of being returned to water held at reference levels method two placed the macroinvertebrates in water that mimics background conductivity levels that occur between storm events to simulate actual measured environmental scenarios (2010 – 2015). This value was derived using a similar method to what was stated above when determining specific conductivity peaks in the dataset. Instead of looking for increases in specific conductivity storm events during the winter months the values between storm events were of interest. Two resting levels were utilized for experimentation, the most frequently experienced and the worst case scenario and these were calculated from the SFNR and BC respectively. The mean value between storm events for each year was taken, and then the mean of the five-year period was calculated to determine the resting levels for the second exposure method.

Salinity Exposures

After collection the macroinvertebrates were allowed to acclimate to their new environment for 72 hours. The 72-hour period is crucial because it allowed separation of any individuals that emerge into adults or do not survive the transfer, and this ensured that after the three day acclimation period individuals remained larvae for the duration of each experimental trial. Water from the reference stream site on Flannery Fork at Rocky Creek Road [36.188075, -81.677577] was collected and utilized to fill the experimental system and to mix the road salt solutions. After experimentation individuals were separated into two categories: 1) individuals who were exposed to elevated conductivity levels and perished; and 2) individuals who were exposed to elevated conductivity levels and survived. Once separated the head capsule length and body length of Ephemeroptera Heptageniidae *Epeorus* were measured under a dissection microscope with an ocular micrometer to account for varying degrees of mortality due to larvae of different developmental stages within the experiment.

Pulse Exposures

A grid was placed within the Frigid Units natural flow through/recycle system, and a random number generator was utilized to place the exposure vessels within the grid. Experimentation utilized a pulse approach to simulate drastic increases in specific conductivity levels that have been recorded, and the experiments were modeled after 96 hour acute toxicity tests [35].

Experimental trials began after the acclimation period and took place for 96 hours. Each pulse took place once every 24 hours, and each trial had a variation in duration and rest periods: exposure to road salt occurred for 1, 6, 12, 18, and 24 hours while resting (for exposure method 1 and 2) occurred for 23, 18, 12, 6, and 0 hours, respectively (for both 2,000 and 10,000 $\mu S\ cm^{-1}$ experimental scenarios). The exposure vessels were removed from a resting tank containing water from upper Flannery Fork, and placed in a 20 cm culture bowl with the respective experimental and resting specific conductivity concentrations. Lethal Concentration of 50% (LC_{50}) was determined from the resulting survivorship counts that occurred at 0, 24, 48, 72, and 96 hours. Every trial had fresh road salt solutions prepared, and each solution was monitored throughout the 96 hour trial with a Thermo handheld multi-parameter water chemistry meter to ensure quality control of the experimental environments. Water in the resting tank was monitored in the same manner, and fresh water from the reference site was added as needed to control conductivity levels. Water in the resting tank was replaced after each experimental trial.

Statistical analysis

Multi-Parameter Water Quality TROLL 9500s were used to collect water parameter data from eight watersheds in the USFNR: pH, temperature, and specific conductivity. Additional parameters calculated include coefficient of variation of temperature, percent impervious and forested area. All water parameter values were calculated from corresponding macroinvertebrate sampling days. Specific conductivity trends were examined to be utilized during mesocosm acute toxicity experiments.

Data were analyzed using SPSS v. 23.0 (IBM Corp, 2015). Continuous variables were tested for normality using Shapiro-Wilkes test. Non-normal variables were identified within our water parameters, diversity values, and stream health values; therefore non-normal statistical measures were utilized for all analyses. I calculated Shannon (H), Simpson (D) Diversity Index values, richness (S), evenness (J), family level North Carolina Benthic Macroinvertebrate Biological Index (NCBI), and genus level NCBI using the macroinvertebrate identification, tolerance values, and abundances for each collection. Spearman correlation matrices were performed to determine if family and genus NCBI, Shannon diversity index and environmental parameters were significantly correlated. Spearman correlation matrices determined that Simpson Index (0.913; $p<0.001$), richness (0.908; $p<0.001$), and evenness (0.620; $p<0.001$) values were significantly and positively correlated to Shannon Diversity. Due to this Shannon Diversity was the diversity representative value for the remaining analyses.

In order to determine the impact of environmental factors on family and genus NCBI, and Shannon diversity a partial least squares regression analysis was conducted (PLS, JMP v. 10.3 Pro, SAS Institute, Cary, NC, USA). The analysis utilized the NIPALS method with k-fold cross validation (k=8). Family and genus NCBI, and Shannon Diversity were selected as dependent variables while temperature, coefficient of variation of temperature, pH, specific conductivity, percent impervious area, and percent forested area were selected as independent predictor variables.

To visualize the differences in macroinvertebrate community structure between the sub watersheds of the USFNR, nonmetric multidimensional scaling (NMS) using Sorenson (Bray-Curtis) distance measures and site as a grouping variable was utilized (PC-ORD v. 6, Gleneden Beach, OR, USA). Joint plots were fitted to the NMS ordination plot displaying the relationship of environmental factors to the structure of the ordination. Pearson’s r and Kendall’s tau correlation coefficients were calculated to quantify the relationship and ‘fit’ of environmental variables to the macroinvertebrate ordination along NMS axes 1 and 2. Multi-response permutation procedure (MRPP) analysis was then used to determine whether macroinvertebrate community structures were significantly similar or different among watersheds using Sorenson (Bray-Curtis) distances.

Statistical analyses were performed using SPSS v. 23 (IBM, 2015). Student’s t-test was utilized to determine the difference between pulse exposures resting at reference levels and realistic background specific conductivity levels for ‘common’ and ‘worst case scenario’ specific conductivity levels. Generalized linear models were utilized to examine the relationship between increased pulse exposure duration and decreased survival of individuals. Two scenarios were examined, 1) a common specific conductivity level that is experienced during the winter months

(2010-2015); and 2) the highest specific conductivity level that is experienced during the winter months (2010-2015) represented as ‘worst case scenario’.

Summary of Results

In the Upper South Fork of the New River, in Boone, North Carolina, we examined benthic macroinvertebrate collections that were taken from eight watersheds. All watersheds experience varying degrees of road salt pollution; four (Middle Fork, South Fork, Boone Creek, and Hodges Creek) experience specific conductivity ranges (2010-2015 mean) from 122-440 µS cm^{-1} and four (Flannery Fork, Winklers Creek, Goshen Creek, and East Fork) experience ranges from 31-67 µS cm^{-1} serving as reference sites. Family and genus NCBI were significantly higher (i.e. less healthy) at sites that experienced increased specific conductivity means from 2010-2015 when compared to reference sites; while Shannon Diversity values were significantly lower at sites that experienced increased specific conductivity means from 2010-2015. Spearman correlations showed that both pH and coefficient of variation of temperature had significant correlations with family and genus NCBI, while specific conductance, percent impervious area, and percent forested area had significant correlations with family and genus NCBI and Shannon Diversity values. Partial least squares regression indicated coefficient of variation of temperature, specific conductivity, percent impervious area, and percent forested area were significant predictors in variation among family level health and genus level health between the samples; while it indicated that specific conductivity, percent impervious area, and percent forested area was a significant predictor in variation of Shannon Diversity between the samples. NMS joint plots indicated that there were three distinct groups within the sub watersheds based on overall organism abundance. However, the variation among these three different groups cannot be explained by the six environmental parameters that were taken into consideration for this study.

The data suggests that aquatic macroinvertebrate health and overall diversity of the sub watersheds, on the family and genus level, are strongly influenced by changes in the coefficient of variation of temperature, specific conductivity, percent impervious area, and percent forested area. The changes in health and diversity seem to be directly linked to decreased forest cover, increased impervious surface, variations of temperature experienced in streams and the increased levels of specific conductivity that are being experienced on an annual basis. Variations in temperature are probably most influential in summer months, and specific conductivity levels in the winter months. I elaborate further on the data in Chapter 1.

Furthermore, I conducted mesocosm road salt exposures that were based on data collected from a long term water monitoring study. These exposures examine the impact of salinity pulses, and also the impact of realistic specific conductivity fluctuations that were experienced by the aquatic organisms during the winter months. The exposure treatment focused on the impact of the salinity increase alone for different time regimes (resting at reference levels between increased salinity treatments), and the impact of the salinity increase with increased salinity levels (‘common’ realistic resting ≈400 µS cm^{-1}, ‘worst case scenario’ realistic resting ≈900 µS cm^{-1}) between treatments for different time regimes (this was done to model the impact of a ‘realistic’ salinity event that may be experienced in rivers). When examining our ‘worst case scenario’ level of 10,000 µS cm^{-1}, the pulse alone and pulse with resting at elevated concentrations were significantly different from one another for each time regime (1, 6, 12, 18 hrs) p=0.007, $1.99*10^{-7}$, $2.65*10^{-6}$, 0.0002, respectively. When comparing the two scenarios there was a decrease in survivorship of 12.5-41.25%. Increased duration to salinity exposures of

10,000 µS cm^{-1} has a significant negative impact on survivorship. When examining our 'common scenario' level of 2,000 µS cm^{-1}, the pulse alone and pulse with resting at elevated concentrations were not significantly different from one another for each time regime (1, 6, 12, 18 hrs) p=N/A, 0.10, 0.28, 0.28, respectively. When comparing the two scenarios there was a decrease in survivorship of 3.8-6.25%. When examining the impact of increased (time) exposure to salinity pulses on survivorship. Increased duration to salinity exposures of 2,000 µS cm^{-1} had no significant negative impact on survivorship. However, there is a negative trend between increased exposure to salinity and decreased survivorship.

The data suggests that aquatic macroinvertebrate survivorship will decrease over time when exposed to increased salinity concentrations. Both salinity levels (2,000 and 10,000 µS cm^{-1}) experienced this trend although one was to a much lesser degree. I elaborate further on the data in Chapter 2.

Future Directions

My research on the impact of specific conductivity on stream health, diversity, and overall abundance is not the first of its kind, but it is the first of its kind to be conducted in a high elevation southern Appalachian watershed utilizing water parameter data taken from a long term monitoring project to examine the impact of salinity pulses on intolerant aquatic macroinvertebrates. Statistical evidence was provided showing that increased impervious area, decreased forested area, increased variation of daily temperature and increased specific conductivity have a significant impact when predicting worsened stream health (as indicated by NCBI with both family and genus level macroinvertebrate identification), and decreased diversity of streams in the USFNR. These trends can be identified immediately when comparing streams that experience more land use related impact (storm water runoff in the summer, snowmelt runoff in the winter) to reference streams that experience little to no land use related impact. These results are consistent with what is known regarding the impact of increased temperature variation and increased specific conductivity on biology and ecology of streams [3, 10, 25, 36]. Due to the process of bank storage, Boone is experiencing increased specific conductivity levels throughout the year due to the infiltration of snowmelt runoff polluted with road salt [37].

My research on the impact of 'realistic' salinity pulses on survivorship using Ephemeroptera Heptageniidae was the first of its kind. Statistical evidence showed that survivorship decreased as exposure to salinity pulses increased in duration. These studies were conducted utilizing an acute toxicity methodology. Understanding what will happen utilizing a chronic toxicity methodology would be extremely beneficial. As Appalachian State University and the town of Boone grow, this will lead to an increase in percent impervious surface area and will consequently lead to greater variation of temperature within the stream and increases in specific conductivity throughout the year, especially during the winter months [38, 39]. The growth will lead to salinity spikes that are more intense in both concentration and duration, but there are steps that can be taken to ensure that these impacts will be lessened over time. The elimination of road salt use in most communities is not feasible because it is easy to use, effective, and has relatively low cost [3]. The best mitigation action for dealing with road salt is preventative and proactive measures [40]. Investing in green infrastructure during construction around aquatic ecosystems will help mitigate issues in the future when dealing with contaminants, and it will help to keep the percentage of impervious surface low [41]. Preventing alterations to the stream bed are critical to keeping stream – ground water (GW) interactions in place, and if these have already been disrupted then restoration of the stream – GW interactions will be extremely beneficial [39]. These interactions are extremely beneficial to the lotic ecosystems and the surrounding environment.

