CHANGES TO WATER CHEMISTRY AND IMPLICATIONS FOR SENSITIVE AQUATIC BIOTA IN SOUTHERN BLUE RIDGE STREAMS

A Thesis
by
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Abstract

AN EXAMINATION OF CHANGES TO WATER CHEMISTRY AND DISTRIBUTION OF SENSITIVE AQUATIC BIOTA IN SOUTHERN BLUE RIDGE STREAMS

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Freshwater systems, which constitute a mere 2.5% of Earth's total water, are increasingly impacted by abiotic and biotic factors. Changes to land use and other anthropogenic stressors are widely understood to drive the alteration of freshwater ecosystems. The Southern Blue Ridge is among North America's most biologically diverse regions and is home to the forested headwaters of the Tennessee and Ohio drainages. I examined long-term (~60 years) water quality and land use (18 years) datasets to assess which broad-scale changes in water chemistry may be correlated with recent declines of aquatic species in this region. My in- depth analysis of water chemistry from 80 Hydrologic Unit Code (HUC10) watersheds revealed that only a limited number of watersheds (41-60) had sufficient long-term water chemistry measurements for statistical analyses. Even fewer exhibited data robust enough to discern seasonal trends over time at the HUC10 scale. Spearman correlations suggest most water chemistry parameters examined increased in value over the last 5 decades (DO concentration, total dissolved solids, pH, and specific conductivity). The most consistent trend was increased pH across all watersheds over time for up to 8 months of the year.

Dissolved oxygen percent saturation (DO% Sat), or how efficiently oxygen is held and used in the aquatic environment, appears to have declined over time. Mixed effects models were used to untangle the variation driving measurement values. Results of model fitting suggest year was the best predictor for all water chemistry parameters. Declines in pH measurements had a strong negative relationship with increasing urbanization, further highlighting the influence of disturbance and development on water quality. Beyond water chemistry, my investigation extended to the ecological domain, and I examined watershed occupancy trends across multiple decades (1900-2010). My results demonstrated consistent declines in occupancy for 9 of 11 species, and 63% of watersheds experienced a loss of 1 or more study species, underscoring the ecological impact of altered aquatic conditions. Decline in occupancy between decades was significant for all 3 freshwater fish species examined. Some of the taxa included in my analysis are some of the most vulnerable to water quality changes and represent a diverse assemblage of endemic freshwater mollusks, amphibians, arthropods, and fishes. From my analysis, the French Broad-Holston Basin contains some of the most urbanized watersheds, reflected in high mean ion concentrations, low DO% Sat, and the largest number of focal species lost during the last 100 years than any other basin. These findings emphasize the vulnerability of various native and endemic aquatic species to water quality changes. My study is the first to show widespread changes in streams that are generally considered to be at low risk of being impacted by anthropogenic and climatic disturbance. My research offers valuable insights into the dynamic interactions between environmental factors, urbanization, and aquatic ecosystems. It also emphasizes the pressing need for effective surface water resource protection and management to ensure the resilience of the Southern Blue Ridge region in the face of emerging water challenges.

Acknowledgments

I would first like to thank my Advisor, Dr. Michael Gangloff, and the Aquatic Conservation Research Lab for taking me on as a student and colleague acting as a sounding board every step of the way through this project. I would also like to thank my committee members, who challenged me in my Ichthyology and Biostatistics courses, along with thoughtful comprehensive exam questions. I am forever grateful to my committee for shaping me into the scientist I am today. A heartfelt appreciation for Dr. Lynn Siefferman and her time and effort spent building my writing and data analytics skills. I would like to acknowledge my sources of support from the Segal AmeriCorps Education Award, James C. Greene Fellowship in the Sciences, and Office of Student Research Grant. This would not have been possible without my profound experience in the Appalachian State Biology Department. I am forever grateful for the halls of Rankin leading me to Cristina Sanders, a mentor and shining example of what it looks like to achieve your goals despite the odds. It also led to my first experience in the field with Andy Hill, which has blossomed into one of my most treasured support systems. Thank you, Dr. Carol Babyak, for all her shared knowledge of chemistry and environmental toxicology, our time in the lab certainly helped prepare me for a project of this magnitude. Thanks to Luke Etchison and Lori Williams at North Carolina Wildlife Resources Commission for assistance with species selection. Thanks to Vincent Santini, Josh Platt, and the Geography and Planning Department for help with ARC GIS.

Dedication

I dedicate this work to my family, who shaped me into the fiercely kind and determined woman I am today. Without their loving foundation and contagious spirit for exploring the outdoors, I can say with confidence that I would not be seeking a career in the environmental sector. They jokingly say, “Hannah’s been in college since she was born!” and there is some truth, my parents had me early in their undergraduate careers and would often take me to lectures. I am honored to write that my parents recently celebrated 25 years of marriage and successfully raised three children while furthering their own educations and careers. I am also uniquely privileged to thank both sets of grandparents for their influence in fostering my interest in the natural world and sciences. Both of which I have been lucky enough to witness celebrate 50 years of marriage. “Our women are water. Soft enough to hold in your hand. Powerful enough to carve out the land itself.” ~Anonymous

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Foreword

This thesis will be submitted to Water Research, a peer-reviewed international journal. This thesis has been formatted according to the style guide for publication in this journal

Introduction

Freshwater systems make up less than 2.5% of all water on Earth but they are fundamental for the survival of many species, including humans (Jackson et al., 2001; Oki and Kanae, 2006). Freshwater systems are highly dynamic and include numerous important linkages in the global hydrological cycle. Natural hydrologic patterns vary extensively over long-time intervals in their spatial and temporal distributions (Gordon et al., 2004). These cycles influence nearly every facet of life and have become increasingly altered by anthropogenic inputs and activities (McGrane, 2016; Jackson et al., 2022). Identifying and interpreting changes in water quality within freshwater systems can indicate spatial and temporal alterations of waterbodies that help inform future water resource management approaches.

The Southern Blue Ridge (SBR) region in eastern North America includes parts of some of the oldest mountain ranges in the world, with some peaks dating to 480 Mya (Glover et al., 1983). The Appalachian Mountains collect and store rainfall, contributing to overall hydrologic flow and connectivity that is critical to maintaining healthy aquatic habitats. The SBR spans five southern states and is home to a complex network of rivers, streams, and reservoirs. Four major river basins (the French Broad-Holston, Kanawha, Upper Tennessee, and Middle Tennessee-Hiwassee) drain the Interior of the SBR region.

These drainage basins are characterized by steep, forested headwaters, and narrow, more developed valleys. Because these watersheds are ancient and were largely unglaciated, they support a high number of endemic freshwater biota (Meyer et al., 2007; Jenkins et al., 2015). These streams provide high-quality water resources for agriculture, industry, fisheries, and many other ecosystem services for human populations in this region (Omernik and Griffith, 1991; Krieger 2001; Viviroli et al., 2007).

Land use patterns in the SBR are dynamic (Tong and Chen, 2002). Historically water quality in the SBR region was affected by agriculture, mining, and logging (Gragson and Bolstad, 2006). The region was heavily logged and deforested in the 19th century but underwent extensive replanting in the last century is and is now predominantly forested (Kennedy, 2013; Blanton and Hossain, 2020; Jackson et al., 2022). Forested watersheds are essential to protect water quality, by absorbing water, filtering runoff, and holding soils in place, thus preventing erosion (Neary et al., 2009). Today, although many freshwater streams and key elements of biological diversity are protected by environmental statutes, freshwaters face threats from less obvious stressors that include ex-urban development, invasive species, introduction of novel diseases, and emerging use of toxicants (Neves et al., 1997; Graf and Cummings, 2007; Webster et al., 2012). Anthropogenic activities frequently alter hydrologic cycles and contribute to degradation of riparian and in-stream habitats and surface water quality (McGrane, 2016).

Loss of forest cover, especially at the landscape scale, may fundamentally disrupt the natural nutrient cycling and sediment dynamics in headwater streams (Dahlgren and Driscoll, 1994; Swank et al., 2001). Headwater streams in southeastern North America are primarily heterotrophic and rely on external inputs of woody debris and leaf litter to fuel in-stream processes (Warren et al., 2016; Thoms et al., 2017). This also means that headwaters are highly dependent on the diversity present within the terrestrial environment to carry out these processes (Mosher et al., 2015). Streams in the SBR are naturally ion poor and are characterized by low pH values and have low acid-neutralizing capacity (ANC), which means that SBR streams are less buffered and tend to be more acidic than streams in adjoining physiographic provinces such as the Valley and Ridge and Piedmont (Argue et al., 2012). Recent and historical surveys have documented widespread changes to the water chemistry and the surrounding landscape of Blue Ridge streams (Scott et al., 2002; Clinton and Vose, 2006; Webster et al., 2012; Hill et al., 2016; Kellner et al., 2018).

Although the mechanisms remain unclear, these changes appear to mirror those observed at continental scales in North America and beyond (Kaushal et al., 2019; Olson, 2019; Weyhenmeyer et al., 2019; Murphy, 2020; Stets et al., 2020; Wu et al., 2021; Zhi et al., 2023). Kaushal et al. (2018) coined the term Freshwater Salinization Syndrome (FSS) to describe the effects of well-documented and regulated natural and anthropogenic influences on stream physicochemical parameters compounded by a suite of cryptic stressors across a watershed.

These stressors include discharge from small-scale wastewater treatment facilities, thermal pollution, agricultural field runoff, and road de-icing agents that are believed to lead to spatially widespread increases in pH, specific conductance, salinity, temperature, dissolved oxygen, and concentration increases for a range of ions in surface waters worldwide (Paul and Meyer, 2001; Dodds et al., 2013; Leach and Moore, 2019; Moore et al., 2019; Kaushal et al., 2020; Bhide et al., 2021; Galella et al., 2021). Changes in solutes can produce freshwater syndromes, such as FSS, that are often associated with increases in total dissolved solids (inorganic and organic substances suspended in water), salinity, and alkalinity trends in riverine systems as influenced by a suite of natural and anthropogenic inputs (Stets et al., 2014; Kaushal et al., 2018; Wu et al., 2021). Salts increase the corrosive nature of water which often results in costly damages to infrastructure and putting drinking water at risk (Vineis et al., 2011; Cooper et al., 2014).

During the past several decades, watersheds across the SBR have experienced dramatic losses of some aquatic species (e.g., hellbenders, mussels) from significant portions of their ranges. The exact causes of these declines are likely due to a combination of stressors, including habitat degradation, point, and nonpoint sources of pollution, climate change, and the introduction of non-native species (Swank et al., 2001; Peters and Meybeck, 2009; Caldwell et al., 2016). Because multiple stressors are involved, it can be challenging to pinpoint a direct cause for a particular decline. However, there is a consensus that sensitive aquatic species are experiencing widespread range reductions due in part to changes in habitat and water quality, particularly evident for freshwater fish (Brungs et al., 1978; Cope et al., 2021). Population sizes, occupied areas, or in some cases both, have decreased for many historically widespread taxa in this region including numerous species of freshwater mussels (Johnson et al., 2014; Ostby et al., 2016; Pandolfi et al., 2022), Eastern hellbenders (Pugh et al., 2016) and fishes (Pugh et al., 2020).

My study incorporates a multi-faceted approach to elucidate relationships among changes to water chemistry, land use, and occupancy of four different groups of freshwater taxa endemic to SBR watersheds. Using data analytics techniques, I examined changes in water chemistry across more than 5 decades in this region to identify spatial patterns at the watershed and basin scales that might help illuminate drivers of enigmatic species declines (Conrads and Roehl Jr., 2010). I aim to untangle the complex interplay between anthropogenic inputs, abiotic and biotic processes, via creation of spatial and temporal profiles of water chemistry measurements at the watershed scale. Findings from my exploratory analysis may highlight changes in water quality over time and associated impacts of urbanization in SBR watersheds. My study will help inform management and conservation of freshwater resources, native aquatic species, and land use in the SBR.

Methods

Study Sites

The French Broad-Holston, Upper Tennessee, Middle Tennessee, and Kanawha basins fall within the Humid Temperate Domain and have similar patterns of climate, vegetation, and aquatic communities (Fig. 1) (Bailey, 1994; Cleland et al., 1997). All streams within the Interior (i.e., Mississippi) Basin are part of the Central Appalachian Broadleaf Forest Province (M221) ecoregion and are characterized by watersheds that are generally forested and steeply sloped. Small portions of the study area fall within the Southeastern Mixed Forest (231) and the Eastern Broadleaf Forest provinces (221). (Cleland et al., 1997; 2007). Most focal streams in this study drain catchments dominated by crystalline lithology (Bailey, 1994; Cleland et al., 1997; Spencer, 2017). Geologic differences among watersheds can influence baseline water chemistry parameters (Cleland et al., 1997; Kaushal et al., 2013; Kaushal et al., 2018).

Water Chemistry Data

I obtained water quality data in September 2022 from the Water Quality Portal (WQP), a public database supported by the collaborative efforts of the United States Environmental Protection Agency (EPA), the United States Geological Survey (USGS), and the National

Water Quality Monitoring Council (NWQMC). I compiled water chemistry records for pH, temperature (^{o}C), spC (temperature standardized, µS/cm @ 25^{o} C, and raw field measurements, µS/cm), total dissolved solids (TDS, ppm), dissolved oxygen (DO, % Sat and mg/l) for my four major study basins. All water chemistry parameters were provided a unique hydrologic unit code (HUC) at the basin (10,596 km^2) and watershed (588 km^2) level based on geolocation of collected water quality measurements. This hierarchical drainage system was initiated by USGS to create a standardized system for reporting and collecting water data.

Records for pH, specific conductance, DO, and temperature spanned a total of 10 decades with dates ranging from May 1930 to August 2021. I aggregated data at the basin level (HUC6) and then further grouped at a finer spatial scale representative of the watershed level (HUC10). Statistical outliers (i.e., those >2 standard deviations from the mean) were then removed, and normality assumptions for correlations were visually and statistically assessed using histograms, Q-Q plots, box plots, Kolmogorov-Smirnov, and linear residual regression to test for homoscedasticity (IBM SPSS Statistics 28.0). Most parameters appeared non-normal in distribution and monotonic in pattern when the measurement was plotted against (see statistical analysis).

I split the data output by month to control for the variation in seasonality. I imported water quality data to ArcGIS Desktop 10.8.2 and associated with the base layer using x and y coordinates based on the GCS_WGS_1984 geographic coordinate system. Water quality observations were then spatially joined to the Watershed Boundary Dataset: HUC 10s by ESRI, based on GPS coordinates to aggregate data at the watershed level (avg. 58,792.7 ha) within their respective basins (avg. 2,744,351.4 ha, NWQMC, 2020). I separately analyzed water chemistry measurements associated with available land use years. I identified a total of 80 HUC10 watersheds within the SBR region and 63 HUC10 watersheds had available water quality data.

My final data matrix, after removal of duplicates and outliers, included 173,396 individual water quality measurements from the four river basins. Earliest measurements ranged from 1930 (TDS), to 2021 (Temp, DO, pH, spC µS/cm, and TDS). I imported water chemistry, land use, and species datasets to IBM SPSS Statistics for descriptive and statistical analysis. Watersheds varied widely in available water quality data; the final matrix included HUC10s with 6 to 76 years of data. I excluded datasets with n<30 observations per parameter from the descriptive analyses.

I had to take special precautions with the parameter pH, because it is not valid to calculate an arithmetic mean as the average pH value. Due to the logarithmic scale, I computed mean pH measurements to properly calculate descriptive statistics. This was done by first converting pH values to hydrogen ion concentrations by taking the antilogarithm using the formula $[H+] = 10^{(-pH)}$. The hydrogen ion concentration was then averaged and converted back to the pH logarithmic scale (Wetzel, 2001).

Occupancy Data

I compiled occurrence data using current and historical species observations mined from Portal Access to Wildlife Systems (PAWS), Vertnet, and the Global Biodiversity Information Facility (GBIF). All data used in the occupancy modeling was sourced from publicly available resources and included museum records, research-grade citizen science data, and North Carolina Wildlife Commission survey records. I selected 11 species with native ranges that included the Ohio and Tennessee drainages that were included in the 2020 Addendum 1

North Carolina Wildlife Action Plan (NCWAP) and/or the Regional Species of Greatest Conservation Need (RSGCN) published by the Southeast Association of Fish and Wildlife Agencies (SEAFWA) (Rice et al., 2019; NCWRC, 2020).

I consulted Wildlife Diversity Biologist, Lori Williams, and the Western Region Aquatic Wildlife Diversity Coordinator, Luke Etchison, at the North Carolina Wildlife Resources Commission (NCWRC) to compile the final species list (Table 1). They also reviewed occurrence data for all fish and amphibian species included in my study. I imported occurrence data in Microsoft Excel and spatially joined them in ArcGIS Desktop 10.8.2 using x and y coordinates from observation locations with the Watershed Boundary Dataset: HUC10s by ESRI.

Using presence absence data for modeling species naïve occupancy can help researchers understand changes in species distribution patterns, especially in cases where species are data-limited or difficult to sample (MacKenzie et al., 2006). Naïve occupancy assumes perfect detectability at all sites and can provide valuable insights when sampling effort or methods are unknown (Ewing and Gangloff, 2016). These approaches can be powerful tools for estimating changes in the extent of occurrence and occupied area of species, but they assume data are binary (i.e., presence/absence data), sites are independent, detection probabilities are homogenous, and there are no false positives or negatives in the dataset (MacKenzie et al., 2006).

I sorted species data according to watershed and converted the number of sightings to binary data (occupied HUC10= 1 and unoccupied HUC10= 0). Data were compiled across 13 decades (1900-2020) for each watershed to examine past and present spatial occupancy across the SBR study watersheds. I backfilled data based on the assumption that all presently occupied sites were also historically occupied. This is a generally conservative assumption because of the historical distribution for the selected species within the SBR watersheds. I used occupancy modeling to observe changes in occupancy and detectability at the HUC10 scale and to compare historical versus current HUC10 occupation for selected study species. I created naïve occupancy models for eleven different aquatic species in four taxonomic groups (fishes, mussels, crayfishes and amphibians, Table S16).

Land use Data

I used percent urban/impervious land cover as a proxy for anthropogenic disturbance at the HUC10 watershed scale. I obtained these data for each watershed from the National Land Cover Database (NLCD) 2001-2019. I clipped the raster file for each year (2001, 2004, 2006, 2008, 2011, 2013, 2016, 2019) to the HUC10 polygon, specifying watershed area, in order to join land use and water chemistry data in the same data table. I converted the watershed percent impervious surface (%) to an annual average percentage using the ‘zonal statistics’ tool in ArcGIS Desktop 10.8.2 for available watersheds within the study region (Figure 18).

I then spatially joined the data using the geoprocessing tool “clip” based on x, y coordinates to represent the HUC6 and HUC10 scale. The Watershed Boundary Dataset was used to define the perimeter and x, y tolerance set to 1 meter (USGS, 2023). I used ArcGIS Desktop 10.8.2 to join the percent urban imperviousness as a column in the final water chemistry attribute, defined by year and HUC10 code. I delineated the HUC10 watersheds in ArcGIS Desktop 10.8.2 using the Watershed Boundary Dataset, overlaid with digital elevation model (DEM).

Watersheds included in my study ranged in area from 284 to 1576 km^2, while mean elevation ranged from 251 to 1159 m (Table 3). My final matrix included the percentage of urban impervious surface which ranged from 6.4% to 47.7% in the forested SBR region from 2001-2019. My attribute table for watersheds in the four Interior Basin drainages was exported to Microsoft Excel for further analysis. I assessed this truncated dataset for normality, outliers, duplicates, and it was visualized in both Jamovi and SPSS. I used the NLCD representing the 8-year series to conduct statistical analyses to test for potential effects of urbanization on water chemistry.

Statistical Analysis

I used Spearman rank correlations to analyze temporal trends in monthly data by examining the relationship between year and a suite of water quality parameters including pH, spC µS/cm, temp, DO (ppm & % Sat), and TDS (Conover and Iman, 1981). I examined water chemistry trends within each month across year to account for seasonal variation. All 2-tailed analyses were performed at $\alpha = 0.05$. I considered correlation coefficients less than 0.3 to be weak and they were not included in further analyses (Argue et al., 2012). From the results, excluded watersheds having <3 decades of data and an average of <10 data points per year, due to my interest in examining long-term trends and the high number of significant relationships among water chemistry parameters.

I analyzed species data using McNemar's test in SPSS to determine if HUC10 occupancy has changed over time from 1990-2010. McNemar's is a commonly used statistical test for comparing paired binary data and is particularly useful in wildlife monitoring and management (Hines et al., 2014). McNemar's chi-square test compares the proportion of discordant pairs to the expected proportion under the null hypothesis of no difference between the survey methods or decadal time periods. A significant result ($\alpha \leq 0.05$) suggests that there is a significant change in the distribution of the species or taxonomic group over time (MacKenzie et al., 2006).

I used linear mixed-effects models to assess fixed and random effects for multiple variables in the relationship between percent urban impervious surface and water chemistry parameter measurements (Temp, pH, DO, spC, TDS). These models apply methods that are an extension to the typical linear model, such as linear regression, and are typically used when faced with complex sample design (i.e., longitudinal data, repeated measures, multi-level data) and non-normal data (Pinheiro and Bates, 2000). This approach allowed me to look at what model terms best fit the associated water chemistry parameter with effects of year and percentage urban imperviousness, based on the nested structure of the measurements associated with the 8-year interval NLCD data (Bolker, 2015).

The restricted maximum likelihood method (REML) was applied to all models to correct bias attributed to the variance components in multi-classified data and all mixed model analyses were performed Jamovi 2.2.5. (Jamovi Project, 2021). I used the Bonferroni correction in my post hoc analyses to control for type 1 errors within the data (Schwarz, 1978). In the model, water chemistry measurements were the dependent variable, year was included as a factor, and % urban imperviousness represented the covariate. Watershed location and unique GPS coordinates were cluster variables in the model fitting. Due to the strong interaction with month and my research focus on detecting long-term changes in measurements and attempting to identify location driven vs. time driven influence on water chemistry parameters, the variable month was excluded from my model fitting.

Fixed effects included in my model included year, % urban imperviousness, and the interaction term year * % imperviousness (Sullivan et al., 2021). I applied the fixed omnibus test, where a higher F value indicates a better model fit and if the p-value is ≤ 0.05, the null hypothesis is rejected, and the fixed predictor variable has a statistically significant effect on the response variable(s) (Bolker, 2015). I estimated the proportion of variance explained by both fixed and random effects (conditional R^2, R^2c) using the base language R with the GAMLj package: General analyses for linear models in Jamovi (Gallucci, 2019; R Core Team, 2021; Jamovi Project, 2021). In summary, the results of a fixed omnibus test in a mixed-effects model analysis provide information about whether the collective set of fixed predictors has a significant impact on the dependent variable.

I included HUC10 and GPS (unique lat/long) as the random effects in my model to account for variation in land use and water quality measurements associated with spatial distribution within the structured data. Variance within the data was explained by the random intercept for each effect, interpreted by the SD, Variance, and Intraclass Correlation Coefficient (ICC) ranging from 0-1, where a larger ICC value indicates higher variation due to the random effect of GPS (sampling location), or HUC10 (watershed).

I used the Akaike Information Criteria (AIC) to assess overall goodness-of-fit for candidate models in my study, where lower AIC equates to the best fitting model (Akaike, 1974). I ran the null models for each dependent variable to determine if the best model was superior to only accounting for the random variable effects. Models within two delta AIC were considered competitive and will be discussed in furth analysis (Burnham and Anderson, 1998; Arnold, 2010; Harrison et al., 2018). The choice of the best model depends on the specific parameter being modeled, and it is important to consider both model fit and complexity when selecting the most appropriate model. Overall model fit in my study was assessed using AIC, Δ AIC, and calculated model weight to assess the fixed and random effects to draw relationships between variables and associated variability within the data (Wagenmakers and Farrell, 2004; Portet, 2020).

Results

Overview

At the basin level (HUC6) positive linear trends were detected for DO ppm, pH, and TDS ppm. Of the 80 HUC10s investigated, only a portion (41-60) had long-term water chemistry data (>30) at the watershed level (Table 2). An even smaller number of HUC10s (6-22) had robust enough measurements over time to detect seasonal trends in the data. Correlative data suggest most watersheds have experienced increased temperature, DO ppm, spC, and pH measurements over the last several decades. The most apparent trend was the increase in pH over time across all watersheds; nearly all correlations had a moderate to strong positive relationship over time. The only decrease over time was for DO% Sat, where most of the correlations had a moderate to strong negative relationship for several months of the year.

Water Temperature

Water temperature data were available from 59 HUC10 watersheds across 70 years. The mean temperature was 13.5° C (SD= 6.2, N= 42,288) and watershed means ranged from 11.1° C in Fox Creek (Kanawha) watershed to 16.9° C in the Chickamauga Lake (Middle Tennessee-Hiwassee) watershed (Table 3; Table 4). Mean temperatures across the 4 HUC6 basins ranged from 12.7° C to 14.2° C with the lowest reported from the Kanawha Basin and the highest from the Middle Tennessee-Hiwassee (Fig. 2; Fig. 3).

