A HABITAT ASSESSMENT OF RARE AND ENDANGERED SPECIES IN THE UPPER LITTLE TENNESSEE RIVER BASIN

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial requirements for the degree of Master of Science

By

James L. Miles

Director: Dr. Keith Gibbs

Assistant Professor of Geosciences and Natural Resources

College of Arts and Sciences

Committee Members: Dr. Thomas Martin, Department of Biology

Dr. Luke Etchison, North Carolina Wildlife Resources Commission

May 2024

ACKNOWLEDGEMENTS

I would like to extend thanks to my thesis director, Dr. Keith Gibbs, for his continual support, guidance, and friendship throughout this study. I also extend gratitude to Dr. Thomas Martin and Dr. Luke Etchison for their thoughts and insights, review of the manuscript, friendship, and serving as members of the advisory committee.

I would like to extend a special thanks to Sonja Himes for her invaluable assistance in manuscript review and friendship. I would also like to acknowledge Trevor Alexander and the 2022 Gibbs' Summer Research Team for field assistance.

Special thanks are also extended to the WCU Graduate School, Tennessee Valley Authority, and the Water Resources Research Institute of the UNC System for the funds needed to complete this project.

Finally, I would like to extend a special thanks to God, my family, and my friends for providing continual support and encouragement throughout my academic career and all aspects of my life.

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ABSTRACT

The Southeastern United States is known for rich aquatic diversity, especially within its fish and mussel species. Decreases in diversity have been associated with the degradation and fragmentation of aquatic habitats essential to these diverse communities of aquatic organisms. Quantification and characterization of habitat use of imperiled fish and mussels are vital to fully understanding these species, with the hopes of preserving and possibly reintroducing them into their historic range where suitable habitat still exists. Throughout this study, we identified, assessed, and compiled habitat availability in sites across the Little Tennessee River Basin upstream of Fontana Reservoir. I used geospatial mapping and multivariate statistical analyses to develop habitat models for species of greatest conservation need to identify potential reintroduction sites. These models have been developed for several fish species such as Stonecat (*Noturus flavus*), Spotfin Chub (*Erimonax monachus*), and the undescribed Sicklefin Redhorse (*Moxostoma sp.*). Mussels of interest include the Tennessee Clubshell (*Pleurobema oviforme*), Appalachian Elktoe (*Alasmidonta raveneliana*), and Slippershell (*Alasmidonta viridis*). We used an Analysis of Similarity (ANOSIM) test to detect significant differences between mainstem sites above and below impoundments and tributary sites. The Principal Components Analysis (PCA) test identified pebble diameter, gravel, sand, and bedrock percentages as being influential to differences among sites. These characteristics could prove to be limiting habitat factors for translocating some species. This information can be used by management organizations to further support the conservation needs of these species.

INTRODUCTION

The Southeastern United States has the richest fish and mussel diversity and the highest number of endemic fishes in North America north of Mexico (Layzer & Scott 2006; Warren et al. 2000). However, over the years, aquatic diversity has decreased significantly within the region, putting much of the aquatic fauna at risk of being threatened, endangered, or becoming extinct. Extinction rates of freshwater taxa have increased greater than those of terrestrial taxa (Burkhead 2012; Haag & Williams 2014). Of the 258 described and undescribed fish species in North Carolina, 31% (79 species) are listed at the state or federal level as either endangered, threatened, special concern, or significantly rare due to continued threats from anthropogenic activities (Tracy et al. 2020; Warren et al. 2000). Of the 302 native mussel species in North America, over 70% of these are considered threatened and endangered due to habitat disturbances, particularly because mussels have limited mobility (Daniel et al. 2017; Graf & Cummings 2021). The characterization of habitat use in imperiled fish and mussels is vital to fully understanding these species to preserve and possibly reintroduce them into their historic range where suitable habitat exists (Gibbs et al. 2014).

Habitat modeling has been used to characterize habitat availability for freshwater and estuarine species, usually to predict human impacts on aquatic ecosystems (Vadas & Orth 2001). Habitat availability consists of different environmental factors that species prefer or depend on, such as water temperature, conductivity, pH, water velocity, and depth. These parameters, combined with habitat quantity and the structural arrangement and accessibility of suitable habitat, are essential for successful river rehabilitation and species conservation (Radinger & Wolter 2015). These models aid fisheries and aquatic biologists in locating and developing vital environmental factors to promote future restoration plans for rare and endangered aquatic species of most conservation need.

The unique, varied, and dynamic conditions of lotic ecosystems promote a diversity of life forms and life-history strategies. Habitat variables within a lotic system that are potential limiting factors for a variety of species are depth, average stream velocities, average substrate size, and percent cover (Vadas & Orth 2001). Aquatic species can be found in various habitats and some move among various habitats throughout their life cycle (Nowak et al. 2004). One example is the Spotfin Chub (*Erimonax monachus*) which during their juvenile stage, thrive in areas of swift current, but use a variety of substrates as adults (USFWS 2014). Though many species may have broad habitat requirements, many have been negatively influenced by anthropogenic habitat degradation. Anthropogenic stressors on the environment may include increased runoff and streambank erosion, which deposits excess sediment and nutrients into the streambed. These causes could affect individual species through habitat destruction and displacement or disrupt their life histories altogether. These effects could immediately affect individual species, but if the affected species are a keystone or foundational species, the overall effects could be profound on multiple species or community dynamics (Layzer & Scott 2006; Warren et al. 2000).

Examples of keystone or foundational species being driven from their habitats due to modification include native mussels and fishes. Stream and riverine mussel species are known as ecosystem engineers, improving habitat for other aquatic species by providing substrate for algae and insect larvae to attach to, and filter finer substrates and algae for clearer, cleaner water (Vaughn & Hakenkamp 2001). Many of our native fishes also play a crucial role as ecosystem engineers. Factors responsible for the decline of native fishes in the United States are attributable to pervasive, complex habitat degradation across the landscape, resulting in decreased populations and fragmented ranges (Waldman & Quinn 2022). With the drastic effects that land use has had on many of our aquatic ecosystems, there is a need for habitat modeling in stream and riverine ecosystems. These models allow fisheries scientists to take the first steps to protect and preserve our vast and numerous aquatic species.

State and federal institutions continually give protection to aquatic species due to ongoing population declines (International Union for Conservation of Nature (IUCN) 2022). Within the Little Tennessee River Basin alone, 12 species of mussels and 20 species of fishes have either state and/or federal protection (Little Tennessee Native Fish Conservation Partnership 2015; 2018). Among these species, several fish and mussels are found in the Upper Little Tennessee River Basin upstream of Fontana Reservoir. These species include Spotfin Chub (*Erimonax monachus*), Sicklefin Redhorse (*Moxostoma sp.*), Stonecat (*Noturus flavus*), Appalachian Elktoe (*Alamidonta raveneliana*), Tennessee Clubshell (*Pleurobema oviforme*), and Slippershell (*Alasmidonta viridis*).

Spotfin Chub (*Erimonax monachus*) is a native minnow (Family Leuciscidae) found within clear, warm water rivers and streams in the Tennessee River Drainage. This species was historically found across five different states, including Alabama, Georgia, North Carolina, Tennessee, and Virginia, but is now restricted to several disjunct river systems such as the Little Tennessee River in North Carolina (Etnier & Starnes 1993; Jenkins & Burkhead 1994). The species gained federal listing in 1977 under the U.S. Endangered Species Act, resulting in ecological studies of the species to aid in restoration efforts (U.S Fish and Wildlife Service 2014). Spotfin Chub frequently use run habitat over boulder and bedrock substrates from the spring to fall and transition to pool habitats with sandy substrates or tributary systems in the winter (McLarney & Meador 2019; Kanno et al. 2012; Russ 2006).

Sicklefin Redhorse (Catostomidae: *Moxostoma sp.*) is an undescribed, imperiled sucker found within its restricted range of the Hiwassee and Little Tennessee River systems. Historically, the species was thought to occur in many, if not all, rivers and large streams of the Blue Ridge Mountains portion of the Hiwassee and Little Tennessee (Favrot 2009; U.S. Fish and Wildlife Service 2013). This species, along with others of its family, are of high conservation concern but have yet to receive much insight and attention from the scientific community (Ivasauskas 2017). Currently, the species is listed as threatened by the North Carolina Wildlife Resources Commission and endangered by the Georgia Department of Natural Resources (Albanese 2020; North Carolina Wildlife Resources Commission 2021). Habitat ecology and life history studies have shown that the species uses various habitat types depending on the age of the fish and spawning cycles. Adults were noted to occur in moderately deep to shallow river channels with swift currents and coarse substrates such as boulder and bedrock. Juveniles of the species were known to prefer moderate to deep pools with large boulder crevices and slow-moving currents (Favrot 2009; Stowe 2014).

Stonecat (Ictaluridae: *Noturus flavus*) is a small catfish native to the eastern parts of North America. While this madtom ranges as far north as the Great Lakes and west to the Mississippi River, little is known about the species in the Little Tennessee River Basin (Barrett

2006; Sanchez (n.d.)). This species is listed as state endangered in North Carolina and is only found in the French Broad and Little Tennessee River basins (Tracy et al. 2020). Little research on the species' habitat preferences in the region exists. However, using the research of the species found in other parts of its range and other members of the genus *Noturus*, we can get a better understanding of what possible habitat this species uses. Research shows that stonecats use riffle and run habitat with gravel, cobble, and boulder substrates under a variety of gentle to fast-moving flows. It is also noted that this species uses large, flat boulders/bedrock in riffles and pools during spawning periods (Barrett 2006; Brewer & Rabeni 2008; Walsh & Burr 1985; Wells et al. 2020).

The Appalachian Elktoe (*Alasmidonta raveneliana*) is a federally endangered mussel species in western North Carolina and eastern Tennessee. There is little known information about its historical range; however, researchers suggest that the species once occupied a majority of rivers and streams in the upper Tennessee River Basin (U.S Fish and Wildlife Service 2022). In Tennessee, the species is known only to occur in a small section of the Nolichucky River near the North Carolina state line (U.S. Fish and Wildlife Service 2022). The species received its federal listing in 1994 in large part due to its fragmented distribution, scattered across the Little Tennessee River system, Pigeon River system, Mills River, and Little River of North Carolina, and the Nolichucky River system of North Carolina and Tennessee (U.S. Fish and Wildlife Service 2011). This species has been documented using small and medium-sized streams and rivers in well-forested watersheds with cool, clean, moderate to fast-flowing water. Appalachian Elktoe are known to mostly occur around riffles, runs, and shallow pools with low proportions of finer sediments in sand or gravel substrate mixed with cobble, boulders, and/or bedrock (Pandolfi et al. 2022; U.S. Fish and Wildlife Service 2011).

The Slippershell (*Alasmidonta viridis*) and Tennessee Clubshell (*Pleurobema oviforme*) are listed species in the Little Tennessee River Basin. The Slippershell is listed as state-endangered in North Carolina, and the Tennessee Clubshell is also classified as state-endangered in North Carolina but listed as rare in Tennessee (North Carolina Wildlife Resources Commission 2024; U.S Fish and Wildlife Service 2011). Little to no information is available about these species in the region, but of what is noted, these species are usually found to congregate around other mussel species in areas of large, stable substrate with ample interstitial spaces that are relatively free of fine sediment (Schilling et al. 2017). Due to a lack of habitat information for Slippershell and Tennessee Clubshell, Appalachian Elktoe habitat preferences were used to locate suitable habitats for these imperiled species.

The goal of this study was to collect habitat data and develop habitat models that will be used to inform future management decisions for species of greatest conservation need in the Upper Little Tennessee River Basin. Additional study objectives included building a habitat variable database for our study sites and a presence map of our targeted species sampled within the last 10 years. This additional information will be used by management organizations to further support the conservation needs of these species.

