HABITAT CORRELATES OF POPULATIONS OF THE SOUTHERN APPALACHIAN BROOK TROUT (*SALVELINUS FONTINALIS*) IN SOUTH CAROLINA

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

By

Joseph Mark Barnes

Advisor: Dr. Keith Gibbs
WCU Department of Natural Resource Management

Committee Members: Dr. Thomas Martin, WCU Department of Biology
Dr. Joseph Pechmann, WCU Department of Biology

May 2024

TABLE OF CONTENTS

List of Figures……………………………………………………………………………......3
List of Tables……………………………………………………………………………..3
Abstract…………………………………………………………………………………...4
Introduction……………………………………………………………………………….6
Study Area………………………………………………………………………………13
Methods…………………………………………………………………………………20
Results…………………………………………………………………………………...29
Discussion………………………………………………………………………………35
Future Directions and Management Recommendations………………………………...39
References………………………………………………………………………………42

LIST OF FIGURES

Figure 1: Map of South Saluda and North Saluda sub-watersheds……………..……..………...17
Figure 2: Map of the South Saluda sub-watershed with three target streams……..……..……..18
Figure 3: Map of the North Saluda sub-watershed with two target streams……………..…..….19
Figure 4: eDNA results for streams in Table Rock sub-watershed ...…………………………...26
Figure 5: eDNA results for streams in North Saluda sub-watershed ……………………………27
Figure 6: Habitat unit sampling example ……………………………………....………..…28
Figure 7: Bar graph of CPUE for Laurel Creek ……………………………………………...….31
Figure 8: Bar graph of CPUE for South Saluda River…………………………………………...32
Figure 9: Principal Component Analysis………………………………………………………...33
Figure 10: Box and Whisker Plot for Canopy Cover…………………………………………….34
Figure 11: Average Monthly Temperatures of all Target Streams……………………………...38

LIST OF TABLES

Table 1: Stream names and situation of target streams in the Table Rock sub-watershed............19
Table 2: Stream names and situation of target streams in the North Saluda sub-watershed.........20

ABSTRACT

HABITAT CORRELATES OF POPULATIONS OF THE SOUTHERN APPALACHIAN BROOK TROUT (*SALVELINUS FONTINALIS*) IN SOUTH CAROLINA

Joseph Mark Barnes

Western Carolina University (May 2024)

Advisor: Dr. Keith Gibbs

The Brook Trout (*Salvelinus fontinalis*) stands as the only native salmonid species inhabiting the Appalachian Mountains, albeit occupying less than 80% of its historical range due to habitat loss, invasive species, and climate change impacts. I assessed the viability of genetically distinct Southern Appalachian Brook Trout (SABT) populations in South Carolina within the South Saluda sub-watershed (Laurel Creek, Slicking Creek, and South Saluda River) as potential sources for translocations or propagation to restore populations in the North Saluda River sub-watershed (Brice Creek and North Saluda River). Additionally, I investigated habitat correlations between streams with SABT present to streams where SABT are absent. My methodology involved single-pass electrofishing surveys to calculate catch per unit effort (CPUE) in stream reaches above and below potential waterfall barriers, also analyzing instream habitat characteristics, across 31 sites in 5 streams. We quantified habitat types by measuring substrate sizes and percentages, cover availability, large woody debris presence, and canopy cover percentage. While South Saluda River and Laurel Creek supported SABT populations, historical presence of SABT is anecdotal in North Saluda River and Brice Creek. However, within Slicking Creek, SABT were extirpated after an intense wildfire in 2016. Notably, Laurel Creek exhibited the highest average CPUE (2.06 fish/min.), while South Saluda River showed a lower average

CPUE (0.63 fish/min.). I did not detect significant differences in habitat characteristics between sites with or without SABT, but canopy cover reduction was evident in recently extirpated sites in Slicking Creek compared to other sites. Consequently, Laurel Creek emerged as the most suitable source population with appropriate management to preserve genetic integrity. Furthermore, we advise against attempting population reestablishment in Slicking Creek until canopy recovery.