Only 19 out of 59 watersheds with available data exhibited statistically significant temperature trends as revealed by Spearman rank correlation analyses (Table 5). Temporal changes in temperature were detected in the French Broad-Holston (7 HUC10s), Kanawha (2 HUC10s), Middle Tennessee-Hiwassee (3 HUC10s), and the Upper Tennessee Basins (7 HUC10s). However, across all months, very few HUC10s had enough measurements to calculate trends across more than 1-2 months of the year. Among the watersheds exhibiting significant temporal trends, 64.3% were positive indicating warmer temperatures whereas 35.7% appear to have become cooler over the period of record (Table 5).

The most consistent temperature increases occurred in the Headwaters of the North Toe River watershed (French Broad-Holston) for the months April (ρ= 0.46, $p<0.001$, N= 71), May (ρ= 0.40, p= 0.001, N= 66) (Table 5; Fig. 4). Significant warming also occurred for Upper Tellico Lake watershed (Upper Tennessee) (Fig. 5). Mud Creek was one of the watersheds that has experienced cooler stream temperatures over time (Table 5; Fig. 6). Temperature data at this spatial and temporal scale allowed for detection of change in surface water temperature over time.

Dissolved Oxygen

Dissolved oxygen concentration (ppm) data were available for 51 HUC10 watersheds ($n\geq 30$) and the final dataset had an overall mean of 9.8 ppm (SD= 0.24, N =30,804) measurements across 53 years (Table 6; Fig. 7). The mean DO concentration was highest in the Kanawha Basin and lowest in the Upper Tennessee Basin (Table 6). All major basins showed an increasing linear trend of DO ppm over time (Fig. 7).

Significant correlations between DO concentration and time were observed for 24 months in 13 watersheds (Table 7). Relatively strong increases in DO concentration were observed in several French Broad-Holston watersheds including the Mills River in July (ρ= 0.56, $p<0.001$, N= 57), August (ρ= 0.79, $p<0.001$, N= 58), September (ρ= 0.52, $p<0.001$, N= 54), and November (ρ= 0.35, p= 0.01, N= 51), the Doe River in July (ρ= 0.76, $p<0.001$, N= 32) and November (ρ= 0.37, p= 0.002, N= 37). Significant decreases in DO concentration were observed in the Oconaluftee River May (ρ= -0.52, $p<0.001$, N= 81) and August (ρ= -0.62, $p<0.001$, N= 75) and Upper Tellico Lake (ρ= -0.62, $p<0.001$, N= 48) HUCs in the Upper Tennessee Basin. Only two HUCs had declines in DO ppm over time in the French Broad-Holson Basin, Mud Creek during July (ρ= -0.47, p= 0.001, N= 44) and August (ρ= -0.36, p= 0.02, N= 42) and Cane Creek during June (ρ= -0.48, p= 0.001, N= 42) and July (ρ= -0.37, p= 0.005, N= 58). Of the 24 significant correlations observed, 71% were positive and 29% were negative. Moderate to strong increases in DO concentration were observed in watersheds in the French Broad-Holston, Kanawha, and Middle Tennessee-Hiwassee Basins. Moderate to strong negative associations were observed in French Broad-Holston and Upper Tennessee HUC10 watersheds (Table 7).

DO saturation data were available for 41 HUC10 watersheds, across 53 years and the mean DO saturation level was 90.2% (SD= 4.7, N= 13,223) (Table 8; Fig 8). Mean DO saturation was lowest in watersheds in the French Broad-Holston Basin and highest in the Middle Tennessee-Hiwassee Basin HUC10s. At the HUC10 scale, mean DO saturation ranged from 80.2 % in Hominy Creek (French Broad-Holston) to 95.5 % in Spring Creek-French Broad River watershed (Table 8). Spearman correlations for DO saturation were observed for 18 out of the 41 HUC10 watersheds initially included in analysis (Table 9). I observed 44 strong to moderate correlations in the dataset with 36 % exhibiting significant positive relationships between DO saturation and 64 % showing significant negative correlations over time (Table 9).

The most consistent decreases in DO saturation were observed in the French Broad-Holston Basin watersheds including the South Toe River-North Toe River (5 months) with the strongest negative correlations over time observed during the months of June (ρ= -0.71, $p<0.001$, N= 52) and July (ρ= -0.66, $p<0.001$, N= 42). The strongest correlations in the Kanawha Basin were observed in the Little River with increases in March (ρ= 0.51, $p<0.001$, N= 41) and June (ρ= 0.53, $p<0.001$, N= 36), North Fork (ρ= -0.59, $p<0.001$, N= 30) and South Fork New River (ρ= -0.64, $p<0.001$, N= 69) watersheds decreased over time. Moderate decreases in DO saturation were also observed in the Little River HUC10 (Kanawha) during May (ρ= -0.45, p= 0.001, N= 47). The strongest positive correlations were observed in the Mills River (French Broad-Holston Basin) during July (ρ= 0.59, $p<0.001$, N= 36) and August (ρ= 0.76, $p<0.001$, N= 46), the Cane River in August (ρ= 0.58, $p<0.001$, N= 32) and the Headwaters of the North Toe River in August (ρ= 0.50, $p<0.001$, N= 46).

In the Middle Tennessee-Hiwassee Basin, streams in the Ocoee River HUC exhibited a strong negative trend in DO saturation during the May (ρ= -0.51, p= 0.003, N= 33) and December (ρ= -0.63, $p<0.001$, N= 37) and moderate negative correlations in June (ρ= -0.34, p= 0.02, N= 45), October (ρ= -0.43, p= 0.003, N= 48), and November (ρ= -0.45, p= 0.003, N= 43). The Valley River also exhibited a trend of decreasing DO saturation during November (ρ= -0.37, p= 0.04, N= 31). In the Upper Tennessee Basin, the Nantahala River HUC DO saturation showed a strong increase in September (ρ= 0.59, $p<0.001$, N= 30) and moderate increases in January (ρ= 0.38, p= 0.03, N= 31) and November (ρ= 0.44, p= 0.01, N= 34). The Alarka Creek-Little Tennessee River HUC was the only watershed in this drainage that exhibited a decline in DO saturation, as July (ρ= -0.37, p= 0.01, N= 45) DO saturation appears to have decreased over time (Table 9).

pH

pH data (reported as the H+ concentration for statistical analyses) were available from 1945-2021, across a total of 60 HUC10 watersheds (Table 10; Fig. 9). Mean converted H+ concentration across all study basins was 6.4 (SD= 5.8, N= 52,839) and ranged from 6.2 in the Upper Tennessee Basin to 6.8 in the Kanawha Basin (Table 10; Fig. 10). At the watershed scale, minimum pH mean ranged from 3.9 in Headwaters North Toe River (French Broad-Holston) to 6.9 in the Watauga River (French Broad-Holston) (Table 10). Maximum mean pH ranged from 7.0 in the Little River (Upper Tennessee) to 9.8 in Roan Creek (French Broad-Holston) (Table 10).

When analyses were conducted within months, I observed 62 significant correlations between pH and year (Table 11). Of these, 63% displayed significant strong positive trends whereas 34% had significant moderate positive trends. In contrast, only 3% of the significant correlations were negative. Thus, 97% of the watersheds where pH has changed over time experienced increases in pH over the period of record (Table 11). Significant increases in pH were observed for 1-8 months in these HUCs. Spearman rank correlations indicated a total of 22 HUC10s had moderate to strong increases in pH over time, 11 watersheds in the French Broad-Holston, 4 watersheds in the Kanawha, 3 watersheds in the Middle Tennessee-Hiwassee, and 4 watersheds in the Upper Tennessee Basin (Table 11).

In the French Broad-Holston Basin, significant increases in pH were observed in the North Indian Creek-Nolichucky watershed for 8 out of 12 months (Table 11; Fig. 11). In the French Broad-Holston, the Doe River watershed was the only HUC to experience a decline in pH during the month of July (ρ= -0.36, p= 0.04, N= 34). In the Kanawha Basin, the South Fork of the New River had the most consistent increases in pH (6 months), followed by the North Fork New River (4 months) (Table 11). In the Middle Tennessee-Hiwassee Basin, strong positive correlations occurred in the Spring Creek watershed for 5 months and no negative correlations were observed among any watersheds in the basin (Table 11). Lastly, in the Upper Tennessee Basin, the most consistent increases in pH occurred in the Cheoah River, with the strongest positive correlations observed in streams of the Upper Tellico Lake HUC (Table 11). Only one watershed in the Upper Tennessee exhibited a weak negative correlation, Abrams Creek, during July (ρ= -0.38, p<0.001, N= 121).

Dissolved Ions

Temperature-standardized conductivity (i.e., specific conductance at 25°C) data were available for 41 HUC10 watersheds with an overall mean of 69.7 uS/cm (SD= 71.9, N= 6,060, Table 12). Decadal ranges varied by HUC10, but generally covered ~64 years of specific conductance observations. Mean specific conductance values across the 4 drainages ranged from 36.1-110 uS/cm with the highest values observed in the French Broad-Holston Basin watersheds and the lowest in the Upper Tennessee Basin (Table 12). Mean specific conductance at the HUC10 scale ranged from 13.0 uS/cm in Hiwassee River-Chatuge Lake HUC (Middle Tennessee-Hiwassee) to 340 uS/cm in the Richland Creek-Pigeon River HUC (French Broad-Holston) (Table 12). No watersheds had >30 specific conductance measurements per year and so I did not examine temporal trends within watershed due to small sample sizes.

Raw field value conductivity (not temperature-standardized) measurements were available for 41 HUC10 watersheds and had an overall mean of 62.7 µS/cm (SD= 58.6, N=

16,986, Table 13). The most comprehensive dataset ranged 41 years. Means for the HUC6 basins ranged from 24.6 µS/cm in the Upper Tennessee to 86.5 µS/cm in the French Broad-Holston (Fig. 12). At the watershed scale, means ranged from 12.1 µS/cm in Fontana Lake (Upper Tennessee) to 413.8 µS/cm in Cove Creek-Nolichucky River (French Broad-Holston, Table 13).

A total of 6 HUC10 watersheds had significant Spearman correlation coefficients (n=13) with conductivity over time, representing all but one basin, the Middle Tennessee-Hiwassee (Table 14). In the French Broad-Holston Basin, the HUC10 Pigeon River had a moderate positive correlation over time during the month of December (ρ= 0.38, p= 0.03, N= 33). In the Kanawha, 3 watersheds (Fox Creek-New River, Little River-New River, and North Fork New River) had moderate to strong positive relationships for 2-3 months of the year, representing the months from April to November (Table 14). In the Upper Tennessee Basin, Fontana Lake had a strong positive correlation with the month of March (ρ= 0.54, $p<0.001$, N= 192), indicating specific conductance has been increasing over time. Also in the Upper Tennessee Basin, the Lower Tuckasegee River was the only watershed with a strong negative correlation, suggesting that specific conductance has been decreasing over time during the months of January (ρ= -0.64, $p<0.001$, N= 158), June (ρ= -0.55, $p<0.001$, N= 166), November (ρ= -0.53, $p<0.001$, N= 190) and December (ρ= -0.55, $p<0.001$, N= 134).

TDS data were available from 46 HUC10 watersheds mean TDS representing all 4 major basins was 46.4 ppm (SD= 40.3, N= 9,389) (Table 15; Fig.13). Mean basin-scale TDS values ranged from 26.3 ppm in the Upper Tennessee, to 64 ppm in the French Broad-Holston Basin. Measurement data ranged from 1930 to 2021, with most observations starting in SBR watersheds around the 1960’s (Table 15). Mean watershed TDS ranged from 11.1

ppm in Hiwassee River-Chatuge Lake HUC (Middle Tennessee-Hiwassee) to 220 ppm in Cove Creek-Nolichucky River HUC (French Broad-Holston, Table 15). Only three watersheds representing the French Broad-Holston and Kanawha basins had enough data to be included in Spearman rank correlation results of TDS over time (with only 1-2 months of data) (Table 16). This made it challenging to detect temporal changes over time for TDS at the watershed level. However, TDS in Chestnut Creek has increased over time in the Kanawha Basin during July (ρ= 0.35, p= 0.01, N= 51) and September (ρ= 0.48, p= 0.002, N= 38). In the French Broad-Holston Basin the Davidson River increased in TDS over time during the month of April (ρ= 0.48, p= 0.006, N= 31) and decreased in Walnut Creek in September (ρ= -0.50, $p<0.001$, N= 69).

Focal Species

The number of HUC10s occupied changed for 9 of 11 species (Table 17; Fig. 14). The French Broad-Holston Basin experienced the greatest number of watershed occupancy lost (35), followed by the Kanawha (17), Upper Tennessee (11), and the Middle Tennessee-Hiwassee (7). Additionally, 48 HUC10 watersheds appear to have experienced loss of one or more focal species. Of these, 17 HUC10 watersheds experienced a loss of 2-3 focal species. The largest changes in HUC10 occupancy occurred in the French Broad-Holston Basin (Elk River, Sandymush Creek, and Walnut Creek) and the Upper Tennessee Basin (Upper Tellico Lake). However, only fishes exhibited statistically significant changes in HUC-scale occupancy between 1900 and 2010; Tangerine darter, Silver shiner, and Fatlips minnow exhibited significant decreases in occupancy (Exact Sig. 2-tailed = 0.008, < 0.001, and < 0.001, respectively) (Table 17; Fig. 15; Fig. 17).

For all three freshwater fish focal species, occupancy declined most sharply between the decades 2000-2020 (Fig. 15; Fig. 17). There were 33 watersheds with historical Tangerine darter records in 1900, and in 2010, only 25 of those original watersheds were still occupied across the study basins (Table 17). Tangerine darter declines occurred in the following watersheds and basins: French Broad-Holston (Big Laurel Creek and Walnut Creek-French Broad River), Upper Tennessee (Upper Tellico Lake and Fontana Lake), and the Middle Tennessee-Hiwassee Basins (Chickamauga Lake-Hiwassee River, Valley River, Hiwassee Lake-Hiwassee River, and Toccoa River-Blue Ridge Lake) (Fig. 16; Fig. 16).

The Fatlips minnow originally occupied 25 watersheds in 1900, and by 2010 was only detected in 14 watersheds that it originally occupied historically (Table 17). Occupancy data were only available for this species from the French Broad-Holston and Upper Tennessee Basins (Fig. 16). Reductions of the Fatlips minnow were greatest in watersheds of the French-Broad Holston Basin (Fig. 16). In the Upper Tennessee, the only HUC10 with a decline in occupancy from 1900-2010 occurred in the Cullasaja River (Fig. 16).

For the Silver shiner, it is estimated that they occupied 58 watersheds in 1900, but only 26 by 2010 (Table 17). Reductions in occupancy primarily occurred in the French Broad-Holston and Kanawha basins, although records indicate they were present in all the focal study basins in past years (Fig. 16). Declines in occupancy occurred in 15 watersheds in the French Broad-Holston and 13 watersheds in the Kanawha Basin. In the Upper Tennessee Basin, 3 HUCs were identified to have declines in occupancy including Upper Tellico Lake, Upper Tuckasegee River, and Headwaters of the Little Tennessee watershed. In the Middle Tennessee Basin, only the Valley River experienced changes in Silver shiner occupancy (Fig. 16).

Amphibians showed no significant changes in occupancy at the HUC10 scale between 1900 and 2010 (Table 17; Fig. 14). Both Eastern hellbender and Mudpuppy occupancy did not significantly change between the time periods examined, nor did that for crayfishes (Table 17). Freshwater mussel occupancy at the HUC10-scale decreased between 1900 and 2010, but due to low rates of historical occupancy, declines were not statistically significant (Table 17; Fig. 14).

Land Use

The final truncated data set included 44 watersheds in the four major study basins. The French Broad was the most developed basin, and the Upper Tennessee was the least developed (true for 2001 and 2019) (Table 3; Fig. 18). Watersheds in the French Broad-Holston and Middle Tennessee-Hiwassee Basins have seen the most change in percent urban impervious surface in the past 18 years (Table 3; Fig. 18). Mean percent urban impervious surface in 2001 ranged from most developed at 6.4% in Mud Creek (French Broad-Holston) to 0.07% in Abrams Creek watershed (Upper Tennessee). In 2019, Mud Creek (7.8%) was the most developed watershed of the 44 included in the analysis, and again, Abrams Creek (0.1%) watershed was the least developed (Table 3). The only watershed to experience a decline in percent urban impervious surface was Headwaters Little Tennessee River watershed (Upper Tennessee), indicating most watersheds, except for one, have increased in development between 2001 and 2019. On average, less than 2 % of the study basins are developed as of 2019 (Fig. 18).

Mixed effects models were used to identify the relationship between a suite of commonly measured water chemistry parameters and variation driven by time "Year" or land use "% Urban" (Table 18). Variation in measurements for all parameters was best explained by the predictor variable "Year" (Table 18). Random effects were responsible of large portion of the variance within the data, suggesting that site and watershed are important for determining water quality measurements. In this dataset, temperature ($F= 9.46$, $p<0.001$), pH ($F= 53.6$, $p<0.001$), and TDS ($F= 23.4$, $p<0.001$) were significantly increasing over time (Table 19; Fig. 19). Whereas DO concentration ($F= 20.9$, $p<0.001$), DO saturation ($F= 3.23$, $p<0.01$), and specific conductance ($F= 3.88$, $p<0.001$) appear to be significantly decreasing over time (Table 19). The relationship for specific conductance and DO saturation were weak and likely driven by site-specific factors (GPS & watershed) (Table 19). The only statistically significant relationship with impervious land use was pH, became more acidic with increased percent urban landcover ($F= 30.8$, $p<0.001$) (Fig. 20). The interaction term also showed a negative trend and a statistically significant result ($F= 9.74$, $p<0.001$) with relatively large amount of variation attributed to fixed and random effects (Table 19).

Discussion

The results of my study show that over the past century there have been significant changes in water quality that may be responsible for the loss of certain species in Southern Appalachian waterways. Although water quality, especially DO concentration (Fig. 7), has largely improved over the last 60 y, the results of this study and many others suggest that biologically meaningful changes are still occurring and that current statutes may not be protective enough against rising ion concentrations and increasing pH values (Andreen, 2003). Water quality and quantity are critical components of freshwater surface water supplies (Malmqvist and Rundle, 2002; Nagy et al., 2011) and changes to water quality can have significant long-term implications for humans via direct health impacts, economic or recreational impacts, and costs associated with chronic water chemistry issues (Sun and Lockaby, 2012; Dodds et al., 2013; Banzhaf et al., 2016).

The Southern Appalachian Mountains intercept warm, moisture laden air and aggregate this moisture in a network of forested headwater streams (Ellison et al., 2017). These streams in turn form larger rivers that make high-quality freshwater available to an estimated 48.7 million people in larger urban centers (e.g., Asheville, Charlotte, Winston-Salem, Knoxville) in the adjoining Piedmont and Tennessee Valley (Caldwell et al., 2014; Liu et al., 2022). Protecting water quality in streams with montane origins is thus of vital importance to local communities as well as communities that are many kilometers away from their headwaters. Water quality is important to all aspects of human life, and sensitive biota supported by aquatic ecosystems in the Southern Appalachian Mountains are some of the best indicators of changes to water chemistry in streams. The high biological diversity supported by these mountains and streams makes this region important for broader global conservation efforts (Zhu et al., 2022; 2023).

However, significant limitations remain in terms of data availability over broad spatial and temporal scales, especially from publicly available sources (Soranno et al., 2015). My correlative results suggest significant widespread increases in pH, DO ppm, conductivity, and temperature in these montane watersheds over the past ~60 years. Water chemistry parameter trends for the SBR concur with broader continental trends in water quality in the United States (e.g., Dodds and Whiles, 2004; Kaushal et al., 2018; Stets et al., 2020). Significant decreases in DO saturation were observed over a broad time scale at the watershed level but largely unchanged across all major basins (Table 9; Fig. 8). Similar patterns for DO saturation have been observed in several studies in aquatic systems (e.g., Diamond et al., 2021; Zhi et al., 2023). Improvements to DO concentration are likely attributable to improvements in water quality via reduction in pollution inputs resulting from implementation of by the Clean Air and Clean Water Acts, among other environmental policies (Andreen, 2003; Greenstone, 2004).

DO is a key indicator of ecosystem health and overall water quality (Abdul-Aziz and Gebreslase, 2023). This is why most environmental regulations surrounding freshwater use are focused on monitoring and improving DO (Thomann and Mueller, 1987; Williams and Boorman, 2012; Myers et al., 2021). The North Carolina Department of Environmental Quality has established a minimum value of 6 ppm for trout waters and has more stringent regulations for mountain streams compared to other water bodies in other parts of the state (NCAC, 2022). Widespread increases in DO concentration (Fig.7) may indicate recovery from point source pollution in this region or a sign of increased primary productivity (Poudel et al., 2013; Mujere and Moyce, 2018).

The EPA has a minimum value for freshwater DO saturation (>90%), and these data suggest that streams and reservoirs in this region commonly experience saturation levels (typically during the warmer months) that may be stressful to aquatic life (Table 8, Lawson and Jackson, 2021). This is often attributed to increases in primary production from excess nutrients which can lead to eutrophication, an increase in the frequency and length of algal blooms, and episodes of low DO (Birk et al., 2021; Piatka et al., 2021).The inverse trend observed between DO concentration and DO saturation may indicate that there is an increase in the amount of oxygen being introduced to the system, but it is not able to be efficiently used which is indicated by lower saturation (Table 9). The solubility of oxygen decreases with increasing water temperatures and plays a critical role in regulating metabolic processes of organisms in stream systems (Battino et al., 1983; Bernhardt et al., 2018). Combined with rising surface water temperatures, increased oxygen consumption can lead to DO being less efficiently utilized, and an overall decrease in DO saturation (Wetzel, 2001; Diamond et al., 2021; Zhi et al., 2023).

Streams with higher buffering capacity often support more organisms because acidic inputs do not change the pH as much as those with low buffering capacity (Gordon et al., 2004; Argue et al., 2012). I found that~97% of the pH correlations were positive over time, indicating watersheds are becoming less acidic, especially over the past six decades (Table 11). In a regional context, this may be indicative of recovery from acid deposition and mining activity that historically impacted the SBR region (Fig. 9; Fig. 10, Likens et al., 1979; Argue et al., 2012; Cianciolo et al., 2020). Acidic deposition in the past 20 years has shifted from that dominated by sulfuric acid to dominated by nitric acid (Chang et al., 2022). Nitric acid has been documented to be less toxic than sulfuric acid in terrestrial systems (Driscoll and Wang, 2019). However, while nitric acid may be less toxic than sulfuric and not persist for long in aquatic systems, its dissociation product (nitrate) and its involvement in redox reactions contribute to the overall oxidizing power and cycling of nitrogen in aquatic environments (Driscoll and Wang, 2019; Anglada et al., 2020; Lehnert et al., 2021; Edwards et al., 2023).

Many Southern Appalachian watersheds are poorly buffered due to their underlying geology that is comprised largely of crystalline metamorphic rocks. As a result, acidic deposition can more easily lead to changes in stream water pH in Blue Ridge streams compared to streams in other physiographic regions (Neff et al., 2009). I observed notable increases in alkalinity for many headwater streams and this may indicate recovery from acid deposition and impacts from accelerated weathering of diverse carbonate/lime sources from increased runoff (Table 11; Fig. 10, Stoddard et al., 1999; Raymond et al., 2008; Cheng et al., 2014; Kaushal et al., 2013; Stets et al., 2014). Elevated alkalinity levels can exacerbate ammonia toxicity in fish and can have rippling negative consequences for stream diversity (Driscoll and Wang, 2019; Edwards et al., 2023).

DO saturation and pH in SBR watersheds were both strongly affected by land use as measured by percent urban impervious surface (Table 18; Table 19). Declines in pH and DO saturation due to increased urbanization are well-documented (Bierschenk et al., 2019). Additionally, soils may become more alkaline in urbanized catchments, and this may provide a linkage between pH and impervious surface cover (Zhang et al., 2023). This can be due to several mechanisms, but urban areas characterized by increased impervious surfaces and infrastructure may leach basic compounds (e.g., calcium, carbonate) into the soil over time (Yang and Zhang, 2015; Kaushal et al., 2020). Changing soil conditions associated with impervious surfaces is a likely explanation for increased stream alkalinity over time (Fig. 19; Fig. 20, Cheng et al., 2014; Utz et al., 2016; Kaushal et al., 2017).

Increased dissolved salts from human activities can also disrupt the natural balance of cations and anions in the soil, causing pH to rise through complex air, water, and soil interactions (Okur and Orcen, 2020; Hamid et al., 2020; Akhtar et al., 2021). The EPA recommends that surface water pH in this SBR ecoregion fall between 6 and 9 (EPA, 2023). Observed increases in pH and other water chemistry parameters over time agree with regional and global trends highlighted in the literature known to affect freshwater fish diversity (Fig. 9; Fig. 19, Epstein et al., 2018; Tang et al., 2021). Interactions between terrestrial and atmospheric environments may complicate effects of increased solutes on freshwater fish (Baker and Christensen, 1991). Even small changes in pH can impact more sensitive aquatic species or affect biologically mediated processes including denitrification and decomposition (Kilham, 1982; Kaushal et al., 2017).