METHODS

Study Area

The Little Tennessee River Basin is located within the Blue Ridge Mountains of the Southern Appalachians, with its uppermost mainstem stream in Georgia and encompasses an area of approx. 1800 mi^2 (Figure 1) (North Carolina Department of Environmental Quality 2012; 2018). The major tributaries of the basin include the Cheoah, Cullasaja, Nantahala, and Tuckasegee rivers. Approximately 90% of the basin is forested, with less than 5% of the land being comprised of urban/developed use, which is mostly found around the region's major cities of Franklin, Sylva, Cullowhee, Highlands, Bryson City, and Robbinsville (North Carolina Department of Environmental Quality 2018). Due to the area's popularity for tourism and outdoor recreation, development continues to grow within the river basin, converting forested areas and former agricultural areas into retirement and vacation home sites. However, since much of the basin is federally owned, with 49% in the Nantahala National Forest and Great Smoky Mountains National Park, this contributes to much of its undisturbed state (North Carolina Department of Environmental Quality 2012; 2018). The Little Tennessee River Basin supports the richest aquatic species assemblage remaining in the Blue Ridge Mountains, with more than 100 species of fish, as well as crayfish, mussels, snails, and aquatic plants, including many with protected status (NFCA 2015). Much of this diversity is located within the 25-mile stretch of the Little Tennessee River between Franklin and Fontana Lake, rivaling much of the faunal diversity in the state and perhaps even the nation (Little Tennessee River Native Fish Conservation Association 2018; North Carolina Department of Environmental Quality 2012).

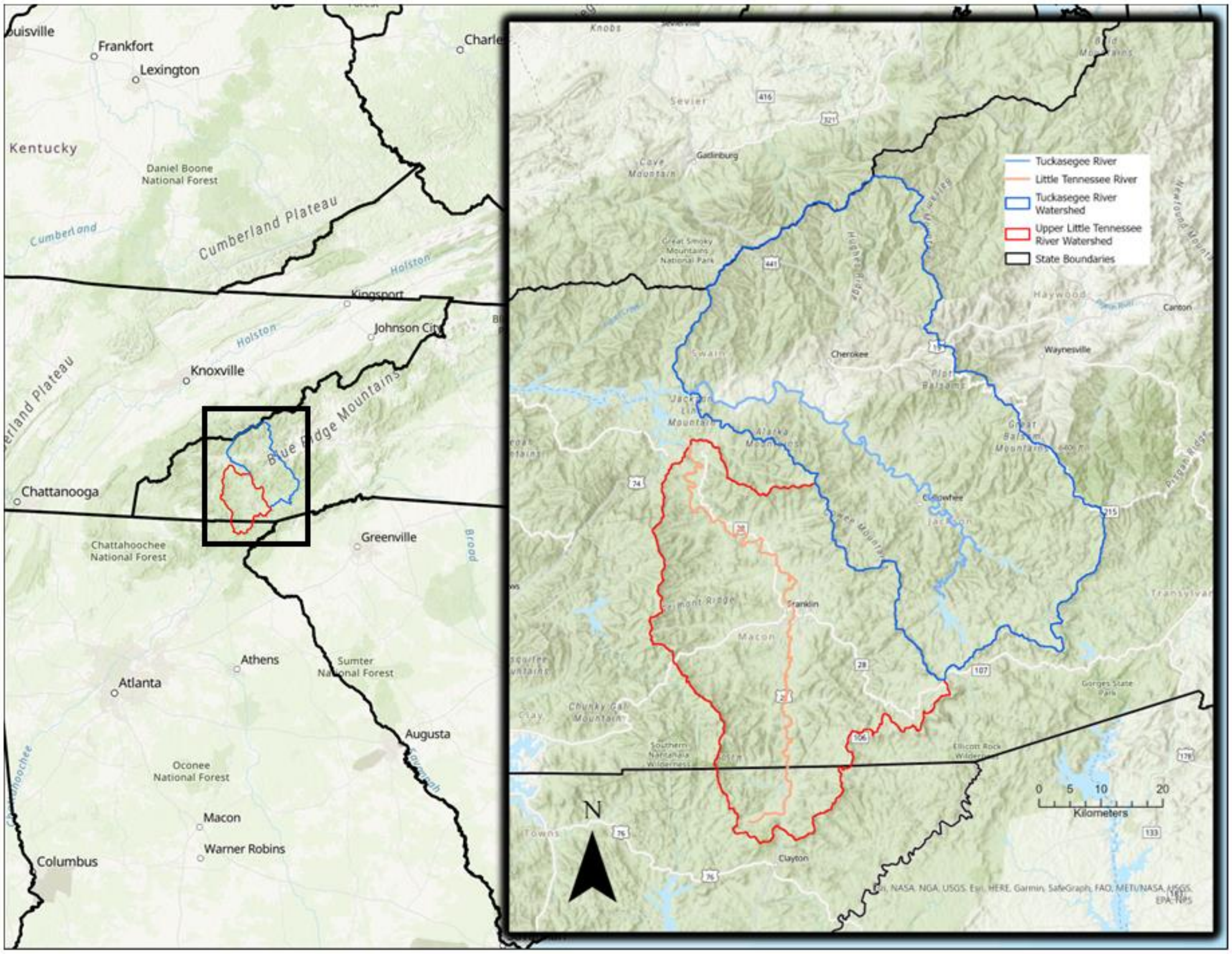

Figure 1. The Tuckasegee River and Little Tennessee River Watersheds map, exhibiting the geographic extents of the watershed and the Little Tennessee River Basin within the Blue Ridge Mountains.

The Tuckasegee River watershed begins in the headwaters of Panthertown and Greenland creeks where it flows northwesterly through Jackson County, North Carolina, transitioning to Swain County, North Carolina before reaching Fontana Lake. Prior to reaching Fontana Lake, the Tuckasegee River is joined by the Oconaluftee River, the largest tributary of the watershed flowing from the Great Smoky Mountains National Park and Qualla Boundary (Figure 1) (WATR NC 2021). The watershed contains some of the most pristine waters in the state. Water quality issues, however, are not uncommon within the watershed, primarily sourced from developmental and agricultural runoff, localized wastewater failures, and stream bank erosion (North Carolina Department of Environmental Quality 2012). Several streams within the watershed, such as Scott's and Savannah Creek, are labeled impaired from the 2022 303(d) list of impaired streams by North Carolina Department of Environmental Quality (North Carolina Department of Environmental Quality 2012; 2022). Within this watershed there is a small low-head dam, Cullowhee Dam, located in the township of Cullowhee, that serves as raw water intake for Western Carolina University and the Tuckasegee Water and Sewer Authority (McGill Associates 2017). Larger portions of agricultural land are often found upstream of the dam while more development and impervious surfaces are found downstream. Upstream hydrologic soils above Cullowhee Dam have low runoff potential due to being largely sand and/or gravel over clay, while soils downstream have more moderately low runoff potential. These soils are made up of 10-20% clay while the rest of the 50-90% are made up of sand and have sandy loam soil types (Table 1) (Mockus et al. 2007).

The Upper Little Tennessee River watershed begins in the headwaters of Rabun County, Georgia flowing north and northwest in North Carolina before being impounded in Fontana Lake (Figure 1). The majority of the river remains free flowing upstream of Fontana Reservoir, except for the tailwater controlled area below Lake Emory Dam located near the town of Franklin, North Carolina (Mainspring Conservation Trust 2012). The mainstem of the Little Tennessee River is very distinct upstream and downstream of Franklin. Upstream reaches of the river meanders through a relatively low gradient valley and is heavily sedimented. This sedimentation along with other water quality issues such as agricultural runoff, stream bank erosion, limited riparian buffers and individual wastewater failures are just a few of the issues that currently and historically plague this watershed (Mainspring Conservation Trust 2012, North Carolina Department of Environmental Quality 2012). These issues are supported by the larger proportions of agricultural and developed lands, and impervious surfaces that can be found above Lake Emory Dam (Table 2) (Mockus et al. 2017). The downstream section from Franklin, however, doubles in size from the confluence of feeder tributary streams, increasing in gradient, before creating the most ecologically intact warm-water river system in the Southern Blue Ridge (Mainspring Conservation Trust 2012).

Habitat Assessment

In 2021 and 2022, we assessed habitat metrics across the Little Tennessee River Basin during the summer months (May-August). These sampling dates were chosen because summer sampling plays a crucial role in locating essential habitats for some species that seasonally or ontogenetically use different habitats (Brewer & Rabeni 2008; Kanno et al. 2012; Stowe 2014).

I chose 39 study sites to quantify aquatic habitat conditions found in the Little Tennessee River Basin (Figure 2). With the aid of the North Carolina Wildlife Resources Commission and Mainspring Conservation Trust personnel, sites were selected because of accessibility and potential habitat for our study species. Sites were located on two major rivers and their tributaries that comprise part of the river basin, including the Upper Little Tennessee River and Tuckasegee watersheds (Table 3). I established 21 transects at 10m intervals to survey 200m reaches at each site to ensure representative sampling of available habitat. Habitat sampling followed standardized methods developed by the US Forest Service (Platts et al. 1983), tailored to match the habitats we encountered.

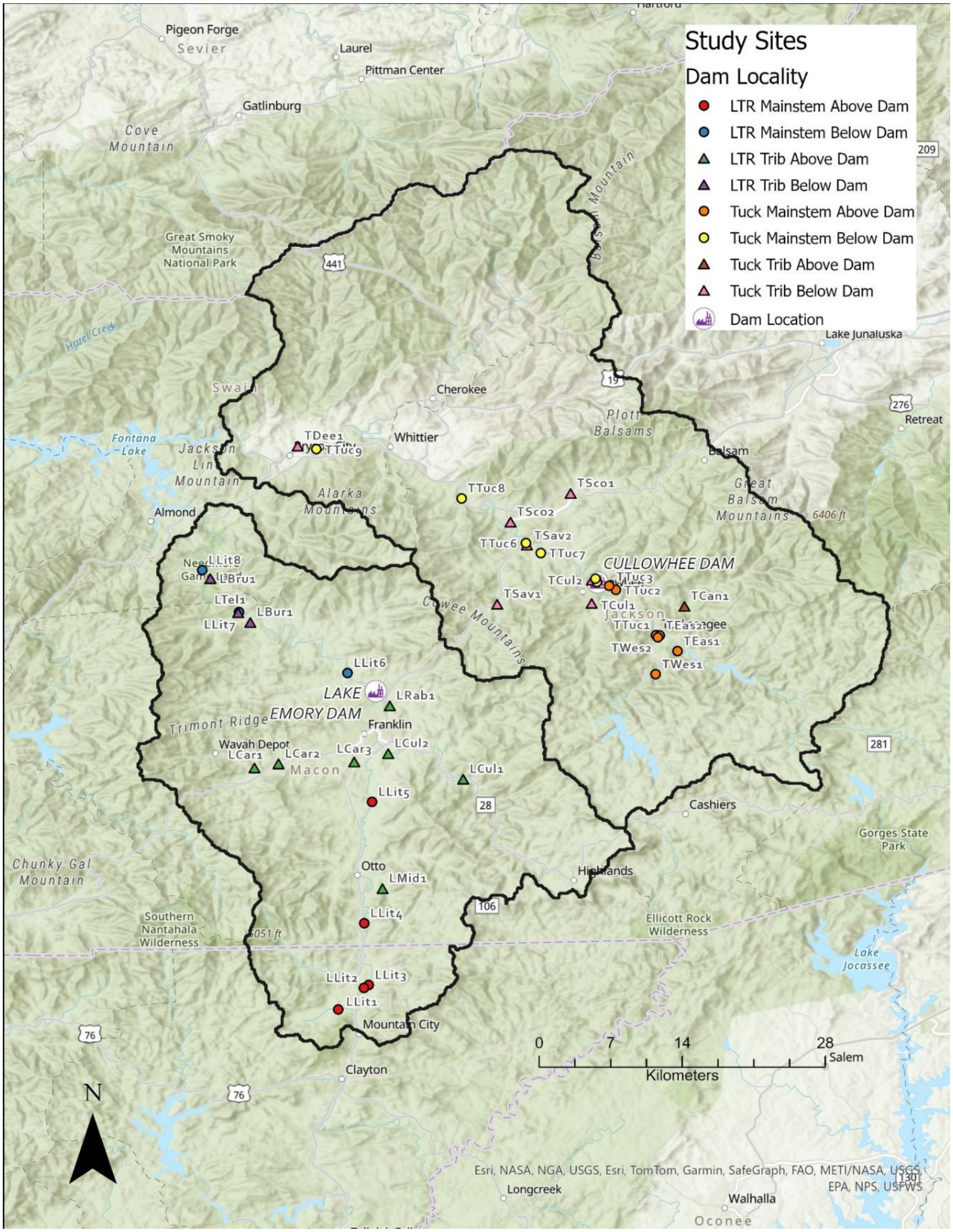

Figure 2. The Little Tennessee River Basin upstream of Fontana Dam, with study sites delineated by locality to Cullowhee and Lake Emory dams along the mainstem Tuckasegee and Little Tennessee rivers and their tributaries.