Introduction:

The United States boasts a significant diversity of 3,000 fish species, found in a full spectrum of freshwater and marine habitats (National Fish Habitat Partnership, 2021). From the salmon-rich waters of the Pacific Northwest to the diverse fish populations of the Great Lakes and the Gulf Coast, U.S. aquatic ecosystems support a range of species adapted to different environmental conditions. Salmonids such as the Chinook Salmon (*Oncorhynchus tshawytscha*) and Rainbow Trout (*Oncorhynchus mykiss*) are prominent in cooler waters, while the American Paddlefish (*Polyodon spathula*) thrives in the Mississippi River basin (Behnke, 2002; Graham, 1997). In the southeastern United States, some species are adapted to warmer waters (Walsh et al.,1997). In addition to widespread species like the Largemouth Bass (*Micropterus salmoides*), this region is home to unique species like the Alabama Shad (*Alosa alabamae*), which migrates up the larger river systems to spawn (Walburg & Nichols, 1967). The variety of habitats from streams to estuaries supports a rich biodiversity, although many species face threats from habitat degradation and water pollution (Smith, 1985). The southern Appalachian Mountain region, generally refers to parts of the states of Virginia, North Carolina, South Carolina, Georgia, Tennessee, Alabama, Kentucky, and West Virginia. This diverse region is characterized by its mountainous terrain and is known for its rich biodiversity (Stephenson & Adams, 1998). This aquatic biodiversity is characterized by a variety of endemic species and high aquatic insect diversity (Morse et al., 1993). This region supports lesser-known but ecologically important species like the Snubnose Darter (*Etheostoma simoterum*), which is only found in clean, fast-flowing streams (Warren et al., 2000). The only salmonid species native to the southern Appalachian Mountains is the Brook Trout (*Salvelinus fontinalis*), which is a key indicator of the health of these ecosystems because it is highly sensitive to changes in water temperature and quality (Petty et al., 2005). Fish diversity across the United States is increasingly under threat

due to a range of anthropogenic factors, including landscape alterations, non-native species introductions, and climate change (Xu et al., 2024). These pressures significantly affect aquatic ecosystems.

Landscape alterations such as agriculture, urban development, deforestation, and the construction of infrastructure like dams and roads can lead to habitat loss, fragmentation, and degradation, which can significantly impact local ecosystems and biodiversity (Baker, 1995; Ekka et al., 2020). Such alterations affect the physical and biological environment, influencing water flow, soil stability, and the availability of habitats for various species (Pess et al., 2008). Some landscape alterations, such as dam building and urban expansion, have disrupted river flow patterns and sediment transport which are critical to the life cycles of species in the same family as the Brook Trout like the Chinook Salmon (*Oncorhynchus tshawytscha*) (Hoekstra et al., 2007). These disruptions can block migration routes essential for spawning, resulting in decreased reproduction and declining populations (Hoekstra et al., 2007).

Non-native species, also known as alien species or exotic species, refer to organisms that are introduced to a region or ecosystem where they are not historically native (Simberloff et al., 2013). Non-native species are often introduced by human activities, either intentionally or accidentally. Many non-native aquatic predatory species can thrive in new environments free of their native predators, competitors, or diseases (Sagoff, 2005). This can lead to significant ecological changes and potentially harmful effects on native biodiversity, including displacement of native species, disruption of local ecosystems, and alteration of habitat (Simberloff et al., 2013). For example, the intentional introduction of Yellowstone Cutthroat Trout (*Oncorhynchus clarkii bouvieri*) in Montana negatively affected the distribution of native Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*) (Dunham et al., 2004).

Climate change refers to significant spatiotemporal changes in the statistical distribution of weather patterns over periods ranging from decades to millions of years (Solomon et al., 2009). It can be a change in average weather conditions or in the distribution of weather around the average conditions (i.e., more or fewer extreme weather events). Modern climate change is largely attributed to the increased levels of atmospheric carbon dioxide produced by the use of fossil fuels and broad deforestation, leading to phenomena such as global warming (Solomon et al., 2009). The effects of climate change on stream ecosystems are anticipated to manifest both directly and indirectly. Direct effects include changes in stream temperature and alterations in runoff patterns and water flow. Indirect impacts are likely to occur through changes in vegetation along stream banks and in surrounding watersheds (Mulholland et al., 2009). Warmer waters lead to thermal stress and lower oxygen levels, making these habitats less hospitable to some species (Petty et al., 2005). The compounding effects of landscape alterations, non-native species, and climate change will inevitably affect the aquatic biodiversity across North America. In terms of conservation, the southern Appalachian Mountains should be placed high on the priority list because this region is a world-renowned "hotspot" for biodiversity that includes over 100 fish species, 2,250 species of vascular plants, 117 species of amphibians and reptiles, 175 species of birds and 65 species of mammals (Clark et al., 2001; Mitchell et al., 1999).