Of the watersheds examined in the water quality analysis, the Little River (Upper Tennessee) was the only watershed to have a mean pH value below 6 (Table 10). Salts, acids, and bases contribute to the dissociation of H^+ and OH^- ions in natural waters and pH is also dependent on carbonic acid and bicarbonate concentrations (Hem, 1985; Wetzel, 2001). The most common source of acidity in unpolluted water is dissolvedCO_2, and higher CO_2 concentrations generally lead to lower pH values over time in freshwater (Gordon et al., 2004).

Conductivity is often used as a proxy for salinity in freshwater systems (Haq et al., 2018). My results indicate conductivity of SBR streams has increased over time (Table 14; Table 16; Fig. 13). These changes may be a result of natural processes, such as precipitation and weathering, but could also result from ex-urban development and increased pressures from growing human populations in the region (Webster et al., 2012). Anthropogenic inputs resulting from impervious surface runoff, road salt application, sedimentation, and industrial chemical and wastewater discharges are all common sources of freshwater salinization and stream impairment (Williams, 2001; Canedo-Arguelles et al., 2013; Kaushal et al., 2018; 2019; 2020). Baseline conductivity measurements are linked to underlying geology and conductivity/conductance in many Tennessee Valley streams (Griffith, 2014). Most freshwater systems range from 10 to 1000 µS/cm, although, it is not uncommon for Blue Ridge Mountain streams to have a specific conductivity of <50 µS/cm (Chapman, 2021).

I did not attempt to untangle relationships between the numerous ions that may have influenced the observed changes in conductivity across many the SBR streams. For conductivity and standard specific conductance, the watersheds Cataloochee Creek-Pigeon River, Richland Creek-Pigeon River, and Headwaters of the Pigeon River (French Broad-Holston) had higher mean measurements and standard exceedances. This likely reflects the large amount of dissolved matter from the high density of industrial polluters present in these watersheds (Neves and Angermeier, 1990; Cho et al., 2011). It also highlights the site-specific nature of variation among the water chemistry measurements. However, laboratory study results suggest that salinization may be one of the biggest threats to aquatic freshwater communities in the region (Dalinsky et al., 2014).

TDS values are highly correlated with increases in dissolved ions and are also commonly used as proxies for salinity (Thomas, 1986; McCleskey et al., 2023). All watersheds included in TDS analyses had mean values well below the EPA threshold of 500 ppm for surface waters within water supply watersheds (EPA, 2023). The Cataloochee Creek-Pigeon River and Richland Creek-Pigeon River watersheds had maximum exceedances beyond the EPA threshold, again likely due to the presence of industrial discharge in these watersheds (Table 15). These HUC10s contains some of the most impacted sites in the Pigeon River, a stream long on the EPA 303d list for impairment due to suspended solids and other factors (Cho et al., 2011). Ongoing efforts to restore sections of the lower Pigeon River combined with the closure of several major factories that discharged into the Pigeon will likely help reduce TDS and conductivity levels in this stream and improve conditions for sensitive native biota (Bartlett, 1996; Mallin et al., 2009).

Occupancy modeling allowed me to detect where and when extirpation may have occurred over time (Fig. 16; Fig. 17, MacKenzie et al., 2006). Based on historical (museum records) and survey data, I determined that 9 out of 11 native species, across 4 taxa, have experienced reductions in occupancy of HUC10 watersheds in the SBR region from 1900-2010 (Table 17; Fig. 14), suggesting that most of the species included in my study are imperiled or in decline. The French Broad-Holston basin appears to have experienced the most species losses, with 13 watersheds having declined in occupancy for 2-3 focal species. The detected declines in species occupancy, accompanied by water quality and land use analysis, helped identify possible drivers of change dominant within study basins.

Statistically significant declines in occupancy occurred for 3 species of freshwater fish (Table 17; Fig. 14; Fig. 15). Cold-water adapted fish have a well-documented behavioral pattern of avoiding non-ideal aquatic habitat (Angermeier, 1995; Morgan et al., 2022). Examination of distributional trends suggest that occupancy is contracting and that connectivity between watersheds may also be declining (Fig. 16; Fig. 17). These changes are likely due to the combined impacts of current and legacy land use in the Southern Appalachians (Scott, 2006). This pattern provides evidence that declines are contributing to range retraction via isolated patch occupancy within watersheds central to the SBR region, as well as in protected and relatively undeveloped watersheds (Turner et al., 2003; Eby et al., 2014; Fig.16). Watershed homogenization can reduce the occupied range of freshwater fish and increase opportunities for colonization by non-native species (Buckwalter et al., 2018; Blouin et al., 2019; Peoples et al., 2020; Sleezer et al., 2021).

Similar to water chemistry, the majority of distributional changes observed occurred in the past 20 years. These declines may be related to the combined impacts of changes in pH, TDS and rising salinity on freshwater systems. Life history and behavioral traits may also explain accelerated declines, in part. For example, Silver shiners are described as an intolerant species and tend to avoid areas with heavy aquatic vegetation and siltation (Tierney, 2016; Burbank et al., 2021). Urbanized watersheds typically have higher levels of sediment inputs, and these may compound impacts of water quality changes on fish by reducing growth and reproduction in these relatively short-lived organisms (Epstein et al., 2018; Burbank et al., 2021).

No change in occupancy was detected for crayfish, likely because they are generally resilient, are widespread in headwater systems, may be difficult to survey and are restricted to a small number of streams (Creed and Reed, 2004; Parkyn and Collier, 2004; Ewing et al., 2016; Table 17). Similarly, amphibian occupancy data are influenced by variable survey methods and both Hellbenders and Mudpuppies may have low detection probabilities (Unger et al., 2021). Eastern hellbenders appear to only have been extirpated from 3 watersheds and Mudpuppies are now extirpated from 2 watersheds (Table 17). However, occupancy modeling does not address changes in population size, which is an aspect of status assessments (Nickerson et al., 2002; Jachowski et al., 2016). For example, although I did not detect changes in watershed occupancy for Eastern hellbenders, other studies suggest that they are becoming increasingly imperiled due to habitat loss and pollution range-wide (Sutton et al., 2023). Additionally, behavioral responses to environmental stress may lead to chronic nest failure for Eastern hellbenders as they appear to trigger a combination of reduced breeding success and filial cannibalism that can lead to recruitment failure (Jachowski and Hopkins, 2018; Diaz et al., 2022; Hopkins et al., 2023). Smaller Hellbender populations composed of older, non-reproducing adults and populations where larvae are unable to survive to adulthood are a major concern for the future of the species (Diaz et al., 2022).

Similarly, declines of freshwater mussels may be more widespread and severe than the data suggest. This may be due, in part, to the fact that I did not differentiate between dead shell and living mussel observations in my analysis (Pandolfi et al., 2022; Table 17; Fig. 14). Historical mussel distributions are well documented in natural history collections and mussel declines have been well documented by field biologists beginning as early as the 1960's (Haag, 2019). Mussel declines are considered enigmatic because they often occur in systems where the rest of the aquatic community remains intact (Ahlstedt et al., 2016; Haag 2019). Mussel populations in cool, unproductive streams such as the SBR may exist at the upstream limits of their distributions in many systems and may thus be more vulnerable to human-caused changes that affect growth or physiological functioning of juveniles and adults (Haag et al., 2019; Sousa et al., 2021). Changes to riparian land use and water quality may also be exacerbate freshwater mussel declines (Pandolfi et al., 2022).

The data examined in this study indicate that more in-depth analysis of water chemistry data is needed to track changes to water chemistry in this region. Moreover, they reveal that even forested headwater catchments in the Southern Appalachians are experiencing changes in water quality that are similar, if less dramatic, than those observed across the southeastern United States (Clinton and Vose, 2006; O'Driscoll et al., 2010; Nagy et al., 2011). This is among the first studies to show widespread changes in streams that are considered to be minimally disturbed and many of these watersheds are considered reference streams by more broadly focused studies of urbanization or agricultural impacts (Price and Leigh, 2006; O'Driscoll et al., 2010; Alnahit et al., 2022).

Among the parameters examined, TDS is of particular concern because it captures numerous ions and compounds, and a rising trend indicates an overall increase in salts and exogenous inputs in headwater streams over time (Johnson et al., 2015; Ondrasek and Rengel, 2021; Kaushal et al., 2021). Additionally, salts are retained in the soil and groundwater and can leach out over time likely driving the long-term increasing trends for associated water chemistry parameters across all seasons (Kelly et al., 2019; Corwin, 2021; Kaushal et al., 2021). Salt pulses can alter aquatic biota growth and reproduction, and lead to homogenized communities comprised of salt-tolerant species (Hintz and Relyea, 2019; Zhao et al., 2021). Salts can also mobilize base cations, carbon, nutrients, and heavy metals in stream systems, causing additional ecosystem level challenges for maintaining surface water quality (Haq et al., 2018; Lazur et al., 2020; Cianciolo et al., 2020).

A combination of salts and the shift of acid deposition to nitric acid composition, may act as a form of nitrogen loading in aquatic systems and may explain elevated levels of TDS and a shift towards more alkaline streams (Li et al., 2016; Cianciolo et al., 2020). This is because nitric acid can react with alkaline substances (such as carbonate minerals), leading to the formation of nitrate ions and potentially increasing the pH of the water and (Kumar and Prabhahar, 2012; Hamid et al., 2019). In addition to salinization, I suspect nutrient loading may be contributing to elevated TDS levels and a shift towards more alkaline conditions in SBR streams.

Parameters associated with dissolved ion concentrations (e.g., conductivity, pH, TDS) have all largely increased while changes to stream temperature and oxygen concentrations have been more equivocal and include watersheds that have seen decreases as well as increases in these parameters. This pattern revealed a complex interplay of water chemistry parameters and their potential consequences for aquatic biota. Models examining water chemistry changes during the last 18 y suggest that these trends may largely be due to increased levels of impervious landcover associated with urban and ex-urban growth and associated impervious surface with pH and TDS showing the most dramatic changes (Table 18; Table 19; Fig. 19; Fig. 20). These changes in water quality and land use may relate to widespread declines seen for many freshwater species (Reid et al., 2019).

Increases in salts and TDS by way of alterations to natural ionic exchange can cause changes in surface and groundwater quality (Jia et al., 2020). At the biotic level, increased salts can inhibit growth and reproduction by way of osmotic regulation stress in both plant and aquatic communities (Hintz and Relyea, 2019; Zhao et al., 2021; Moreira et al., 2023). High TDS is also a known stressor to aquatic life and has been documented to reduce species richness in macroinvertebrate communities, another bioindicator of stream health (Timpano et al., 2010). High pH alone does not have effects on biota except at extreme levels, but it is of particular concern the synergistic effects of increasing salts, temperature, and pH (Kaushal et al., 2021). Ammonia is a common nitrogen compound formed in aquatic systems, the toxicity of ammonia increases with pH, meaning that under alkaline conditions, the adverse effects on aquatic life may be more pronounced (Thurston et al., 1981). Ammonia and salts are toxic to aquatic organisms and may explain stark declines seen in freshwater fish in the last century (Hintz and Relyea, 2019; Zhu et al., 2021; Lawson and Jackson, 2021; Kaushal et al., 2021; Parvathy et al., 2022; Zhang et al., 2023).

Identifying the key watershed characteristics, values, pollution sources, and native aquatic species can help watershed managers create appropriate regulations for managing and sustaining healthy streams (Alnahit et al., 2022). Effective conservation and river management requires consideration of water chemistry, emerging contaminants, and knowledge of historical land use legacies to interpret changes on spatial and temporal scales (Wohl, 2019). Changes in surface water chemistry, such as salts and pH, have direct impacts on aquatic biota livelihood and may lead further species extirpations. Chronic and acute shifts in water quality may cause accelerated weathering of infrastructure, leading to costly repairs and threats to drinking water quality as well as well contamination, septic failure, and sanitary sewer overflows (Vineis et al., 2011; Canedo-Arguelles et al., 2013; Cooper et al., 2014; Kaushal et al., 2018). These changes will likely worsen in the face of climate change and growing population pressures in this region if stakeholders do not change the current trajectory of land and water use in the SBR (Troia et al., 2019; Elsen et al., 2020).

Future Directions and Limitations

Although the goal of my study was to provide a general regional overview of water quality and occupancy in the SBR region, due to data availability and project time constraints (~1 year), analyses were focused on North Carolina datasets. Substantial improvements to data reporting protocols are needed to streamline data submission to State and Federal agencies. Streamlining data entry and management will improve our availability to monitor changing conditions within dynamic freshwater systems across broad spatial scales. Future studies should examine changes to other water chemistry parameters including alkalinity, nitrate, sulfate, and base cations. Utilizing a data analytics framework that allows for systematic exploration of historical datasets with land use, water chemistry, and species data (Chandarana and Vijayalakshmi, 2014). However, understanding and managing changes to river systems are dependent on the continued monitoring of both water chemistry and aquatic species across broad spatial and environmental scales (Arthington et al., 2010). Continuous investments in water research are necessary to understand how to protect water quality in the face of multiple stressors with new and innovative solutions.

Climate change will add additional stress to streams, as rainfall has shifted to more stochastic rainfall events (i.e., intense thunderstorms) interspersed within longer dry periods (Hassan et al., 2020). This results in changes to riverine flows. In this way, climate change can also cause alterations in stream temperature, pH, dilution rates, and salinity, all of which are critical parameters for the survival of aquatic life (Noyes et al., 2009; Staudt et al., 2013). The risk of extreme fluctuations in precipitation regimes are expected to increase in the Appalachian Mountains and these changes will likely influence chemical conditions within numerous freshwater streams that are critical refuges for sensitive or critically endangered biota (Kunkel et al., 2020). Phenological analyses may be useful in understanding the timing of ecosystem processes and determining shifts in seasonal trends of water quality over time (Shuter et al., 2012; Kuczynski et al., 2017).

Snowmelt, for example, often occurs when salamanders and fish are hatching, and it can put a pulse of acidity into the streams and lakes that causes deformities and death of these organisms (Schaefer et al., 1990). If the timing of snowmelt changes due to climate change, then the impact on these organisms will change either for the better (if it occurs before they hatch in the future) or for the worse (if it matches more closely when they hatch; Todd et al., 2010; Haver et al., 2022; Prather et al., 2023). Phenological assessments have the potential to help determine the vulnerability of aquatic species on an individual basis in the face of ongoing climate change and development (Kim and Kanno, 2020). Large-scale shifts in the environmental conditions and community structure of the regions cool, high-elevation watersheds may further threaten the viability of native and endemic species within the region

(Ficke et al., 2007; Zhu et al., 2021). Further examination of seasonal patterns, and deviations, may improve future exploratory analyses of long-term datasets in pursuit of better understanding the anthropogenic impacts on water quality (Palmer and Ruhi, 2019).

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Tables and Figures

Table 1. Species of study interest and current conservation status. Information combined from NC Wildlife Action Plan (2020) and US Fish and Wildlife Service reports on the Federal Register (2023). Highlighting general distribution in Tennessee, and Kanawha Basins and contribution to the Ohio and Mississippi Drainages.

Scientific Name	**Common Name**	**NCWRC**	**USFWS**	**Distribution**
Alasmidonta raveneliana	Appalachian Elktoe	Endangered	Endangered	Upper TN Basin endemic
Alasmidonta viridis	Slippershell Mussel	Endangered		Ohio and Mississippi Drainages
Pleurobema oviforme	Tennessee Clubshell	Endangered	Proposed Endangered	TN Basin endemic
Cambarus reburrus	French Broad River Crayfish	Threatened		Headwaters of French Broad and Savannah Basins
Cambarus eeseeohensis	Grandfather Mountain Crayfish			Headwaters of Linville, and TN Basins
Cambarus georgiae	Little Tennessee Crayfish	Special Concern		Headwaters of the Little TN Basin
Cryptobranchus alleganiensis	Eastern Hellbender	Special Concern	Under Review	Ohio and lower Missouri Basins
Necturus maculosus	Common Mudpuppy	Special Concern		Ohio, Mississippi, and Great Lakes Drainages
Percina aurantiaca	Tangerine Darter			TN Basin endemic
Phenacobius crassilabrum	Fatlips Minnow			Upper TN Basin endemic
Notropis photogenis	Silver Shiner			Ohio, Mississippi, and Great Lakes Drainages

Table 2. Environmental water chemistry parameter descriptive statistics for all four major SBR study basins. N represents the number of samples, N HUC10's indicates the number of watersheds with available data. All values are reported as mean ± standard deviation for each parameter unless otherwise specified.

Parameter	**N**	**N1 Years**	**N HUC10's**	**$\bar{x}$ + SD**
Temp	42,288	70	59	13.5 °C ± 6.2
DO (ppm)	30,804	53	51	9.8 ppm ± 0.24
DO% Sat	13,223	53	41	90.2 % ± 4.7
pH	52,839	76	60	6.4 ± 5.8
spC @ 25°C	6,060	70	41	69.7 µS/cm ± 71.9
spC	16,986	41	41	62.7 µS/cm ± 58.6
TDS	9,389	91	46	46.4 ppm ± 40.3

Table 3. SBR watershed characteristics and water chemistry records. Percent Increase for each watershed was calculated using average impervious surface the formula, ((Original Value-New Value)/Original Value)×100 between the period of record for land use.

HUC6 Basin	HUC10 Watershed	Area (ha)	Mean Elevation (m)	Period of Record	N l Years	% Increase Urban (2001-2019)
French Broad-Holston	Big Laurel Creek	52500	893.4	1956-2016	11	16.2
French Broad-Holston	Cane Creek-French Broad River	60200	746.1	1956-2020	49	39.6
French Broad-Holston	Cane River	62400	1070.7	1956-2021	58	32
French Broad-Holston	Cataloochee Creek-Pigeon River	73300	1068.7	1955-2021	64	12.8
French Broad-Holston	Clear Creek-French Broad River	53300	535.4	1946-2021	50	47.7
French Broad-Holston	Cove Creek-Nolichucky River	106400	492.7	1973-2021	45	13.5
French Broad-Holston	Davidson River-French Broad River	65200	834.2	1954-2020	60	16.4
French Broad-Holston	Doe River	54500	951.0	1967-2021	31	6.5
French Broad-Holston	Elk River	33000	1043.5	1954-2021	22	29.2
French Broad-Holston	Gulf Fork Big Creek	31900	710.6	1976-2021	11	20.1
French Broad-Holston	Headwaters French Broad River	50500	898.6	1956-2020	59	16.2
French Broad-Holston	Headwaters North Toe River	72600	1029.7	1956-2020	53	19.6
French Broad-Holston	Headwaters Pigeon River	65700	1159.4	1954-2020	60	14.4
French Broad-Holston	Hominy Creek	40700	821.1	1954-2020	44	27.1
French Broad-Holston	Ivy Creek	63600	856.4	1956-2019	22	22.4
French Broad-Holston	Mills River-French Broad River	51800	826.7	1956-2020	60	26.1
French Broad-Holston	Mud Creek	43900	710.2	1951-2020	37	21.9

French Broad-Holston	North Indian Creek-Nolichucky River	104100	683.0	1968-2021	54	15.8
French Broad-Holston	Pigeon River	60600	571.9	1957-2021	61	15.7
French Broad-Holston	Richland Creek-Pigeon River	71200	1070.3	1954-2020	59	16.3
French Broad-Holston	Roan Creek	66700	872.7	1974-2021	19	26.5
French Broad-Holston	Sandymush Creek-French Broad River	92800	753.4	1956-2020	59	18.3
French Broad-Holston	South Indian Creek	32100	938.7	1977-2021	20	9.2
French Broad-Holston	South Toe River-North Toe River	102800	994.2	1951-2020	61	20.4
French Broad-Holston	Spring Creek-French Broad River	41900	840.9	1957-2021	29	12.7
French Broad-Holston	Swannanoa River	52200	903.9	1956-2020	54	14.4
French Broad-Holston	Walnut Creek-French Broad River	37600	731.7	1954-2020	60	19.4
French Broad-Holston	Watauga Lake-Watauga River	87100	955.6	1956-2021	61	23.8
French Broad-Holston	Watauga River	105700	616.9	1967-2021	24	14.4
Kanawha	Chestnut Creek-New River	48200	773.1	1930-2021	65	6.4
Kanawha	Elk Creek-New River	45200	869.1	1970-2018	37	14.6
Kanawha	Fox Creek-New River	65600	967.0	1970-2021	52	18
Kanawha	Little River-New River	90100	866.6	1968-2020	53	19.2
Kanawha	North Fork New River	115700	1041.0	1954-2021	58	21.7
Kanawha	South Fork New River	131300	981.0	1955-2020	58	25.1
Middle Tennessee-Hiwassee	Brasstown Creek-Hiwassee River	41700	637.4	1956-2021	38	35.4
Middle Tennessee-Hiwassee	Chickamauga Lake-Hiwassee River	114000	251.1	2002-2021	16	25.4
Middle Tennessee-Hiwassee	Hiwassee Lake-Hiwassee River	60900	617.9	1956-2021	60	25

Middle Tennessee-Hiwassee	Hiwassee River-Chatuge Lake	73100	791.1	1951-2019	36	41
Middle Tennessee-Hiwassee	Nottely River	28400	549.1	1951-2021	31	30.7
Middle Tennessee-Hiwassee	Nottely River-Nottely Lake	82600	680.9	1951-1974	24	39
Middle Tennessee-Hiwassee	Ocoee River	157600	522.6	1951-2021	68	31.8
Middle Tennessee-Hiwassee	Spring Creek-Hiwassee River	110400	484.9	1966-2021	35	28.6
Middle Tennessee-Hiwassee	Toccoa River-Blue Ridge Lake	89100	749.1	1951-2021	25	24.8
Middle Tennessee-Hiwassee	Tusquitee Creek-Hiwassee River	42400	791.8	1956-1981	21	38.7
Middle Tennessee-Hiwassee	Valley River	45500	740.6	1956-2020	59	21.8
Upper Tennessee	Abrams Creek	34200	688.1	1968-2020	31	41
Upper Tennessee	Alarka Creek-Little Tennessee River	79300	797.4	1954-2020	59	22.2
Upper Tennessee	Cheoah River	83900	908.1	1968-2021	53	18.6
Upper Tennessee	Cullasaja River	35900	1021.8	1954-2004	18	24.5
Upper Tennessee	Fontana Lake	65400	858.0	1968-2020	42	17.7
Upper Tennessee	Headwaters Little Tennessee River	76900	853.0	1957-2021	59	18
Upper Tennessee	Little River (601020101)	149100	606.3	1991-2020	30	28.8
Upper Tennessee	Lower Tuckasegee River	56600	972.6	1956-2020	61	24.5
Upper Tennessee	Middle Tuckasegee River	63700	930.2	1956-2018	35	24.9
Upper Tennessee	Nantahala River	68100	1074.1	1956-2020	59	14.7
Upper Tennessee	Oconaluftee River	73700	1136	1956-2019	51	38.9
Upper Tennessee	Tellico River	111200	498.6	1968-2019	32	34.5

Upper Tennessee	Upper Tellico Lake	111600	521.2	1968-2019	36	23.7
Upper Tennessee	Upper Tuckasegee River	92700	1088.5	1954-2020	28	34.9

Table 4. Temperature (°C) descriptive statistics for Hydrologic Unit Code 10, watershed level. The mean temperature is reported with its standard deviation (SD) and range. The period during which temperature data were collected and recorded are available.