Wetted width, habitat type, gradient, depth, bottom velocity, and column velocity within each transect were measured and recorded at 25%, 50%, 75% of the width, and 1 meter from each side of the stream bank. Wetted width was measured at each transect with a TruPulse 200 laser range finder (Laser Technology, Inc., Centenial, CO). Depth, and, bottom and column velocities were measured at each width percentage in each transect with the use of a Hach FH950 portable velocity meter (Hach Company, Loveland, CO) and a top-setting wading rod. Gradient measurements were recorded using the CST/Berger surveyor’s level (Robert Bosch Tool, Inc., Mount Prospect, IL). These values were combined to find each study site's average wetted width, gradient, depth, bottom velocity, and column velocity. The dominant and subdominant substrate composition percentage was also visually assessed and recorded at each depth and velocity point. Percentages of dominant and subdominant substrates were then combined with the sums to find the weighted total of each site. I divided each substrate type by the weighted total and multiplied by 100 to find the weighted percentage of the dominant and subdominant substrates.

The embeddedness of riffles was measured by haphazardly collecting 10 cobble rocks within the reach and measuring the total height and embedded height as indicated by a line of periphyton growth, subtracting the difference, and then taking the average to find the overall embeddedness of a site. Chain roughness was calculated by dropping a 5 m chain parallel to streamflow in 10 haphazard locations in fast-flowing habitats and 10 haphazard locations in slow-flowing habitats. The chain length as it lays on the substrate compared to the total chain length provided a relative measure of bed roughness to reflect any irregularities in the stream channel. Chain roughness measurements for each site were added together and then divided to find the average chain roughness value for each site. Visual habitat assessments were also collected with the use of Modified Stream Visual Assessment Protocols for the Southern Appalachians (SVAP) (Mainspring Conservation Trust (n.d)) and Tennessee Department of Environment and Conservation (TDEC) Moderate to High Gradient Streams Habitat Assessment (TDEC 2021). We used two different stream visual assessments to compare if results were similar or different in habitat scores. This was due to SVAP providing a "simpler" and more layperson-friendly protocol. In contrast, TDEC's protocol is more quantitative and more reflective of methods used by NC DEQ and Tennessee Valley Authority. Each of these habitat metrics provided valuable information for building our habitat model to determine whether our target species could inhabit the more extensive reaches in which our testing sites are located.

Analysis

Multivariate habitat analyses were performed in Primer 7 version 7.0.21 (Clarke & Gorley 2015). Habitat data were separated for analysis by reach level (wetted width, depth, bottom velocity, column velocity, riffle embeddedness, chain roughness, grade, SVAP, and TDEC) or substrate characteristics (bed and boulder counts, pebble diameter, and percentages of bedrock, boulder, cobble, gravel, sand, silt, and woody debris.). Histogram plots were used on both sets of characteristics to determine if any highly skewed data points would need to be transformed to limit distorting effects (Clarke & Gorley 2015). Each value was normalized by subtracting it from the mean and then dividing it by the variable's standard deviation. This allowed all variables to be on a comparable measurement scale (Clarke & Gorley 2015). I also downweighed large values for all variables with a 4^{th} root transformation if habitat variables skewed the dataset.

Principal components analysis (PCA) was used to visually portray how each site compared across the suite of habitat variables. A resemblance matrix was also generated for all habitat variables based on Euclidean distance, which is best suited for environmental variables (Clarke & Gorley 2015). A one-way analysis of similarities test (ANOSIM) was used to test for significant differences among tested factors. These tested factors consisted of groupings based on site locality (ex. Tuckasegee and Little Tennessee River mainstem and tributary sites) and dam locality (ex. upstream or downstream of Cullowhee and Lake Emory dams). A one-way similarity percentages (SIMPER) test was conducted on each set of habitat characteristics to explain variable contribution to site differences. Means plots were also used to visualize differences among variables based on tested factors.

Mapping of Habitat Quality and Species Occurrence

Geospatial modeling was performed using ArcGIS Pro version 3.1 (ESRI 2023). Habitat study sites were compared with known target species occurrence data from the last 10 years and habitat requirements based on literature (e.g., Favrot 2018; Kanno et al. 2012; Schilling et al. 2017; US Fish and Wildlife 2011). Occurrence data from the last 10 years was obtained through North Carolina Wildlife Resources PAWS database, where species were either detected from sampling or were noted to be reintroduced at that location (NCWRC 2024). Reintroduction areas were based on whether habitat requirements were met, and I prioritized sites if they could be used for multiple species reintroductions. Areas were designated as being either “Good,” “Fair,” or “Poor.” “Good” sites described areas that had suitable habitats and had occurrences of more than one desired species. “Fair” sites were areas that had suitable habitats and had either no or at least one of the desired species. Lastly, “Poor” sites were areas that had no suitable habitats or had no desired species currently present. This visualization tool will assist management organizations in delineating what sites would be best suited for reintroductions based on habitat metrics.

RESULTS

Habitat Data- All Sites

Over 50% of the variation among measurement sites were described by the first 3 principal components (Figure 3). The PC1 axis was influenced most by the positively correlated pebble diameter and negatively correlated sandy substrate. The negatively correlated bedrock substrate and positively correlated gravel substrate influenced PC2. Lastly, PC3 was influenced by the positively correlated column velocity and the negatively correlated boulder substrate. Sites in the upper Little Tennessee River (Upper_LTR) were isolated from all other study sites, while the lower Tuckasegee River (Lower_TKR) and Little Tennessee River (Lower_LTR) sites clustered together (Figure 3). Tributary sites also clustered together (Figure 3).

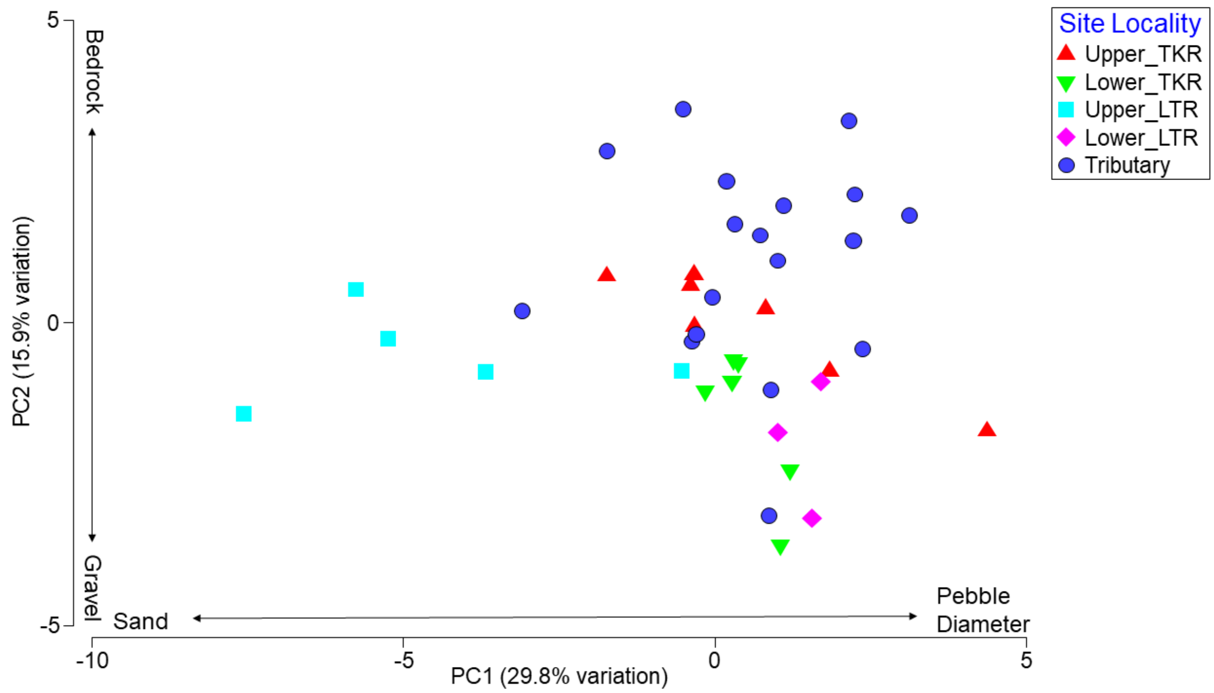

Figure 3. Principal components analysis of all habitat characteristics plotted to display variation and correlation of study sites based on location in the Little Tennessee River Basin and their localized watersheds. The sites in the upper Little Tennessee River (Upper_LTR) were isolated from all other study sites, while the lower Tuckasegee River (Lower_TKR) and Little Tennessee River (Lower_LTR) sites clustered together. The tributary and upper Tuckasegee River (Upper_TKR) sites also show signs of clustering, but to themselves.

Reach Level Habitat Characteristics (Site Locality Factor)

Using a one-way Analysis of Similarities (ANOSIM) test, I observed a significant difference among reach-level habitats based on site locality (p-value= 0.001, R-value = 0.297). I visualized the difference among study sites with distinct clustering of Little Tennessee River (LTR) and Tuckasegee River (TKR) tributary sites in our PCA biplot (Figure 4). The PC1 axis accounted for 29.5% of the variation and was most influenced by a positive correlation of chain roughness and a negative correlation with TDEC habitat scores. A positive correlation of SVAP habitat scores and a negative correlation of wetted width greatly influenced the PC2 axis. In the Tuckasegee River tributary sites (TTrib), column velocity accounted for 13.12% of the variation. In comparison, SVAP habitat scores accounted for 38.50% of similarity in Little Tennessee River tributary sites (LTrib) based on a one-way analysis of similarity percentages (SIMPER) test.