Landscape alterations, introduction of non-native species, and climate change are points of concern for biodiversity in southern Appalachian Mountains. Landscape alterations such as the construction of roads and urban development have fragmented habitats and altered the natural flow of streams (Baker,1995; Ekka et al., 2020). Such habitat fragmentation critically impacts Brook Trout which rely on continuous, undisturbed aquatic corridors. These disruptions reduce the genetic diversity and resilience of populations by limiting their range and access to

breeding areas (Zastavniouk et al., 2017). The introduction of non-native species also poses significant threats to native stream fauna. Another study showed that Eastern Hellbender (*Cryptobranchus alleganiensis*) larvae responded stronger to cues from native salmonids (Brook Trout) than non-native salmonids (Rainbow Trout); this interaction is thought to be driven by the decreased time the non-native salmonids and Eastern Hellbender have coexisted (Gall & Mathis, 2010). In other words, the Eastern Hellbender is likely more susceptible to predation by non-native salmonids when compared to native salmonids. Furthermore, climate change exacerbates these challenges by altering precipitation patterns and increasing air and water temperatures. This leads to changes in stream hydrology and water quality which affect the reproductive cycles and survival of native aquatic species such as Brook Trout. These fish are particularly vulnerable to temperature increases because they require cold, oxygen-rich water to thrive. Rising temperatures can lead to thermal stress and decreased dissolved oxygen levels, significantly impacting Brook Trout populations (Meisner, 1990). The compounding effects of landscape alterations, non-native species, and climate change drive significant negative environmental changes for Brook Trout in the southern Appalachian Mountains (Kazyak et al., 2022).

Brook Trout (*Salvelinus fontinalis*) are widely distributed from northern Canada south to the Appalachian Mountains in the United States (Hudy et al., 2008). They are found in freshwater environments characterized by clear, cold, oxygen-rich waters which are essential for their survival and reproduction (Hudy et al., 2008). These fish prefer stream habitats with abundant cover such as logs, rocks, and overhanging vegetation which provide protection from predators and strong water currents, as well as rich food sources like insects and crustaceans (Curry and Noakes, 1995). Ecologically, Brook Trout are a keystone species in their habitats. They play a crucial role in structuring community dynamics and influencing the food web. As

both predators and prey, Brook Trout impact the populations of other aquatic organisms, helping to maintain the ecosystem (Flebbe et al., 2006). They are opportunistic feeders, predominantly consuming aquatic and terrestrial insects, but their diet can also include smaller fish and other aquatic organisms depending on the availability (Petty et al., 2005). Brook Trout are also known for their sensitivity to environmental changes, particularly changes in temperature, sedimentation and chronic acidification (Neff et al., 2009). They are often used as an indicator species for monitoring the health of freshwater ecosystems. Increases in water temperature and decreases in water quality can lead to habitat loss for Brook Trout, influencing their distribution and abundance in affected areas (Meisner, 1990).

Native Brook Trout found within the southern Appalachian region are commonly referred to as Southern Appalachian Brook Trout (SABT) (Ewing, 2023). The negative effects of landscape alterations, non-native species, and climate change on SABT are exacerbated by the steep terrain and erodible soils of the southern Appalachians (Clark S. et al., 2001). Landscape alterations, such as road construction and deforestation, result in an influx of sediment into streams (Baker,1995; Ekka et al., 2020). The influx of sediment can smother SABT spawning beds, reducing both reproductive success and juvenile survival rates. Increased sediment loads can also degrade water quality, further stressing SABT populations already vulnerable to temperature fluctuations and reduced dissolved oxygen levels (Ficke et al., 2009)

Dams and roads influence SABT habitat fragmentation by creating physical barriers preventing upstream movement (Januchowski-Hartley et al., 2013). SABT access upstream habitats for spawning and as cold refuges during warmer months, critically impacting their life cycles and population dynamics (Petty et al., 2005). Non-native species such as Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo trutta*) are encroaching upon SABT habitats,

exacerbating their decline (Kanno et al., 2016). The introduction of Rainbow Trout (*Oncorhynchus mykiss*) into these ecosystems has led to competition with SABT for resources and habitat, often resulting in the displacement of SABT. Rainbow Trout are more aggressive than SABT and can dominate food resources, pushing Brook Trout out of optimal habitats and into less suitable environments (Larson & Moore 1985). The genetic diversity of SABT not only marks them as unique among Brook Trout populations but also highlights their specialized adaptations to the distinct environmental conditions of the southern Appalachians (Hayes et al., 1996; Kazyak et al., 2015). Understanding these genetic differences is crucial as it informs conservation strategies tailored to preserve the unique lineage of SABT in the face of ongoing environmental pressures.