HUC6	**HUC10**	**N**	**Temp x̄ + SD (range)_**	**Period of Record**
French Broad-Holston	Cane Creek-French Broad River	602	15.4 + 6.1 (1.2-26)	1970-2020
French Broad-Holston	Cane River	708	13.3 + 7.1 (0.0-28)	1968-2020
French Broad-Holston	Cataloochee Creek-Pigeon River	1589	11.9 + 5.7 (-3.5-27)	1967-2020
French Broad-Holston	Clear Creek-French Broad River	486	15.6 + 7.4 (0.8-30)	1969-2021
French Broad-Holston	Cove Creek-Nolichucky River	1220	14.9 + 5.3 (0.2-29)	1974-2021
French Broad-Holston	Davidson River-French Broad River	1517	13.5 + 6.1 (0.0-30)	1968-2020
French Broad-Holston	Doe River	389	12.6 + 6.1 (0.1-25)	1967-2021
French Broad-Holston	Elk River	152	13.6 + 6.1 (0.0-25)	1968-2021
French Broad-Holston	Gulf Fork Big Creek	91	14.6 + 5.8 (3.3-23)	1976-2021
French Broad-Holston	Headwaters French Broad River	628	13.5 + 5.5 (1.0-25)	1968-2020
French Broad-Holston	Headwaters North Toe River	971	13.0 + 6.5 (0.0-27)	1968-2020
French Broad-Holston	Headwaters Pigeon River	1263	14.3 + 6.7 (0.0-30)	1968-2020
French Broad-Holston	Hominy Creek	506	15.4 + 6.6 (0.3-28)	1968-2020
French Broad-Holston	Ivy Creek	76	15.8 + 5.4 (5.0-24)	1967-2019
French Broad-Holston	Mills River-French Broad River	1190	13.6 + 6.0 (0.0-28)	1968-2020
French Broad-Holston	Mud Creek	431	15.3 + 6.0 (2.9-28)	1972-2020
French Broad-Holston	North Indian Creek-Nolichucky River	1721	14.2 + 6.6 (0.0-30)	1968-2021
French Broad-Holston	Pigeon River	1139	14.0 + 6.2 (0.0-27)	1968-2021

French Broad-Holston	Richland Creek-Pigeon River	932	13.4 + 5.7 (0.3-27)	1967-2020
French Broad-Holston	Roan Creek	574	12.8 + 6.0 (0.0-27)	1974-2021
French Broad-Holston	Sandymush Creek-French Broad River	1150	15.2 + 7.0 (0.0-30)	1968-2020
French Broad-Holston	South Indian Creek	153	13.4 + 6.0 (0.4-29)	1977-2021
French Broad-Holston	South Toe River-North Toe River	1292	13.0 + 6.5 (0.0-28)	1968-2020
French Broad-Holston	Spring Creek-French Broad River	156	15.5 + 7.6 (0.9-29)	1968-2021
French Broad-Holston	Swannanoa River	614	13.1 + 6.4 (0.5-28)	1969-2020
French Broad-Holston	Walnut Creek-French Broad River	831	14.8 + 7.0 (0.0-29)	1972-2020
French Broad-Holston	Watauga Lake-Watauga River	1281	13.4 + 6.8 (-0.1-29)	1968-2021
French Broad-Holston	Watauga River	105	13.1 + 6.3 (0.5-25)	1967-2021
Kanawha	Chestnut Creek-New River	1638	13.2 + 7.2 (-1.0-29)	1967-2021
Kanawha	Elk Creek-New River	300	12.6 + 6.8 (0.0-26)	1970-2018
Kanawha	Fox Creek-New River	1187	11.1 + 6.5 (-0.2-27)	1970-2021
Kanawha	Little River-New River	2227	12.9 + 7.0 (-0.2-31)	1968-2020
Kanawha	North Fork New River	879	12.0 + 6.9 (0.0-28)	1968-2021
Kanawha	South Fork New River	1633	13.6 + 6.9 (0.0-29)	1968-2020
Middle Tennessee-Hiwassee	Brasstown Creek-Hiwassee River	338	13.1 + 5.5 (0.0-24)	1968-2021
Middle Tennessee-Hiwassee	Chickamauga Lake-Hiwassee River	55	16.9 + 6.0 (7.0-27)	2002-2021
Middle Tennessee-Hiwassee	Hiwassee Lake-Hiwassee River	751	15.8 + 6.2 (1.0-29)	1968-2021
Middle Tennessee-Hiwassee	Hiwassee River-Chatuge Lake	500	12.5 + 5.0 (0.0.-24)	1951-2019
Middle Tennessee-Hiwassee	Nottely River	273	12.7 + 5.2 (3.5-25)	1951-2021
Middle Tennessee-Hiwassee	Nottely River-Nottely Lake	128	12.9 + 5.6 (3.5-25)	1951-1974

Middle Tennessee-Hiwassee	Ocoee River	2195	14.4 + 5.6 (0.1-29)	1951-2021
Middle Tennessee-Hiwassee	Spring Creek-Hiwassee River	520	14.2 + 5.7 (1.4-27)	1966-2021
Middle Tennessee-Hiwassee	Toccoa River-Blue Ridge Lake	113	12.8 + 4.7 (3.5-23)	1951-2021
Middle Tennessee-Hiwassee	Tusquitee Creek-Hiwassee River	178	13.0 + 5.1 (1.0-25)	1968-1981
Middle Tennessee-Hiwassee	Valley River	798	14.4 + 5.9 (-1.0-29)	1968-2020
Upper Tennessee	Abrams Creek	289	13.4 + 5.0 (2.0-26)	1976-2020
Upper Tennessee	Alarka Creek-Little Tennessee River	912	15.2 + 5.7 (0.0-29)	1968-2020
Upper Tennessee	Cheoah River	705	14.1 + 5.6 (-1.0-29)	1968-2021
Upper Tennessee	Cullasaja River	64	16.4 + 5.4 (4.0-27)	1967-2004
Upper Tennessee	Fontana Lake	225	12.2 + 4.8 (2.8-21)	1968-2020
Upper Tennessee	Headwaters Little Tennessee River	859	13.9 + 5.9 (0.0-28)	1968-2021
Upper Tennessee	Little River	38	11.3 + 4.8 (-3.4-19)	1991-2020
Upper Tennessee	Lower Tuckasegee River	699	13.4 + 6.2 (-3.4-29)	1968-2020
Upper Tennessee	Middle Tuckasegee River	293	15.5 + 5.6 (1.0+26)	1968-2018
Upper Tennessee	Nantahala River	648	11.6 + 5.1 (-1.0-28)	1968-2020
Upper Tennessee	Oconaluftee River	1054	11.4 + 5.4 (-0.2-25)	1968-2019
Upper Tennessee	Tellico River	313	14.6 + 5.9 (0.5-27)	1968-2019
Upper Tennessee	Upper Tellico Lake	538	14.1 + 4.7 (1.9-30)	1968-2019
Upper Tennessee	Upper Tuckasegee River	171	13.4 + 5.2 (1.0-23)	1967-2020

Table 5. Results of Spearman correlation analyses examining the relationship between Temperature (°C) and year of record across each month of the year Spearman's rho (ρ), is listed followed by statistical significance (P), and sample size (N). Data were analyzed at the HUC10 scale and are organized by HUC6. Spearman’s rho represents the correlation coefficient indicating the strength and direction of the monotonic relationship between the variables. The p-value associated with the Spearman correlation coefficient, assessing the statistical significance of the observed correlation.

HUC6/HUC 10	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
French Broad-Holston				-0.72 P < 0.001 N = 66		0.45 P < 0.001 N = 80						
Headwaters North Toe R.				0.46 P < 0.001 N = 71	0.4 P = 0.001 N = 66			0.42 P < 0.001 N = 79	0.34 P = 0.003 N = 76			
Mills River		0.35 P = 0.002 N = 80										
Mud Creek							-0.57 P < 0.001 N = 44	-0.5 P = 0.001 N = 39				
North Indian Creek				-0.33 P < 0.001 N = 129								
Roan Creek								-0.43 P = 0.002 N = 50		0.8 P < 0.001 N =75		
Swannanoa River												0.41

					P = 0.008 N = 41
Kanawha					
Chestnut Creek					-0.37 P < 0.001 N = 99
Fox Creek			-0.38 P = 0.001 N = 68		
Middle Tennessee-Hiwassee					
Hiwassee Lake		0.42 P = 0.001 N = 57			
Ocoee River	0.43 P < 0.001 N = 142				
Spring Creek				-0.36 P = 0.012 N = 49	

Upper Tennessee						
Abrams Creek				0.41 P = 0.004 N = 48		
Alarka Creek-Little TN						0.51 P < 0.001 N = 60
Cheoah River		0.55 P < 0.001 N = 58				
Lower Tuckasegee River	0.46 P < 0.001 N = 58		0.56 P < 0.001 N = 58		-0.55 P < 0.001 N = 71	
Middle Tuckasegee River			-0.5 P < 0.001 N = 58			

Oconaluftee River	0.59 P < 0.001 N = 87		
Upper Tellico Lake		0.8 P < 0.001 N = 50	0.57 P < 0.001 N = 66

Table 6. Dissolved Oxygen (ppm) descriptive statistics for Hydrologic Unit Code 10, watershed level. The mean DO is reported with its standard deviation (SD) and range. The period during which DO data were collected and recorded are available.

HUC6	HUC10	N	DO ppm $\bar{x}$ + SD (range)	Period of Record
French Broad-Holston	Cane Creek-French Broad River	495	9.2 + 1.6 (5.2-16)	1972-2020
French Broad-Holston	Cane River	544	10.4 + 1.8 (6.1-16)	1971-2020
French Broad-Holston	Cataloochee Creek-Pigeon River	807	10.2 + 1.5 (2.0-16)	1971-2020
French Broad-Holston	Clear Creek-French Broad River	221	10.0 + 2.0 (4.6-16)	2001-2021
French Broad-Holston	Cove Creek-Nolichucky River	1004	9.5 + 1.6 (4.3-17)	1999-2021
French Broad-Holston	Davidson River-French Broad River	1437	9.6 + 1.7 (2.6-15)	1971-2020
French Broad-Holston	Doe River	322	10.1 + 1.5 (7.1-15)	1999-2021
French Broad-Holston	Elk River	70	10.0 + 1.6 (7.1-16)	1973-2021
French Broad-Holston	Gulf Fork Big Creek	60	10.4 + 1.6 (7.6-14)	2015-2021
French Broad-Holston	Headwaters French Broad River	585	10.2 + 1.3 (7.8-15)	1970-2020
French Broad-Holston	Headwaters North Toe River	883	10.3 + 1.6 (7.3-15)	1971-2020

French Broad-Holston	Headwaters Pigeon River	1140	9.6 + 2.0 (1.5-17)	1971-2020
French Broad-Holston	Hominy Creek	476	9.2 + 2.2 (0.3-16)	1972-2020
French Broad-Holston	Ivy Creek	45	9.5 + 1.6 (6.6-14)	1971-2010
French Broad-Holston	Mills River-French Broad River	628	9.7 + 1.9 (3.5-15)	1971-2020
French Broad-Holston	Mud Creek	406	9.0 + 1.5 (5.8-14)	1972-2020
French Broad-Holston	North Indian Creek-Nolichucky River	1150	9.6 + 1.7 (4.4-16)	1972-2021
French Broad-Holston	Pigeon River	640	9.4 + 1.8 (4.5-15)	1972-2021
French Broad-Holston	Richland Creek-Pigeon River	775	10.1 + 1.6 (5.7-15)	1972-2020
French Broad-Holston	Roan Creek	524	10.2 + 1.5 (6.7-14)	1999-2021
French Broad-Holston	Sandymush Creek-French Broad River	1032	9.6 + 1.7 (3.4-15)	1970-2020
French Broad-Holston	South Indian Creek	127	10.1 + 1.3 (7.1-13)	1999-2021
French Broad-Holston	South Toe River-North Toe River	706	10.0 + 1.5 (2.9-14)	1971-2020

French Broad-Holston	Spring Creek-French Broad River	115	9.6 + 1.5 (7.2-14)	1972-1981
French Broad-Holston	Swannanoa River	508	9.9 + 1.6 (6.1-16)	1972-2020
French Broad-Holston	Walnut Creek-French Broad River	596	9.8 + 1.7 (6.8-17)	1972-2020
French Broad-Holston	Watauga Lake-Watauga River	1199	10.2 + 1.7 (4.6-16)	1973-2021
Kanawha	Chestnut Creek-New River	1118	10.0 + 1.8 (4.0-16)	1970-2014
Kanawha	Fox Creek-New River	600	10.1 + 1.9 (6.3-15)	1970-2011
Kanawha	Little River-New River	1976	10.2 + 1.8 (5.0-17)	1970-2020
Kanawha	North Fork New River	712	10.4 +1.8 (5.8-16)	1970-2020
Kanawha	South Fork New River	1545	10.1 + 1.8 (5.6-17)	1970-2020
Middle Tennessee-Hiwassee	Brasstown Creek-Hiwassee River	258	9.8 + 1.6 (0.0-14)	1973-2020
Middle Tennessee-Hiwassee	Hiwassee Lake-Hiwassee River	435	9.6 + 1.8 (0.1-17)	1973-2021

Middle Tennessee-Hiwassee	Hiwassee River-Chatuge Lake	113	9.4 + 1.7 (5.2-14)	2001-2019
Middle Tennessee-Hiwassee	Nottely River	118	9.3 + 2.3 (0.0-13)	2001-2019
Middle Tennessee-Hiwassee	Ocoee River	1452	9.9 + 1.7 (3.5-17)	1999-2021
Middle Tennessee-Hiwassee	Spring Creek-Hiwassee River	397	9.4 + 1.9 (2.9-14)	1973-2021
Middle Tennessee-Hiwassee	Tusquitee Creek-Hiwassee River	170	9.1 + 2.6 (1.2-14)	1973-1981
Middle Tennessee-Hiwassee	Valley River	567	10.0 + 1.5 (7.4-14)	1973-2020
Upper Tennessee	Alarka Creek-Little Tennessee River	545	9.7 + 1.6 (6.5-15)	1968-2020
Upper Tennessee	Cheoah River	496	9.9 + 1.4 (6.7-16)	1968-2021
Upper Tennessee	Cullasaja River	37	8.8 + 1.5 (6.2-12)	1968-1975
Upper Tennessee	Headwaters Little Tennessee River	756	9.6 + 1.6 (0.0-14)	1968-2021

Upper Tennessee	Lower Tuckasegee River	488	10.1 + 1.6 (6.4-17)	1968-2020
Upper Tennessee	Middle Tuckasegee River	253	9.2 + 1.7 (3.9-16)	1968-2018
Upper Tennessee	Nantahala River	602	10.1 + 1.2 (6.8-15)	1968-2020
Upper Tennessee	Oconaluftee River	940	10.2 + 1.5 (3.4-16)	1968-2019
Upper Tennessee	Tellico River	164	9.6 + 1.5 (6.9-14)	1999-2019
Upper Tennessee	Upper Tellico Lake	421	9.0 + 1.7 (1.6-14)	1968-2019
Upper Tennessee	Upper Tuckasegee River	146	9.8 + 1.4 (6.8-14)	1968-2020

Table 7. Results of Spearman correlation analyses examining the relationship between DO concentration (ppm) and year of record across each month of the year Spearman's rho (ρ), is listed followed by statistical significance (P), and sample size (N). Data were analyzed at the HUC10 scale and are organized by HUC6.

HUC6/HUC 10	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
French Broad-Holston												
Cane Creek-French Broad				0.51 P < 0.001 N = 57		-0.48 P = 0.001 N = 42	-0.37 P = 0.005 N = 58					
Cove Creek-Nolichucky		-0.45 P < 0.001 N = 66			0.36 P = 0.003 N = 66							
Doe River							0.76 P < 0.001 N = 32				0.37 P = 0.002 N = 37	
Hominy Creek								0.34 P = 0.01 N = 54	0.37 P = 0.01 N = 43			
Mills River							0.56 P < 0.001 N = 57	0.79 P < 0.001 N = 58	0.52 P < 0.001 N = 54		0.35 P = 0.01 N = 51	
Mud Creek							-0.47 P = 0.001 N = 44	-0.36 P = 0.02 N = 42				

HUC6/HUC 10	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Kanawha												
Chestnut Creek												0.45 P < 0.001 N = 69
Fox Creek		0.46 P = 0.003 N = 41		0.34 P = 0.023 N = 45			0.44 P < 0.001 N = 54					
North Fork New River											0.49 P < 0.001 N = 68	
South Fork New River				0.36 P < 0.001 N = 124								
Middle Tennessee-Hiwassee												
Spring Creek					0.38 P = 0.01 N = 45							
Upper Tennessee												
Oconaluftee River					-0.52 P < 0.001 N = 81			-0.62 P < 0.001 N = 75				
Upper Tellico Lake							-0.62 P < 0.001 N = 48					

Table 8. Dissolved Oxygen Percent Saturation descriptive statistics for Hydrologic Unit Code 10, watershed level. The mean DO % Sat is reported with its standard deviation (SD) and range. The period during which DO % Sat data were collected and recorded are available.

HUC6 Name	HUC10 Name	N	DO % Sat $\bar{x}$ + SD (range)	Period of Record
French Broad-Holston	Cane Creek-French Broad River	269	88.1 + 10.9 (58-120)	1970-1996
French Broad-Holston	Cane River	360	93.6 + 7.3 (67-119)	1969-1996
French Broad-Holston	Cataloochee Creek-Pigeon River	418	93.1 + 6.3 (71-120)	1971-1996
French Broad-Holston	Davidson River-French Broad River	911	86.8 + 8.9 (31-116)	1970-1996
French Broad-Holston	Headwaters French Broad River	359	93.4 + 6.2 (67-116)	1970-1996
French Broad-Holston	Headwaters North Toe River	493	92.2 + 6.2 (73-113)	1971-1996
French Broad-Holston	Headwaters Pigeon River	681	86.1 + 13.0 (16-126)	1969-1996
French Broad-Holston	Hominy Creek	181	80.2 + 20.2 (4-113)	1972-1996
French Broad-Holston	Ivy Creek	38	86.5 + 21.6 (7-112)	1968-1975
French Broad-Holston	Mills River-French Broad River	346	85.2 + 12.7 (40-114)	1971-1996
French Broad-Holston	Mud Creek	149	88.9 + 10.0 (64-124)	1972-1996
French Broad-Holston	North Indian Creek-Nolichucky River	224	93.6 + 6.4 (76-114)	1971-2018
French Broad-Holston	Pigeon River	267	86.1 + 11.0 (23-111)	1971-2011
French Broad-Holston	Richland Creek-Pigeon River	296	89.6 + 12.0 (7-121)	1968-1996
French Broad-Holston	Sandymush Creek-French Broad River	648	87.9 + 12.7 (6-119)	1968-1996
French Broad-Holston	South Toe River-North Toe River	427	90.3 + 6.4 (70-118)	1969-1996
French Broad-Holston	Spring Creek-French Broad River	117	95.5 + 6.3 (70-108)	1971-1981
French Broad-Holston	Swannanoa River	284	89.6 + 6.5 (72-106)	1972-1996
French Broad-Holston	Walnut Creek-French Broad River	383	92.3 + 6.9 (63-117)	1972-1996
French Broad-Holston	Watauga Lake-Watauga River	464	94.6 + 8.1 (66-121)	1973-1996
Kanawha	Little River-New River	565	93.1 + 7.3 (70-124)	1970-1996

Kanawha	North Fork New River	333	93.6 + 9.2 (73-129)	1970-1996
Kanawha	South Fork New River	696	91.5 + 8.7 (58-123)	1970-1996
Middle Tennessee-Hiwassee	Brasstown Creek-Hiwassee River	172	94.6 + 9.4 (0-116)	1973-2020
Middle Tennessee-Hiwassee	Hiwassee Lake-Hiwassee River	133	92.3 + 18.5 (1-117)	1973-1994
Middle Tennessee-Hiwassee	Hiwassee River-Chatuge Lake	68	92.4 + 7.1 (69-116)	2001-2019
Middle Tennessee-Hiwassee	Nottely River	71	84.7 + 23.4 (0-110)	2001-2019
Middle Tennessee-Hiwassee	Ocoee River	557	93.9 + 8.2 (28-121)	2001-2021
Middle Tennessee-Hiwassee	Spring Creek-Hiwassee River	166	81.4 + 15.3 (43-120)	1973-2013
Middle Tennessee-Hiwassee	Tusquitee Creek-Hiwassee River	170	84.7 + 21.0 (13-116)	1973-1981
Middle Tennessee-Hiwassee	Valley River	346	92.8 + 6.9 (73-117)	1973-1996
Upper Tennessee	Alarka Creek-Little Tennessee River	342	91.4 + 7.2 (71-115)	1968-1996
Upper Tennessee	Cheoah River	283	94.1 + 6.9 (68-117)	1968-1996
Upper Tennessee	Cullasaja River	36	87.6 + 10.2 (62-117)	1968-1975
Upper Tennessee	Headwaters Little Tennessee River	494	90.9 + 6.1 (73-126)	1968-2021
Upper Tennessee	Lower Tuckasegee River	283	92.3 + 8.3 (20-118)	1968-1996
Upper Tennessee	Middle Tuckasegee River	222	89.1 + 9.9 (44-115)	1968-1994
Upper Tennessee	Nantahala River	403	93.8 + 7.2 (74-116)	1968-1996
Upper Tennessee	Oconaluftee River	208	93.3 + 6.9 (57-113)	1968-1996
Upper Tennessee	Upper Tellico Lake	248	84.2 + 12.9 (18-113)	1968-2018
Upper Tennessee	Upper Tuckasegee River	112	92.2 + 8.1 (70-113)	1968-1981

Table 9. Results of Spearman correlation analyses examining the relationship between DO percent saturation and year of record across each month of the year. Data were analyzed at the HUC10 scale and are organized by HUC

HUC6/HUC 10	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
French Broad-Holston												
Cane Creek-French Broad				-0.35 P = 0.03 N = 40								
Cane River		-0.61 P < 0.001 N = 31						0.58 P < 0.001 N = 32				-0.38 P = 0.04 N = 31
Cataloochee Creek-Pigeon										0.38 P = 0.02 N = 38		
Headwaters French Broad							-0.49 P < 0.001 N = 49					
Headwaters North Toe							-0.46 P = 0.003 N = 40	0.50 P < 0.001 N = 46	0.47 P = 0.001 N = 44	-0.38 P = 0.017 N = 38		
Headwaters Pigeon R.		-0.36 P = 0.007										

HUC6/HUC 10	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
		N = 55										
Mills River							0.59 P < 0.001 N = 36	0.76 P < 0.001 N = 46	0.44 P = 0.009 N = 34			
Sandymush Creek				-0.36 P = 0.016 N = 45								
South Toe-North Toe				-0.37 P = 0.036 N = 32		-0.71 P < 0.001 N = 52	-0.66 P < 0.001 N= 42		-0.42 P = 0.006 N = 42			-0.49 P = 0.004 N = 33
Walnut Creek					-0.49 P = 0.005 N = 31	-0.45 P = 0.009 N = 32	-0.46 P = 0.007 N = 34	-0.49 P = 0.002 N = 40				
Watauga Lake-Watauga R.	-0.39 P = 0.022 N = 35	0.48 P = 0.002 N = 40										0.33 P = 0.027 N = 44
Kanawha												
Little River		0.35 P = 0.025 N = 40	0.51 P < 0.001 N = 41		-0.45 P = 0.001 N = 47	0.53 P < 0.001 N = 36						
North Fork New River					-0.59 P < 0.001 N = 30							

HUC6/HUC 10	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
South Fork New River		0.36 $P = 0.013$ $N = 46$			-0.64 $P < 0.001$ $N = 69$							
Middle Tennessee-Hiwassee												
Ococee River					-0.51 $P = 0.003$ $N = 33$	-0.34 $P = 0.022$ $N = 45$				-0.43 $P = 0.002$ $N = 48$	-0.45 $P = 0.003$ $N = 43$	-0.63 $P < 0.001$ $N = 37$
Valley River											-0.37 $P = 0.039$ $N = 31$	
Upper Tennessee												
Alarka Creek-Little TN							-0.39 $P = 0.009$ $N = 45$					
Nantahala River	0.38 $P = 0.033$ $N = 31$								0.59 $P < 0.001$ $N = 30$		0.44 $P = 0.009$ $N = 34$	

Table 10. pH descriptive statistics for Hydrologic Unit Code 10, watershed level. The mean H+ content is reported as converted pH value with its standard deviation (SD) and range. The period during which pH data were collected and recorded are available.