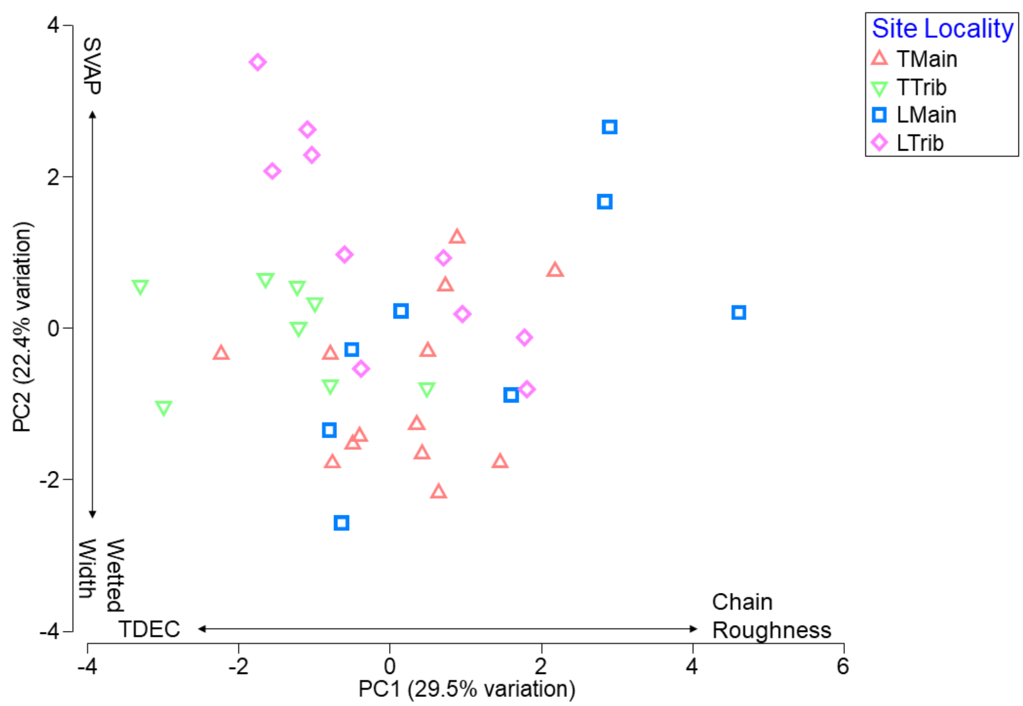

Figure 4. Principal components analysis of reach level habitat characteristics plotted to display variation and correlation of study sites based on location in the Little Tennessee River Basin and their localized watersheds. Distinct clustering of the Tuckasegee River mainstem (TMain) and tributary (TTrib) was observed on the PCA biplot. Additional clustering was also observed in the Little Tennessee mainstem (LMain) sites but not in the tributaries (LTrib).

Substrate Habitat Characteristics (Site Locality Factor)

I found a significant difference among substrate variables by site locality (p-value = 0.002, R-value = 0.158). These variables differed between the Tuckasegee River (TKR) and Little Tennessee River (LTR) mainstem sites. At the same time, there was no detectable difference between the Tuckasegee River and Little Tennessee River tributaries. PC1 accounted for 39.6% of the variation and was influenced by a positive correlation of pebble size and a negative correlation of sand percentage. PC2 accounted for 20.5% of the variation, influenced by a positive correlation of gravel percentage and a negative correlation of bedrock counts (Figure 5), reflecting the differences observed from our ANOSIM. I observed clustering of the upper LTR mainstem sites along PC1 due to a predominance of sand substrate at those sites. The three variables that contributed most to the similarities among all LTR mainstem sites included boulder (14.98%), gravel (11.91%), and cobble (11.85%) substrates.

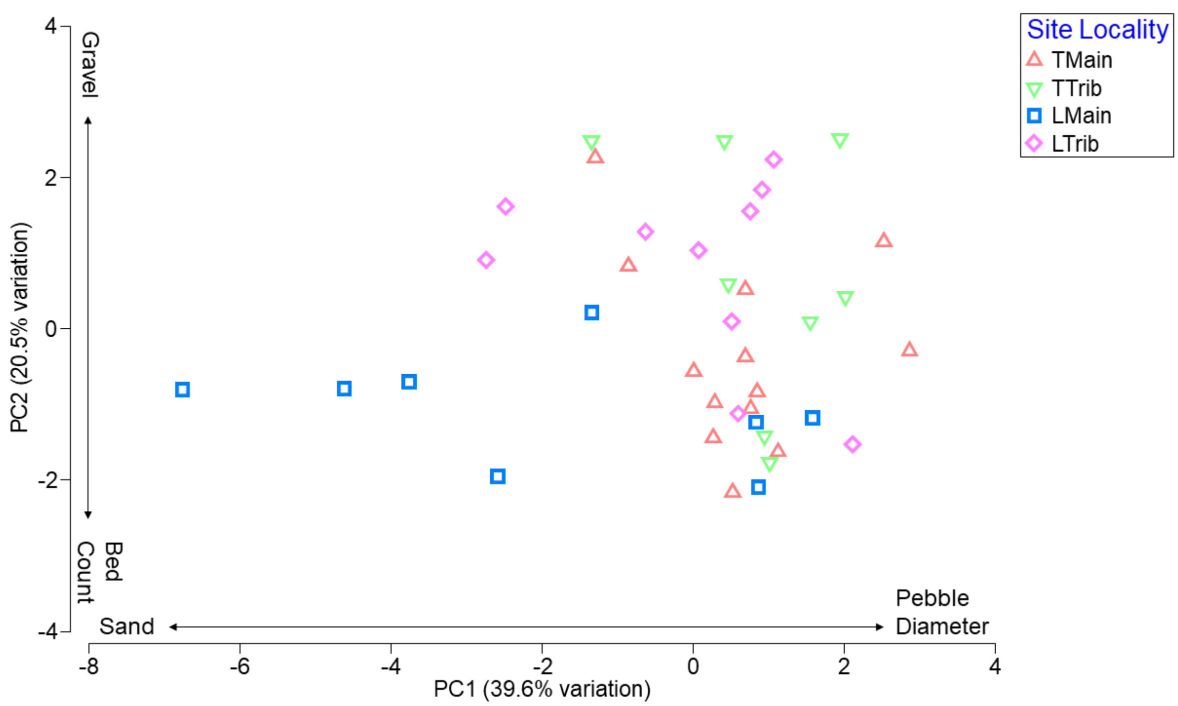

Figure 5. Principal components analysis of substrate habitat characteristics plotted to display variation and correlation of study sites based on location in the Little Tennessee River Basin and

their localized watersheds. Clustering of the Little Tennessee mainstem sites (LMain) was observed due to a predominance of sand substrates and smaller substrates at these sites.

Reach Level Habitat Characteristics (Mainstem Sites/Dam Locality Factor)

I found a significant difference (p-value = 0.001, R-value = 0.323) among study sites using reach level characteristics when grouped by location relative to a dam (i.e., above or below). Among pairwise tests, there was a significant difference for all groupings except the sites in the mainstem Tuckasegee River above Cullowhee Dam (TKR_Above) compared to the mainstem Little Tennessee River sites below Lake Emory Dam (LTR_Below). PC1 accounted for 39.1% and PC2 accounted for 22.5% of the variation. The TDEC habitat variable had a positive correlation, while riffle embeddedness (RE) had a negative correlation on PC1. Whereas, SVAP habitat variable had a positive correlation, while column velocity (CV) had a negative correlation on PC2 (Figure 6). I observed clustering of the Little Tennessee River sites above Lake Emory Dam (LTR_Above) on the left of the PC1 axis, primarily driven by the larger quantities of embedded riffles and low scoring of TDEC habitat assessments. The clustering of Tuckasegee River sites below Cullowhee Dam (TKR_Below) positively correlated on the PC1 axis were largely influenced by the higher-scored TDEC ratings and reduced riffle embeddedness (Figure 7; Figure 8). The clustering of the LTR above dam sites shared the most similarities in the variables of RE (26.84%), depth (13.25%), and TDEC (8.29%). While the variables depth (18.44%), CV (13.82%), and RE (11.21%) influenced the similarities between the TKR below dam sites.

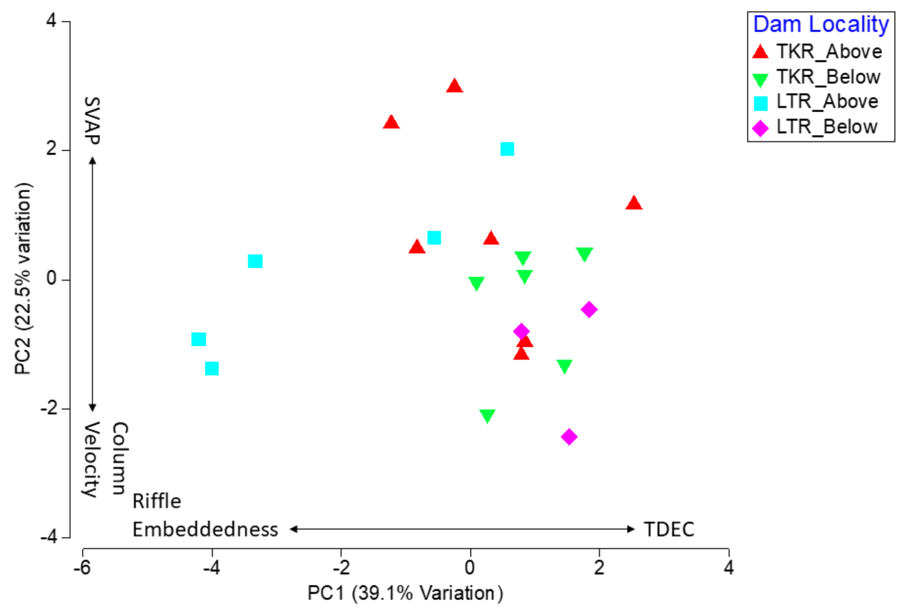

Figure 6. Principal components analysis of reach level habitat characteristics plotted to display variation and correlation of mainstem study sites based on dam location in the Little Tennessee River Basin and their localized watersheds. Clustering of the Little Tennessee River sites above dams (LTR_Above) is isolated to the left of the PC1 axis while clustering of Tuckasegee River below dam sites (TKR_Below) was to the right of the axis. Additional clustering was observed in the Little Tennessee River below dam sites (LTR_Below) and Tuckasegee River above dam sites (TKR_Above).

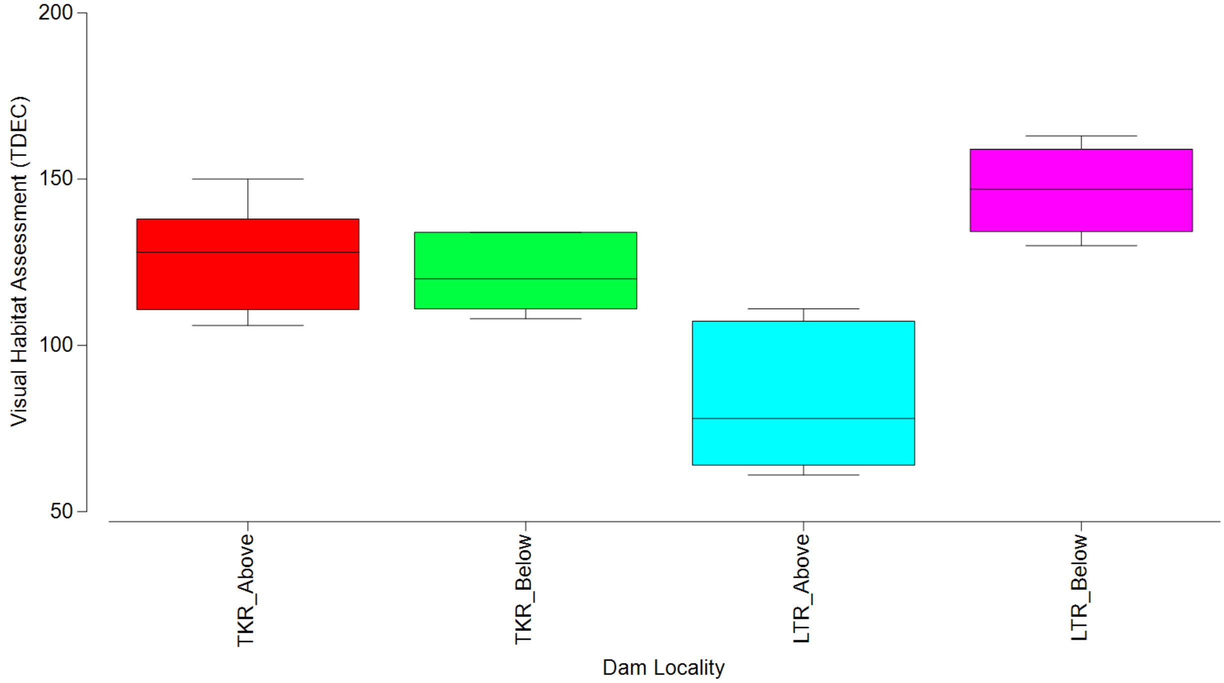

Figure 7. Visual habitat assessment scores for TDEC measurements based on dam locality of study site locations in the Little Tennessee River Basin. The Tuckasegee River sites (TKR_Above and TKR_Below) are shown to have similarities in scores, whereas the Little Tennessee River below dam sites (LTR_Below) have the highest habitat quality and the Little Tennessee River above dam sites (LTR_Above) have the lowest habitat quality.