There are significant genetic differences between northern strain Brook Trout and southern Appalachian Brook Trout measured by both allozymes (Stoneking et al., 1981) and microsatellites from mitochondrial and nuclear DNA (Hayes et al., 1996; Kazyak et al., 2015). These genetic differences suggest a long history of isolation and local adaptation. Genetic variation of SABT can also be significant within SABT populations and in streams that are geographically close in proximity (Weathers et al., 2019). One pivotal study by Kazyak et al. (2015) used microsatellite DNA markers to examine the genetic structure of SABT populations throughout their range. They concluded that each subpopulation of SABT both within and among streams possesses unique genes and morphology because of varying influences from genetic drift, selection, and phenotypic plasticity (Kazyak et al., 2015). Barriers, such as waterfalls and cascades, also fragment populations, leading to unique mitochondrial DNA and nuclear DNA signatures in populations above and below barriers (Whiteley et al., 2013). The fragmentation from barriers influences populations below the barriers that are exposed to higher temperatures

and non-native fish while those populations above the barriers do not have those selection pressures (Meisner, 1990; Moore et al., 1986). This may be another reason that we see such strong genetic differences between above-barrier and below-barrier populations.

These genetic distinctions are crucial for conservation, as they highlight the need for region-specific management practices that acknowledge the unique genetic makeup and ecological requirements of SABT. Preservation of genetic diversity within the species is essential for resilience and adaptability to changing environmental conditions, and safeguarding against the loss of valuable traits (Habera, & Moore, 2005).

Populations of SABT within South Carolina are considered to be rear-edged populations (Pregler et al., 2018). Rear-edged populations, typically found in the farthest reaches of a species' distribution, face unique challenges due to their isolation and small population sizes (Hampe & Petit, 2005). Populations of SABT found within South Carolina had some of the lowest genetic diversity ever recorded for SABT (Pregler et al., 2018), which is a common symptom of rear-edged populations (Davis and Shaw 2001; Hampe and Petit 2005). Pregler et al. (2018) determined that SABT populations in South Carolina displayed a combination of genetic signatures from both southern and northern populations. Of the 18 patches analyzed, six demonstrated evidence of genetic mixing due to historical stocking efforts. Three of these patches had high levels of hatchery genetic admixture, with values significant enough to suggest they may predominantly consist of hatchery descendants. Conversely, the remaining 12 patches showed no indications of genetic influence from hatchery stocks (Pregler et al., 2018). Using the genetic characterization of SABT from Pregler et al. (2018), my study locations coincide with three of the 12 patches of SABT, all from the Santee drainage within South Carolina, that had

retained their native genetic integrity (i.e., South Saluda River, Slicking creek, and Laurel Creek).

With an increasingly recognized threat of habitat loss and genetic vulnerability facing SABT, I partnered with Greenville Water Utility to undertake fish distribution assessments. This collaboration had several objectives, including: (1) assessment of Southern Appalachian Brook Trout populations to identify source streams that can be used for potential reintroduction projects and (2) characterization of instream habitats of both streams with extant populations and streams where reintroductions are possible.

Study Area:

Greenville (SC) Water Utility owns and manages both the South Saluda and North Saluda sub-watersheds, which are roughly 15.5 miles (25 km) apart, and combined area of approximately 30,000 acres (Austin & Krueger, 2014). The South Saluda sub-watershed is 10,000 acres situated between the North Carolina and South Carolina border and Table Rock State Park. The North Saluda sub-watershed is approximately 20,000 acres along the North Carolina border in northern Greenville County (Fig.1). The vegetation across the two sub-watersheds is primarily dominated by mature deciduous forest hosting widespread species such as Scarlet Oak (*Quercus coccinea*) and Chestnut Oak (*Quercus prinus*) with limited coverage of coniferous trees such as Pitch Pine (*Pinus rigida*) and Virginia Pine (*Pinus virginiana*) (Austin & Krueger, 2014). The riparian zones are now primarily dominated by hardwoods since the extirpation of the Eastern and Carolina hemlocks (*Tsuga canadensis*, *Tsuga caroliniana,* respectively), Rhododendron (*Rhododendron maximum*) and Mountain Laurel (*Kalmia latifolia*) fill the open riparian habitat. The streams are categorized as high-gradient systems with south facing slopes. In the northern hemisphere, south facing slopes receive significantly more

exposure to solar radiation making them warmer and drier then north facing slopes (Bennie et al., 2008). The streams in the South Saluda sub-watershed flow northwest to southeast (Fig. 2), and North Saluda sub-watershed streams flow southwest to northeast (Fig. 3).