HUC6 Name	HUC10 Name	N	pH x̄ + SD (range)	Period of Record
French Broad-Holston	Cane Creek-French Broad River	650	6.7 + 6.4 (5.2-8.5)	1956-2020
French Broad-Holston	Cane River	782	6.9 + 6.7 (5.7-9.2)	1956-2020
French Broad-Holston	Cataloochee Creek-Pigeon River	3129	6.5 + 6.2 (4.6-8.7)	1955-2020
French Broad-Holston	Clear Creek-French Broad River	383	7.1 + 6.6 (5.4-8.9)	1946-2021
French Broad-Holston	Cove Creek-Nolichucky River	1005	7.7 + 7.4 (6.0-9.1)	1974-2021
French Broad-Holston	Davidson River-French Broad River	1726	6.4 + 6.2 (4.9-9.0)	1954-2020
French Broad-Holston	Doe River	335	7.2 + 6.9 (5.9-9.0)	1967-2021
French Broad-Holston	Elk River	161	7.0 + 6.7 (5.9-9.2)	1954-2021
French Broad-Holston	Gulf Fork Big Creek	92	7.4 + 7.2 (6.7-8.3)	1976-2021
French Broad-Holston	Headwaters French Broad River	738	6.5 + 6.4 (5.4-8.4)	1956-2020
French Broad-Holston	Headwaters North Toe River	1070	6.6 + 5.4 (3.9-8.7)	1956-2020
French Broad-Holston	Headwaters Pigeon River	1497	6.7 + 6.4 (5.2-9.0)	1954-2020
French Broad-Holston	Hominy Creek	475	6.5 + 5.6 (4.3-8.8)	1954-2020
French Broad-Holston	Ivy Creek	81	6.9 + 7.0 (6.3-8.1)	1956-2019
French Broad-Holston	Little Pigeon River	175	5.7 + 5.1 (4.0-7.0)	1976-2019
French Broad-Holston	Mills River-French Broad River	1319	6.5 + 6.2 (4.9-8.7)	1956-2020
French Broad-Holston	Mud Creek	386	6.5 + 6.2 (5.2-8.8)	1951-2020
French Broad-Holston	North Indian Creek-Nolichucky River	1585	7.1 + 6.7 (5.3-9.3)	1968-2021
French Broad-Holston	Pigeon River	2066	6.1 + 5.8 (4.2-8.7)	1957-2021
French Broad-Holston	Richland Creek-Pigeon River	1031	6.9 + 6.8 (6.0-8.3)	1954-2020
French Broad-Holston	Roan Creek	560	7.5 + 7.2 (6.1-9.8)	1974-2021

French Broad-Holston	Sandymush Creek-French Broad River	1366	6.9 + 6.7 (5.6-9.2)	1956-2020
French Broad-Holston	South Indian Creek	137	7.1 + 6.8 (5.9-9.5)	1999-2021
French Broad-Holston	South Toe River-North Toe River	1417	6.4 + 5.8 (4.6-9.2)	1951-2020
French Broad-Holston	Spring Creek-French Broad River	226	6.7 + 6.6 (5.7-8.8)	1957-2021
French Broad-Holston	Swannanoa River	703	6.7 + 6.5 (5.4-8.4)	1956-2020
French Broad-Holston	Walnut Creek-French Broad River	992	6.9 + 6.5 (5.2-8.9)	1954-2020
French Broad-Holston	Watauga Lake-Watauga River	1383	7.0 + 6.8 (5.7-9.1)	1956-2021
French Broad-Holston	Watauga River	47	7.6 + 7.6 (6.9-8.6)	1967-2021
French Broad-Holston	West Prong Little Pigeon River	226	5.3 + 5.0 (4.0-7.1)	1994-2019
Kanawha	Chestnut Creek-New River	1663	6.8 + 6.5 (5.3-9.5)	1945-2021
Kanawha	Elk Creek-New River	276	6.8 + 6.5 (5.6-8.8)	1970-2018
Kanawha	Fox Creek-New River	1255	6.6 + 6.5 (5.4-9.2)	1970-2021
Kanawha	Little River-New River	2046	6.8 + 6.6 (5.4-9.6)	1968-2020
Kanawha	North Fork New River	835	6.8 + 6.3 (4.9-9.0)	1954-2021
Kanawha	South Fork New River	1613	6.9 + 6.7 (5.7-9.4)	1955-2020
Middle Tennessee-Hiwassee	Brasstown Creek-Hiwassee River	389	6.6 + 5.8 (4.5-9.0)	1956-2021
Middle Tennessee-Hiwassee	Chickamauga Lake-Hiwassee River	55	7.1 + 7.1 (6.3-8.3)	2002-2021
Middle Tennessee-Hiwassee	Hiwassee Lake-Hiwassee River	847	6.7 + 6.6 (5.5-9.3)	1956-2021
Middle Tennessee-Hiwassee	Hiwassee River-Chatuge Lake	385	6.5 + 6.2 (5.3-8.4)	1958-2019
Middle Tennessee-Hiwassee	Nottely River	166	6.4 + 6.3 (5.5-8.1)	1957-2021
Middle Tennessee-Hiwassee	Ocoee River	1657	6.6 + 5.9 (4.4-9.3)	1957-2021

Middle Tennessee-Hiwassee	Spring Creek-Hiwassee River	531	6.6 + 6.4 (5.2-8.4)	1966-2021
Middle Tennessee-Hiwassee	Toccoa River-Blue Ridge Lake	41	6.3 + 6.2 (5.5-9.3)	1957-2021
Middle Tennessee-Hiwassee	Tusquitee Creek-Hiwassee River	189	6.5 + 6.4 (5.4-7.9)	1956-1981
Middle Tennessee-Hiwassee	Valley River	880	6.8 + 6.5 (5.3-8.9)	1956-2020
Upper Tennessee	Abrams Creek	1192	6.2 + 5.4 (4.0-8.4)	1968-2020
Upper Tennessee	Alarka Creek-Little Tennessee River	709	6.6 + 6.3 (5.0-8.9)	1954-2020
Upper Tennessee	Cheoah River	834	6.5 + 6.1 (4.7-8.3)	1968-2021
Upper Tennessee	Cullasaja River	101	6.4 + 6.4 (5.6-7.4)	1954-2004
Upper Tennessee	Fontana Lake	889	6.2 + 5.5 (4.0-7.5)	1968-2020
Upper Tennessee	Headwaters Little Tennessee River	959	6.6 + 6.4 (5.2-7.8)	1957-2021
Upper Tennessee	Little River	2046	5.6 + 5.6 (4.5-7.0)	1991-2020
Upper Tennessee	Lower Tuckasegee River	3207	5.7 + 5.5 (4.2-8.7)	1956-2020
Upper Tennessee	Middle Tuckasegee River	408	6.7 + 6.5 (5.3-9.1)	1956-2018
Upper Tennessee	Nantahala River	782	6.6 + 6.4 (5.3-8.7)	1956-2020
Upper Tennessee	Oconaluftee River	2129	6.1 + 5.7 (4.6-8.8)	1956-2019
Upper Tennessee	Tellico River	212	6.7 + 6.5 (5.7-8.8)	1968-2019
Upper Tennessee	Upper Tellico Lake	621	6.5 + 6.3 (5.2-8.9)	1968-2019
Upper Tennessee	Upper Tuckasegee River	179	6.4 + 6.1 (5.0-7.8)	1954-2020

Table 11. Results of Spearman correlation analyses examining the relationship between pH and year of record across each month of the year Spearman's rho (ρ), is listed followed by statistical significance (P), and sample size (N). Data were analyzed at the HUC10 scale and are organized by HUC6.

HUC6/HUC 10	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
French Broad-Holston												
Cane River			0.57 P < 0.001 N = 69		0.57 P < 0.001 N = 63				0.44 P < 0.001 N = 64			
Cove Creek-Nolichucky	0.61 P < 0.001 N = 69								0.42 P < 0.001 N = 113			
Doe River							-0.36 P = 0.04 N = 34	0.51 P < 0.001 N = 39				
Headwaters North Toe R.				0.39 P < 0.001 N = 76	0.65 P < 0.001 N = 76				0.75 P < 0.001 N = 79			
Headwaters Pigeon River							0.36 P < 0.001 N = 113					
Hominy Creek							0.36 P = 0.01 N = 49					

HUC6/HUC 10	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
North Indian Creek-Noli.	0.52 P < 0.001 N = 113	0.51 P < 0.001 N = 121	0.74 P < 0.001 N = 138	0.63 P < 0.001 N = 127	0.66 P < 0.001 N = 129	0.42 P < 0.001 N = 129	0.57 P < 0.001 N = 146				0.57 P < 0.001 N = 124	
Roan Creek				0.63 P < 0.001 N = 30				0.57 P < 0.001 N = 47	0.47 P < 0.001 N = 68			
Swannanoa River								0.35 P = 0.01 N = 51				
Walnut Creek				0.36 P = 0.002 N = 71		0.42 P < 0.001 N = 81			0.50 P < 0.001 N = 99			
Watauga Lake-Watauga R.	0.62 P < 0.001 N = 99								0.35 P < 0.001 N = 124			
Kanawha												
Chestnut Creek-New R.			0.54 P < 0.001 N = 113			0.51 P < 0.001 N = 112			0.37 P < 0.001 N = 112			
Fox Creek-New R.						0.60 P < 0.001 N = 64						

HUC6/HUC 10	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
North Fork New River			0.49 $P < 0.001$ $N = 61$					0.43 $P = 0.002$ $N = 50$	0.54 $P < 0.001$ $N = 69$			0.36 $P = 0.01$ $N = 51$
South Fork New River	0.64 $P < 0.001$ $N = 124$	0.51 $P < 0.001$ $N = 124$	0.60 $P < 0.001$ $N = 124$					0.63 $P < 0.001$ $N = 129$	0.52 $P < 0.001$ $N = 142$		0.61 $P < 0.001$ $N = 112$	
Middle Tennessee-Hiwassee												
Hiwassee Lake		0.54 $P < 0.001$ $N = 57$					0.46 $P < 0.001$ $N = 69$		0.56 $P < 0.001$ $N = 66$			
Spring Creek	0.60 $P < 0.001$ $N = 49$		0.71 $P < 0.001$ $N = 49$		0.34 $P = 0.009$ $N = 58$		0.41 $P = 0.003$ $N = 51$		0.78 $P < 0.001$ $N = 50$			
Valley River					0.40 $P < 0.001$ $N = 75$						0.53 $P < 0.001$ $N = 76$	
Upper Tennessee												
Abrams Creek							-0.38 $P < 0.001$ $N = 121$					
Cheoah		0.34 $P = 0.007$		0.54 $P < 0.001$	0.48 $P < 0.001$					0.52 $P < 0.001$		

HUC6/HUC 10	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
		N = 61		N = 64	N = 66					N = 71		
Fontana Lake											0.69 $P < 0.001$ N = 61	
Upper Tellico Lake			0.56 $P < 0.001$ N = 75					0.67 $P < 0.001$ N = 65		0.82 $P < 0.001$ N = 71		

Table 12. Specific Conductance (*µS/cm* at 25°C) descriptive statistics for Hydrologic Unit Code 10, watershed level. The mean spC is reported with its standard deviation (SD) and range. The period during which spC data were collected and recorded are available.

HUC6 Name	**HUC10 Name**	**N**	**spC at 25°C $\bar{x}$ + SD (range)**	**Period of Record**
French Broad-Holston	Cane Creek-French Broad River	84	54.1 + 31.9 (6-161)	1956-2005
French Broad-Holston	Cane River	90	29.9 + 14.5 (10-69)	1956-1986
French Broad-Holston	Cataloochee Creek-Pigeon River	571	123.3 + 192.9 (6-1740)	1955-2019
French Broad-Holston	Clear Creek-French Broad River	198	121.6 + 102.6 (12-530)	1968-2011
French Broad-Holston	Cove Creek-Nolichucky River	122	249.4 + 149.5 (14-474)	1973-2011
French Broad-Holston	Davidson River-French Broad River	154	58.7 + 54.1 (9-225)	1954-1997
French Broad-Holston	Doe River	68	74.3 + 28.3 (13-125)	1967-2011
French Broad-Holston	Elk River	52	51.3 + 12.4 (30-84)	1954-2011
French Broad-Holston	Headwaters French Broad River	82	15.8 + 2.4 (12-26)	1956-1997
French Broad-Holston	Headwaters Pigeon River	111	19.2 + 4.2 (6-32)	1954-2014
French Broad-Holston	Mills River-French Broad River	102	84.0 + 52.1 (6-225)	1956-2002
French Broad-Holston	North Indian Creek-Nolichucky River	216	74.8 + 67.6 (10-430)	1968-2011
French Broad-Holston	Pigeon River	285	218.3 + 189.1 (11-1050)	1957-2011
French Broad-Holston	Richland Creek-Pigeon River	143	340.0 + 338.0 (11-1870)	1954-1982
French Broad-Holston	Roan Creek	34	96.9 + 29.6 (37-140)	1974-1988
French Broad-Holston	Sandymush Creek-French Broad River	165	101.3 + 43.1 (27-291)	1956-2004
French Broad-Holston	South Toe River-North Toe River	105	28.3 + 14.1 (10-69)	1951-2014
French Broad-Holston	Spring Creek-French Broad River	93	78.7 + 41.3 (10-210)	1957-2011
French Broad-Holston	Swannanoa River	151	43.6 + 52.1 (14-417)	1956-2010
French Broad-Holston	Walnut Creek-French Broad River	513	92.1 + 35.6 (8-235)	1956-2004
French Broad-Holston	Watauga Lake-Watauga River	33	59.2 + 13.2 (33-83)	1956-1973

French Broad-Holston	Watauga River	60	81.2 + 22.1 (44-125)	1967-1982
Kanawha	Chestnut Creek-New River	336	47.4 + 7.8 (25-85)	1949-1986
Kanawha	Little River-New River	65	45.5 + 6.1 (36-72)	1968-1973
Middle Tennessee-Hiwassee	Brasstown Creek-Hiwassee River	98	35.3 + 10.8 (19-78)	1956-2001
Middle Tennessee-Hiwassee	Hiwassee Lake-Hiwassee River	32	23.2 + 3.5 (19-37)	1956-1973
Middle Tennessee-Hiwassee	Hiwassee River-Chatuge Lake	434	13.0 + 3.7 (7-55)	1958-2011
Middle Tennessee-Hiwassee	Nottely River	69	50.9 + 132.5 (19-833)	1957-2001
Middle Tennessee-Hiwassee	Nottely River-Nottely Lake	34	26.2 + 4.2 (19-38)	1957-1974
Middle Tennessee-Hiwassee	Ocoee River	380	153.0 + 202.8 (14-1110)	1957-2015
Middle Tennessee-Hiwassee	Spring Creek-Hiwassee River	109	30.0 + 11.2 (16-85)	1966-1988
Middle Tennessee-Hiwassee	Toccoa River-Blue Ridge Lake	33	18.3 + 2.5 (15-26)	1957-1974
Upper Tennessee	Alarka Creek-Little Tennessee River	342	26.4 + 4.7 (14-38)	1954-2015
Upper Tennessee	Cullasaja River	52	18.6 + 4.4 (11-29)	1954-2004
Upper Tennessee	Headwaters Little Tennessee River	116	95.2 + 104.9 (7-367)	1957-2011
Upper Tennessee	Little River	43	16.6 + 3.5 (12-26)	1999-2011
Upper Tennessee	Lower Tuckasegee River	133	36.5 + 28.1 (9-160)	1956-2019
Upper Tennessee	Middle Tuckasegee River	66	56.5 + 67.1 (17-400)	1956-1973
Upper Tennessee	Nantahala River	68	17.1 + 7.1 (7-42)	1956-2014
Upper Tennessee	Tellico River	141	28.8 + 35.4 (8-315)	1968-2004
Upper Tennessee	Upper Tellico Lake	77	24.6 + 11.3 (9-88)	1968-2011

Table 13. Specific Conductance (µS/cm) descriptive statistics for Hydrologic Unit Code 10, watershed level. The mean spC is reported with its standard deviation (SD) and range. The period during which spC data were collected and recorded are available.

HUC6 Name	HUC10 Name	N	spC x̄ + SD (range)	Period of Record
French Broad-Holston	Cane Creek-French Broad River	105	42.3 + 21.5 (27-152)	2008-2020
French Broad-Holston	Cane River	86	50.6 + 10.4 (9-84)	2006-2020
French Broad-Holston	Cataloochee Creek-Pigeon River	1695	17.9 + 37.4 (-2-672)	1993-2020
French Broad-Holston	Clear Creek-French Broad River	41	68.3 + 14.8 (38-108)	2001-2006
French Broad-Holston	Cove Creek-Nolichucky River	406	413.8 + 132.2 (33-811)	1999-2009
French Broad-Holston	Davidson River-French Broad River	226	17.7 + 5.5 (10-38)	2008-2020
French Broad-Holston	Doe River	75	65.5 + 32.2 (17-146)	1999-2007
French Broad-Holston	Headwaters French Broad River	105	16.1 + 3.5 (8-27)	2008-2020
French Broad-Holston	Headwaters North Toe River	194	70.5 + 26.2 (24-176)	2006-2020
French Broad-Holston	Headwaters Pigeon River	205	226.9 + 336.3 (12-2000)	2008-2020
French Broad-Holston	Hominy Creek	182	68.5 + 14.3 (32-110)	2008-2020
French Broad-Holston	Mills River-French Broad River	180	27.0 + 34.2 (11-200)	2007-2020
French Broad-Holston	Mud Creek	161	66.8 + 13.2 (30-121)	2007-2020
French Broad-Holston	North Indian Creek-Nolichucky River	278	249.4 + 187.8 (13-593)	1999-2019
French Broad-Holston	Pigeon River	1173	24.0 + 43.1 (0-557)	1993-2020
French Broad-Holston	Richland Creek-Pigeon River	203	51.8 + 16.5 (9-187)	2008-2020
French Broad-Holston	Roan Creek	228	114.3 + 40.9 (18-260)	1999-2009
French Broad-Holston	Sandymush Creek-French Broad River	198	53.8 + 13.8 (23-117)	2008-2020
French Broad-Holston	South Toe River-North Toe River	194	59.6 + 59.9 (8-164)	2000-2020
French Broad-Holston	Swannanoa River	95	69.5 + 27.2 (14-241)	2008-2020
French Broad-Holston	Walnut Creek-French Broad River	115	57.0 + 16.5 (28-117)	2008-2020

French Broad-Holston	Watauga Lake-Watauga River	369	71.4 + 47.2 (8-272)	2001-2020
Kanawha	Chestnut Creek-New River	1064	48.3 + 40.3 (0-500)	1979-2021
Kanawha	Fox Creek-New River	1317	27.8 + 24.8 (5-500)	1979-2021
Kanawha	Little River-New River	815	51.1 + 23.3 (1-500)	1979-2020
Kanawha	North Fork New River	518	41.0 + 24.7 (13-120)	1987-2021
Kanawha	South Fork New River	471	90.0 + 45.4 (13-247)	2008-2020
Middle Tennessee-Hiwassee	Hiwassee Lake-Hiwassee River	79	39.3 + 53.2 (19-279)	1999-2020
Middle Tennessee-Hiwassee	Ocoee River	223	51.5 + 50.4 (12-362)	1999-2009
Middle Tennessee-Hiwassee	Valley River	72	49.9 + 13.3 (25-89)	2008-2020
Upper Tennessee	Abrams Creek	1154	40.6 + 39.4 (1-161)	1993-2020
Upper Tennessee	Alarka Creek-Little Tennessee River	102	29.5 + 6.3 (13-47)	2007-2020
Upper Tennessee	Cheoah River	87	30.5 + 12.1 (11-98)	2008-2020
Upper Tennessee	Fontana Lake	725	12.1 + 3.0 (5-27)	1993-2020
Upper Tennessee	Headwaters Little Tennessee River	88	23.7 + 3.7 (12-31)	2008-2020
Upper Tennessee	Lower Tuckasegee River	2349	13.0 + 2.8 (2-28)	1991-2020
Upper Tennessee	Nantahala River	98	14.2 + 4.7 (8-30)	2008-2020
Upper Tennessee	Oconaluftee River	1064	17.8 + 11.8 (0-190)	1993-2018
Upper Tennessee	Tellico River	65	52.3 + 47.9 (1-185)	1999-2009
Upper Tennessee	Upper Tellico Lake	148	22.4 + 34.5 (6-338)	1993-2009
Upper Tennessee	Upper Tuckasegee River	33	14.7 + 5.5 (8-22)	2007-2020

Table 14. Results of Spearman correlation analyses examining the relationship between Specific Conductance (μS/cm) and year of record across each month of the year Spearman's rho (ρ), is listed followed by statistical significance (P), and sample size (N). Data were analyzed at the HUC10 scale and are organized by HUC6.

HUC6/HUC 10	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
French Broad-Holston												
Pigeon River												0.38 P = 0.03 N = 33
Kanawha												
Fox Creek-New R.						0.56 P < 0.001 N = 52		0.37 P = 0.003 N = 63				
Little River-New R.				0.49 P < 0.001 N = 58	0.52 P < 0.001 N = 80						0.44 P < 0.001 N =76	
North Fork New River										0.40 P < 0.001 N =76	0.38 P = 0.03 N =33	
Upper Tennessee												
Fontana Lake			0.54 P < 0.001 N =192									
Lower Tuckasegee	-0.64					-0.55					-0.53	-0.55

HUC6/HUC 10	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
	P < 0.001 N = 158					P < 0.001 N = 166					P < 0.001 N = 190	P <0.001 N = 134

Table 15. Total Dissolved Solids (ppm) descriptive statistics for Hydrologic Unit Code 10, watershed level. The mean TDS is reported with its standard deviation (SD) and range. The period during which TDS data were collected and recorded are available.

HUC6 Name	HUC10 Name	N	TDS $\bar{x}$ + SD (range)	Period of Record
French Broad-Holston	Cane Creek-French Broad River	148	41.4 + 18.2 (10-105)	1956-2005
French Broad-Holston	Cane River	117	30.6 + 9.1 (10-49)	1956-2006
French Broad-Holston	Cataloochee Creek-Pigeon River	1166	106.0 + 114.2 (4-1390)	1955-2021
French Broad-Holston	Clear Creek-French Broad River	230	55.8 + 25.6 (2-180)	1946-2021
French Broad-Holston	Cove Creek-Nolichucky River	260	220.3 + 83.3 (11-408)	1973-2021
French Broad-Holston	Davidson River-French Broad River	300	42.9 + 32.8 (10-157)	1954-1997
French Broad-Holston	Doe River	123	48.1 + 23.0 (10-101)	1967-2021
French Broad-Holston	Elk River	87	39.2 + 10.7 (14-65)	1954-2021
French Broad-Holston	Headwaters French Broad River	161	17.7 + 2.9 (11-28)	1956-1997
French Broad-Holston	Headwaters North Toe River	47	28.1 + 5.3 (20-54)	1956-2006
French Broad-Holston	Headwaters Pigeon River	211	18.5 + 3.8 (10-28)	1954-2002
French Broad-Holston	Hominy Creek	31	35.1 + 4.5 (29-48)	1954-1997
French Broad-Holston	Mills River-French Broad River	189	60.6 + 31.4 (5-157)	1956-2002
French Broad-Holston	Nantahala River	103	17.0 + 5.0 (8-36)	1956-1973
French Broad-Holston	North Indian Creek-Nolichucky River	409	112.6 + 104.4 (10-304)	1968-2021
French Broad-Holston	Pigeon River	1167	111.9 + 68.3 (0-390)	1957-2021
French Broad-Holston	Richland Creek-Pigeon River	244	200.1 + 202.3 (10-1390)	1954-1982
French Broad-Holston	Roan Creek	179	57.5 + 40.8 (10-460)	1974-2012
French Broad-Holston	Sandymush Creek-French Broad River	215	70.5 + 27.0 (35-189)	1956-1997
French Broad-Holston	South Indian Creek	34	50.2 + 131.6 (10-569)	2005-2021
French Broad-Holston	South Toe River-North Toe River	142	28.0 + 10.1 (10-49)	1951-1986

French Broad-Holston	Spring Creek-French Broad River	141	62.9 + 20.9 (30-143)	1957-1997
French Broad-Holston	Swannanoa River	176	30.4 + 28.1 (8-218)	1956-2010
French Broad-Holston	Walnut Creek-French Broad River	752	65.5 + 22.9 (25-189)	1954-1997
French Broad-Holston	Watauga Lake-Watauga River	111	51.4 + 35.3 (10-158)	1956-2021
French Broad-Holston	Watauga River	54	63.0 + 15.7 (31-96)	1967-2021
Kanawha	Chestnut Creek-New River	628	38.0 + 5.5 (25-54)	1930-1986
Kanawha	Little River-New River	91	34.4 + 6.8 (10-57)	1968-2007
Kanawha	North Fork New River	53	39.5 + 7.5 (28-61)	1954-1973
Kanawha	South Fork New River	58	43.9 + 71.4 (21-470)	1955-2016
Middle Tennessee-Hiwassee	Brasstown Creek-Hiwassee River	62	20.6 + 3.4 (15-31)	1956-1973
Middle Tennessee-Hiwassee	Chickamauga Lake-Hiwassee River	54	43.0 + 12.9 (6-74)	2006-2021
Middle Tennessee-Hiwassee	Hiwassee Lake-Hiwassee River	62	20.6 + 3.4 (15-31)	1956-1973
Middle Tennessee-Hiwassee	Hiwassee River-Chatuge Lake	134	11.1 + 6.5 (5-40)	1958-1986
Middle Tennessee-Hiwassee	Ocoee River	322	55.9 + 44.1 (3-284)	1957-2021
Middle Tennessee-Hiwassee	Spring Creek-Hiwassee River	139	23.2 + 8.2 (3-62)	1966-2018
Middle Tennessee-Hiwassee	Tusquitee Creek-Hiwassee River	38	17.9 + 2.9 (11-27)	1956-1972
Middle Tennessee-Hiwassee	Valley River	59	27.4 + 7.3 (16-48)	1956-2015
Upper Tennessee	Alarka Creek-Little Tennessee River	83	21.2 + 3.9 (12-32)	1954-1973
Upper Tennessee	Cheoah River	36	19.9 +3.4 (14-29)	1968-1973
Upper Tennessee	Cullasaja River	101	17.3 + 4.1 (9-28)	1954-1974
Upper Tennessee	Headwaters Little Tennessee River	69	22.5 + 4.4 (15-36)	1957-1973
Upper Tennessee	Lower Tuckasegee River	183	36.7 + 23.2 (7-137)	1956-2000
Upper Tennessee	Middle Tuckasegee River	132	43.8 + 47.7 (16-390)	1956-1973
Upper Tennessee	Tellico River	148	27.4 + 21.7 (6-120)	1968-2019
Upper Tennessee	Upper Tellico Lake	140	21.5 + 20.8 (6-250)	1968-2014

Table 16. Results of Spearman correlation analyses examining the relationship between Total Dissolved Solids (ppm) and year of record across each month of the year Spearman's rho (ρ), is listed followed by statistical significance (P), and sample size (N). Data were analyzed at the HUC10 scale and are organized by HUC6.