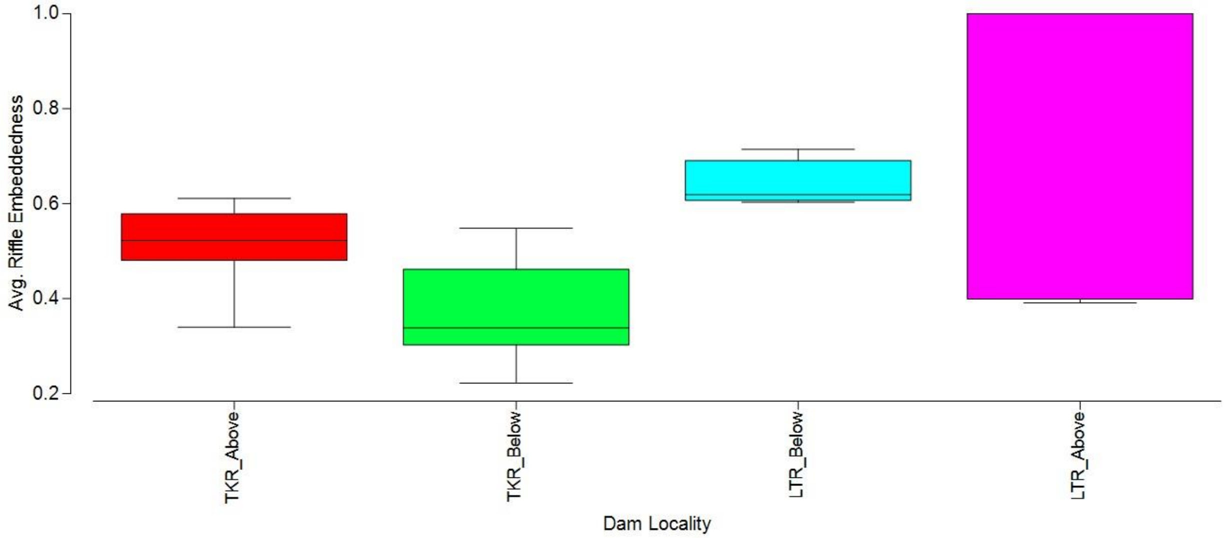

Figure 8. Riffle embeddedness measurements for study site locations in the Little Tennessee River Basin. Tuckasegee River sites (TKR_Above and TKR_Below) have a range of low riffle embeddedness among their sites, while the Little Tennessee River below dam sites (LTR_Above and LTR_Below) having slightly higher embeddedness, and lastly, the Little Tennessee River above dam sites (LTR_Above) had a very wide range of embeddedness with some slight being embedded to some riffles being fully embedded with sediment.

Reach Level Habitat Characteristics (Tributary Sites/Dam Locality Factor)

I found a significant difference among tributaries (p-value = 0.006, R-value = 0.343) based on the dam locality. This significant difference focused on the Tuckasegee River sites below Cullowhee Dam and Little Tennessee River sites below Lake Emory Dam. Further support for this difference was associated with a PCA for reach-level characteristics in tributaries, with 37.6% of the variation among sites represented on the PC1 axis and 29.1% on the PC2 axis. Eigenvectors that I observed to have the most influence on the PC1 axis were the positively correlated depth and the negatively correlated SVAP habitat variables. A positively correlated SVAP habitat variable and a negatively correlated column velocity influenced the PC2 axis. Sites in Little Tennessee River (LTR) clustered as did sites in the Tuckasegee River (TKR)

(Figure 9). Using a SIMPER test, the most significant differences in TKR and LTR below dam sites were SVAP (25.18%), column velocity (18.62%), and gradient (12.85%). For the TKR and LTR above dam sites, column velocity (32.64%), bottom velocity (24.37%), and SVAP scores (13.17%) accounted for most differences.

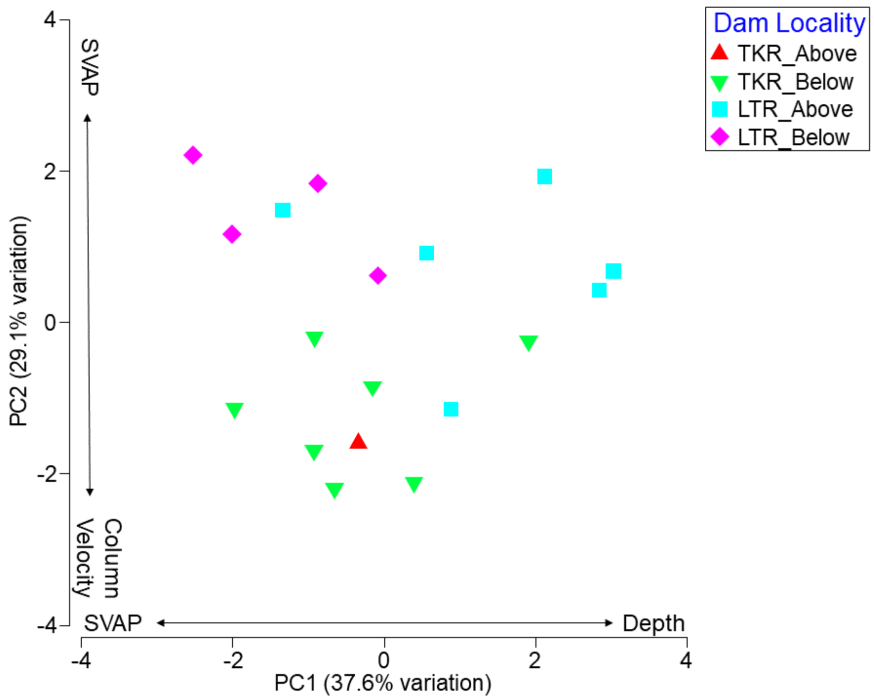

Figure 9. Principal components analysis of reach level habitat characteristics plotted to display variation and correlation of tributary study sites based on dam location in the Little Tennessee River Basin and their localized watersheds. Clustering of sites is observed with the Little Tennessee River sites (LTR_Above and LTR_Below) being grouped together versus the Tuckasegee River sites (TKR_Above and LTR_Below) being grouped separately.

Habitat Substrate Composition (Mainstem sites/Dam Locality Factor)

I detected a significant difference among mainstem sites (p-value 0.001, R-value = 0.319). I found these significant differences for all testing groups except the Tuckasegee River

(TKR) below Cullowhee Dam versus the Little Tennessee River (LTR) below Lake Emory Dam sites. A PCA further supported my findings, with 55.1% of the variation being associated with the PC1 axis and 16.1% of the variation being with the PC2 axis. Eigenvectors associated with these axes showed that pebble diameter was positively correlated with PC1 and sand percentages negatively correlated. Cobble was positively correlated to PC2 and bedrock percentages were negatively correlated (Figure 11; Figure 12). I observed clustering of LTR above dam sites to be isolated from others on the left side of the PC1 axis due to large quantities of sandy substrates. However, the LTR and TKR below dam sites were to the right of the PC1 axis and were clustered together, having a similar range of pebble diameters (Figure 10). The most influential variables causing similarities and isolation of the LTR above dam sites were gravel (11.21%), boulder (15.53%), and cobble (11.21%).

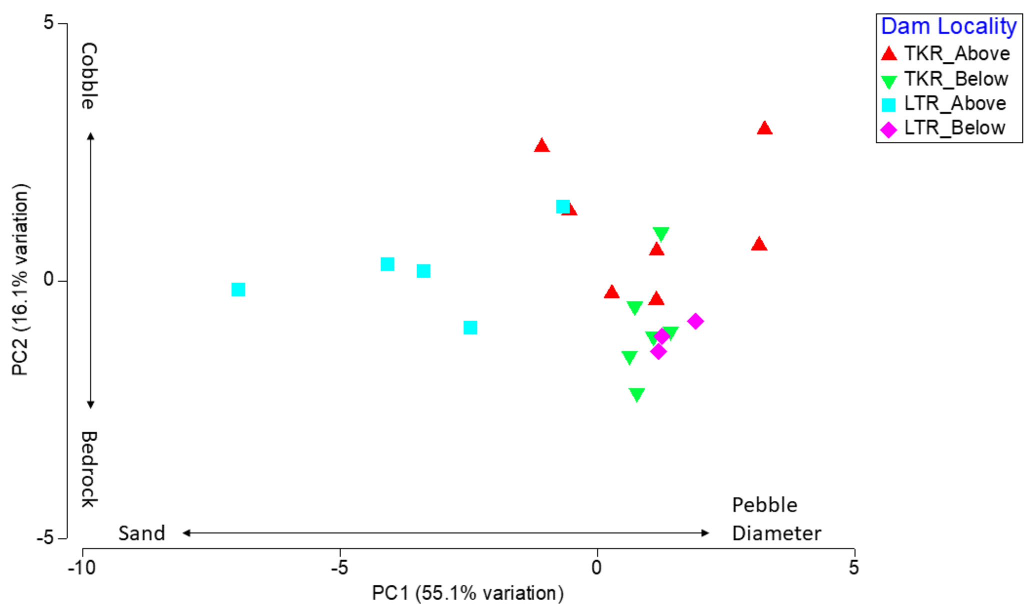

Figure 10. Principal components analysis of substrate habitat characteristics plotted to display variation and correlation of mainstem study sites based on dam location in the Little Tennessee River Basin and their localized watersheds. Clustering of sites are observed with the Little

Tennessee River above dam sites (LTR_Above) being grouped together on the left of the PC1 axis versus the Tuckasegee (TKR_Below) and Little Tennessee River sites below dams (LTR_Below) being grouped together on the right of the axis.