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Chapter 1

Running Head: Water parameter effects on macroinvertebrates in the USFNR

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Specific conductivity, temperature, and pH effects on stream health, diversity and abundance in the Upper South Fork of the New River

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Abstract

In many regions of the United State current winter road management practices requires that deicing agents be applied to roads before or during each of the winter snow and ice events. Long term water monitoring projects have indicated significant salinization of surface and ground water bodies raising concern from scientists about the impact on aquatic organisms and drinking water quality. Boone, NC is unique in that it has a winter climate comparable to southern New England due to its high elevation in the Southern Appalachian Mountains (982 m), precipitation (132 cm) and quickly developing urban center. Eight headwater streams originating in Boone combine to form the Upper South Fork of the New River. Data collected from these eight streams with sondes from 2010 to 2015 provided water chemistry data (temperature represented by coefficient of variation of temperature, pH, and specific conductivity) and impervious and forested area were determined and correlated to NC DEQ Qual 4 benthic macroinvertebrate collections for which NC biology index (BI), Shannon diversity index, family and genus richness were calculated. Four of the streams have increased impervious area along with elevated specific conductivity levels due to road salt in the winter, and four of the streams have less impact and serve as reference streams. Spearman correlations indicate that the coefficient of variation of temperature, specific conductivity, percent impervious and forested area all have significant moderate correlations with family and genus NCBI values, while specific conductivity, percent impervious and forested area have significant strong correlations with Shannon Diversity. Partial least squares regression indicated that coefficient of variation of temperature, specific conductivity, percent impervious and forested area were significant predictors of observed variation in macroinvertebrate health of the family and genus level. Specific conductivity, percent impervious and forested area were significant predictors of observed variation in macroinvertebrate diversity. The study suggests that variation in temperature throughout the

seasons, and elevated specific conductivity levels have an overall negative impact on macroinvertebrate communities in the headwaters of Boone, NC.

Keywords: Upper South Fork of the New River, road salt, specific conductivity, stream health, macroinvertebrates

Introduction

Watershed urbanization is a process that involves the removal of riparian vegetation and the replacement of natural land cover with pavement, buildings, and urban infrastructure [1]. Urban land cover in the U.S. has increased from 61,000 km^2 in 1945 to 247,000 km^2 in 2007 [2]. This has long been described as a major detriment to overall stream water quality by impacting stream temperature and stream conductivity [3, 4]. Increases in both population growth and migration into urban areas further exacerbate the negative impacts on water resources and further compromise water quality [5]. Many studies have examined the direct effects of urbanization on stream temperatures, and conductivity levels. Urbanization also leads to reductions in riparian vegetation that provide shading to the stream, allochthonous energy inputs and leads to an increase in storm water runoff following rain events [6, 7]. There is a positive relationship between aquatic ecosystem exposure to road salt and the increase in urban development with time, which leads to increased temperatures during summer storm events and road salt application during snow/ice events [2, 8].

The Upper South Fork of the New River in Boone in the Blue Ridge Mountains of northwestern North Carolina, USA (Figure 1). Boone has an elevation of 982 meters which consequently leads to enhanced precipitation, and a winter climate in Boone that compares more closely to southern New England [9]. Boone receives an average of 88.9 centimeters of snowfall annually, and this leads to a unique microclimate when compared to the rest of North Carolina or the southeastern US region [9]. The USFNR watershed has experienced an increase in impervious surface area due to the 26 percent population growth experienced in the past ten years (2005 to 2015) [10]. Boone experiences many storm events that cause river levels to rise quickly and these levels can be sustained for prolonged periods. A rapid increase in stream stage can lead to infiltration of surface water into the stream banks; this phenomenon is known as bank storage, which contributes to the maintenance of baseflow in rivers after a flow event [11, 12]. If the stream water is able to overtop the banks and flooding occurs over a large area of land surface, then widespread recharge to the water table can take place throughout the flooded area [11, 12]. In situations like this the amount of time it takes for recharged floodwater to return to the stream via ground water flow could be weeks, months, or years [12]. Floodwater recharge occurring during the winter ‘salt-season’ can lead to ground water contamination that could take months to years for dilution to occur [13]. This phenomenon is of concern to aquatic ecotoxicologists because salinization has major adverse implications for the aquatic life that live in urbanized streams.

To better understand the relationship between increased urbanization, storm runoff and water contamination during the ‘salt-season’ the Appalachian Aquatic Science Research team (AppAqua) deployed data sondes starting in 2010. The long-term monitoring effort has focused on gathering water physiochemical and Global Information Systems (GIS) data to monitor impacts of intense development on the water quality in the unique USFNR watershed. Utilizing the long-term monitoring data we sought to add to current research on the impact of road salt and temperature on macroinvertebrates due to increased impervious surface area by asking the following questions: (1) How do the water parameters (specific conductivity, temperature, and pH), and the surrounding environmental conditions (impervious and forested surface area) impact overall stream health, diversity, and abundance of aquatic macroinvertebrates in the Upper South Fork of the New River? (2) Is there a water parameter (specific conductivity, temperature, or pH) that serves as a better predictor of the variation in overall stream health, diversity, and abundance of aquatic macroinvertebrates? Finally, a comment on cost/benefits of the use of Family vs Genus level identification for macroinvertebrates is included.

Materials and Methods

Site description

The headwater streams of the USFNR were chosen for this study due to their proximity to Boone and the range of measured conductivities, rates of development and impervious surfaces. They include: Boone Creek (BC), East Fork of the New River (EFNR), Flannery Fork Creek (FF), Goshen Creek (GC), Hodges Creek (HC), Middle Fork of the New River (MFNR), Winklers Creek (WC), and the South Fork of the New River proper (SFNR; represented as State Farm). At each site the Appalachian Aquatic Science Research team (AppAqua) manages In-Situ Inc. Multi-Parameter Water Quality TROLL 9500s (data sondes) are located at the outlets of each stream (Figure 2).

Macroinvertebrate identification & water parameter data

Macroinvertebrate collections were performed by student groups at ASU and the North Carolina Division of Water Quality from the watersheds around Boone (n=40, Figure 2). Collections from ASU utilized the Qual 4 Method, four collections are made: one riffle-kick, one sweep, one leaf-pack, and a visual search of submerged woody debris and stones [14]. This method was designed to be conducted only in small streams, which are defined as having a drainage area ≤ 3.0 square miles, and all macroinvertebrates are included in calculations to represent the community after they were identified down to genus and species (if possible with dichotomous key) [14, 15, 16]. Collections from the North Carolina Division of Water Quality were identified to species. North Carolina biotic index (NCBI), represent the relative tolerance of the benthic community to the presence of general stressors on a scale from 0.0 to 10.0, with the lower values indicating pristine conditions and higher values indicating stress [14]. North Carolina biotic index values were calculated for each collection utilizing tolerance values on both the Family and Genus level.

Shannon Diversity Index (H), Simpson (D) Diversity Index, richness (S), and evenness (J) were calculated for each collection.

AppAqua manages nine In-Situ Inc Multi-Parameter Water Quality TROLL 9500s (data sondes, n=9) that are located on the headwater streams of the USFNR (Figure 2). Water parameter data collection began in July 8, 2010 and this study utilized data from August 22, 2010 to January 31, 2017. Parameters were recorded every fifteen minutes by the data sondes and included temperature (°C), specific conductivity (µS cm^{-1}), and pH. Additional parameters, percent impervious and forested area, were calculated utilizing 1 meter resolution in ArcMap v. 10.3 for each watershed within the USFNR. Each macroinvertebrate collection was paired with its corresponding water parameter data on a daily basis by utilizing the mean. The percent impervious and forested area for each watershed was given a single representative value for the study (August 22, 2010 to January 31, 2017). Temperature, pH, and specific conductivity were measured at the time of each macroinvertebrate collection incase an issue existed with the sonde data.

The coefficient of variation was calculated for temperature of each sample due to the fact that there were an uneven number of samples taken between seasons, and seasonality was not accounted for thus the coefficient of variation was calculated as a remedy.

Statistical analysis

Multi-Parameter Water Quality TROLL 9500s (n=9) were used to collect water parameter data from eight watersheds in the USFNR as stated above: pH, temperature, and specific conductivity. Coefficient of variation was calculated utilizing temperature to account for changes due to seasonality.

Data was analyzed using SPSS v. 23.0 (IBM Corp, 2015). Continuous variables were tested for normality using Shapiro-Wilke tests. Non-normal variables were identified within our water parameters, diversity values, and stream health values; therefore non-normal statistical measures were utilized for all analyses. Shannon Diversity Index (H), Simpson Diversity Index (D), richness (S), evenness (J), family and genus NCBI values were calculated using the identified macroinvertebrates and abundances for each collection (n=40). Spearman correlation matrices determined that Simpson Diversity Index (0.913; $p<0.001$), richness (0.908; $p<0.001$), and evenness (0.620; $p<0.001$) values were significantly and positively correlated to Shannon Diversity Index. Thus, the Shannon Diversity Index was utilized in all remaining analyses as a dependent variable. To determine if there is a significant correlation between family and genus NCBI, Shannon diversity index and environmental parameters Spearman correlation matrices were performed. Strength of Spearman correlations were determined utilizing the following scale: 0.00-0.19, “very weak”; 0.20-0.39, “weak”; 0.40-0.59, “moderate”; 0.60-0.79, “strong”; 0.80-1.0, “very strong” [17]

To determine the impact of environmental factors (pH, temperature, coefficient of variation of temperature, specific conductivity, percent impervious and forested area) on family and genus NCBI, and Shannon diversity a partial least squares regression analysis was conducted (PLS, JMP v. 10.3 Pro, SAS Institute, Cary, NC, USA). The analysis utilized the NIPALS method with k-fold cross validation (k=8). Family and genus NCBI and Shannon Diversity were selected as dependent variables while temperature, coefficient of variation of temperature, pH, specific conductivity, percent impervious area, and percent forested area were selected as independent predictor variables.

To visualize the differences in macroinvertebrate community structure between the watersheds of the USFNR nonmetric multidimensional scaling (NMS) using Sorenson (Bray-Curtis) distance measures and site as a grouping variable was utilized (PC-ORD v. 6, Gleneden Beach, OR, USA). Joint plots were fitted to the NMS ordination plot displaying the relationship of environmental factors to the structure of the ordination. Pearson's r and Kendall's tau correlation coefficients were calculated to quantify the relationship and "fit" of environmental variables to the macroinvertebrate ordination along NMS axes 1 and 2. Multi-response permutation procedure (MRPP) analysis was then used to determine whether macroinvertebrate community structures were significantly similar/different among watersheds using Sorenson (Bray-Curtis) distances within the NMS ordination. Indicator species analysis (ISA) was then utilized to determine what taxonomic groups were most responsible for the ordination grouping between sites in the NMS analysis [18]. There were a total of 227 taxonomic groups from the monitoring effort but only 179 could be compared between the sites due to 48 taxonomic groups being present in a single sample.

It is important to note that regarding Spearman correlations, PLS regression analysis, and NMS ordination that the temperature, pH, specific conductivity, percent impervious and percent forested area had a sample size of 40. However, the data sonde in Hodges Creek was installed in 2016 and therefore the coefficient of variation of temperature could be calculated for only one Hodges Creek sample resulting in a sample size of 38. This also means that some values used in the study were measured during the time of collection and not taken from the long term sonde data. This is acknowledged and understood that the impact of specific conductivity and the coefficient of variation of temperature on macroinvertebrate stream health, diversity, and overall abundance may be influenced.