HUC6/HUC 10	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
French Broad-Holston												
Davidson River				0.48 P = 0.006 N = 31								
Walnut Creek									-0.50 P < 0.001 N = 69			
Kanawha												
Chestnut Creek							0.35 P = 0.012 N = 51		0.48 P = 0.002 N = 38			

Table 17. McNemar's test used for statistical analysis of watersheds occupied by study focal species over time (1900-2010). If the p-value (probability value) associated with a statistical test is less than or equal to 0.05, it is considered statistically significant.

Common Species Name	**Decade**	**Occupied HUC10**	**McNemar's Value (Exact Sig. 2-tailed)**
Eastern hellbender	1900	41	0.25
	2010	38	
Mudpuppy	1900	10	0.5
	2010	8	
Tangerine darter	1900	33	0.008
	2010	25	
Silver shiner	1900	58	<.001
	2010	26	
Fatlips minnow	1900	25	<.001
	2010	14	
Little Tennessee crayfish	1900	8	No change
	2010	8	
Grandfather Mountain crayfish	1900	5	No change
	2010	5	
French Broad River crayfish	1900	12	0.125
	2010	8	
Tennessee clubshell	1900	8	0.25
	2010	5	
Appalachian elktoe	1900	14	1.0
	2010	13	
Slippershell	1900	10	0.63
	2010	5	

Table 18. Results of the best fit models to explain variation in water chemistry parameters measurements. Predictors included: percent urban impervious surface (% Urban), year and interactions between % Urban and year. For all models, random effects included site location and stream drainage. All models were tested against the null model. This table includes only models with 2AIC of the best model (lowest AIC).

Parameter	Model	K	AIC	ΔAIC	Akaike Weight
Temperature °C	Year	1	46393	0	0.3439
	Year + % Urban	2	46394	1	0.3317
	Year + % Urban + Year * % Urban	3	46394	1	0.3244
Total Dissolved Solids (mg/l)	Year	1	12227	0	0.5023
	Year + % Urban	2	12227	0	0.4977
Specific Conductance (uS/cm)	Year	1	32349	0	0.5167
	Year + % Urban	2	32350	1	0.4833
pH	Year + % Urban + Year * % Urban	3	11568	0	1.0000
Dissolved Oxygen (ppm)	Year	1	22668	0	0.5247
	Year + % Urban	2	22670	2	0.4753
Dissolved Oxygen (% Sat)	Year + % Urban	2	3095	0	0.5111
	Year	1	3096	1	0.4889

Table 19. Effect of predictors on each water chemistry parameter. These results show only the parameters of the top models. Degrees of freedom are calculated using the Satterthwaite method.

Parameter	**Predictor**	**Relationship**	**F**	**df**	**p**	**R^2c**
Temperature °C	Year	+	9.46	75,280	<0.001	0.08
Dissolved Oxygen (ppm)	Year	-	20.9	74,586	<0.001	0.14
Dissolved Oxygen (% Sat)	Year	-	3.23	5,82	0.01	0.92
	% Urban	-	2.72	1,8	0.138	
pH	Year	+	53.59	79,168	<0.001	0.67
	% Urban	-	30.78	1,871	<0.001	
	Year ✻ % Urban	-	9.74	78,769	<0.001	
Specific Conductance (uS/cm)	Year	-	3.88	73,173	<0.001	0.97
Total Dissolved Solids (mg/l)	Year	+	23.4	71,009	<0.001	0.71

Figure 1. Study basins at the HUC6 scale (Basin) outlined in red. Smaller shaded regions represent watersheds at the HUC10 scale. Dark blue shading represents areas with water quality data and over lapping species data. While the lighter blue regions are watersheds with only species data available. These highlighted watersheds were used for further descriptive and statistical analysis.

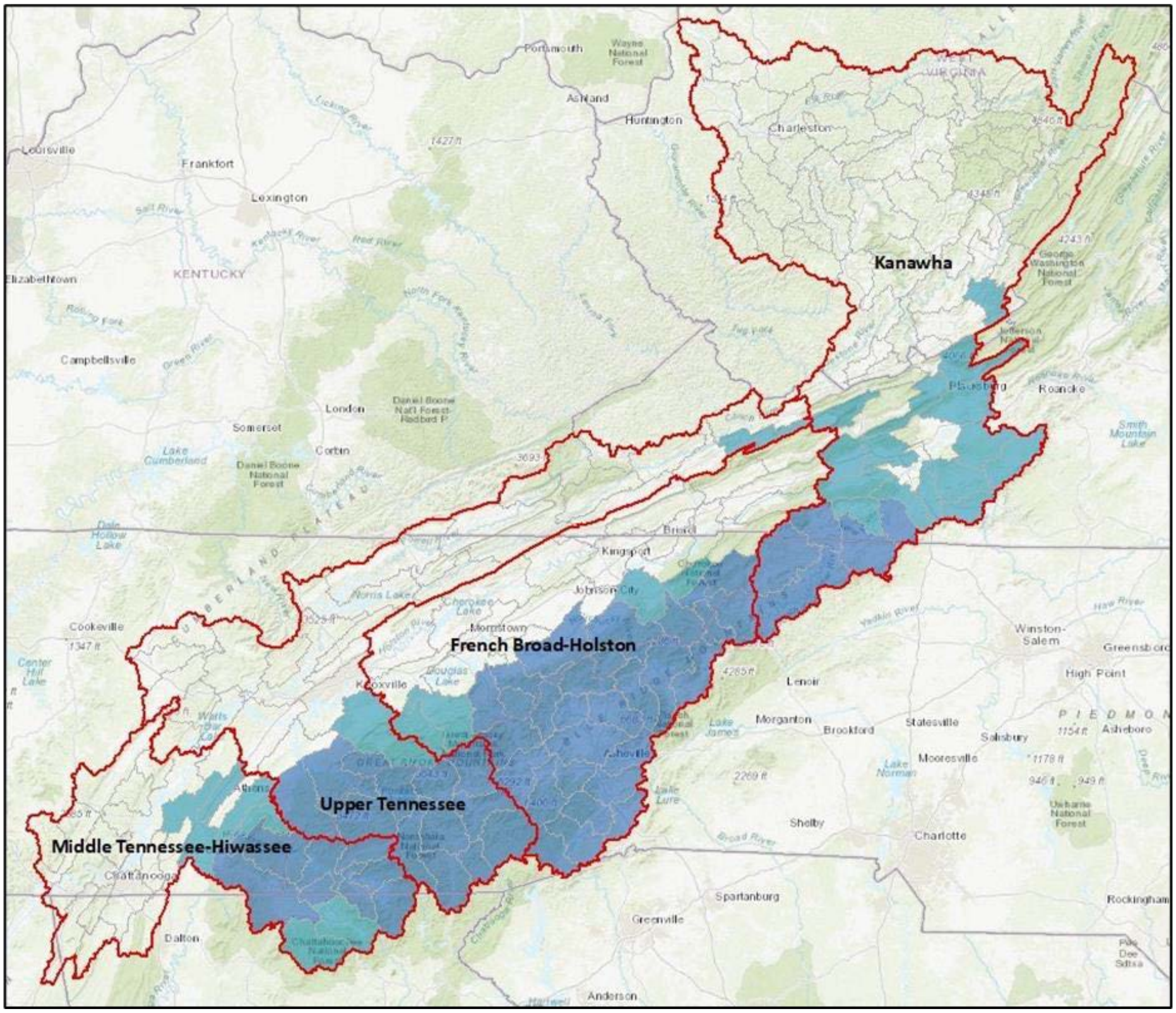


Figure 2. Mean temperature at basin scale (across all four major basins). No strong changes in temp were detected across all 4 major Basins. 59 watersheds, 70 years. Mean temp 13.5°C (n= 42,288).

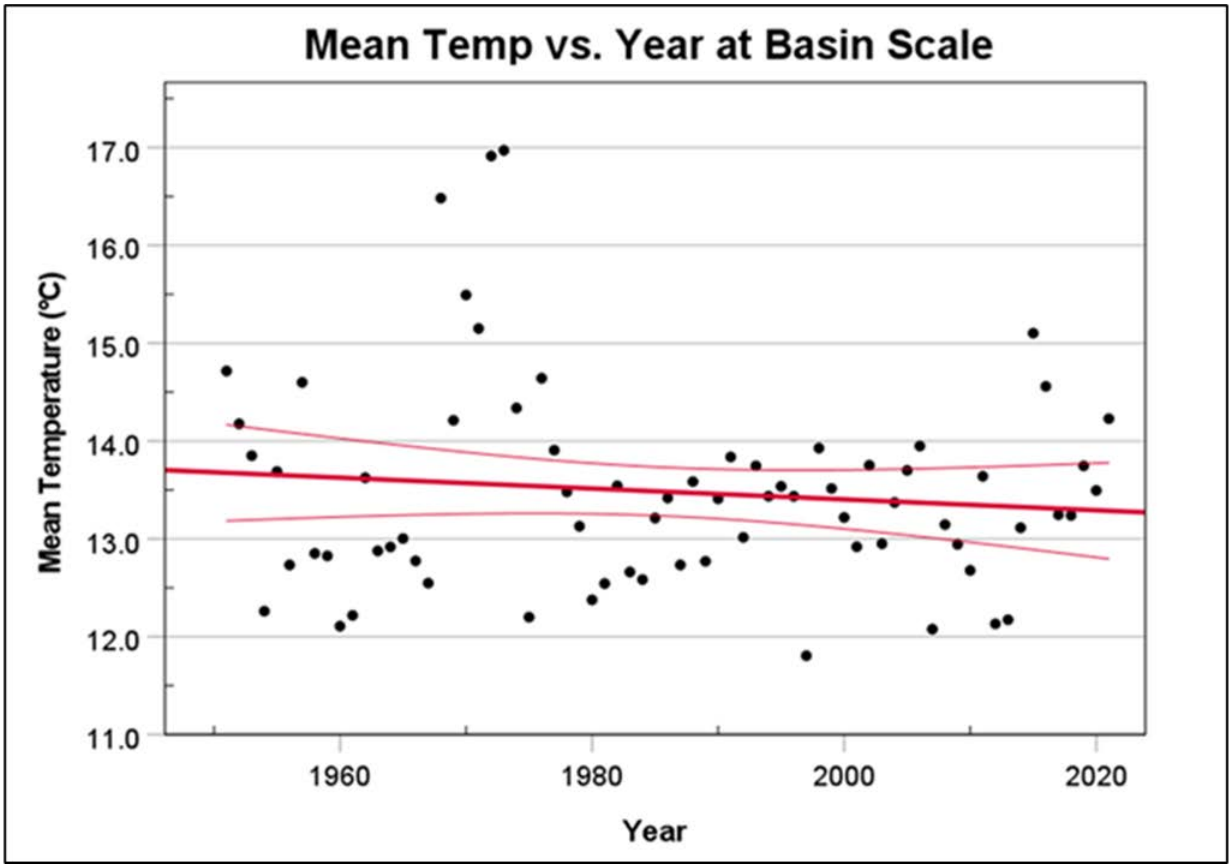


Figure 3. Mean basin temperature over time split by HUC6 basin. Upper Tennessee, Kanawha, and French Broad-Holston Basins appear to be decreasing in temperature over time. While the Middle Tennessee-Hiwassee has been increasing over the last ~60 years.

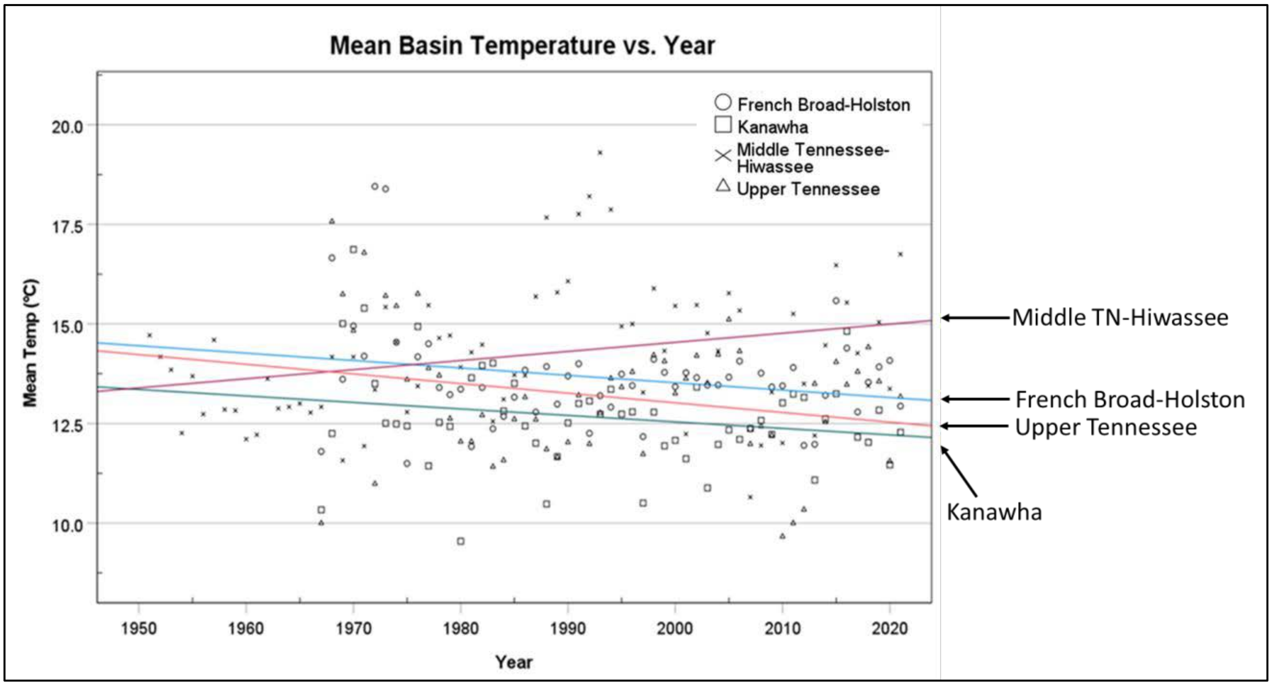


Figure 4. Mean annual temperature over time by month. Watershed example is representative of visual patterns accompanied by watersheds with consistent and significant increases in temperature over time. The Headwaters North Toe River (French Broad-Holston Basin) has increased in temperature in April and May over the past five decades. August and September have been increasing in temperature over the last six decades for this HUC10.

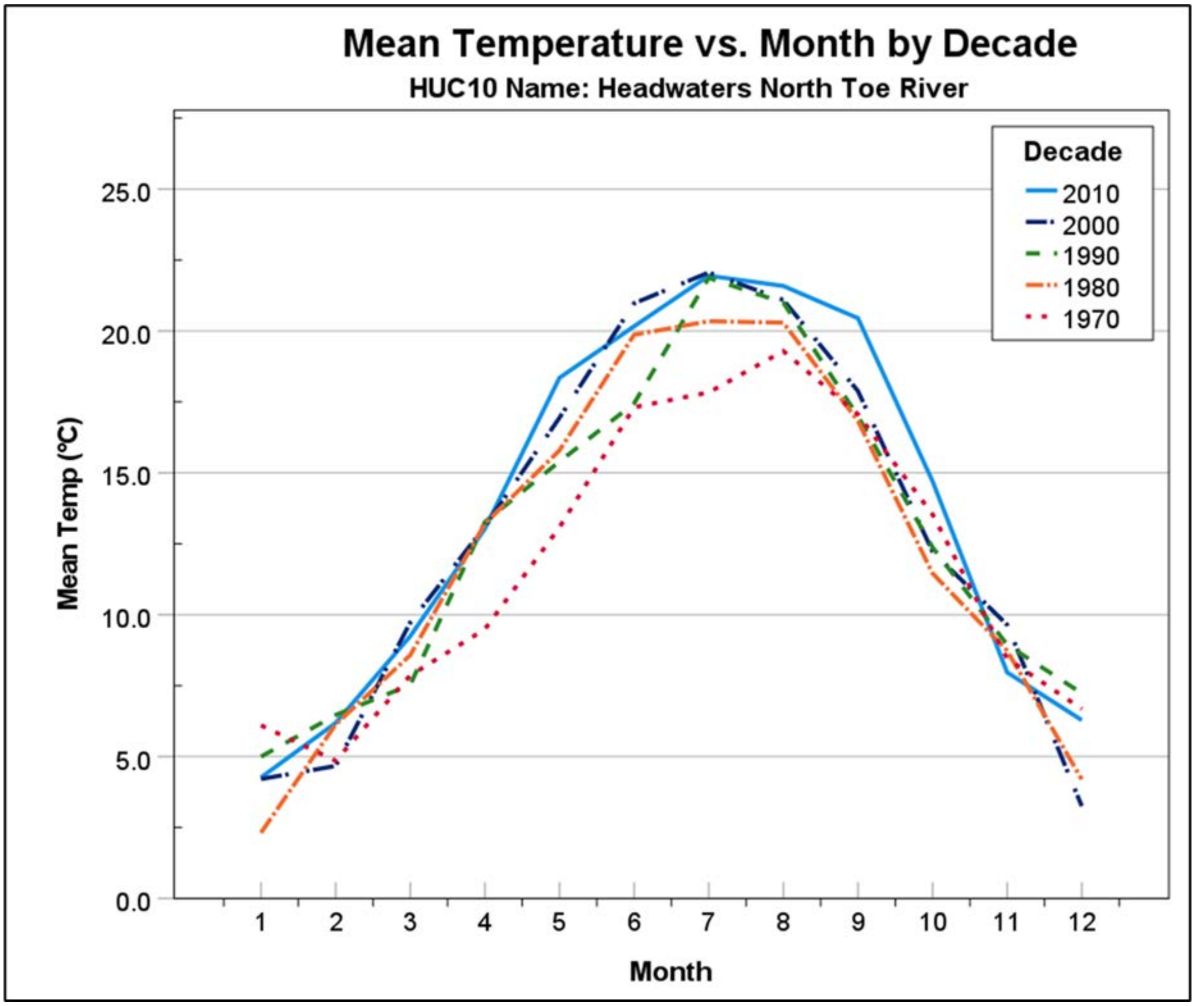


Figure 5. Mean annual temperature over time by month. Increases in temperature are more dramatic in other watersheds, such as Upper Tellico Lake (Upper Tennessee). Warming trends primarily occurred between April and September annually.

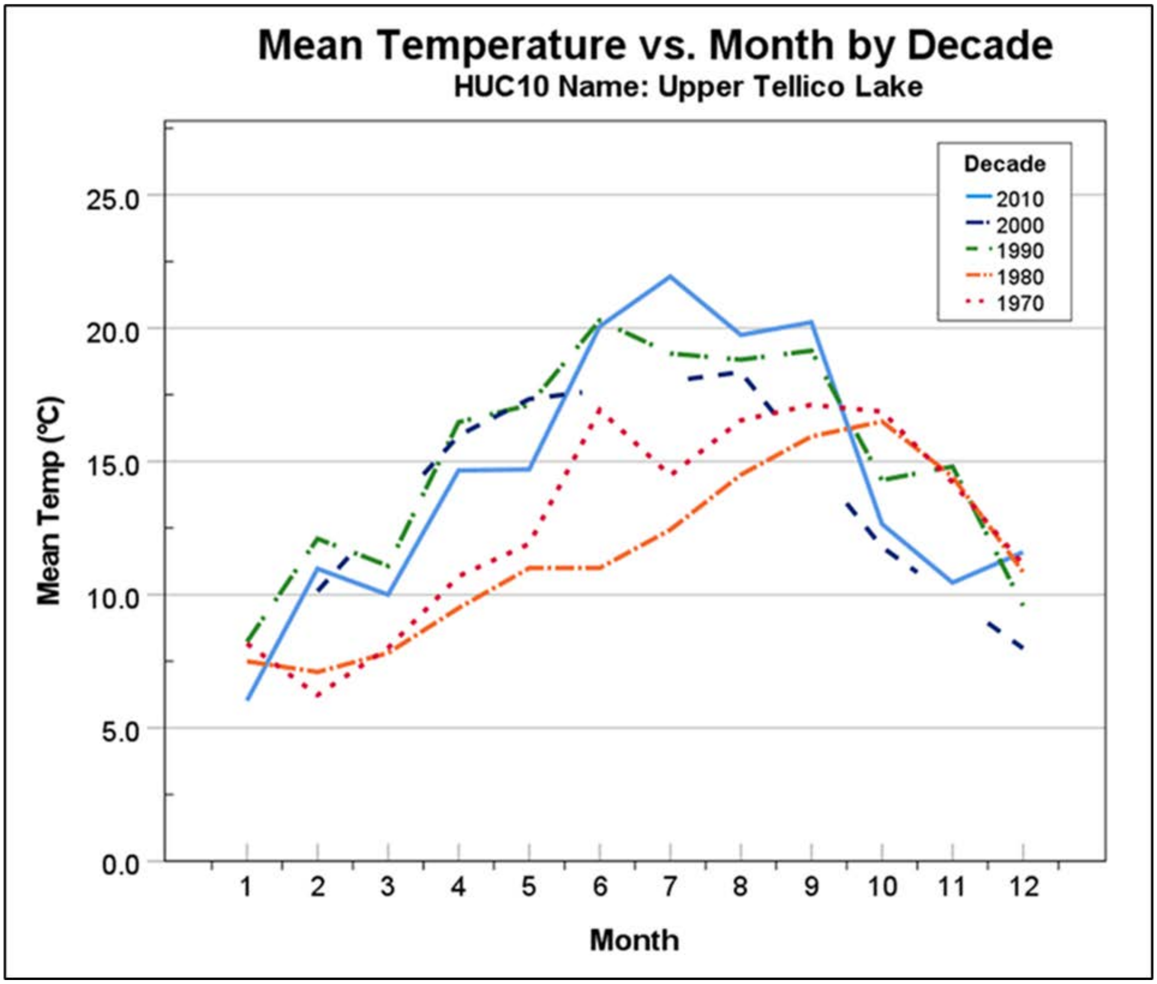


Figure 6. Mean annual temperature over time by month. Mud Creek (French Broad-Holston) was the only watershed to show cooling trends over the past four decades. This primarily occurred during the months of July and August. This suggests recovery may be occurring within this particular watershed.

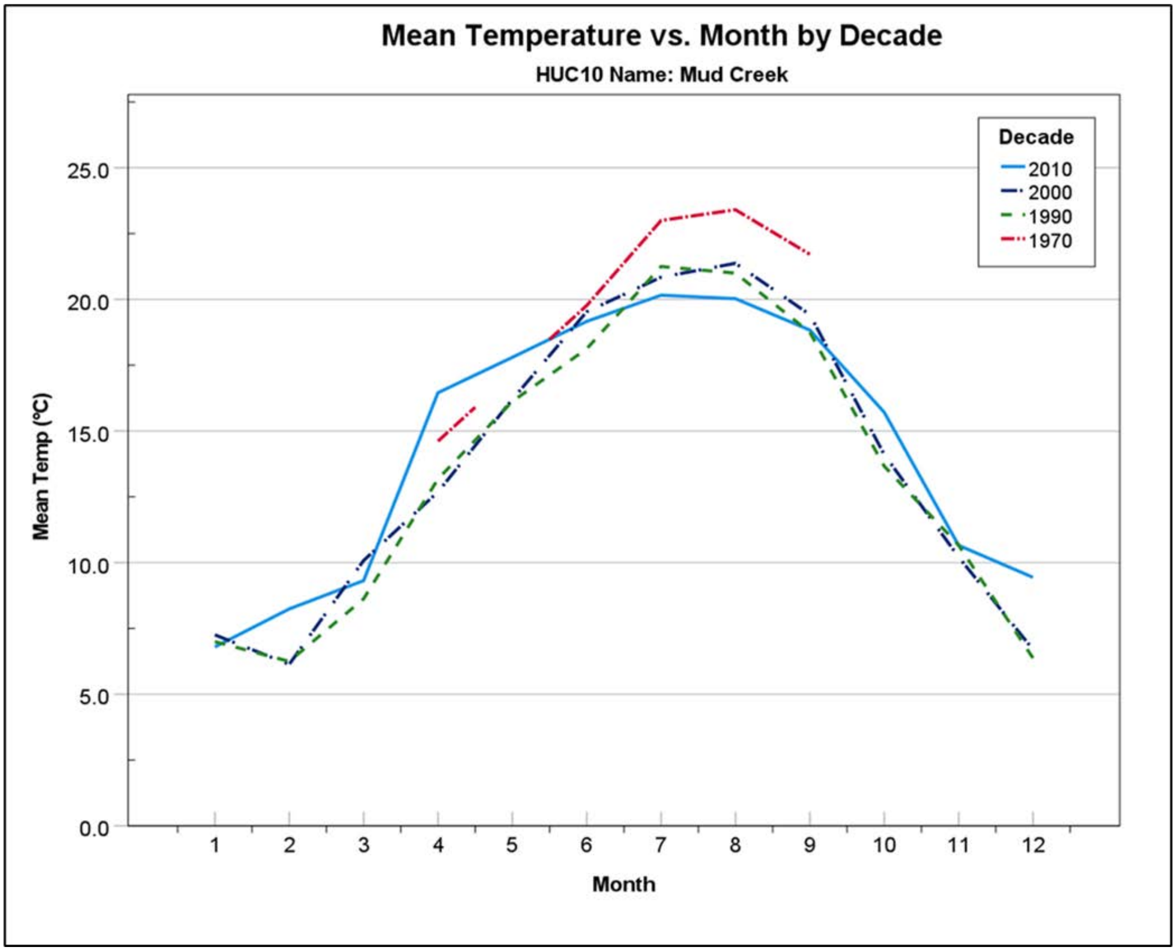


Figure 7. Mean DO ppm at basin scale (across all four major basins) appears to be increasing over time. (51 watersheds, 53 years) Mean DO: 9.9 ppm (n=30,804).