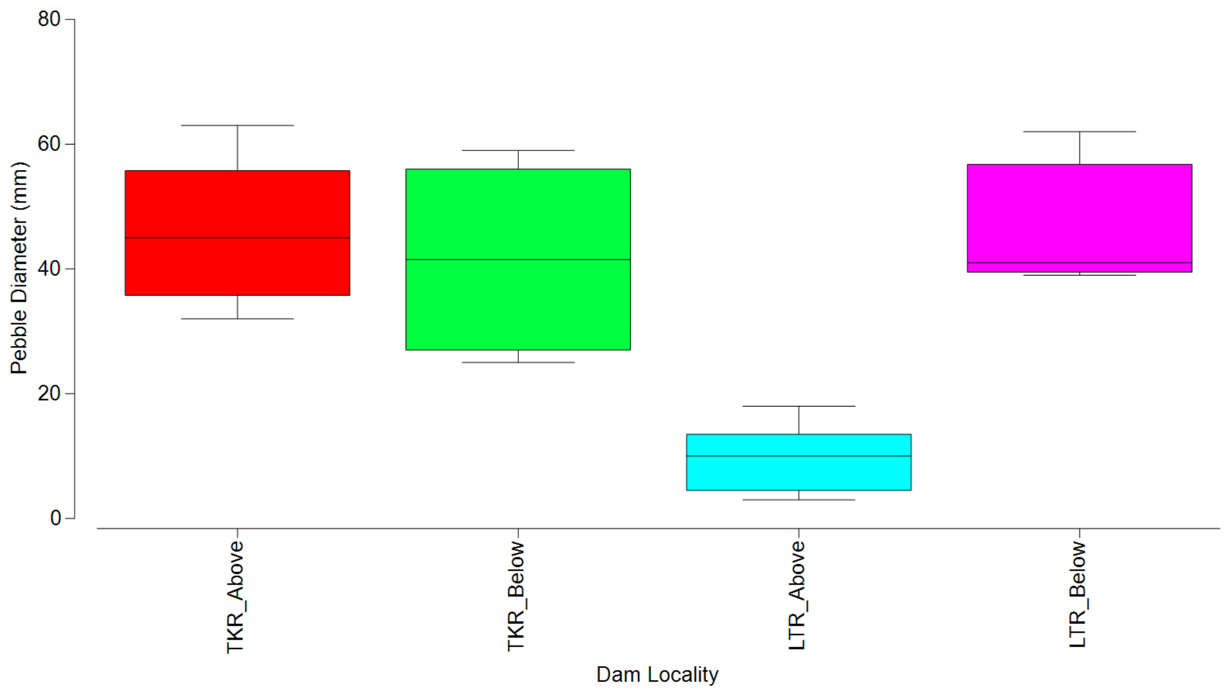

Figure 11. Pebble diameter measurements for mainstem study sites based on dam locality factorization in the Little Tennessee River Basin. Study site groups from the Tuckasegee River above dam (TKR_Above), below dam (TKR_Below), and the Little Tennessee River below dam (LTR_Below) sites had similar ranges of pebble diameters, except for the Little Tennessee River above dam sites (LTR_Above), which had a range of smaller grain sized substrates.

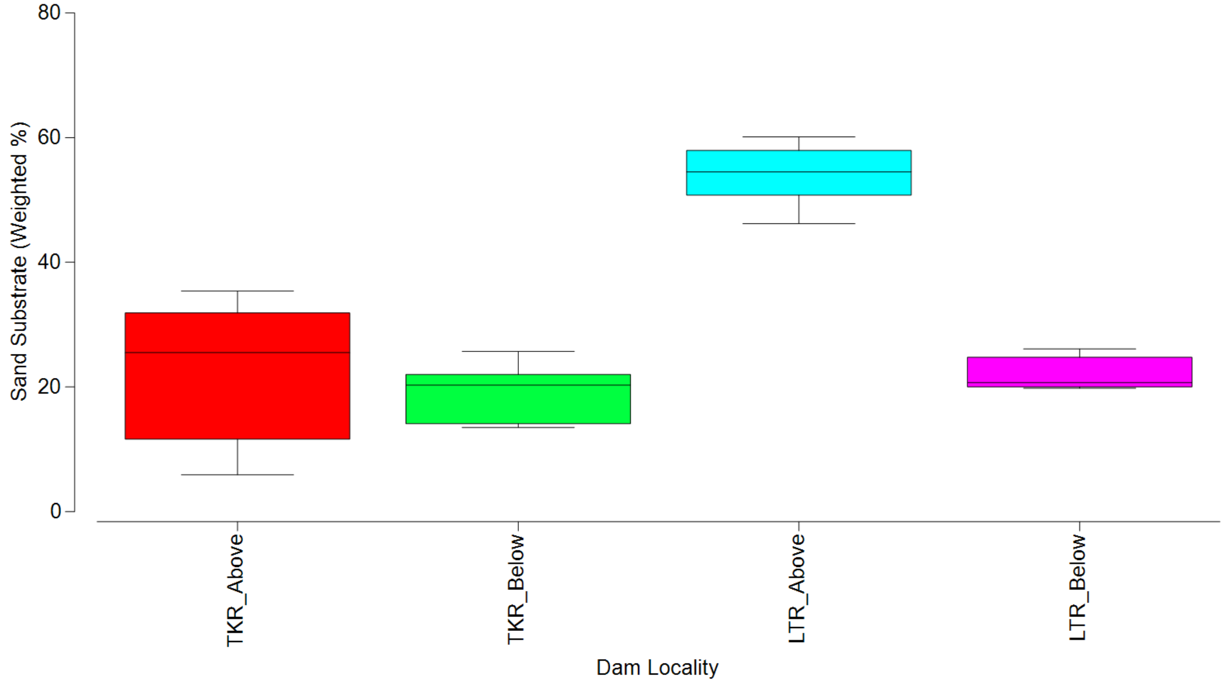

Figure 12. Sand substrate percentages for mainstem sites based on dam locality factorization in the Little Tennessee River Basin. Little Tennessee River above dam sites (LTR_Below) had the highest percentages of sand versus that of the other study site groups. Other sites like the Tuckasegee River above dam (TKR_Above), below dam (TKR_Below), and the Little Tennessee River below dam (LTR_Below) shared similarities among sand substrate percentages.

Habitat Substrate Composition (Tributary Sites/Dam Locality Factor)

I observed no detectable differences among tributary study sites using dam locality (p-value = 0.069, R-value = 0.16). Additionally, I did not observe distinct clustering in our PCA (Figure 13). The PC1 axis accounted for 33.9% of the total variation, while the PC2 axis accounted for 27.2%. Using the eigenvectors of the PCA, the PC1 axis was most influenced by positively correlated bedrock counts and negatively correlated gravel percentages. I found that positively associated silt and negatively associated woody debris percentages influenced the PC2 axis.

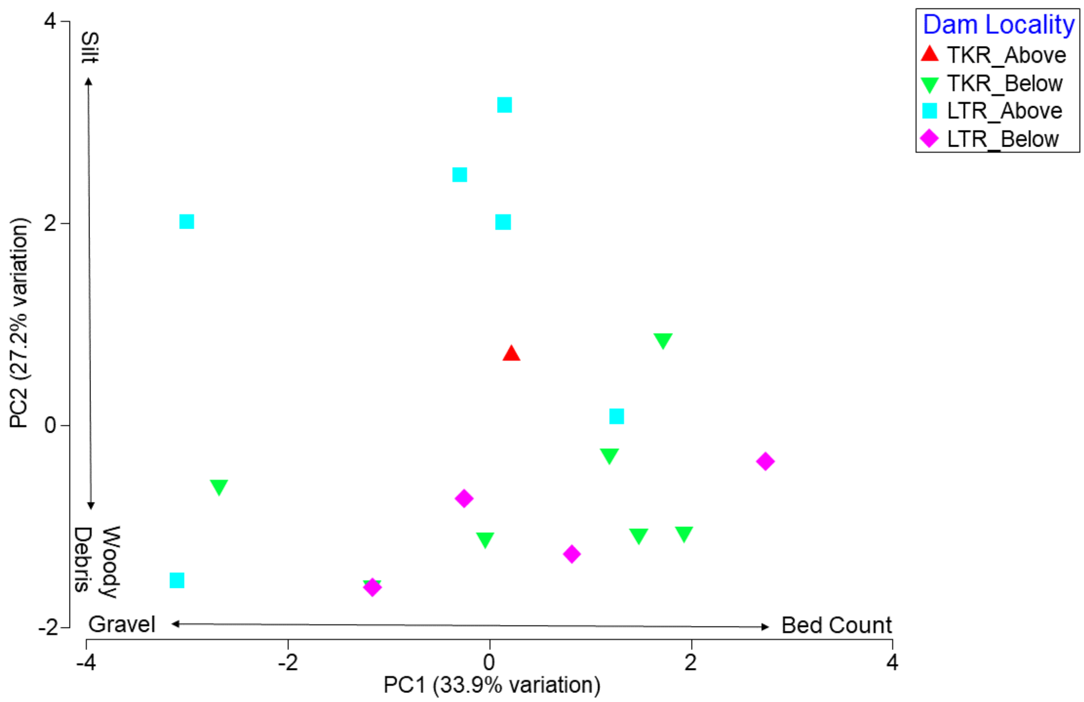

Figure 13. Principal components analysis for substrate variables in tributary sites based on dam locality factorization in the Little Tennessee River Basin. No distinct clustering was observed between my Tuckasegee River above dam (TKR_Above), below dam (TKR_Below) and the Little Tennessee River above dam (LTR_Above), and below dam (LTR_Below) sites.

Geospatial Mapping of Habitat Quality and Species Occurrence

With occurrence data for my target species and habitat data we collected, I was able to choose areas best suited for reintroductions through mapping (Figure 14) (NCWRC 2024). Due to the lack of occurrence data for the tributary systems, with the exception of the Cullasaja and Cartoogechaye Rivers, and more habitat data for our mainstem sites compared to tributary sites, mainstem reaches were largely used for my reintroduction map. However, since there were no significant differences associated with substrate characteristics and only minor differences in reach level characteristics, species that used tributaries in one watershed should be able to use the tributaries from the other. Among the mainstem systems, Little Tennessee River and Tuckasegee River sites below dams were among the most suitable habitats, due to the availability of favorable habitats, and many of my study species are already found in these areas or could potentially exist there. However, the Tuckasegee River sites above Cullowhee Dam come with a mix of suitable habitat designations. The areas between Cullowhee Dam and the confluence of the East and West Fork Tuckasegee River have fair habitat conditions and occupancy of at least one of the study species. Areas above the confluence of the East and West Forks of the Tuckasegee River have unsuitable habitat conditions and lack of desired study species. This was the same as in the Little Tennessee River sites above Lake Emory dam, where unsuitable habitat conditions were prominent, and a lack of the desired study species were observed within the mainstem reach. However, it should be mentioned that since some study species were detected in Cartoogechaye and Cullasaja, which are feeder streams of the upper Little Tennessee River, some areas that have lacked sampling efforts could hold the desired species. The Cartoogechaye and Cullasaja rivers have fair habitat conditions for my study species and at least one of the study species is currently known to exist in both river reaches.