Results

We examined benthic macroinvertebrate collections that were taken from eight sub watersheds of the USFNR (Table 1), and there were a total of 227 taxonomic groups in total throughout all 40 samples (Family, Genus, and species). The collections were correlated to four water physiochemical and two habitat parameters to examine their impact on overall health (family and genus NCBI), diversity (Shannon Diversity Index), and community abundance. Spearman correlations (Table 2) showed that coefficient of variation of temperature (CV) had a significantly positive correlation with family and genus NCBI (r_s=0.415, and 0.461 respectively); pH had a weak positive correlation with genus NCBI (r_s=0.339); specific conductivity and percent impervious area had a significantly positive correlation with family and genus NCBI (r_s=0.543, 0.572; r_s=0.529, 0.580 respectively) while showing a significantly negative correlation with H (r_s=-0.728; r_s=-0.651); percent forested area had a significantly negative correlation with family and genus NCBI while showing a significantly positive correlation with H (r_s= -0.311, -0.391, 0.522 respectively); temperature was not significantly correlated with any health or diversity values, and data is not shown here.

A partial least squares regression (PLS) was conducted with six factors (four water parameters, two habitat parameters) to examine the impact on macroinvertebrate diversity and health. A PLS regression model of observed variation showed that the six factors accounted for 53.34%, 48.38%, and 36.91% of the cumulative variation in family and genus NCBI, and Shannon Diversity, respectively (Table 3). Multiple variables were important predictors of family and genus NCBI and Shannon Diversity Index (Table 3). Specific conductivity (VIP = 1.27), percent impervious area (VIP = 1.11), coefficient of variation of temperature (VIP = 1.10), and percent forested area (VIP = 0.99) were significant predictors with the PLS model of family NCBI

(Table 3). Specific conductivity (VIP = 1.31), coefficient of variation of temperature (VIP = 1.27), percent impervious area (VIP = 1.11), percent forested area (VIP = 0.94) were significant predictors with the PLS model of genus NCBI (Table 3). Percent impervious area (VIP = 1.403), specific conductivity (VIP = 1.400), and percent forested area (VIP = 1.14) were significant predictors with the PLS model of Shannon Diversity (Table 3). Temperature and pH were not significant predictors with the PLS model (Table 3).

NMS ordination plots revealed three communities are present within the eight sub watersheds, but the samples that make up the SFNR overlap two of these communities. Joint plots revealed that specific conductivity and percent impervious area had more influence over community 1 found in the Boone Creek sub watershed and community 2 made up Winklers Creek, Flannery Fork, and Goshen Creek sub watersheds. Coefficient of variation of temperature, temperature, and percent forested area had more influence over community 3 made up of Middle Fork, East Fork, and Hodges Creek. South Fork of the New River overlapped with community 2 and 3 (Figure 3). Pearson and Kendall correlations quantified the correlation of each environmental variable and the ordination of community data along each NMS axis. These correlations found that the influence of the environmental variables over the communities within the watersheds was not significant (Table 4). MRPP analysis showed significant differences for 57% of pairwise watershed comparisons (Table 4). The Individual Species Analysis (ISA) showed that a total of ten taxonomic groups, out of the 179 that were compared, were statistically significant in explaining the ordination grouping shown by the NMS ordination plot (Table 5). The ISA showed that there are three taxa that help explain the clustering experienced in ordination group 2, and why these three watersheds within ordination group 2 are different from the rest (Table 5). These individuals include *Stenonema modestum* (Ephemeroptera Heptageniidae), *Acroneuria* *abnormis* (Plecoptera Perlidae), and *Haploperla* (Plecoptera Chloroperlidae) (Table 5). There are five taxonomic groups that help explain the clustering experienced in ordination group 3, and why these three watersheds separate out from the rest (Table 5). These individuals include *Macdunnoa* (Ephemeroptera Heptageniidae), *Stenacron* (Ephemeroptera Heptageniidae), *Arigomphus* (Odonata Gomphidae), *Acroneuria* (Plecoptera Perlidae), and *Diplectrona* (Trichoptera Hydropsychidae) (Table 5). The SFNR overlapped with ordination group 2 and 3, and there are two taxonomic groups that help explain why the SFNR overlaps with so many watersheds and does not cluster into one group over the other (Table 5; Figure 3). These individuals include *Dixa* (Diptera Dixidae), and *Centroptilum* (Ephemeroptera Baetidae) (Table 5).

Discussion

Watershed urbanization has long been seen as a detriment to stream water quality by impacting stream temperature and conductivity by the removal of riparian vegetation and the replacement of natural land cover with pavement, buildings, and urban infrastructure [1, 2, 3]. In Boone, NC aquatic life in urbanized streams experience extreme changes in temperature during the summer months (associated with short-term fish kills, Tuberty, per. com.), and increases in specific conductivity during the winter months. This study evaluated these variables and highlights the impact of these changes in water quality on the macroinvertebrate communities of the headwater streams in the Upper South Fork of the New River (USFNR). Due to the nature of the macroinvertebrate sampling (utilizing the Qual-4 Method) all taxonomic groups were taken into account during analyses [14].

Spearman correlations revealed that the percentages of impervious and forested area were significantly correlated to family and genus NCBI and Shannon Diversity Index. Increasing percent impervious surface area leads to decreases in riparian vegetation around streams, increased population densities of tolerant species or those benefiting from the environmental changes, increased storm runoff, and increased need for salt application during the winter months. Streams around Boone with higher percent impervious area had worse health values (both family and genus NCBI) with an overall lower diversity of macroinvertebrates, while streams with higher percent forested area had healthier macroinvertebrate communities with an overall higher diversity. I see the same trend followed when examining the indicators of increased impervious area, i.e. coefficient of variation of temperature and specific conductivity. The PLS model revealed that the coefficient of variation of temperature significantly impacted the range of family and genus NCBI values, while specific conductivity significantly impacted the range of NCBI and Shannon Diversity Index values. These results indicate that both the coefficient of variation and specific conductivity are important factors for predicting the health and diversity that you will experience in a stream. This is a substantial result and an aspect that can be utilized for predicting health and diversity of macroinvertebrate communities when examining the differences in seasonality.

The data suggests that specific conductivity has an overall greater impact on the health and diversity of streams compared to the coefficient of variation of temperature; therefore, in the headwater streams of the USFNR, specific conductivity is a better predictor of macroinvertebrate stream health, and diversity. However, the distinction between these two factors could be characterized with an experimentally designed macroinvertebrate sampling and water monitoring effort. The unevenness between samples collected in the summer months versus winter months require the analyses to rely on the coefficient of variation of temperature. This factor is crucial and it shows that watersheds with high variability within the daily temperature mean lead to decreased stream health, while watersheds with more stable daily temperature means have overall improved health. However, if seasonality was tested evenly between the summer and winter months I would expect that temperature would be a far greater influence over health and diversity during the summer months, and specific conductivity would have the greater influence during the winter months. Boone receives a large amount of precipitation in both the summer and winter months, which lead to increased temperature levels in the summer months followed by increased specific conductivity levels in the winter months.

Bank storage is a phenomenon that is leading towards a mixture of thermal and salinity pollution, especially when examining summer months in the future. Road salts have been found to persist in ground water (GW) and surface water (SW) well after the time of application, often into the summer months and in some locations (such as Boone) the elevated specific conductivity levels can be experienced throughout the year [19, 20]. Dilution is the sole solution for road salt as a pollutant, and in regions that experience high levels of winter precipitation the use of road salt will begin again before complete dilution or flush of the salt from GW can occur [21]. Due to this issue it is important to examine the impact of each factor on stream health, and diversity. The NMS ordination, regarding the overall abundance of macroinvertebrates, showed that out of the eight watersheds in Boone there are three distinct community groupings. Boone Creek makes up ordination group 1 that has no overlap with any other watersheds, indicating that the macroinvertebrate community found in this watershed is drastically different than the remaining seven, and this is something that can be explained because Boone Creek contains the highest percent impervious area in the USFNR, 33.44%. The large amount of impervious area leads to high variation in temperature throughout the year as well as having the highest elevated specific conductivity levels of any watershed in Boone, which leads to a highly tolerant assemblage of macroinvertebrates that cannot be matched by any other watershed (Figure 3). Ordination group 2 consists of Winklers Creek, Flannery Fork, and Goshen Creek and these three watersheds fall within one another. The macroinvertebrate communities found within these three watersheds are very similar, and this is expected since these streams flow from protected and well forested lands on the Blue Ridge Parkway managed by the US National Park Service (Figure 3). Ordination group 3 consists of Middle Fork, East Fork, and Hodges Creek. The Middle Fork and East Fork overlap with one another but they also share overlap with the reference stream Winklers Creek. The ISA shows what specific organisms are responsible for the clustering and separation experienced between ordination groups 2 and 3, while also showing what organisms are causing the SFNR to overlap so much with all of the watersheds except for Boone Creek. Hodges Creek and Middle Fork are two streams in the USFNR that experience high impervious area percentages along with temperature and specific conductivity pollution, and the reason why they overlap and ordinate more with reference streams is likely due to the influence of a large number of pristine unnamed feeder streams.

An explanation for the ordination assemblages we see are source-sink dynamics. There are many small healthy streams with low impervious area that feed into the more impacted watersheds that are serving as source populations and contributing healthy individuals to the sink populations [22]. This may explain how highly-sensitive macroinvertebrates can be found in collections from the more impacted streams, like Hodges Creek, and highlights the importance of protected areas in maintaining the health of the watershed as a whole [23]. When analyzing the parameter data the expectation was that Hodges Creek and Boone Creek would have similar macroinvertebrate communities, but as stated previously the source-sink dynamics complicate the communities [22]. The ISA is a good indicator of what organisms may be contributing to the source-sink dynamics experienced by the watersheds that have the higher impervious area percentages.

This study supports what previous experiments have shown, regarding the negative impact of temperature and specific conductivity on aquatic macroinvertebrates. In the USFNR watershed of Boone the most influential factor for driving health and diversity of streams is specific conductivity. With continued monitoring and a study with an experimental design it should show that pollutants in the USFNR are more seasonally driven, as discussed earlier with summer months being impacted more by temperature and winter months being more impacted by specific conductivity.

Our data suggest that there is a distinction between examining communities on a family versus genus taxonomic level. When organisms are identified to genus the structure of that community is understood with far greater detail, and the health/diversity values are more accurate. If an investigator were to identify down to species level this would only enhance the accuracy of the study to reveal the true community structure and allows more significant correlation between the biological and physiochemical characteristics of the streams. There has long been a debate about whether this is worth the extra effort, and I believe that taking the time to train individuals to complete this work will be extremely beneficial to future efforts. The learning curve is very steep, but once the work is put in on the front end the difference in time between identifying to family, genus, and species becomes less onerous.

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Tables

Table 1. Mean water parameter and biotic data along with habitat parameter data for each sub watershed within the USFNR.

Sub Watershed	Coefficient of Variation (Temperature)	pH	Specific Conductivity (µS cm^{-1})	Temperature (^{0}C)	Percent Impervious	Percent Forested	Family NCBI	Genus NCBI	Species Richness
Middle Fork	3.17	7.18	105.77	19.04	15.72	69.24	2.94	2.98	19
Flannery Fork	1.99	6.81	33.29	9.62	5.26	80.54	3.12	2.88	31
Winklers Creek	3.14	6.78	33.72	13.62	4.15	87.55	3.27	3.02	24
South Fork	5.89	6.86	152.95	12.97	16.59	66.71	3.74	3.79	28
Boone Creek	3.08	7.28	372.33	11.56	33.44	57.33	4.11	4.00	9
Goshen Creek	0.90	6.55	47.32	15.91	11.31	73.16	3.22	2.82	19
East Fork	2.07	6.70	161.07	9.30	11.33	56.00	2.98	2.91	23
Hodges Creek	7.69	6.81	277.33	14.37	22.03	65.16	4.67	5.10	10

Table 2. Spearman correlations comparing stream health (Family and Genus level NCBI) and diversity values (Shannon Diversity Index) to water and habitat parameter data, n = 40.