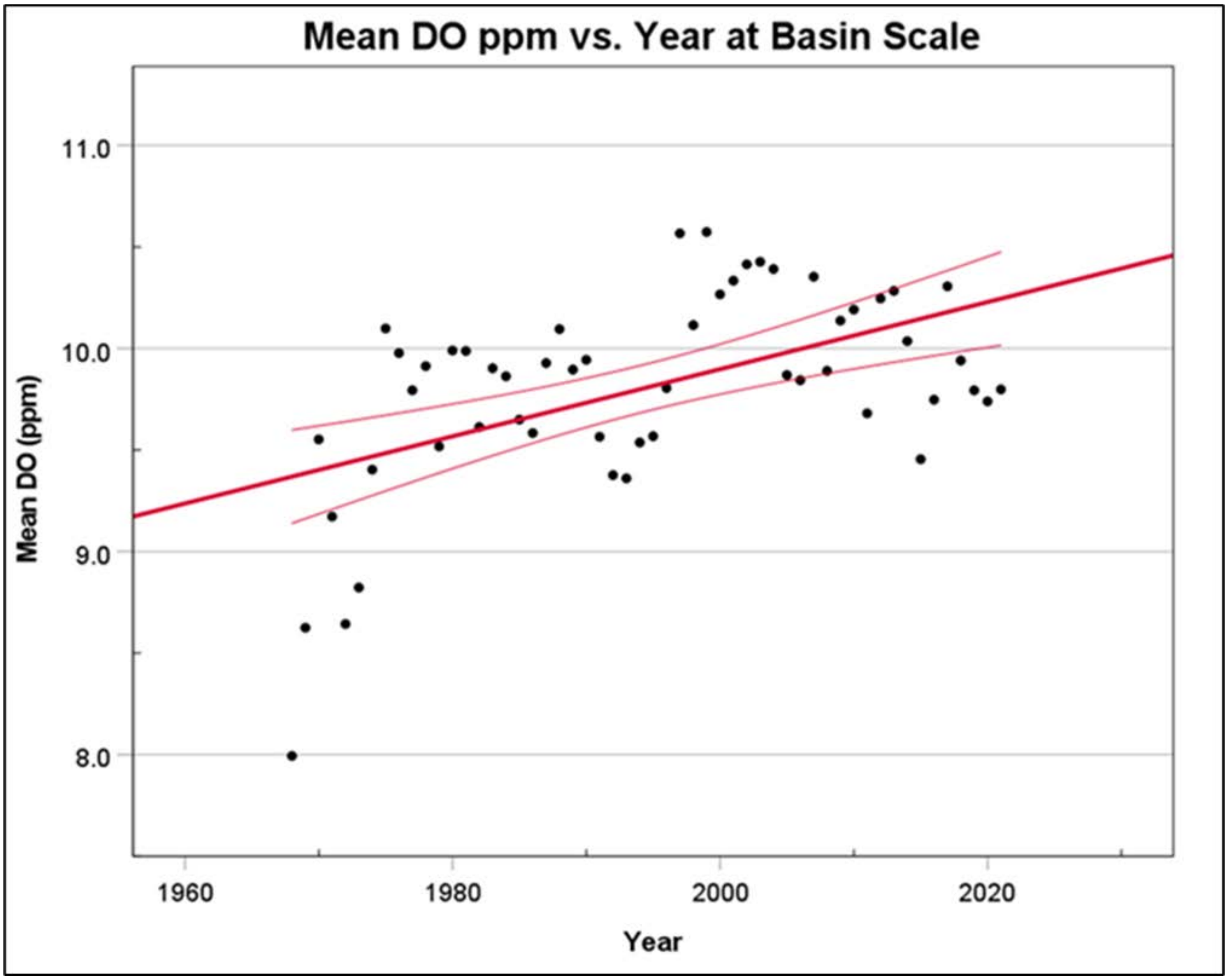


Figure 8. Mean DO% Sat at basin scale (across all four major basins). No strong linear trends were detected for DO% Sat across all major Basins. 41 watersheds, 53 years. Mean DO% Sat: 91.1% (n=13,223).

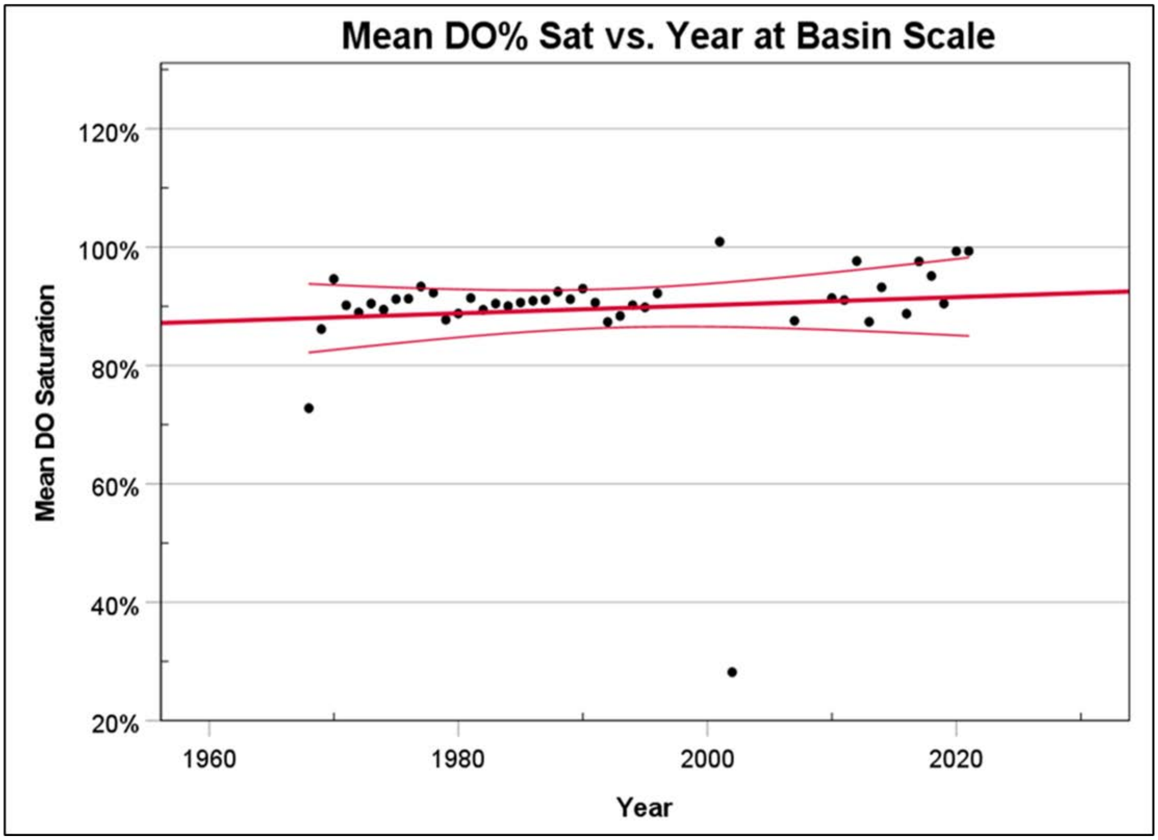


Figure 9. pH at basin scale (across all four major basins). No strong changes in pH were detected across 4 major basins, although slight linear trend appears. 60 watersheds, 76 years (n= 52,839).

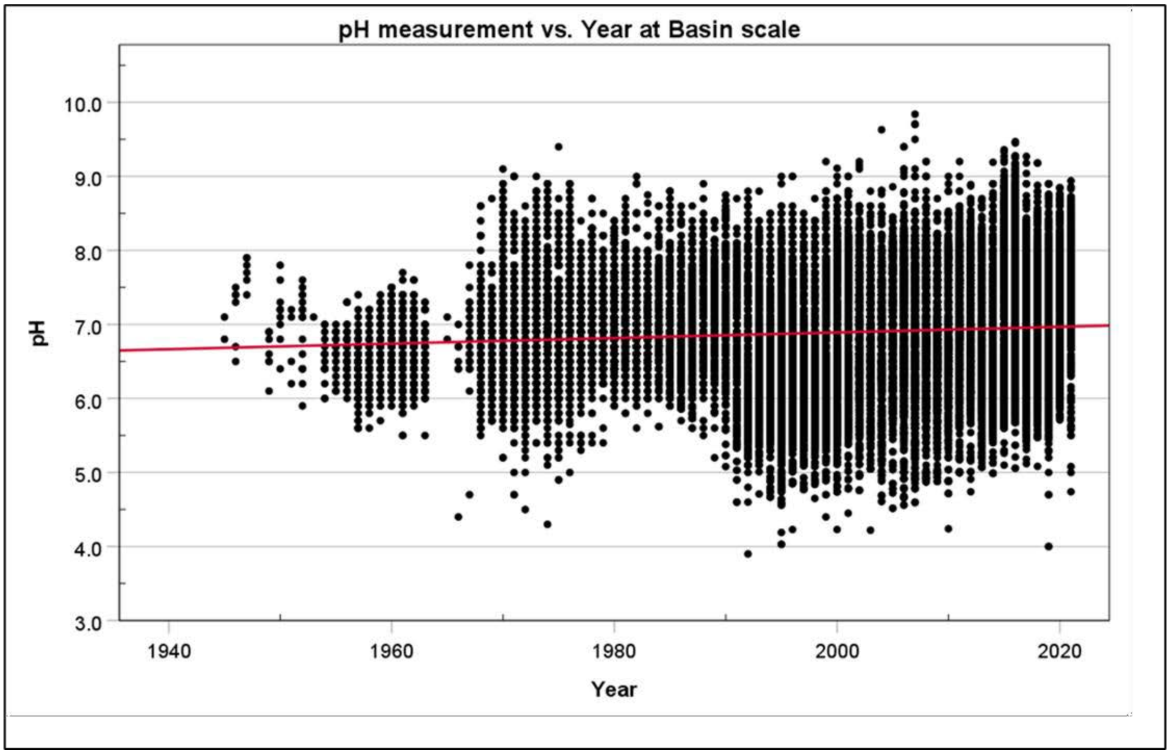


Figure 10. pH trends by major HUC6 basin. All major basins except for the Upper Tennessee appear to have watersheds that are increasing in pH over time.

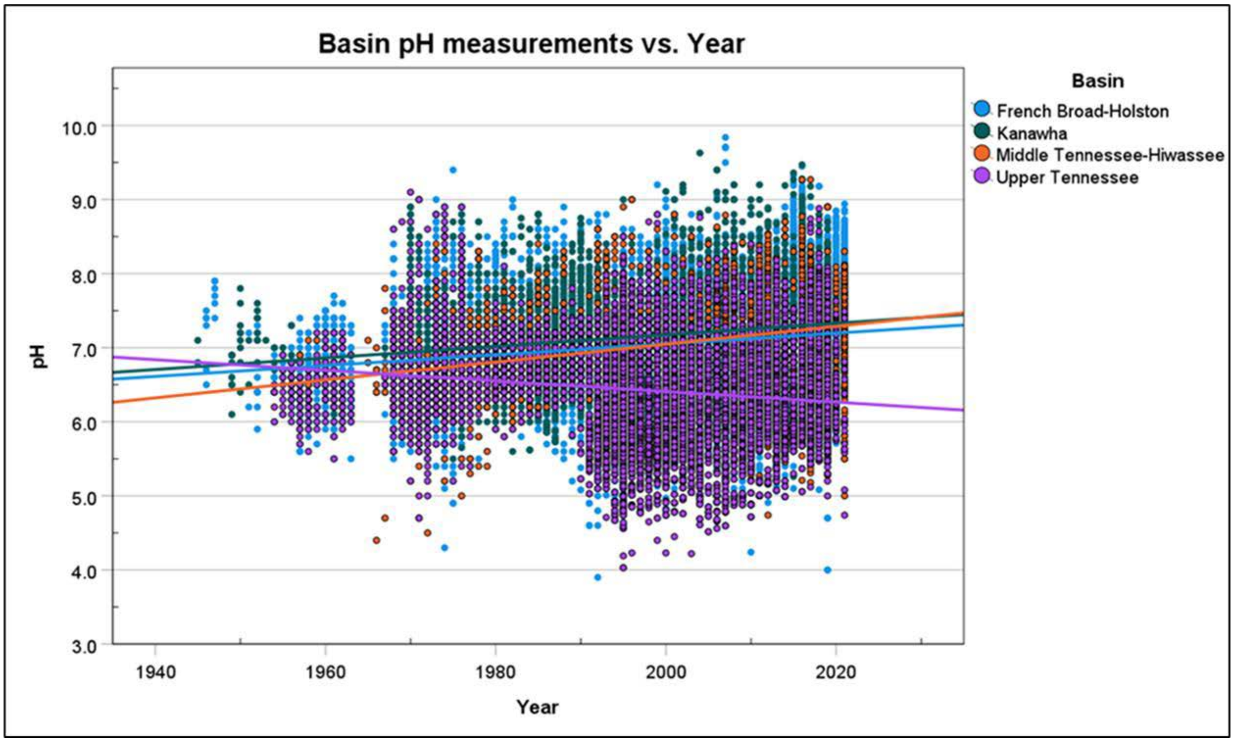


Figure 11. pH measurements over time at HUC10 watershed level. North Indian Creek-Nolichucky River (French Broad-Holston) is an example of what trends in watershed with strong correlations over time visually appear when graphed. Similar trends in a large majority of watersheds exhibited increases in pH suggesting widespread changes in pH over the last two decades.

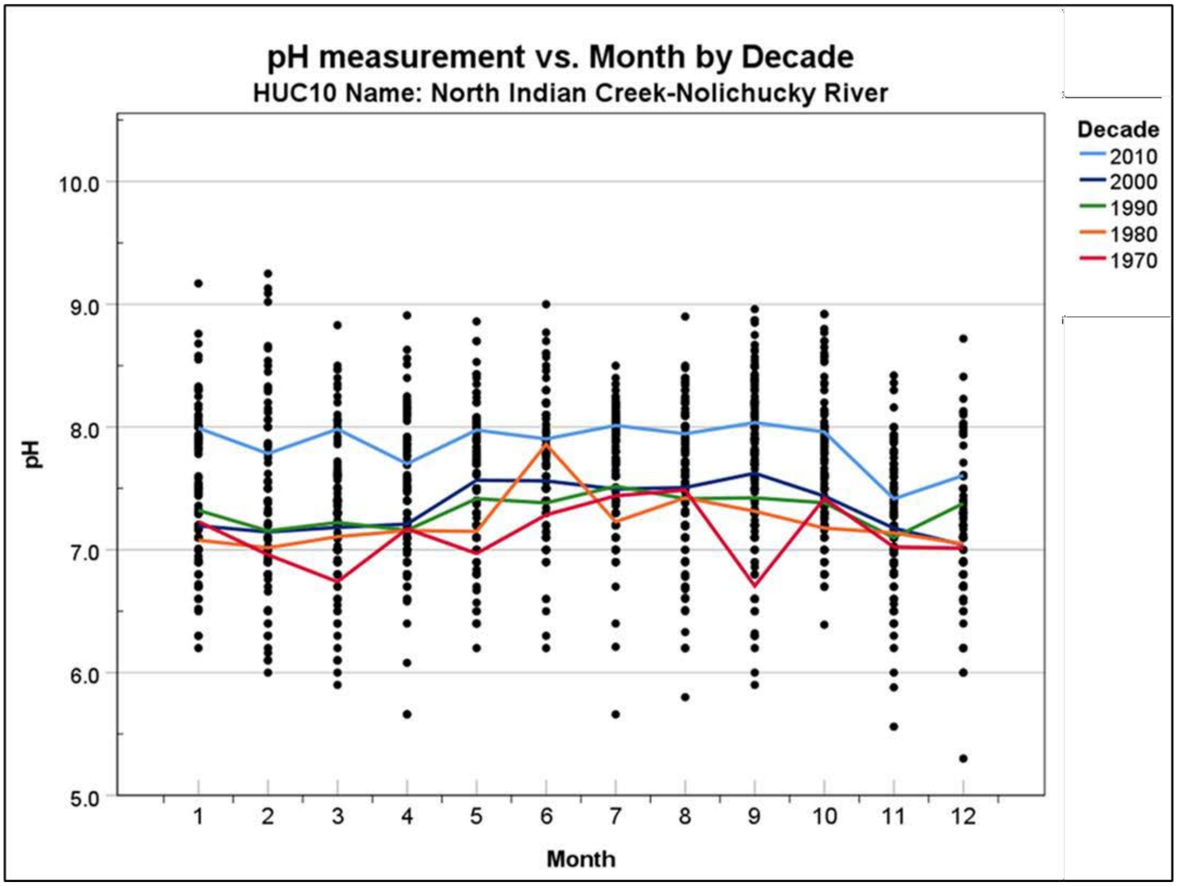


Figure 12. Mean spC over time at basin HUC6 scale (across all major basins). Largely no trend across all 4 major basins. 41 watersheds, 41 years. Mean spC: 62.7 µS/cm (n= 16,986).

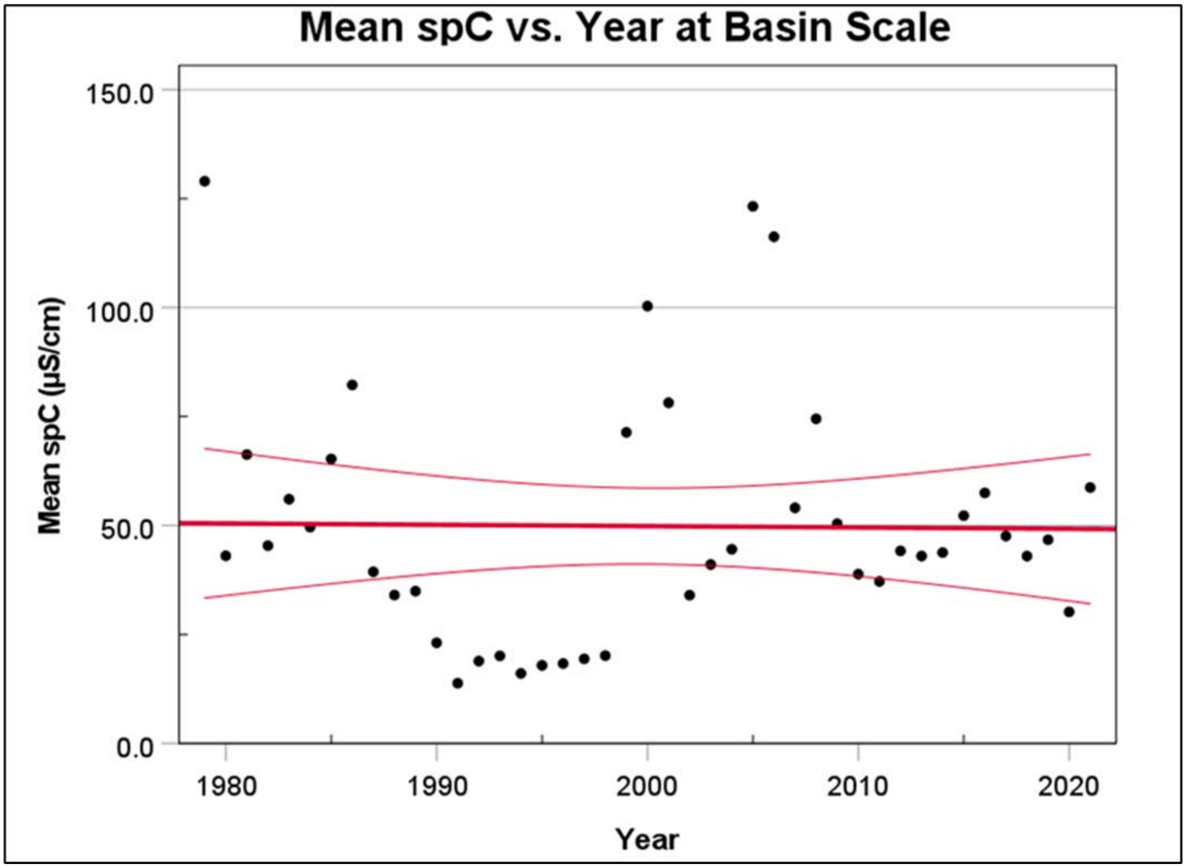


Figure 13. Mean TDS over time at HUC6 basin scale (across all major basins). Increasing over time. 46 watersheds, 91 years. Mean TDS: 46.4 ppm (n= 9,389).

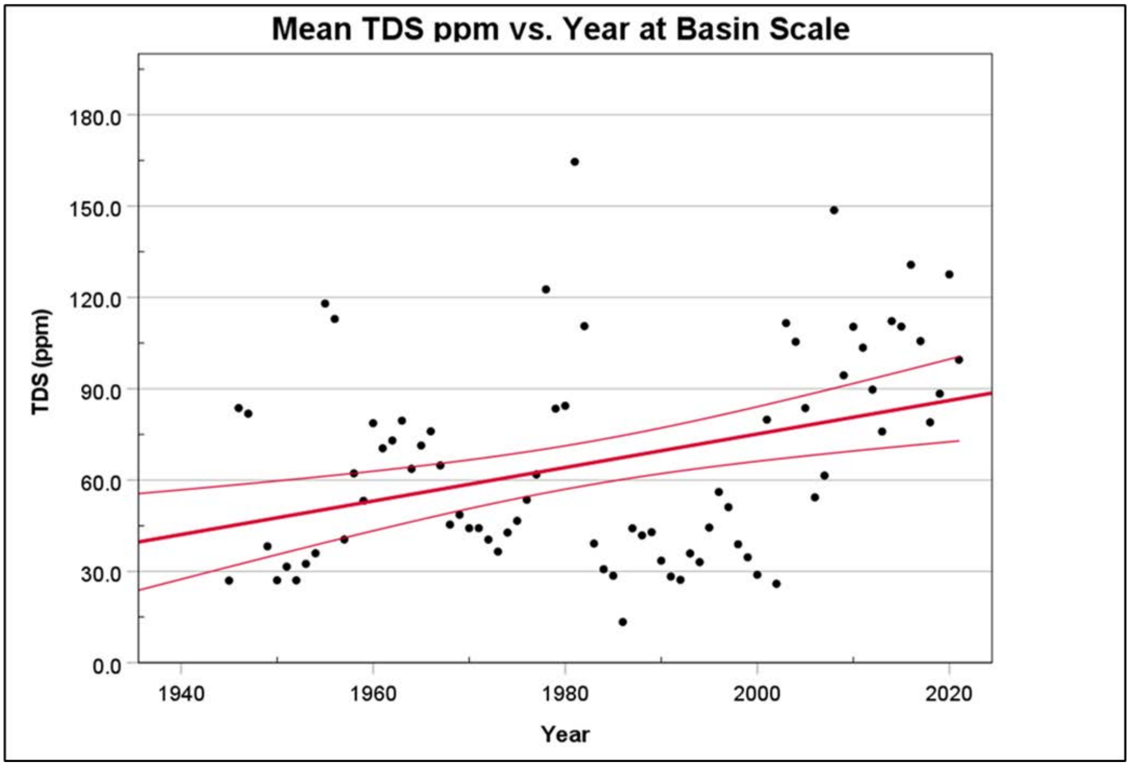


Figure 14. Change in study taxa occupancy over the last century for eleven species representing four sensitive taxonomic groups that are native or endemic to Southern Appalachian Streams.