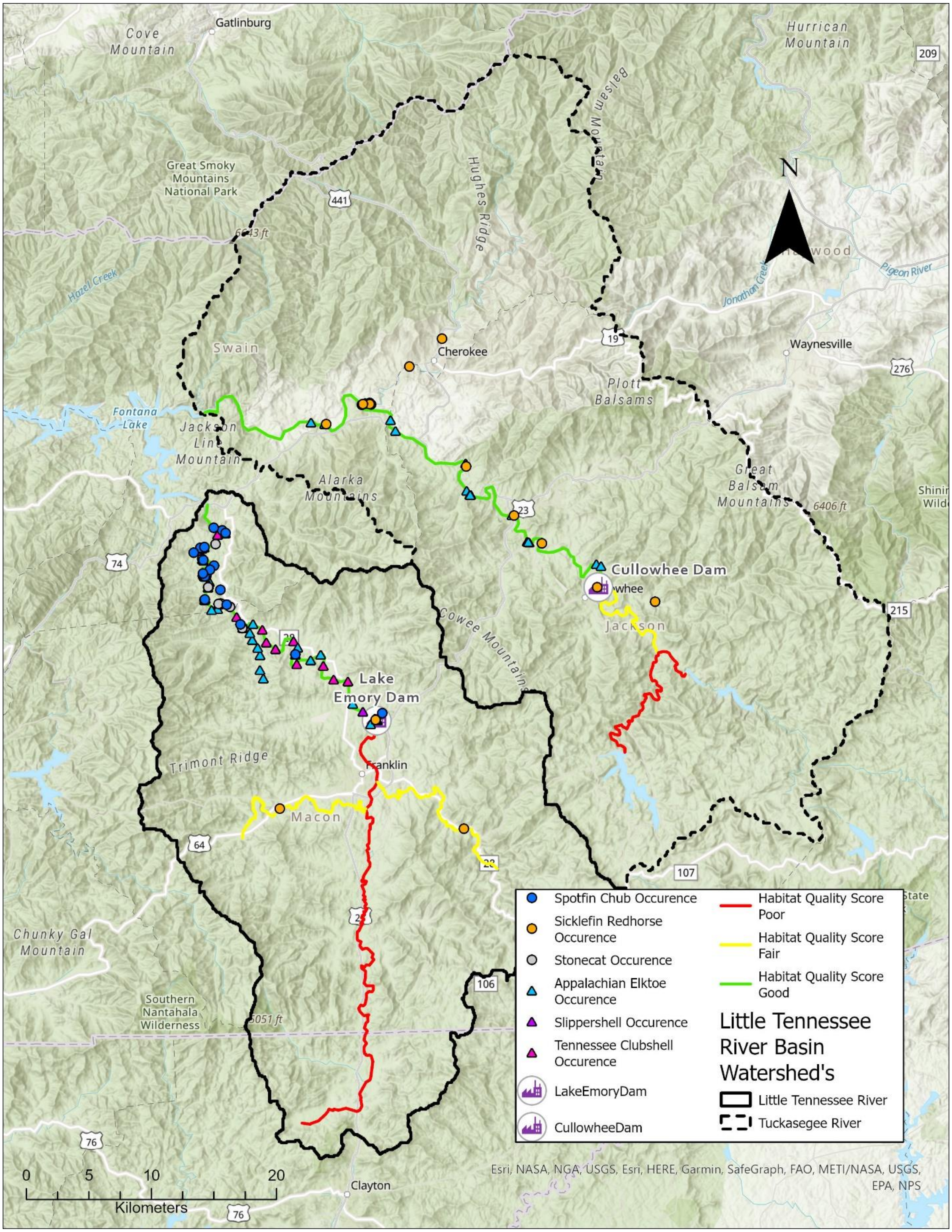
Spotfin Chub Occurence
Sicklefin Redhorse Occurence
Stonecat Occurence
Appalachian Elktoe Occurence
Slippershell Occurence
Tennessee Clubshell Occurence
LakeEmoryDam
CullowheeDam
Habitat Quality Score Poor
Habitat Quality Score Fair
Habitat Quality Score Good
Little Tennessee River Basin Watershed's
Little Tennessee River
Tuckasegee River
Cullowhee Dam
Lake Emory Dam
N
0 5 10 20
Kilometers
Esri, NASA, NGA, USGS, Esri, HERE, Garmin, SafeGraph, FAO, METI/NASA, USGS, EPA, NPS
Gatlinburg
Cherokee
Waynesville
Franklin
Clayton
Great Smoky Mountains National Park
Southern Nantahala Wilderness
Fontana Lake
Swain
Jackson
Macon
Trimont Ridge
Cowee Mountains
Alarka Mountains
Plott Balsams
Great Balsam Mountains
Hughes Ridge
Balsam Mountain
Cove Mountain
Hurrican Mountain
Chunky Gal Mountain
Jackson Line Mountain
Hazel Creek
Jonathon Creek
Pigeon River

Figure 14. Habitat quality scoring map for the Little Tennessee River Basin including the Little Tennessee and Tuckasegee River watersheds. Habitat quality scores were based on available suitable habitat and occurrence data for desired species in the last 10 years, which resulted in reaches being color-coded as “Good”, “Fair”, or “Poor”. The locations of dams and study species found within the study were also labeled on the map.

DISCUSSION AND CONCLUSION

The Little Tennessee River Basin upstream of Fontana Reservoir is a complex and diverse riverine system home to a large diversity of aquatic life (Little Tennessee River NFCA 2018). I observed significant differences for multiple reach-level and substrate composition habitat variables when sites were grouped by location. Chain roughness, habitat assessments (SVAP and TDEC), wetted width, pebble diameter, sand, gravel, and bedrock substrate percentages were important habitat variables across both watersheds. These habitat variables are crucial to overall habitat stability and aquatic organism well-being. For example, pebble diameter and chain roughness give scientist’s significant information on the streambed's overall condition and whether there is any available space for fish or mussels to feed on or hold to during high-flow events. Habitat assessments like SVAP and TDEC also share significant importance in riverine health by allowing researchers to assess the conditions of various riverine variables through a rapid snapshot assessment at a relatively low cost (Maddock 1999). Additionally, many of our study species, including Appalachian Elktoe, Spotfin Chub, and Sicklefin Redhorse, strongly depend on specific undisturbed natural substrate compositions like bedrock and gravel (Favrot 2009; Kanno et al. 2012; Rondel 2019)

I observed significant differences across reach level and substrate characteristics for all mainstem sites in the Tuckasegee and Little Tennessee River watersheds based on their site locations relative to their respective dams. An increased quantity of smaller substrates and lower habitat assessment scores primarily influenced the isolation of Little Tennessee River sites upstream of Lake Emory Dam. Within these areas, there is evidence that farming and historical industrial and logging practices largely contributed to mass streambed erosion and runoff, pushing large amounts of finer sediments into these systems, and causing the loss of riparian zones (Mainspring Conservation Trust 2012, North Carolina Department of Environmental Quality 2012; Table 2). The similarities between the Tuckasegee and Little Tennessee River mainstem sites focus on the areas found downstream of Cullowhee Dam and Lake Emory Dam. Variables I observed that contributed to this were similar riffle embeddedness, TDEC habitat scores, and pebble diameters. Within these sites, most of the riverine habitats are intact, healthier, and lack an overabundance of smaller substrates, unlike the Upper Little Tennessee. The cause of this similarity is likely due to the top-release dams found within these river reaches. The Cullowhee Dam on the Tuckasegee and Lake Emory Dam and Little Tennessee Rivers were built in the late 1920-30s, and act as a substrate barrier from upstream runoff due to their top of dam releases (Mainspring Conservation Trust 2012; McGill Associates 2017). With the dams acting as substrate barriers, along with smaller quantities of anthropogenic disturbance and a largely forested landscape, these conditions make for favorable, natural riverine habitats in the lower Tuckasegee and Little Tennessee rivers (Appendix 1; 2).

The Tuckasegee River upstream of Cullowhee Dam to the confluence of the East and West Fork of the Tuckasegee River also had favorable conditions similar to that of the lower Little Tennessee River below Lake Emory. These sites have similar ranges of riffle embeddedness, gradient, and TDEC assessment scores. However, one issue of the East and West Fork dams that we do not see in the Lake Emory dam is that these two dams use a scheduled bottom release method for power generation. This type of release could inhibit many species as these river sections receive more altered stream discharge, temperature regime, and increased water depth (Zarri et al. 2022).

I observed significant differences across reach-level characteristics within our tributary sites for the Little Tennessee River and Tuckasegee watersheds based on dam locality. However, I did not observe significant differences across substrate habitat variables. Among reach-level characteristics, depth, SVAP, and column velocity influenced the observed site differences. However, bedrock counts, gravel, silt, and woody debris percentages influenced site similarities among substrate characteristics. Differences in reach level characteristics for tributaries could be mainly due to the varying size of tributary streams like Caney Fork Creek in the Tuckasegee River watershed versus that of the Cullasaja River found in the Little Tennessee watershed. This change in stream size further reflects why there were influential differences in the reach level variables of water depth, velocity, and surrounding riparian areas by SVAP. However, similarities in substrate habitat indicate how these streams are found in similar areas of geography and elevation while having less overall anthropogenic disturbance than that of the mainstem sites.

Through this study, I was able to identify and assess habitat conditions across different study sites within the Little Tennessee and Tuckasegee River watersheds of the Little Tennessee River Basin. This study will help set baselines of habitat conditions within these watersheds to assist in the planning of reintroductions for at-risk species and watershed-wide habitat databases for long-term monitoring. Similar to our results, a study that quantified habitat for Chinook Salmon in a glaciated watershed noted that habitat variables like flow and gradient were vital in locating probable habitat across the landscape (Bidlack et al. 2014). However, their study acquired the habitat data using GIS software and satellite data instead of real-time physical data. Another similar study focused on building a watershed model for bull trout on the South Fork Boise River (Benjankar et al. 2018). Using physical characteristics like stream velocity and water temperature in their watershed model allowed researchers to predict water availability and fish habitat within their targeted watershed (Benjankar et al. 2018). While these habitat characteristics are similar to variables gathered in my study, they were also acquired through large national habitat datasets and models.

Habitat variables are only one component needed to determine suitability for species reintroductions. Temperature and water chemistry are vital for species success and were not continuously collected during this project. Water temperature is a vital habitat variable as it affects a fish's metabolic rate, energy balance, and behavior, along with determining whether species can live in certain areas during parts of the year or trigger spawning events (Volkoff & Ronnestad 2020). Kanno et al. (2012) stated that Spotfin Chub will be more selective of higher stream velocities during warmer temperatures due to a lack of available oxygen. Sicklefin Redhorse initiate spawning in the upper Hiwassee River basin when a mean temperature reaches 17.5° C (Favrot 2009). Higher water temperatures related to other environmental conditions, such as drought or climate change, can dramatically affect localized fish populations. Malone et al (2021) noted that drought events were associated with fish assemblage changes in Little River and Cataloochee Creek after one year of a drought occurrence. Water chemistry metrics are also crucial in species health, as examined by Jarvis (2011) on Appalachian Elktoe in the Upper Little

Tennessee River Basin. Jarvis noted that copper and zinc levels could exceed freshwater mussel thresholds during storm events due to elevated sediment levels from runoff and stream erosion.

There are still knowledge gaps for several of our study species that need to be addressed before or during reintroductions. For instances, Cathcart et al. (2019) noted the importance of distance to upstream tributaries, flow, and habitat availability on migratory suckers. I recommend pairing the information gathered during my study with other species distribution models or habitat suitability methods like Maximum Entropy (MaxEnt). MaxEnt is a popular method used for animal habitat suitability projects based on large-scale habitat variable databases (Daniel et al. 2017; Radinger & Wolter 2015). It is favorable to pair this method with a study like ours to merge real-time ground truthing variables to a larger scale habitat suitability project. Holder et al. (2020) paired traditional habitat sampling and MaxEnt modeling to create species distribution models for 19 species on the remote North Slope of Alaska, which provided critical species data for those data poor regions. Based on my findings, more work is needed in species needs and distribution before some of these study species should be reintroduced. However, these species can be restored to these extirpated reaches and thrive in their native habitat when these knowledge gaps are addressed.

MANAGEMENT RECOMMENDATIONS

Overall recommendations for habitat management in the Little Tennessee River Basin consists of restoring the upper mainstem system of the Little Tennessee River and maintaining and monitoring the conditions of the lower Little Tennessee River and all the Tuckasegee River. The upper Little Tennessee River mainstem areas would need to undergo the removal of a large sum of smaller substrates like sand and silt to bring balance substrate composition in a majority of stream reaches. It would also be beneficial to promote and restore riparian zones along the stream to prevent any ongoing stream erosion that may occur. Within the upper Tuckasegee River mainstem, coordination with power generation needs to better mimic natural seasonal stream discharges would benefit migratory fishes by allowing better access to spawning habitat.

Upstream of Fontana Reservoir, Spotfin Chub is currently only found within the lower Little Tennessee River below Lake Emory Dam. However, support for possible translocations in the lower Tuckasegee River based on similarities in substrate types and composition while also overlapping in some reach-level characteristics is likely based on my study. This species prefers boulder/bedrock substrates with medium to high velocity and depth. Since this species is a crevice spawner, they rely on these substrates to spawn and, after spawning, move into smaller tributaries during fall and winter (Kanno et al. 2012; U.S Fish & Wildlife Service 2012). Two studies support that areas with higher bedrock counts are often associated with better habitat suitability and higher estimated abundance in a river reach (Doll et al. 2020a; 2020b). These habitat characteristics are reflected within both of our study systems. However, there is a lack of information on the tributary use of the species, so further research is needed before reintroduction. Prevention of habitat degradation is essential for the species to persist in the Little Tennessee River Basin due to its reliance on minimally altered stream habitat in forested areas and adverse effects caused by fine suspended sediments (Perkin et al. 2019; Sutherland & Meyer 2007).