			Mean Temperature	Coefficient of Variation (Temperature)	Mean pH	Mean Specific Conductivity (μS cm^{-1})	Percent Impervious Area	Percent Forested Area
Spearman's rho	Family BI Values	Correlation Coefficient	-0.179	0.415**	0.310	0.543**	0.529**	-0.311*
		Sig. (2-tailed)	0.270	0.010	0.052	0.000	0.000	0.050
		N	40	38	40	40	40	40
	Genus BI Values	Correlation Coefficient	-0.077	0.461**	0.339*	0.572**	0.580**	-0.391*
		Sig. (2-tailed)	0.636	0.004	0.033	0.000	0.000	0.013
		N	40	38	40	40	40	40
	Shannon Diversity Index	Correlation Coefficient	0.109	-0.259	-0.221	-0.728**	-0.651**	0.522**
		Sig. (2-tailed)	0.503	0.116	0.171	0.000	0.000	0.001
		N	40	38	40	40	40	40

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3. Partial least squares (PLS) regression with Family, and Genus NCBI, and Shannon Diversity Index as dependent variables.

Partial Least Squares Regression							
Dependent Variable	Independent Variable	VIP	Number of VIP>0.8	Method	Number of Factors	Percent Variation Explained for Cumulative X	Percent Variation Explained for Cumulative Y
Family NCBI	Coefficient of variation	1.10*	4	NIPALS	6	100	53.34
	Temperature	0.69					
	pH	0.71					
	Specific Conductivity	1.27*					
	Percent Impervious Area	1.11*					
	Percent Forested Area	0.99*					
Genus NCBI	Coefficient of variation	1.27*	4	NIPALS	6	100	48.40
	Temperature	0.53					
	pH	0.53					
	Specific Conductivity	1.31*					
	Percent Impervious Area	1.11*					
	Percent Forested Area	0.94*					
Shannon Diversity Index	Coefficient of variation	0.49	3	NIPALS	6	100	36.91
	Temperature	0.52					
	pH	0.51					
	Specific Conductivity	1.400*					
	Percent Impervious Area	1.404*					
	Percent Forested Area	1.14*					

*VIP>0.8

Table 4. Multi-response permutation procedure by watershed for macroinvertebrate abundance and diversity using Bray-Curtis distance measures. Pearson’s r and Kendall’s tau correlations to determine significant relationships between environmental factors and the ordination of different watersheds. Shown on following page.

Multi-response Permutation Procedure (MRPP)		
Comparison	A value	P value
1 vs. 2	0.101	0.005**
1 vs. 3	0.085	0.001**
1 vs. 4	0.070	0.020*
1 vs. 5	0.172	<0.001**
1 vs. 6	0.138	0.003**
1 vs. 7	0.043	0.080
1 vs. 8	0.042	0.040*
2 vs. 3	-0.018	0.908
2 vs. 4	0.044	0.060
2 vs. 5	0.080	0.002**
2 vs. 6	0.008	0.351
2 vs. 7	0.041	0.102
2 vs. 8	0.040	0.064
3 vs. 4	0.026	0.043*
3 vs. 5	0.080	<0.001**
3 vs. 6	0.005	0.284
3 vs. 7	0.023	0.084
3 vs. 8	0.026	0.041*
4 vs. 5	0.112	<0.001**
4 vs. 6	0.072	0.022*
4 vs. 7	0.039	0.140
4 vs. 8	0.021	0.217
5 vs. 6	0.080	0.005**
5 vs. 7	0.102	0.003**
5 vs. 8	0.075	0.007**
6 vs. 7	0.099	0.026*
6 vs. 8	0.060	0.055
7 vs. 8	0.005	0.40

Pearson and Kendal Correlations with Ordination Axes						
Environmental Variable	Axis 1			Axis 2		
	r	r^2	tau	r	r^2	tau
Specific Conductivity	0.241	0.046	0.001	-0.031	0.001	-0.045
Temperature	-0.249	0.062	-0.169	0.137	0.019	0.072
pH	-0.005	0.000	0.005	-0.028	0.001	-0.010
CV	-0.213	0.045	0.045	-0.228	0.052	-0.182
Impervious Area	0.309	0.095	0.078	-0.085	0.007	-0.040
Forested Area	-0.019	0.000	0.020	-0.014	0.000	-0.086

*. Significant at the 0.05 level.

**. Significant at the 0.01 level.

Table 5. Indicator species analysis (ISA) for the eight water sheds in Boone, NC to examine which macroinvertebrate taxonomic groups are most constant and abundant in the groups that have been found to differ in composition via the NMS ordination and the MRPP analysis.

Taxonomic Group	Tolerance Value (TV)	Max Group	Corresponding Watershed	Observed Indicator Value (IV)	P value
Diptera, Dixidae *Dixa*	2.5	4	South Fork of the New River	40.0	0.033*
Ephemeroptera, Baetidae *Centroptilum*	3.8	4	South Fork of the New River	40.0	0.030*
Ephemeroptera, Heptageniidae *Macdunnoa*	4	7	East Fork of the New River	62.7	0.003**
Ephemeroptera, Heptageniidae *Stenacron*	3.58	1	Middle Fork of the New River	46.8	0.025*
Ephemeroptera, Heptageniidae *Stenonema modestum*	5.8	3	Winklers Creek	45.8	0.041*
Odonata, Gomphidae *Arigomphus*	4	8	Hodges Creek	37.5	0.049*
Plecoptera, Perlidae *Acroneuria*	1.9	1	Middle Fork of the New River	66.3	0.004**
Plecoptera, Perlidae *Acroneuria abnormis*	2.1	2	Flannery Fork	50.0	0.018*
Plecoptera, Chloroperlidae *Haploperla*	1.4	2	Flannery Fork	46.7	0.024*
Trichoptera, Hydropsychidae *Diplectrona*	2.3	7	East Fork of the New River	62.7	0.007**

*. Significant at the 0.05 level.

**. Significant at the 0.01 level.

Figures

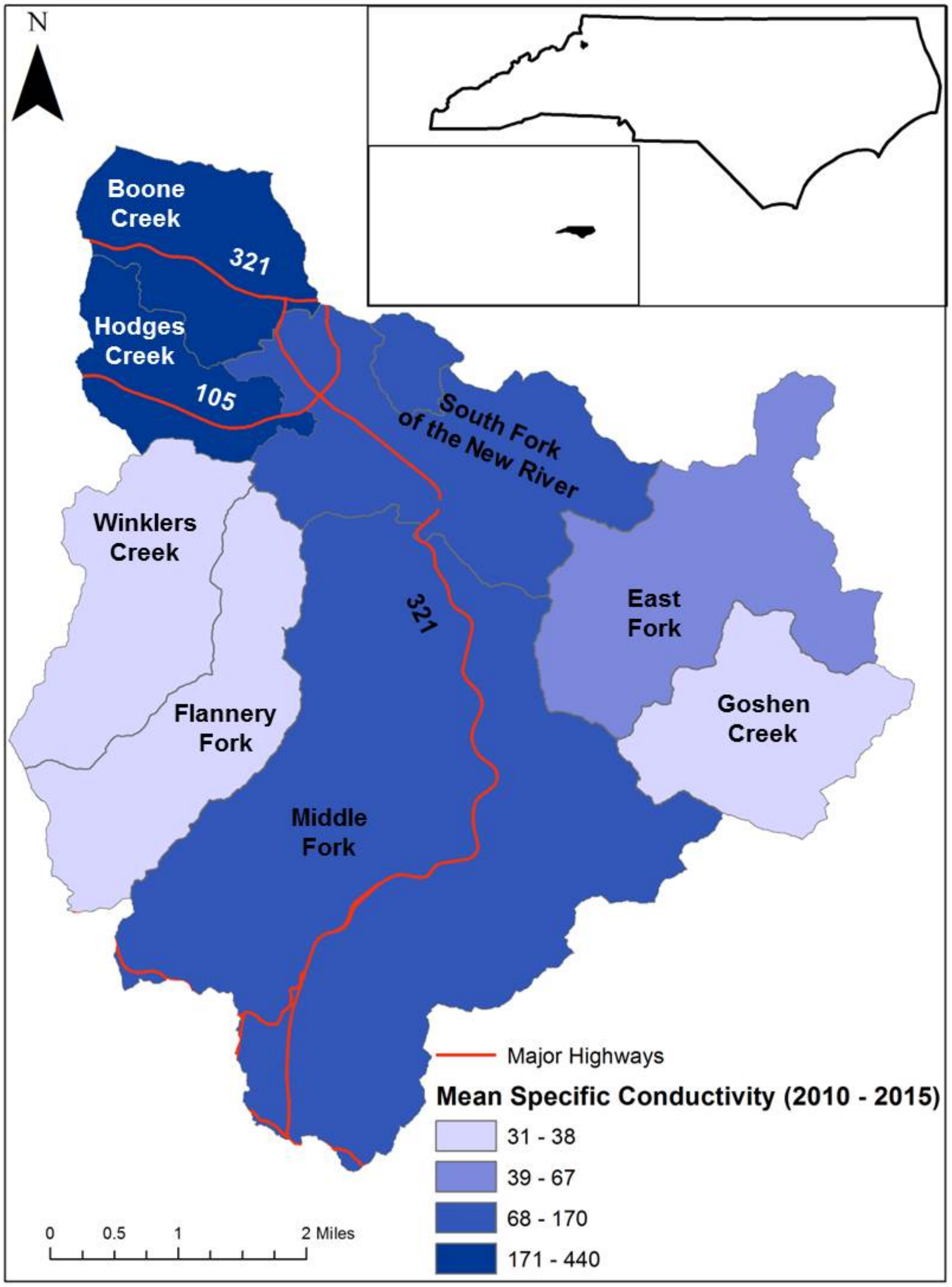

Figure 1. Map identifying the USFNR and sub watersheds within North Carolina and the United States. The map displays mean specific conductivity for the nine major watersheds of the USFNR (from 2010 to 2015), and major highways. Monitoring for Hodges Creek began in 2016, and the mean value displayed for Hodges creek is from November 2016 to February 2017.