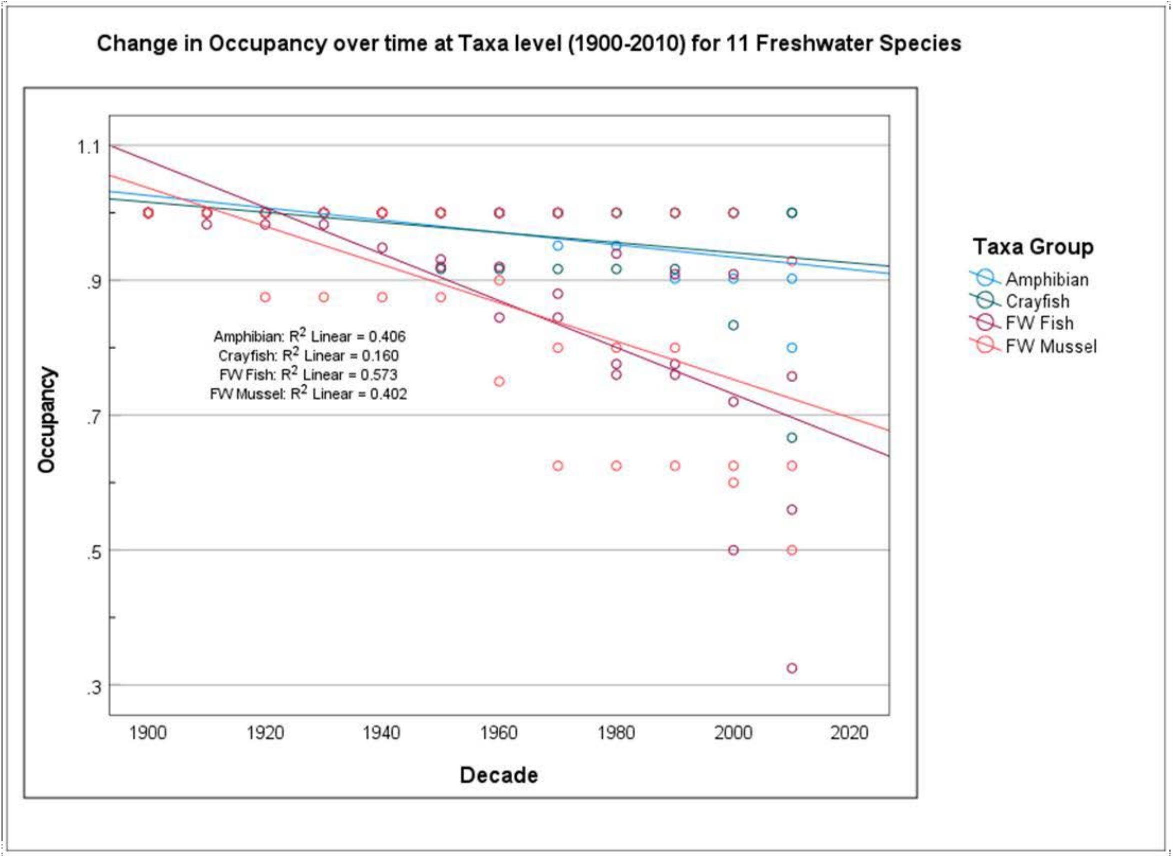


Figure 15. Change in freshwater fish occupancy for three native fish species in SBR watersheds over time (1900-2010). Freshwater fish represent the only taxonomic group to have statistically significant change in watershed occupancy over time. The Silver shiner shows the steepest decline in watershed site occupancy, followed by Fatlips minnow, and the Tangerine darter.

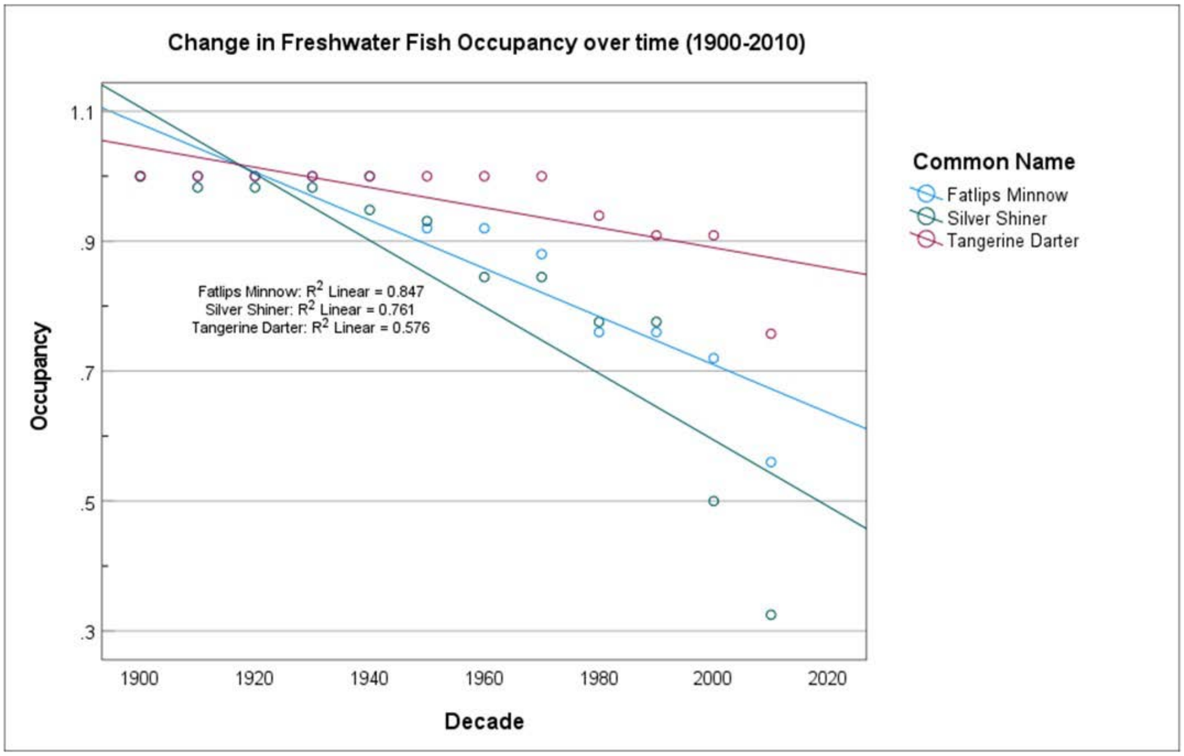


Figure 16. Graphical results of spatial distribution and associated changes in watershed (HUC10) occupancy over time for three native freshwater fish species to the parts of the French Broad-Holston, Upper Tennessee, Middle-Tennessee Hiwassee, and Kanawha Basins.

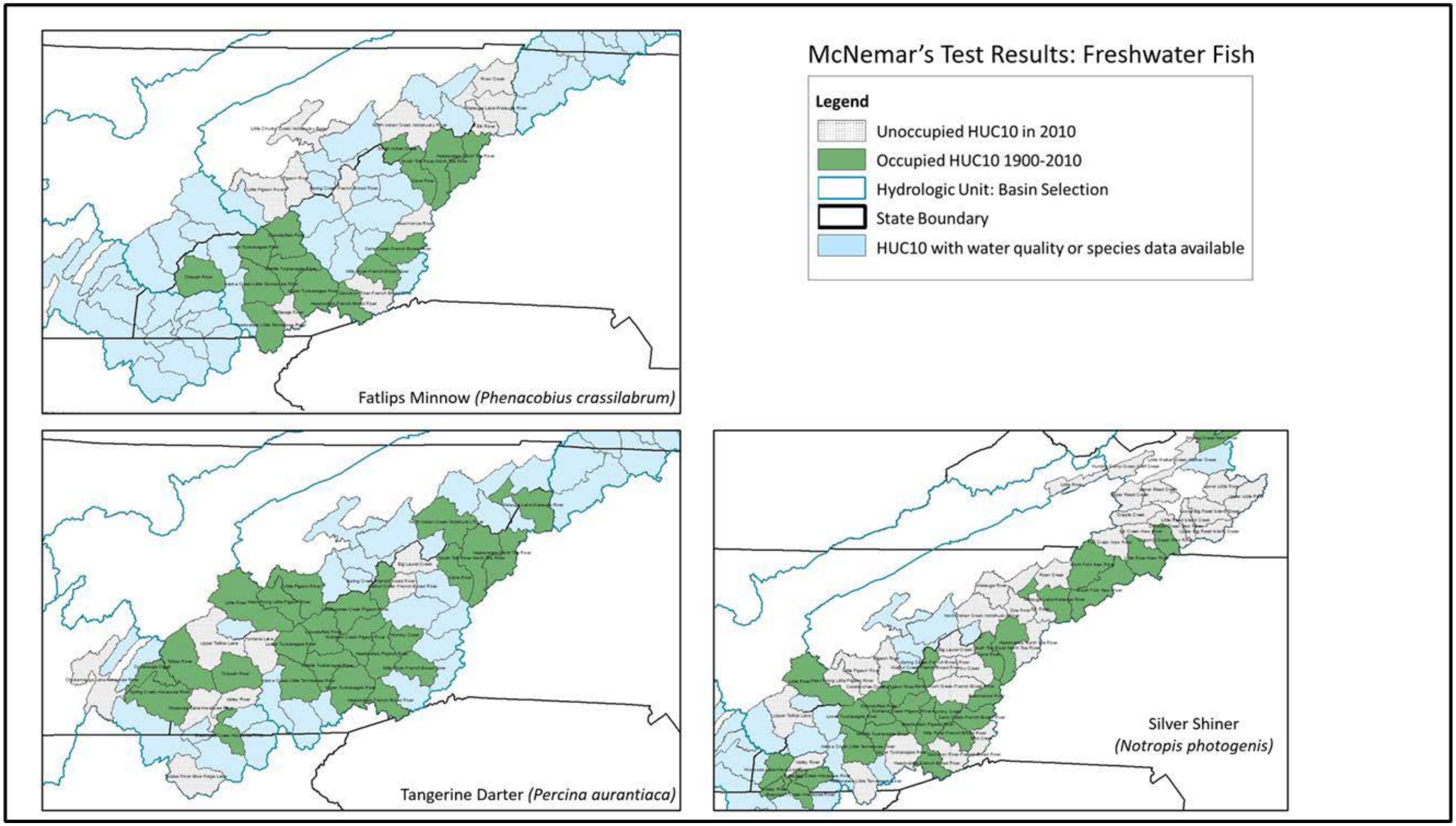


Figure 17. Heatmap of areas experiencing most losses across major study basins for three study fish species over time. Blue and red regions highlight regions that have experienced the most change in watershed occupancy for freshwater fish.

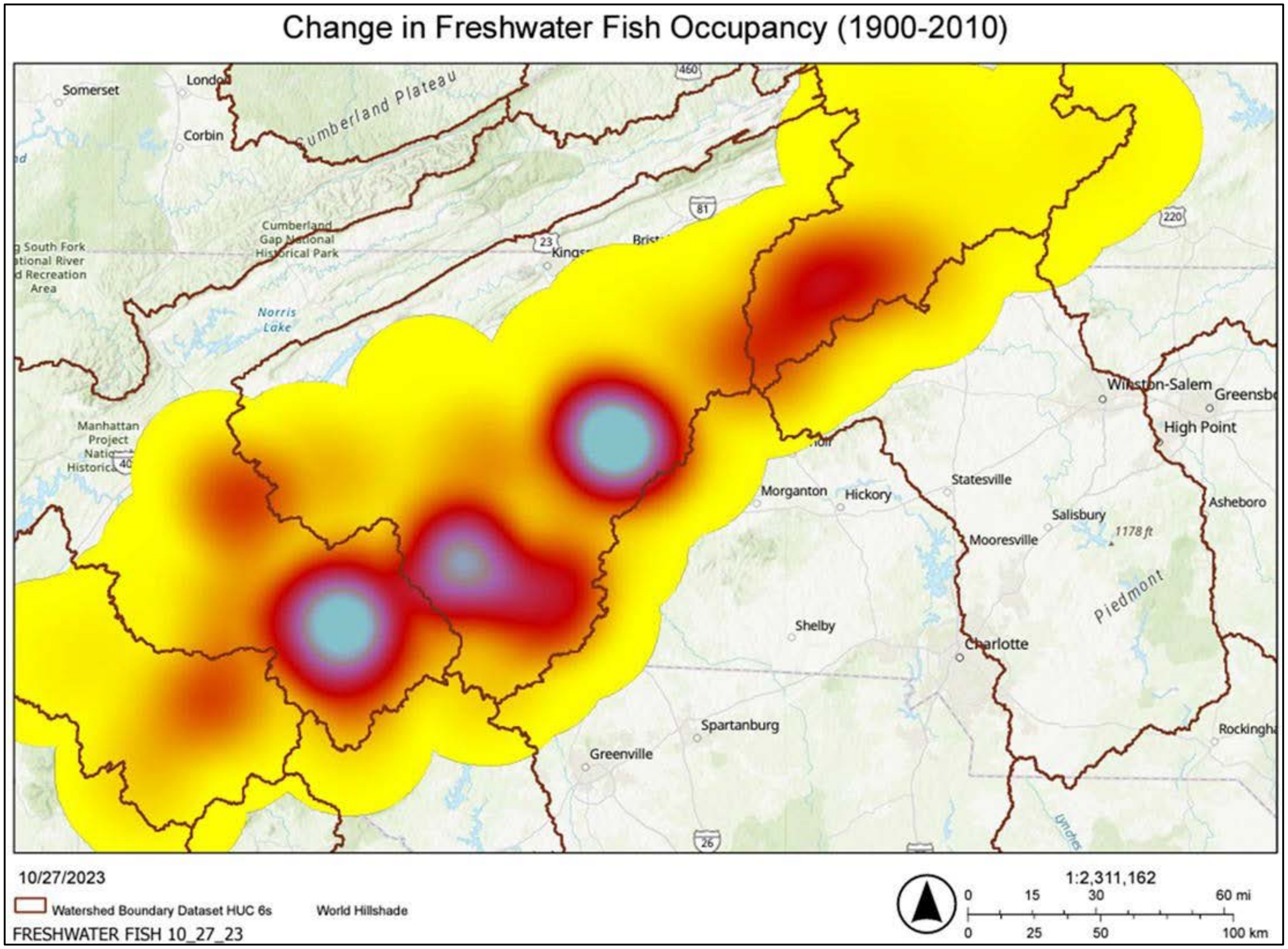
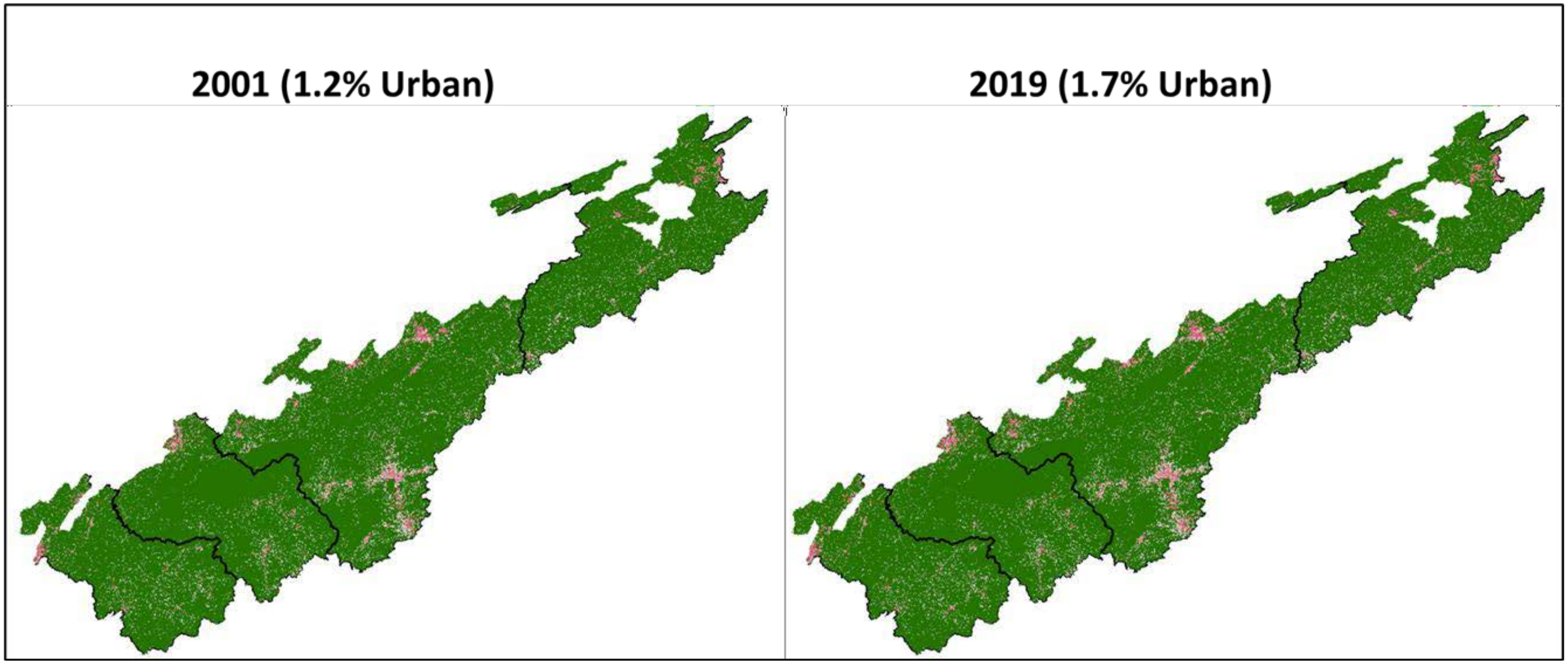


Figure 18. Change in mean percent urban imperviousness in SBR basins from National Land Cover Dataset (NLCD). The French Broad-Holston Basin had the highest concentration of developed watersheds.



2001 (1.2% Urban)

2019 (1.7% Urban)

Figure 19. pH values over time. Representative of the smaller dataset used for mixed effect model fitting. pH appears to be increasing over time (2001-2019). N=9,361.

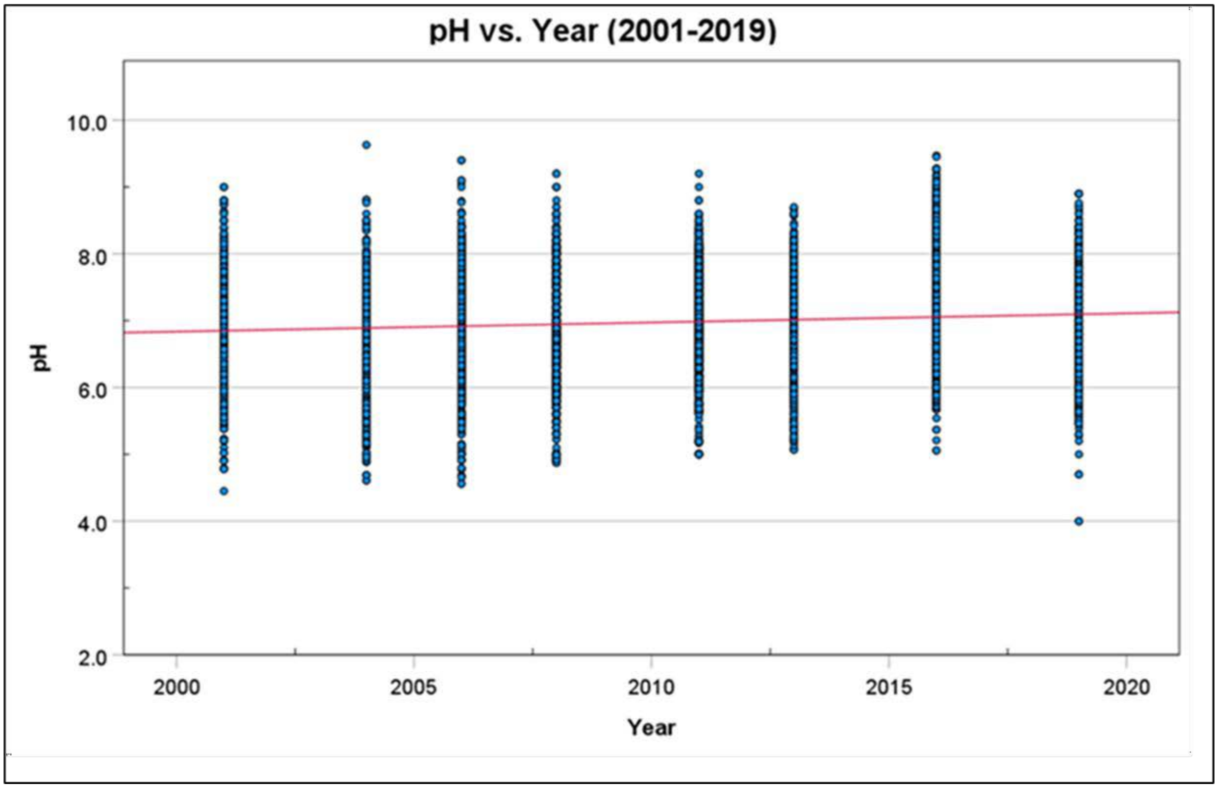
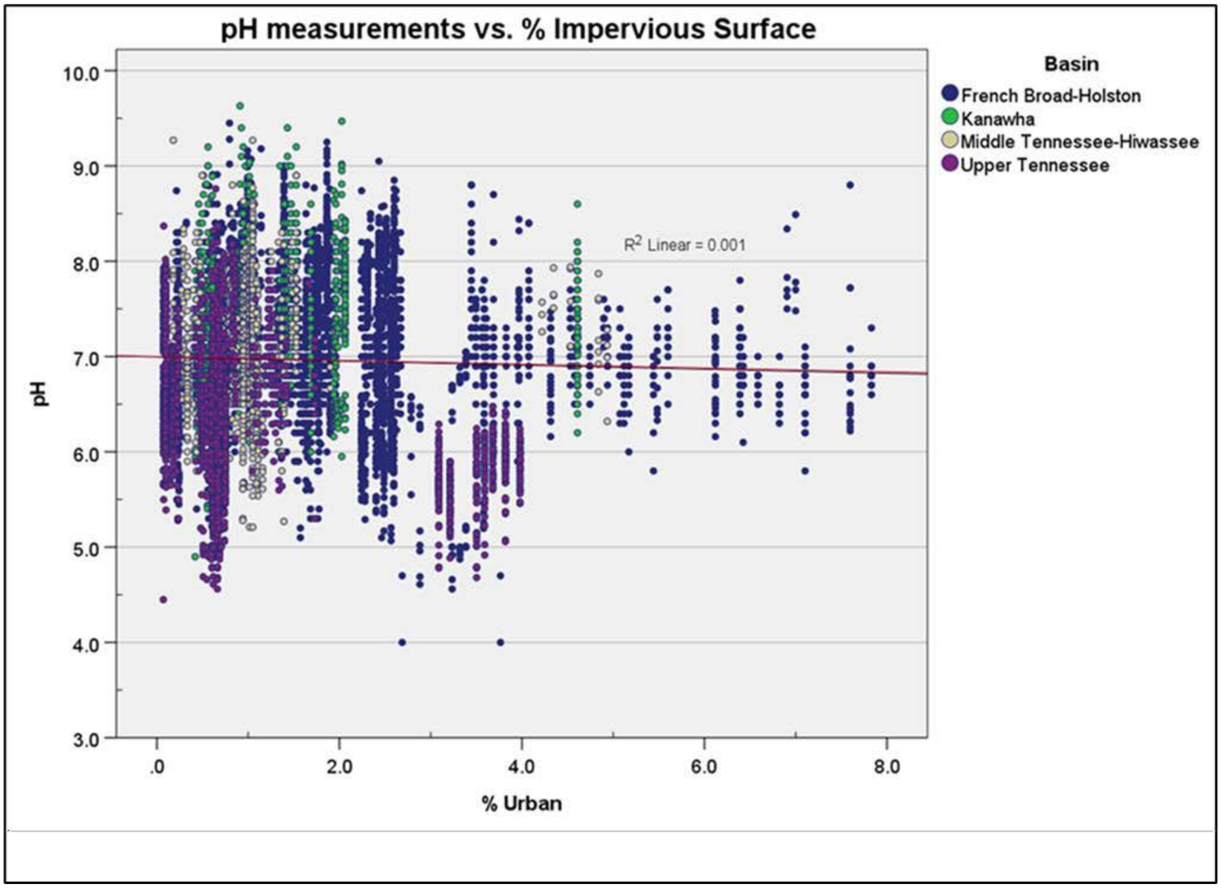


Figure 20. pH values over time plotted against increasing percent impervious surface. Representative of the smaller dataset used for mixed effect model fitting (2001-2019). There is a slight linear trend towards more acidic surface water and increasing urban impervious surface. The French Broad-Holston Basin appears to be the most developed in terms of % urban. The Upper Tennessee Basin has some of the most acidic pH measurements on record.



Vita

Hannah Christine Woodburn was born in Greensboro, North Carolina, to Martha and Jonathan Woodburn. She graduated from Appalachian State University with her undergraduate degree in December 2019. She is dedicating her life to the study of freshwater systems and how to protect them, cherish them, and enjoy their countless offerings. After three and a half years in graduate school, a global pandemic, a change of project, and countless hours of research, lab, and fieldwork, one of many part-time jobs turned into a full-time career in the field. She graduated in December 2023 from Appalachian State University with an M.A. in General Biology. After graduation, she plans to remain in Boone, continuing her work as a community organizer and staff scientist with a regional environmental non-profit.