The Sicklefin Redhorse is currently found within both studied Little Tennessee River Basin watersheds. However, they are found in small quantities and restricted ranges in both areas. This is mainly due to fragmented habitats and lack of spawning areas caused by anthropogenic landscape manipulation. Habitat used by the species is nonrandomly chosen, consisting of swift currents, shallow depths, and coarse substrates like boulders and bedrock (Favrot 2009). Researchers have noted that regulated rivers, like the Tuckasegee River can either expand or restrict how much seasonal habitat is available depending of scheduled flow rates (Fisk et al. 2015). It would benefit this species to remove, relict or damaged dams (e.g., Cullowhee Dam) and assure proper seasonal river flows because this species needs river connectivity, natural flow levels, and seasonally appropriate water temperatures (Favrot & Kwak 2018; Fisk et al. 2015). It would also be beneficial if further research were focused on locating and researching prime spawning habitats. Identifying and protecting such areas would benefit all species, in fact.

Stonecats are currently only found within the lower Little Tennessee River of the two studied watersheds. However, as previously mentioned for Spotfin Chub, the lower Little Tennessee and Tuckasegee rivers have favorable habitats in both reach level and substrate characteristics for both species. The Stonecat and other members of the genus *Noturus* prefer shallow depths, moderate stream velocities, and coarser substrates (Brewer & Rabeni 2008; Etnier and Starnes 1993; Wells et al. 2020). I recommend reintroducing this species into the lower Tuckasegee River while also protecting and preserving the population in the lower Little Tennessee River. This could be accomplished by ensuring adequate habitat is maintained by adequate flow and reduced siltation from runoff (Brewer and Rabeni 2008; Trautman 1981).

The mussels of this study are primarily located in the lower Little Tennessee River, except for the Appalachian Elktoe population in the lower Tuckasegee River. Most of these mussel populations co-occur in similar well-forested habitats with low proportions of fine sediments (Pandolfi et al. 2022; Schilling et al. 2017). These habitats consist of shallow, moderate to fast-moving water with coarse substrates that are relatively silt-free (Schilling et al. 2017; US Fish & Wildlife 2011). The lower Tuckasegee and Little Tennessee rivers share favorable habitats in reach level and substrate characteristics for all three mussels. Best management practices for these species consist of reintroductions to the lower Tuckasegee River while promoting better habitat protection and runoff abatement measures across both watersheds. These actions will help prevent excessive amounts of smaller substrates like sand and silt from land-use activities reaching these water bodies and maintain water quality standards to promote the restoration of multiple species with overlapping native ranges (Jarvis et al. 2011; U.S. Fish and Wildlife Service 2011). Gangloff and Feminella (2007) noted that high sheer stress caused by high flows was indicative of low mussel abundance in Appalachian streams. Better partnerships with stakeholders throughout the basin to manage more favorable stream flow conditions and better floodplain connectivity would create suitable sheer stress conditions for mussel abundance and richness.

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APPENDIX

Table 1. USGS StreamStats Data for the Tuckasegee River Watershed, with a breakdown of the differences between the whole watershed and the area above and below the Cullowhee Dam (U.S. Geologic Survey 2019).

Parameter Code	Parameter Description	Unit	Tuck All	Tuck Above Dam	Tuck Below Dam	Percent Difference Above_Below
DRNAREA	Area that drains to a point on a stream	square miles	663	207	456	-120%
LC11BARE	Percentage of barren from NLCD 2011 class 31	percent	0.1	0.1	0.1	0%
LC11CRPHAY	Percentage of cultivated crops and hay, classes 81 and 82, from NLCD 2011	percent	3	3.7	2.6	30%
LC11DEV	Percentage of developed (urban) land from NLCD 2011 classes 21-24	percent	6.2	4.9	6.7	-37%
LC11FOREST	Percentage of forest from NLCD 2011 classes 41-43	percent	88.4	87.7	88.7	-1%
LC11GRASS	Percent of area covered by grassland/herbaceous using 2011 NLCD	percent	0.6	0.7	0.5	29%
LC11IMP	Average percentage of impervious area determined from NLCD 2011 impervious dataset	percent	0.7	0.3	0.8	-167%
LC11SHRUB	Percent of area covered by shrubland using 2011 NLCD	percent	1.2	1.4	1.1	21%
LC11WATER	Percent of open water, class 11, from NLCD 2011	percent	0.5	1.5	0.4	73%
LC11WETLND	Percentage of wetlands, classes 90 and 95, from NLCD 2011	percent	0.1	0.1	0.1	0%
PROTECTED	Percent of area of protected Federal and State owned land	percent	0.8	1	0.7	30%
SSURGOA	Percentage of area of Hydrologic Soil Type A from SSURGO	percent	32.1	62	18.5	70%
SSURGOB	Percentage of area of Hydrologic Soil Type B from SSURGO	percent	61.1	26.1	76.9	-195%
SSURGOC	Percentage of area of Hydrologic Soil Type C from SSURGO	percent	3.7	7	2.2	69%
SSURGOD	Percentage of area of Hydrologic Soil Type D from SSURGO	percent	2.1	3.1	1.6	48%

Table 2. USGS StreamStats Data for the Upper Little Tennessee River Watershed, with a breakdown of the differences between the whole watershed and the area above and below the Lake Emory Dam (U.S. Geological Survey 2019).

Parameter Code	Parameter Description	Unit	LTR All	LTR Above Dam	LTR Below Dam	Percent Difference Above_Below
DRNAREA	Area that drains to a point on a stream	square miles	448	309	139	55%
LC11BARE	Percentage of barren from NLCD 2011 class 31	percent	0.1	0.1	0.1	0%
LC11CRPHAY	Percentage of cultivated crops and hay, classes 81 and 82, from NLCD 2011	percent	7.1	7.4	6.4	14%
LC11DEV	Percentage of developed (urban) land from NLCD 2011 classes 21-24	percent	8.6	9.7	6.15	37%
LC11FOREST	Percentage of forest from NLCD 2011 classes 41-43	percent	81.2	79.8	84.3	-6%
LC11GRASS	Percent of area covered by grassland/herbaceous using 2011 NLCD	percent	1.3	1.3	1.3	0%
LC11IMP	Average percentage of impervious area determined from NLCD 2011 impervious dataset	percent	0.9	1.1	0.4	64%
LC11SHRUB	Percent of area covered by shrubland using 2011 NLCD	percent	1.5	1.5	1.5	0%
LC11WATER	Percent of open water, class 11, from NLCD 2011	percent	0.2	0.1	0.4	-300%
LC11WETLND	Percentage of wetlands, classes 90 and 95, from NLCD 2011	percent	0.1	0.1	0.1	0%
PROTECTED	Percent of area of protected Federal and State owned land	percent	1.0	1.0	1	0%
SSURGOA	Percentage of area of Hydrologic Soil Type A from SSURGO	percent	27.2	30.3	20.3	33%
SSURGOB	Percentage of area of Hydrologic Soil Type B from SSURGO	percent	65.1	62.0	71.9	-16%
SSURGOC	Percentage of area of Hydrologic Soil Type C from SSURGO	percent	4.9	4.9	4.9	0%
SSURGOD	Percentage of area of Hydrologic Soil Type D from SSURGO	percent	2.2	2.3	1.9	17%

Table 3. Habitat study sites for 2021 and 2022, labeled by site code, description, GPS coordinates, and date of sampling.

Site Code	Study Site	Latitude	Longitude	Date of Sampling
LBru1	Brush Creek (Above Little Tennessee Confleunce)	35.318589	-83.616335	8/10/2022
LBur1	Burningtown Creek (Lower)	35.2798	-83.48126	6/14/2022
LCar1	Cartoogyche River (Upper)	35.15236	-83.47747	7/23/2021
LCar2	Cartoogyche River (Mtn Hope Baptist)	35.155484	-83.456502	7/6/2022
LCar3	Cartoogyche River (Lower)	35.157008	-83.38843	7/7/2021
LCul1	Cullasaja River (Upper)	35.14187	-83.29454	7/14/2021
LCul2	Cullasaja River (Lower)	35.16441	-83.36038	7/8/2021
LMid1	Middle Creek (Above Little Tennessee Confluence)	35.045665	-83.36505	8/3/2022
LRab1	Rabbit Creek (Above Lake Emory Confluence)	35.206536	-83.358647	7/21/2022
LTel1	Tellico Creek (Above Little Tennessee Confluence)	35.288399	-83.491942	8/9/2022
LLit1	Little Tennessee River (Parkdale Access)	34.939358	-83.403939	7/29/2022
LLit2	Little Tennessee River (Kelly's Creek Rd. Bridge)	34.958467	-83.381355	7/15/2022
LLit3	Little Tennessee River (Before Darnell Creek Confluence)	34.960965	-83.377168	6/28/2022
LLit4	Little Tennessee River (Tryphosa Rd.)	35.015265	-83.381219	7/5/2022
LLit5	Little Tennessee River (Prentiss Rd. Bridge)	35.122041	-83.374186	7/1/2022
LLit6	Little Tennessee River (Sanderstown Bridge)	35.23544	-83.39568	6/6/2022
LLit7	Little Tennessee River (Tellico Creek Access)	35.288855	-83.491293	6/29/2022
LLit8	Little Tennessee River (Needmore Suspension Bridge)	35.325825	-83.523401	7/26/2022
TCan1	Caney Fork (Community Center)	35.29402	-83.099688	6/27/2022
TCul1	Cullowhee Creek (Upper/CVS)	35.29657	-83.18122	5/25/2022
TCul2	Cullowhee Creek (Above Confluence)	35.31705	-83.18098	5/9/2022
TDee1	Deep Creek (Above Tuck Confluence)	35.434944	-83.439642	7/12/2022
TEas1	East Fork Tuckasegee River (Circle Cove)	35.25457	-83.10589	6/10/2022
TEas2	East Fork Tuckasegee River (Before Confluence)	35.26836	-83.12129	6/15/2022
TSav1	Savannah Creek (Upper)	35.29583	-83.26428	6/1/2022
TSav2	Savannah Creek (Savannah/Tuck Confluence)	35.34785	-83.23817	5/19/2022
TSco1	Scotts Creek (Upper)	35.39327	-83.19977	6/2/2022
TSco2	Scotts Creek (Above Confluence)	35.36809	-83.25259	5/17/2022
TWes1	West Fork Tuckasegee River (Powerplant Bridge)	35.23447	-83.12495	6/9/2022
TWes2	West Fork Tuckasegee River (Before Confluence)	35.266864	-83.122923	6/16/2022
TTuc1	Tuckasegee River (After East/West Confluence)	35.26884	-83.12457	6/13/2022
TTuc2	Tuckasegee River (High Ground/Above Wayehutta Bridge)	35.308661	-83.160016	6/21/2022
TTuc3	Tuckasegee River (Wayehutta Bridge)	35.312325	-83.165704	7/11/2022
TTuc4	Tuckasegee River (Below Cullowhee Dam)	35.315484	-83.176681	6/20/2022
TTuc5	Tuckasegee River (Below Cullowhee Creek Confluence)	35.318338	-83.178074	7/11/2022
TTuc6	Tuckasegee River (Below Savannah Creek Confluence)	35.349603	-83.239181	7/8/2022
TTuc7	Tuckasegee River (Webster Baptist Church)	35.340834	-83.226109	7/14/2022
TTuc8	Tuckasegee River (Barker's Creek Access)	35.389013	-83.295434	7/18/2022
TTuc9	Tuckasegee River (Bryson City Access)	35.432393	-83.42315	7/25/2022