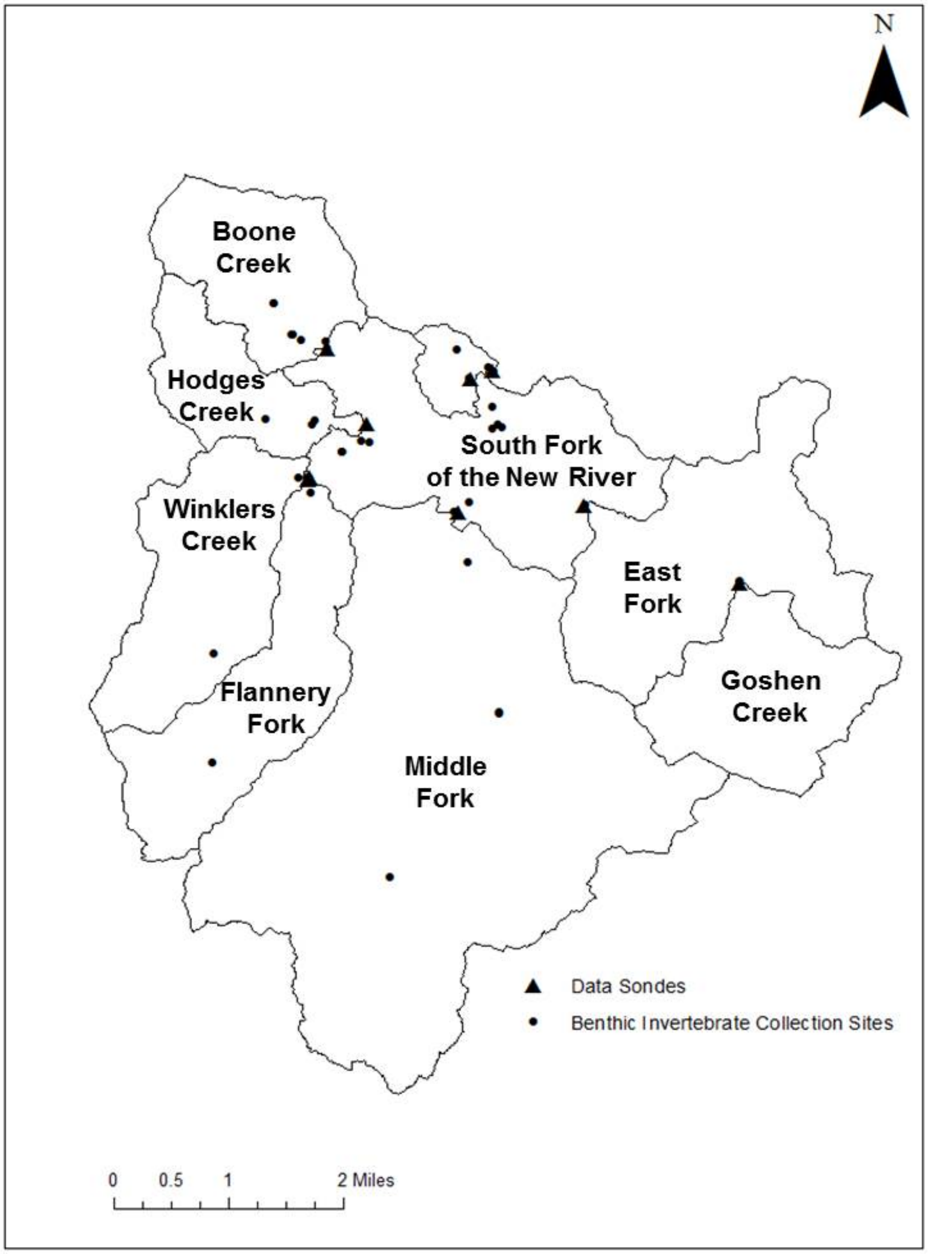

Figure 2. Map identifying the location of In-Situ Inc. Multi-Parameter Water Quality TROLL 9500s (data sondes, n=9), and the location of macroinvertebrate collection sites (n=40) within the USFNR watersheds.

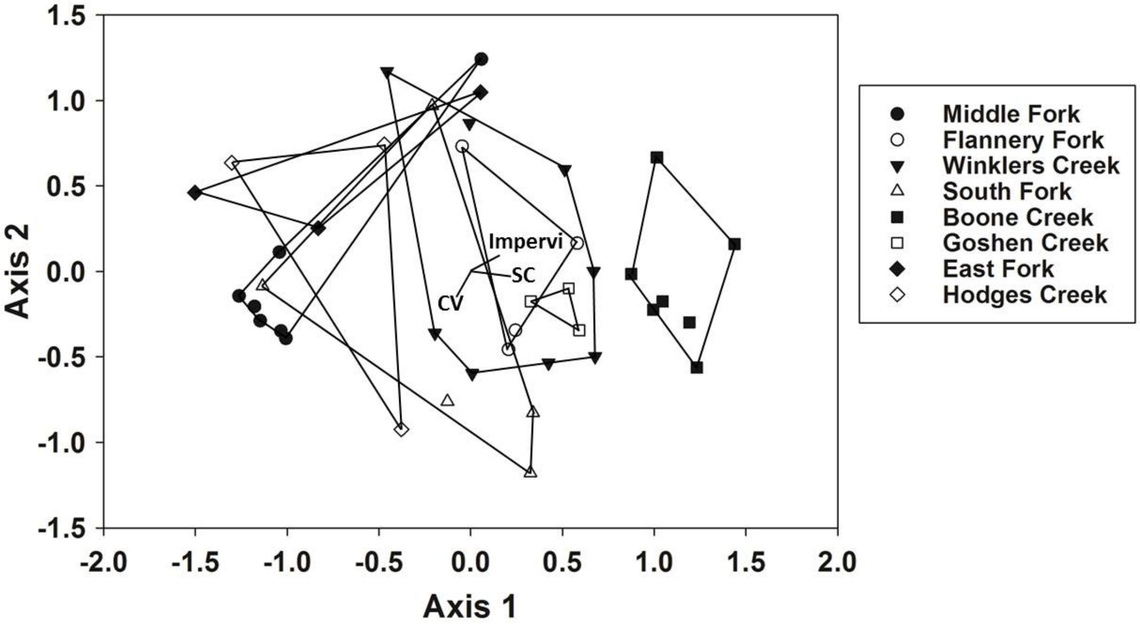

Figure 3. NMS ordination joint plot of six environmental variables on the community structure from the different watersheds within Boone.

Chapter 2

Running Head: Effects of episodic road salt pulses on macroinvertebrates

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Effects of winter road salt application and episodic pulses on Southern Appalachian headwater stream macroinvertebrates

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Abstract

The impact of pulse NaCl exposure on aquatic macroinvertebrates survival were examined utilizing the most frequently experienced specific conductivity spikes (2,000 µS cm^{-1}) and the worst case scenario spikes (10,000 µS cm^{-1}) experienced in Boone Creek through the winter months from 2010 to 2015, in the Upper South Fork of the New River watershed (USFNR) in Boone, NC. Exposures to the spikes alone occurred along with exposures that aimed to simulate environmental concentrations. To simulate episodic winter runoff conditions, after the salt spike the levels were dropped to 400 µS cm^{-1} and 900 µS cm^{-1} for the most frequently experienced levels and the worst case scenario levels, respectively. The 2,000 µS cm^{-1} pulse resting at reference levels (<50 µS cm^{-1}) reduced survivorship up to 83.75% ± 1.8, and 2,000 µS cm^{-1} resting at 400 µS cm^{-1} reduced survivorship up to 73.75% ± 4.6. The 10,000 µS cm^{-1} resting at reference levels (<50 µS cm^{-1}) reduced survivorship up to 10% ± 3.2, and 10,000 µS cm^{-1} resting at 900 µS cm^{-1} reduced survivorship up to 28.75% ± 6.3. This study suggests that as pulse duration increases for all four scenarios we see a decrease in overall macroinvertebrate survival. An LC_{25} was reached with the 2,000 µS cm^{-1} resting at 400 µS cm^{-1} during the 18 hour pulse duration, and an LC_{50} was reached with the 10,000 µS cm^{-1} resting at 900 µS cm^{-1} during the 6 hr pulse duration. Our research suggests that specific conductivity levels measured in Boone, NC are having a negative impact on overall macroinvertebrate health in the headwater streams of the Upper South Fork of the New River.

Keywords: road salt, freshwater salinization, pulse exposure, acute toxicity, Upper South Fork of the New River

Introduction

Snow and ice-covered roads during the winter months are hazardous to the public, and increase risk of injury along with negatively impacting economic activity [1]. Historically, the application of road salt has been relied on to provide safe passage for people traveling during winter storm events [1, 2, 3]. Road salt is one of the favored methods for the deicing of roadways because it is easy to use, effective, and has relatively low cost [1, 2, 3, 4]. A study conducted by Marquette University investigated the number of highway incidents that occurred during winter-road conditions and the effectiveness of road salt in reducing collisions. The results supported the conclusion that road-salt reduced the amount of crashes and the cost of injuries and accidents by 88% and 85% respectively [1]. A study conducted by the University of Waterloo also investigated the impact on winter-road collisions when solely using road salt for winter-road maintenance, and the combination of road salt with plowing for winter-road maintenance [5]. A 51% reduction in the collision rate occurred when road salt was used alone, and a 65% reduction in the collision rate occurred when road salt was used in conjunction with plowing [5]. Although the reduction in collisions in the Marquette University study is less impressive, there is a definite negative relationship between road salt used for winter-road maintenance and the amount of winter-road collisions [5].

There is a positive relationship between aquatic ecosystem exposure to road salt and the increase in urban development with time [4, 6]. Urban land cover in the U.S. has increased from 61,000 km^2 in 1945 to 247,000 km^2 in 2007 [4]. The increase in urbanization will consequently lead to the increase in road salt application for deicing during snow/ice events. Application logs starting in 2006 and ending in 2011 show an average of 19.5 million metric tons of road salt were applied each year to Northern US roads [4]. After road salt is applied it becomes incorporated with melting snow/ice and washed into the nearest body of water. The resulting increase in chloride levels has been observed in rivers, groundwater, inland lakes, and even the Laurentian Great Lakes [4]. The elevated salt concentrations can exert a negative impact on the health of the aquatic organisms in aquatic ecosystems [7, 8, 9].

Salinity is the property of water which results from the shared input of all disassociated mineral salts and the most common contributors are matrix ions that include NaCl [3]. Salinity can be measured in many ways but the most common method is conductivity. Conductivity, on the most basic level, is the ability of a substance to conduct an electrical current [3, 10, 11]. There is a positive correlation between the concentration of dissolved ions in a body of water and the ability to pass an electrical current therefore, as the concentration of dissolved ions in the water increase it will lead to a higher measured conductivity value, and vice versa, and is measured utilizing microSiemens per centimeter ($\mu S\ cm^{-1}$) [3]. The standardized method for recording is specific conductivity which is a measurement that is made at or corrected to $25^{o}C$ [3, 10].

Salinity exposure, whether it be naturally occurring in a marine ecosystem or occurring in a freshwater ecosystem as a pollutant, impacts aquatic organisms (fish, amphibians, mussels, and invertebrates) via direct contact [3]. Aquatic organisms have an especially higher risk of exposure due to the fact that their respiratory structures (gills on outside of abdomen, etc.) are in direct contact with any dissolved ions that are in the water. These specific structures are utilized to transport nutrient ions, and control their individual ionic and osmotic balance [12, 13, 14, 15]. These cell membrane specific structures and functions only work within a range of salinities, and the range of salinities will be directly tied to the evolutionary history of the specific organism [3, 12, 16].

Salinity toxicity experimentation has led to the establishment, by the Environmental Protection Agency (EPA), of freshwater aquatic life standards. Chronic exposure is defined as a four day average, and the water quality standard established for this is 300 µS cm^{-1} [3, 4, 9]. Acute exposure is defined as a one hour average, and the water quality standard established for this is approximately 1,720 µS cm^{-1} [3, 4, 9].

It is important to note that these water quality standards were established using conductivity levels from regions of the country that experience mountain mining, hydraulic fracturing, and de-icing road salt practices (Eastern KY, SW Va, SW West Va). These standards have been scrutinized and tested to ensure they will help protect freshwater aquatic organisms. Studies have shown that the chronic toxicity benchmark provides a reasonable level of protection for most aquatic insect groups. However, near the chronic benchmark certain taxonomic groups are impacted greater than others. Mayflies are very sensitive to elevated conductivity levels, and near the chronic toxicity benchmark there was an increase in mayfly drift, a reduction in community metabolism, and a reduction in the abundance of certain mayfly groups (Baetidae and Heptageniidae) [17]. Increased conductivity values (> 1,200 µS cm^{-1}, less than the acute toxicity benchmark) were shown to have major restructuring effects on stream benthic communities [17]. Investigators have shown that standards derived from exclusively laboratory experiments may not be protective of natural benthic communities. However, Clements and Kotalik (2016) established that the chronic toxicity benchmark set by the EPA is reasonably protective of aquatic insect communities in naturally low-conductivity streams.

The Upper South Fork of the New River in Boone in the Blue Ridge Mountains of northwestern North Carolina, USA (Figure 1). Boone has an elevation of 982 meters which consequently leads to enhanced precipitation, and a winter climate in Boone that compares more closely to southern

New England [18]. Boone receives an average of 88.9 centimeters of snowfall annually, and this leads to a unique microclimate when compared to the rest of North Carolina or the southeastern US region [18]. Water physiochemical measurements have been recorded by data sondes managed by the Appalachian Aquatic Science Research team, otherwise known as AppAqua, in multiple drainages since 2010 in the USFNR. Due to the large snowfall totals and frequency during the winter months, which results in road salt applications, the average specific conductivity for a given winter month in Boone can range from 400 μS cm^{-1} (December, 2014 average) to 1249 μS cm^{-1} (February, 2015 average) within streams that are highly impacted by urbanization and development. For our study, a 'specific conductivity storm event' is any increase in conductivity that is ≥ 15% within an hour, and the duration of the storm will be determined by how long it takes for the conductivity readings to return to background levels. This rate of change was selected, because once this characteristic occurred the storm event would surpass either the EPA chronic toxicity 4-day average or the acute toxicity 1-hour average concentration of ambient water quality criteria for conductivity toxicity.

A conductivity pulse exposure is a fluctuating, intermittent, exposure peak that varies with any given amount of time [19]. Given that yearly conductivity averages, and conductivity pulses are being experienced well above the EPA standard for freshwater biota in Boone. Examining the impact of these phenomena on headwater aquatic macroinvertebrates will be informative. This study will examine the impact of pulse salinity exposure on aquatic macroinvertebrates. Throughout the winters in Boone, there are frequent spikes in specific conductivity directly after a snow storm (Tuberty unpublished). These spikes have reached levels up to 12,083 μS cm^{-1} (as stated above), but levels this high may only occur for as short as 15 minutes and then drop down to 1000 – 3000 μS cm^{-1} which will persist for hours. Organisms are exposed to pollutants in the environment in an episodic nature, and a standard exposure experiment may not sufficiently show the impact of high but short lived conductivity concentrations [20]. Conducting pulse exposures will allow a more realistic measure of what the macroinvertebrates experience under these conditions. Specifically, we sought to answer the following questions: (1) Throughout the winters of 2010 to 2015, what are the cumulative distributions of each specific conductivity pulse event experienced in Boone Creek and the South Fork of the New River; (2) What are the impacts of pulse exposure experiments with the highest values observed and the most frequently experienced specific conductivity values observed and (3) Do increases in pulse exposure duration have an increased negative impact on survivorship?

Materials and Methods

Site description

The headwater streams of the USFNR were chosen for this study due to their proximity to Boone and the range of measured conductivities, rates of development and impervious surfaces. They include: Boone Creek (BC), East Fork of the New River (EFNR), Flannery Fork Creek (FF), Goshen Creek (GC), Hodges Creek (HC), Middle Fork of the New River (MFNR), Winklers Creek (WC), and the South Fork of the New River proper (SFNR; represented as State Farm). The two streams that were utilized for this study were Boone Creek, and SFNR. The Boone Creek sub watershed represents a heavily developed stream, while the SFNR represents a moderately developed watershed (Figure 1). Both experience exposure to winter road management.

Specific conductivity cumulative distribution analysis

Once the streams have been selected the data is scanned to look for any conductivity storm events that match the characteristic of ≥15% increase in one hour. When the storm event is found the data from the beginning till the end (when conductivity returns to background levels) was extracted. This process will continue until every storm event has been identified during the monitored years (2010 – 2015) for each stream. After each storm event has been isolated the data was broken down into 1-hour increments, and then the mean of each 1-hour storm event was calculated. The cumulative distribution was determined from this new dataset. It displayed different specific conductivity levels and how many hours each stream spent at the respective concentration ranges and help determine the different conductivity levels that were utilized for the pulse exposure trials [20, 21]. To simulate an environmentally relevant scenario, realistic background specific conductivity levels were identified by recording specific conductivity values between winter storm events. The mean value between storm events for each year was taken, and then the mean of each year was calculated (2010 – 2015) to determine the resting levels for the second exposure method. The background resting level calculated for the SFNR was paired with the most frequently experienced specific conductivity spike, and the level calculated for Boone Creek was paired with the worst case scenario specific conductivity spike.

Salinity pulse exposures

Insects from the order Ephemeroptera with a focus on the family Heptageniidae were used for the exposure experiments. Individuals were be identified by morphotype in the field with a focus on collecting *Epeorus* and *Maccaffertium* alive with kick seines, washing into shallow tubs, and removing them with Nitex mesh and transporting them to the lab on ice and/in aerated water. After collection the macroinvertebrates were allowed to acclimate to the experimental environment for 72 hours. Both macroinvertebrates and water used in experimentation (both to fill the experimental system, and mix road salt solutions) were collected from the reference stream site on Flannery Fork at Rocky Creek Road (36.188075, -81.677577). After experimentation individuals were separated into two categories: 1) individuals who were exposed to elevated conductivity levels and perished, or 2) individuals who were exposed to elevated conductivity levels and survived. Once separated, head capsule length and body length were measured under a dissection microscope with an ocular micrometer to account for varying developmental stages within the experiment.

Exposures took place in a custom built Frigid Units natural flow through system at 12^{0}C. Custom built Nitex Screen (600 µm) and VWR glass petri dish exposure vessels were used to contain macroinvertebrates (n = 10). There were a total of eight replicates for each exposure scenario with four negative control replicates, a total of 12 exposure vessels. A random number generator was utilized to place the exposure vessels within the grid in the Frigid Units natural flow through/recycle system.

Exposures were modeled after 96 hours acute toxicity tests [22]. Each trial had a variation in duration in both exposure to road salt and resting at either reference levels or increased specific conductivity levels to simulate expected environmental scenarios (Table 1). Twenty centimeter culture bowls held 1 liter of the experimental road salt solutions (2,000 and 10,000 µS cm^{-1}) and the resting elevated solutions (400 and 900 µS cm^{-1}). Examining the impact of the road salt pulse alone required that between exposures, organisms are removed from and returned to clean reference water. Examining the impact of an expected environmental scenario required that between exposures, organisms are removed and returned to elevated specific conductivity water. Clean reference water, culture bowls with experimental specific conductivity levels, and culture bowls with elevated resting specific conductivity levels were all contained in the Frigid Units natural flow through system at 12°C. Lethal Concentration of 50% (LC_{50}) was determined from the resulting survivorship counts that occurred at 0, 24, 48, 72, and 96 hours. Every trial had fresh sodium chloride solutions prepared, and each solution was monitored throughout the 96 hour trial with a handheld multi-parameter water chemistry meter to ensure quality control of the experimental environments. Water in the resting tank was monitored in the same manner, and fresh water from the reference site was added as needed to control conductivity levels. Water in the resting tank was replaced after each experimental trial.

Statistical analysis

Microsoft Excel 2010 was utilized to analyze specific conductivity data gathered by the data sondes. Specific conductivity spikes were broken down into 1-hour increments, and the average of each hour was taken and then a cumulative distribution was created for all specific conductivity spikes (2010-2015). A watershed was given a designated amount of hours that it experienced a specific conductivity range during the winter months of 2010-2015. The same process was replicated but rather than analyze spikes the specific conductivity levels between spikes were analyzed, and a realistic resting conductivity level was designated for ‘common’ and ‘worst case scenario’.

Statistical analyses were performed using SPSS v. 23 (IBM, 2015). A student’s t-test was utilized to determine the difference between the pulse of road salt alone and the impact of a simulated environmental scenario where resting between pulse exposures occurs at ‘common’ and ‘worst case scenario’ specific conductivity levels and not reference levels. Generalized linear models were utilized to examine the relationship between increased pulse exposure duration and decreased survival of individuals in the simulations of an environmental scenario. Two scenarios were examined, 1) a common specific conductivity level that is experienced during the winter months (2010-2015); 2) the highest specific conductivity level that is experienced during the winter months (2010-2015) represented as worst case scenario.

Results

Specific conductivity cumulative distribution analysis

After examining cumulative distribution data from the two impacted streams (BC, SFNR) two conductivity levels were chosen for experimentation (2,000 and 10,000 µS cm^{-1}). These two levels were chosen because in the most impacted sub basin (BC) there are five instances during 2010 to 2015 that the conductivity went above 10,000 µS cm^{-1} (Table 2). Using this as an exposure level represented the worst case scenario for streams that are facing greater impacts from development and thus being impacted by de-icing road salts in the winter. In Boone Creek the water experienced conductivity levels between 1,000 – 3,000 µS cm^{-1} for approximately 677 hours, and the South Fork of the New River experienced conductivity levels between 1,000 – 3,000 µS cm^{-1} for approximately 96 hours (Table 2). The middle point of this specific conductivity range, 2,000 µS cm^{-1}, represented the most frequently experienced concentration. Between the peaks the baseline specific conductivity was analyzed during the winter months through 2010 to 2015 to get an idea of what the specific conductivity levels are experienced in order to simulate an environmental scenario. The mean baseline specific conductivity for the SFNR was 359.94 µS cm^{-1} and for experimentation this value was rounded up to 400 µS cm^{-1}, and this value was paired with the most frequently experienced level, 2,000 µS cm^{-1}. The mean baseline specific conductivity for Boone Creek was 815.49 µS cm^{-1} and for experimentation this value was rounded up to 900 µS cm^{-1}, and this value was paired with the worst case scenario concentration, 10,000 µS cm^{-1}.

Salinity pulse exposures

The pulse alone exposure concentration of 2,000 µS cm^{-1} had 100% survival at 1 hour duration pulse, 95% survival at 6 hour duration pulse, 90% survival at 12 hour duration pulse, 80%

survival at 18 hour pulse duration, and 83% survival at 24 hour pulse duration. The generalized linear model (GLM) describes a significant (p = 0.026), as the duration of the pulse alone increases the survivorship significantly decreases. The pulse alone exposure concentration of 10,000 µS cm^{-1} had 100% survival at 1 hour duration, 70% survival at 6 hour duration, 91.25% survival at 12 hour duration, 75% survival at 18 hour duration, and 10% survival at 24 hour duration. The GLM for the pulse alone is not significant (p = 0.124), therefore, as the duration increases there is not a significant decrease in survivorship.

The simulation of an environmental scenario of 2,000 µS cm^{-1} pulse and resting at 400 µS cm^{-1} had 100% survival at 1hour pulse and 23 hour resting duration, 88.75% survival at 6 hour pulse and 18 hour resting duration, 86.75% survival at 12 hour pulse and 12 hour resting duration, and 73.75% survival at 18 hour pulse and 6 hour resting duration. The GLM for the simulated environmental scenario is not significant (p = 0.153), as the duration increased there was not a significant decrease in survivorship. The simulation of an environmental scenario of 10,000 µS cm^{-1} pulse and resting at 900 µS cm^{-1} had 87.5% survival at 1 hour pulse and 23 hour resting duration, 52.5% survival at 6 hour pulse and 18 hour resting duration, 51.25% survival at 12 hour pulse and 12 hour resting duration, and 28.75% survival at 18 hour pulse and 6 hour resting duration. The GLM for the simulated environmental scenario is significant (p = 0.001), as the duration increases the survivorship significantly decreases.

Size comparison of* Ephemeroptera Heptageniidae *Epeorus

We see a significant difference when comparing the head lengths and body lengths of individuals of Ephemeroptera Heptageniidae *Epeorus* that survived and did not (Figure 4). When examining the individuals exposed to the 2,000 µS cm^{-1} there is not a significant difference in head length (survived: 1.10 mm ± 0.03, perished: 1.02 mm ± 0.04; p=0.135) but there is a significant difference in body length (survived: 4.53 mm ± 0.07, perished: 4.15 mm ± 0.10; p=0.004). The data indicates that individuals who survived were significantly larger than those who did not survive. The same trend follows when examining the size data for the 10,000 µS cm^{-1}. The data shows that individuals who survived the exposures were significantly larger in both head length (survived: 1.19 mm ± 0.02, perished: 1.10 ± 0.02; p=0.0015) and body length (survived: 4.86 mm ± 0.07, perished: 4.08 mm ± 0.07; p=8.16*10^{-13}) than those who did not, and in comparison to the size data for 2,000 µS cm^{-1} there is a much greater difference in body length. We see that the individuals who perished are on average smaller than those who survived in 2,000 µS cm^{-1} (Figure 4).

Discussion

Salinization of streams is considered to be one of the greatest threats to the integrity of freshwater ecosystems and is recognized as a stressor of global concern [9, 23]. Natural variation in specific conductivity is strongly influenced by local geology, patterns of precipitation and evaporation, but anthropogenic sources can significantly increase concentrations in streams more than natural variation [17, 24]. Many studies have examined the toxic impact of salinization on a number of different aquatic organisms. The effect levels include testing the efficacy of the EPA chronic aquatic life benchmark [17], reduced density/diversity and increased drift [23], survival of limnephilid caddisflies (96 hr LC_{50}) [25], mean survival of many insect species (72 hr LC_{50}) [26], survival and emergence of *Chironomus riparius* [27], and the impact of growth of early instar *Neocloeon triangulifer* [28]. This study focused on the effect of environmentally relevant specific conductivity levels recorded the USFNR through the winters of 2010 to 2015, and examined the impact of acute pulse road salt exposures. There is a significant relationship that exists between exposure to increased pulse duration and decreased survivorship (Figure 2, Figure 3).

The scenario of 2,000 $\mu S\ cm^{-1}$ is the specific conductivity spike that was experienced most frequently during the winters of 2010 to 2015. Examining the impact of the pulse alone and then comparing it to the simulated environmental scenario allows us to see if survivorship increases when macroinvertebrates have a chance to recover with freshwater. The results of 2,000 $\mu S\ cm^{-1}$ resting at <50 $\mu S\ cm^{-1}$ and 2,000 $\mu S\ cm^{-1}$ resting at 400 $\mu S\ cm^{-1}$ are statistically similar (Table 3, Figure 2). When the background salinity levels are low, then the salinity pulse levels are responsible for the reduction in survivorship. The environmental simulation lead to an LC_{25} during the 18 hour pulse, and this is a significant reduction in sensitive taxa within the headwater streams of the USFNR (Table 3). The impact is likely to get worse if usage of deicing road salt increases and expands its range to the more pristine streams as the population of the town continues to grow. Through the use of the GLM statistics we see that there are inconsistencies between the 2,000 and 10,000 µS cm^{-1} data, and this is possibly due to the collection methodology. Collecting organisms for experimentation from the environment will result in variation amongst the trials. However, when examining the results there is an obvious negative trend that is occurring and we can conclude that as macroinvertebrates are exposed to increased pulse durations of 2,000 µS cm^{-1} that there is a decrease in survivorship overall (Figure 2).

The scenario of 10,000 µS cm^{-1} is a specific conductivity spike that is experienced rarely and has a short duration. Utilizing this level for experimentation was to demonstrate what could happen to sensitive taxa if specific conductivity pulses increase in intensity and duration due to increased population and impervious area. Variations in survival are high when organisms experience an extreme pulse and then are exposed to low salinity levels, i.e. 10,000 µS cm^{-1} resting at <50 µS cm^{-1}, which increases the ability of organisms to recover from these extreme salinity spikes (Table 3, Figure 3). When organisms experience an extreme pulse and then are exposed to elevated salinity levels, i.e. 10,000 µS cm^{-1} resting at 900 µS cm^{-1}, the decrease in survivorship remains constant. Increased background salinity levels coupled with extreme salinity pulses will have an increased negative impact on aquatic ecosystems.

Both 2,000 and 10,000 µS cm^{-1} experience the same trend in survivorship with the simulated environmental scenario. Survivorship decreases from the 1 hour to the 6 hour pulse, but survivorship between the 6 hour pulse and the 12 hour pulse for both concentrations are not significantly different. Then after the pulse of 18 hours we see another decrease in survivorship. The fact that both concentrations follow this same trend is interesting, and indicates that there is likely a physiological threshold of stress between 12 and 18 hours that leads to a greater decrease in survivorship. This may indicate that specific conductivity spikes of 12 hours or less in duration before returning to background specific conductivity levels could limit mortality among aquatic macroinvertebrates.

Salinity toxicity has a greater impact on macroinvertebrates who are less mature. There is a greater difference between individuals who survived and perished in the 10,000 compared to 2,000 µS cm^{-1}. As expected, the data indicates that macroinvertebrates of a younger instar are more susceptible to pollutants than the more mature individuals of the population [29, 30, 31, 32]. This could lead to an overall loss of less mature instar individuals during the winter months with both the common and worst case specific conductivity levels [30, 31, 32].

As stated earlier the use of road salt is something that will not be replaced in the near future due to cost and effectiveness. Therefore a way to help reduce the amount of road salt in the streams would be improving management practices. Training employees who handle road salt during the application process to decrease road salt usage [33]. This training would aim to “utilize the minimum amount of material necessary to achieve the desired outcome” by following the 4-R’s (right material, right amount, right place, and right time) [30]. Through education the amount of salt spread could decrease because employees would know the proper technique and science behind the method, and it would also help to retrain employees with the mindset that, “more is better”. Salt management plans (SMPs) can also ensure that maintenance agencies have a strategic tool to provide safe, efficient, and cost-effective road management [33, 34]. Making sure that all staff and personnel (including hired contractors) follow the protocol is another proactive measure to keep excess salt from the streams. Key components of a well-executed SMP would include: a thorough statement of policy and objectives, varying situational analyses (on-road use, salt-vulnerable areas, sand and salt storage sites, detailed snow disposal sites, and employee training), and up to date documentation regarding the application of road salt.

This study supports previous experiments, that increased road salt concentration in streams has a negative impact on aquatic invertebrate survivorship [17]. Due to the fact that pollutants are introduced into the environment via pulses we felt it was important to investigate this aspect of environmental exposure. Our data suggest that with an acute toxicity model the most frequently observed and worst case scenario salinity pulses in Boone can lead to a 26% or 90% survivorship reduction, respectively. Utilizing an identical methodology, future work could focus on the impact of the chronic toxicity of specific conductivity pulses on aquatic macroinvertebrates. Understanding the impact of specific conductivity pulses on an acute and chronic level would be valuable to road management crews when dealing with future road salt management plans to minimize adverse impacts to aquatic environments.

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35.

Tables

Table 1. Experimental design used to evaluate *Epeorus* and *Maccaffertium* response to pulse exposures of road salt. Each exposure replicate had an n = 8.

Target Road Salt Concentration (µS cm^{-1})	Actual Road Salt Concentration (µS cm^{-1})	Exposure x Duration (hr)	Days of Exposure	Reference Resting (µS cm^{-1})	Reference Resting Duration (hr)	Realistic Resting (µS cm^{-1})	Realistic Resting Duration (hr)
2,000	2,017	1 x 1	1, 2, 3, 4	< 50	23	400	23
2,000	2,017	1 x 6	1, 2, 3, 4	< 50	18	400	18
2,000	2,014	1 x 12	1, 2, 3, 4	< 50	12	402	12
2,000	2,005	1 x 18	1, 2, 3, 4	< 50	6	403	6
2,000	2,000	1 x 24	1, 2, 3, 4	< 50	0	N/A	0
10,000	9,960	1 x 1	1, 2, 3, 4	< 50	23	900	23
10,000	10,050	1 x 6	1, 2, 3, 4	< 50	18	902	18
10,000	10,036	1 x 12	1, 2, 3, 4	< 50	12	901	12
10,000	10,041	1 x 18	1, 2, 3, 4	< 50	6	901	6
10,000	10,050	1 x 24	1, 2, 3, 4	< 50	0	N/A	0

Table 2. Specific conductivity cumulative distributions for Boone Creek and the South Fork New River. From this data values were chosen to simulate the most common and worst case environmental scenarios (2,000 and 10,000 µS cm^{-1}).

Boone Creek (2010-2015)			South Fork of the New River (2010-2015)		
Specific Conductivity Range (µS cm^{-1})	Total Hours	Cumulative Percentage	Specific Conductivity Range (µS cm^{-1})	Total Hours	Cumulative Percentage
100-200	9	0.42	0-100	9	0.63
201-300	72	3.76	101-200	266	19.19
301-400	101	8.46	201-300	294	39.71
401-500	206	18.03	301-400	249	57.08
501-600	237	29.04	401-500	189	70.27
601-700	227	39.59	501-600	133	79.55
701-800	178	47.86	601-700	78	85.00
801-900	136	54.18	701-800	50	88.49
901-1000	101	58.88	801-900	35	90.93
1001-2000	481	81.23	901-1000	31	93.09
2001-3000	196	90.33	1001-2000	91	99.44
3001-4000	99	94.93	2001-3000	5	99.79
4001-5000	54	97.44	3001-4000	3	100
5001-6000	29	98.79			
6001-7000	13	99.40			
7001-8000	5	99.63			
8001-9000	3	99.77			
9001-10000	2	99.86			
>10000	3	100			

Table 3. Generalized linear model significance examining to see if there is a significant decrease in survivorship as pulse duration increases, for all treatment scenarios.

Treatment	Exposure x Duration (hrs)	% Survival	GLM Significance	2,000 µS cm^{-1} vs. 2,000 resting at 400 µS cm^{-1}	10,000 µS cm^{-1} vs. 10,000 resting at 900 µS cm^{-1}
2,000 µS cm^{-1} pulse alone	1 x 1	100 ± 0	p = 0.026*	p = N/A	p = 0.007**
	1 x 6	95 ± 1.8		p = 0.106	p = <0.001**
	1 x 12	90 ± 3.7		p = 0.287	p = <0.001**
	1 x 18	80 ± 3.2		p = 0.287	p = <0.001**
	1 x 24	83.75 ± 1.8			
2,000 µS cm^{-1} with 400 µS cm^{-1} rest	1 x 1	100 ± 0	0.153		
	1 x 6	88.75 ± 4.4			
	1 x 12	86.25 ± 5.3			
	1 x 18	73.75 ± 4.6			
10,000 µS cm^{-1} pulse alone	1 x 1	100 ± 0	0.124		
	1 x 6	70 ± 2.6			
	1 x 12	91.25 ± 3.9			
	1 x 18	75 ± 7.3			
	1 x 24	10 ± 3.2			
10,000 µS cm^{-1} with 900 µS cm^{-1} rest	1 x 1	87.5 ± 4.5	0.001**		
	1 x 6	52.5 ± 4.5			
	1 x 12	51.25 ± 3.9			
	1 x 18	28.75 ± 6.3			

*. Significant at the 0.05 level.
**. Significant at the 0.01 level.

Figures

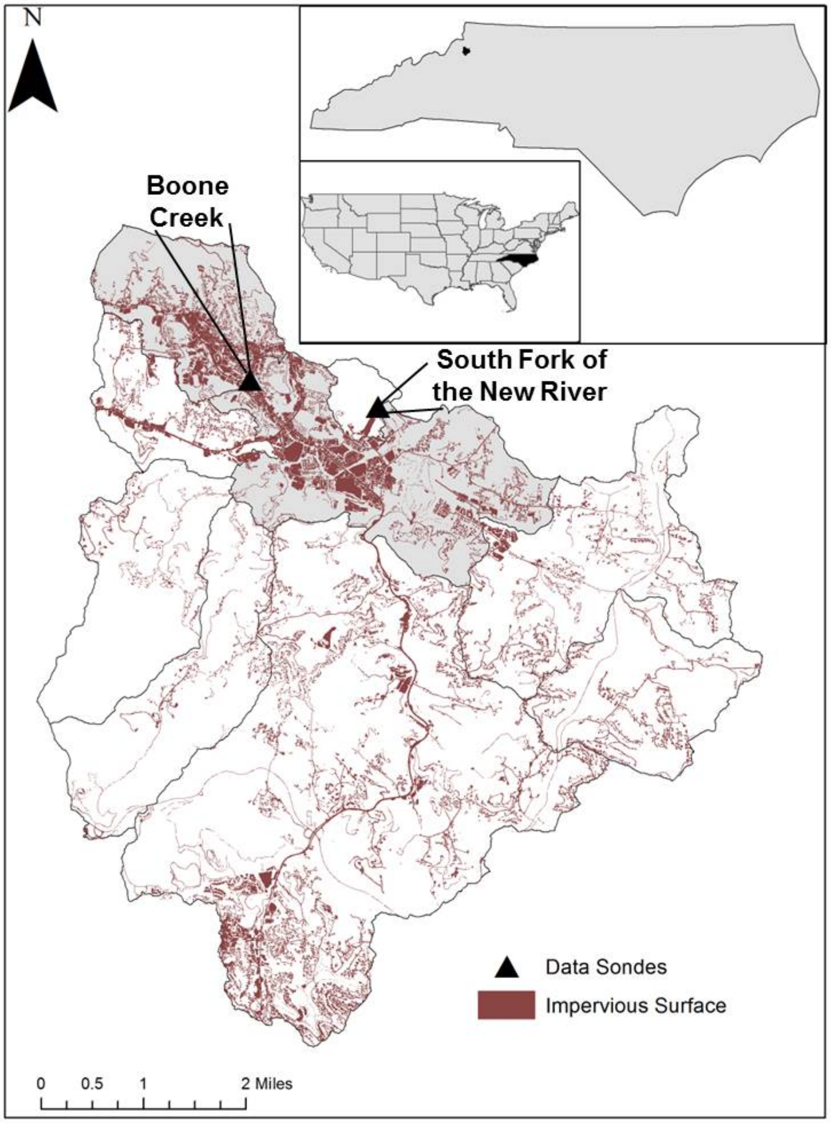

Figure 1. Map identifying the USFNR sub watersheds within North Carolina and within the United States. The map displays the impervious surface of Boone, NC. Boone Creek, the South Fork of the New River and the location of the data sondes.

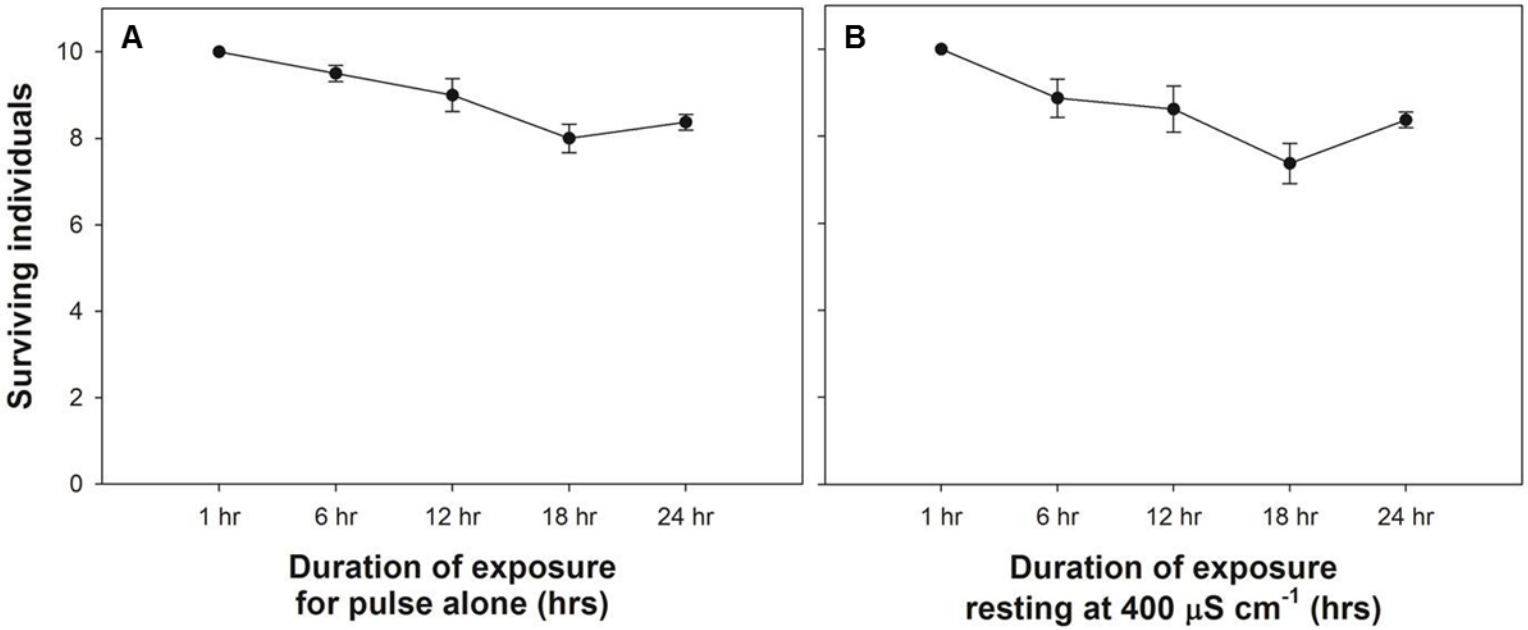

Figure 2. Surviving individuals of the varying durations of the (A) 2,000 µS cm^{-1} pulse alone and the (B) 2,000 µS cm^{-1} pulse with resting at 400 µS cm^{-1} to simulate environmental scenarios of the USFNR. The bars for each trial represent standard error. The GLM significance for (A) p=0.026, and the GLM significance for (B) p=0.153.

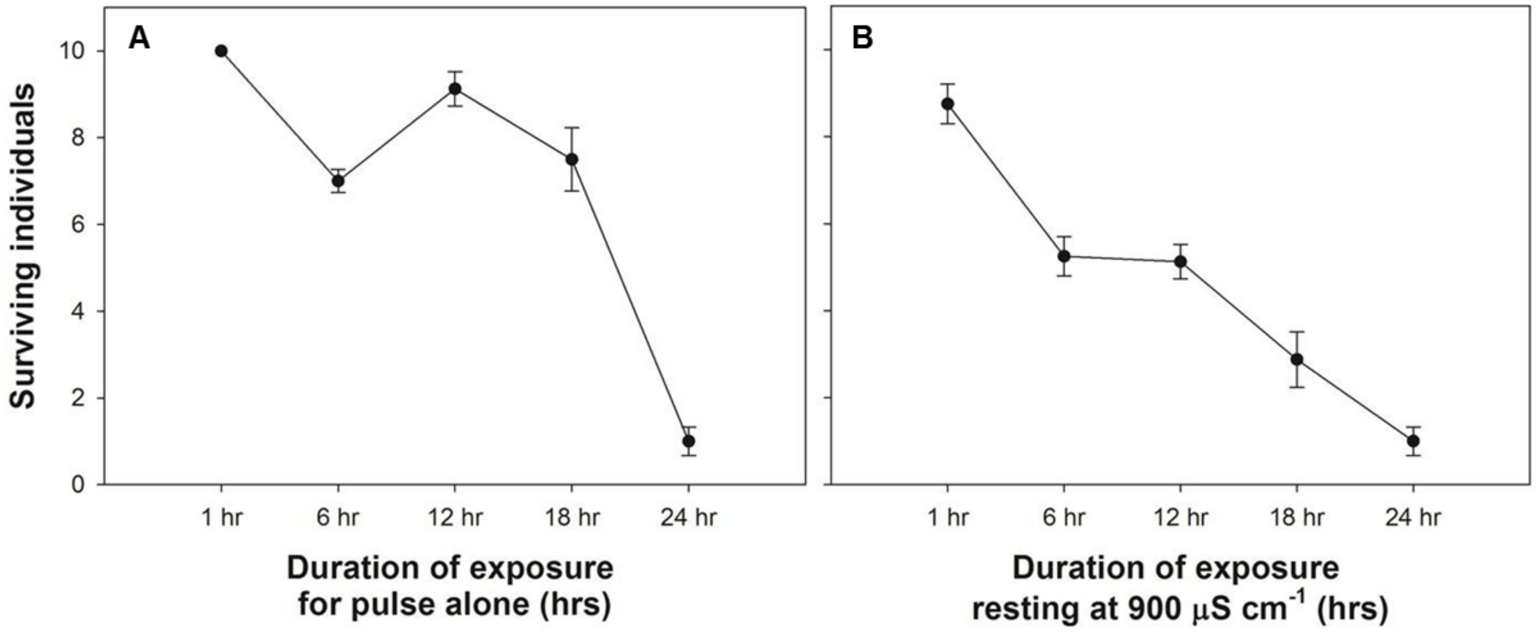

Figure 3. Surviving individuals of the varying durations of the (A) 10,000 µS cm^{-1} pulse alone and the (B) 10,000 µS cm^{-1} pulse with resting at 900 µS cm^{-1} to simulate "worst case" environmental scenarios from the USFNR. The bars for each trial represent standard error. The GLM significance for (A) p=0.124, and the GLM significance for (B) p=0.001.

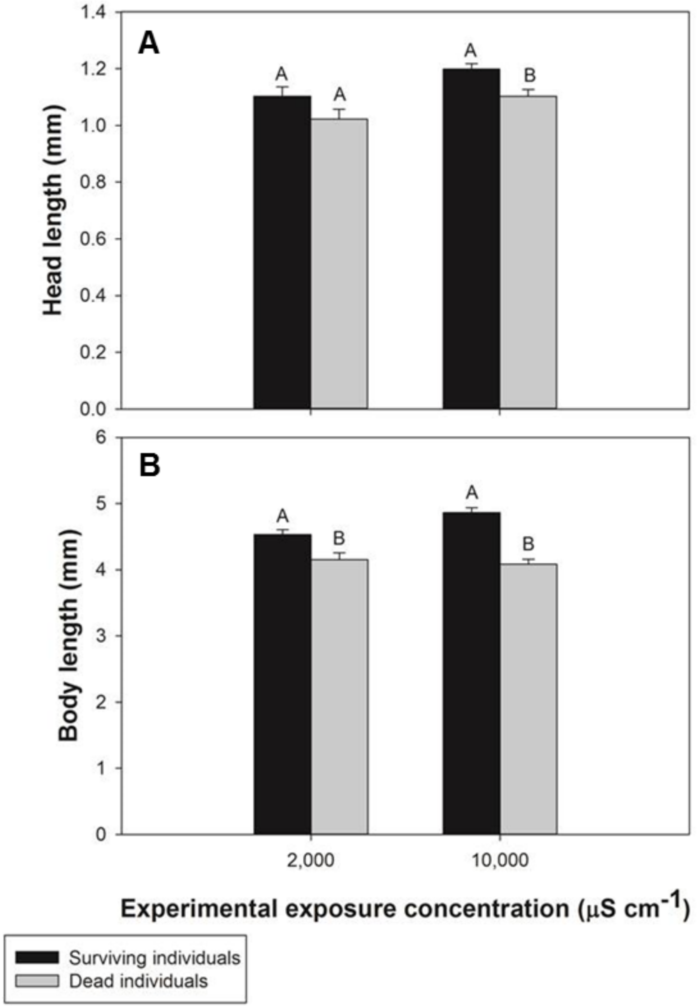

Figure 4. Comparison of (A) head length and (B) body length between individuals of Ephemeroptera Heptageniidae *Epeorus* following the pulse exposure(s). All individuals from the various treatment durations of the environmental simulations were combined for 2,000 (n=260) or 10,000 μS cm^{-1} (n=406). Bars with different letters represent significant difference ($p<0.05$) by a T-test, and each has standard error bars.

Vita

Matthew Joseph Fleetwood was born in Rutherford, North Carolina to Brian and Linda Fleetwood. He graduated from East Rutherford High School in Bostic, North Carolina in May 2009 and enrolled in Appalachian State University, Boone, North Carolina to study Cell/Molecular Biology the following fall. In December 2013, he was awarded a Bachelor of Science degree in Biology with a concentration of Cell/Molecular Biology. After graduating, he began work as a research assistant in a Chemistry lab at Appalachian State University. The fall of 2014 he started a Master of Science degree in Ecology with a focus on Aquatic Eco-Toxicology at Appalachian State University. The M.S. from Appalachian State University was awarded in May 2017.

In his free-time, Mr. Fleetwood is an avid endurance athlete with a focus on running and cycling both road and trail. He resides in Boone, North Carolina.