Greenville Water Utility first purchased the South Saluda sub-watershed in the early 1920s then later bought the North Saluda sub-watershed in the early 1950s. Greenville Water Utility managed the land themselves until 1993. In 1993, a conservation easement between Greenville Water Utility and the South Carolina Chapter of The Nature Conservancy was approved. Since the purchase of the two sub-watersheds, tree harvesting has taken place; however, the extent and practices are unknown (Austin & Krueger, 2014). Nonetheless, there are still patches of forests that could be considered old growth forests with trees dating to over 250 years old (Austin & Krueger, 2014). Greenville Water Utility and South Carolina Department of Natural Resources continue to maintain the South Saluda and North Saluda sub-watersheds. Since 1993, the only major disturbances are from access roads and a forest fire started in Table Rock State Park in 2016 (South Carolina Forestry Commission, 2021).

Three out of five of our target streams are located within the South Saluda sub-watershed including Laurel Creek, South Saluda River, and Slicking Creek. Although the streams are geographically close, they exhibit varying SABT distribution. All three streams have supported genetically unaltered populations of SABT (Table 1) in the past (Pregler et al., 2018).

While Laurel Creek provides habitat for both RBT and SABT, in the uppermost reaches, we observed populations of SABT without any encroachment from RBT. As we moved downstream, we encountered a series of plunge pools of varying sizes, some less than a meter in height and others exceeding 3 meters in height. Neither SABT nor RBT were detected within these reaches. Downstream of these plunge pools, as the gradient became more gradual, we

detected RBT. Thus, these plunge pools in Laurel Creek appear to serve as a barrier and that, combined with the absence of non-native fish species, renders Laurel Creek a suitable candidate as a template stream for our study.

The South Saluda River (SSR) has a distinctive pattern of headwater streams originating from gradual slopes, transitioning into a series of cascades and falls in lower reaches before flowing into Table Rock Reservoir (Austin & Krueger, 2014). I am uncertain of the effectiveness of the falls and cascades as barriers as it hosts populations of RBT and SABT above the falls and cascades.

Slicking Creek historically harbored populations of SABT (Pregler et al., 2018). However, following wildfires in 2016, recent observational data indicates the extirpation of SABT from the creek. Historically, Slicking Creek, spanning approximately 6 miles (9.6 km), boasted SABT populations with individuals ranging from 250 mm in total length in the headwaters up to 380 mm in larger pools (Rankin & Geddings, 1992). Slicking Creek has no documented history of stocking (Rankin & Geddings, 1992). Despite the presence of large SABT, densities were lower in Slicking Creek compared to the South Saluda River and Laurel Creek (Rankin & Geddings, 1992). Anecdotal observations suggest a reduction in canopy cover in Slicking Creek since the 2016 fires.

Two of our five target streams: Brice Creek and the North Saluda River are located in the North Saluda sub-watershed (Fig. 3). Neither Brice Creek (BC) nor the North Saluda River (NSR) currently have persisting populations of SABT. The only historical evidence of their presence is limited to anecdotes from locals that have spoken with Greenville Water Utility. Given that current populations of SABT persist roughly 24 km away in the South Saluda sub-watershed, these two streams are being analyzed for possible reintroduction of SABT.

Brice Creek (BC), stretching approximately 5 km, is a potential site for SABT reintroductions, owing to its distinctive features. Notably, BC has a substantial waterfall, greater than 10 m, that serves as a formidable barrier and prevents upstream fish migration. While Rainbow Trout (RBT) populations thrive in the lower reaches of BC, there are no RBT above the waterfall (Rankin & Geddings, 1992). This absence of RBT above the waterfall renders BC an appealing candidate for SABT reintroduction. The presence of such a significant natural barrier, coupled with the lack of competing RBT populations, underscores Brice Creek's potential suitability for SABT reintroduction. However, the viability of translocation hinges on ensuring that habitat characteristics above the waterfall remain comparable to those of LC. If such similarities persist, BC presents a promising opportunity for SABT restoration efforts.

The North Saluda River has only RBT, and it has a more traditional arrangement of high slopes in the headwaters with a declining gradient as the water flows towards the lower reaches (Austin, & Krueger, 2014; Rankin, & Geddings, 1992). The North and South Saluda rivers are the largest streams in this study; they are approximately twice the width of other targeted streams.

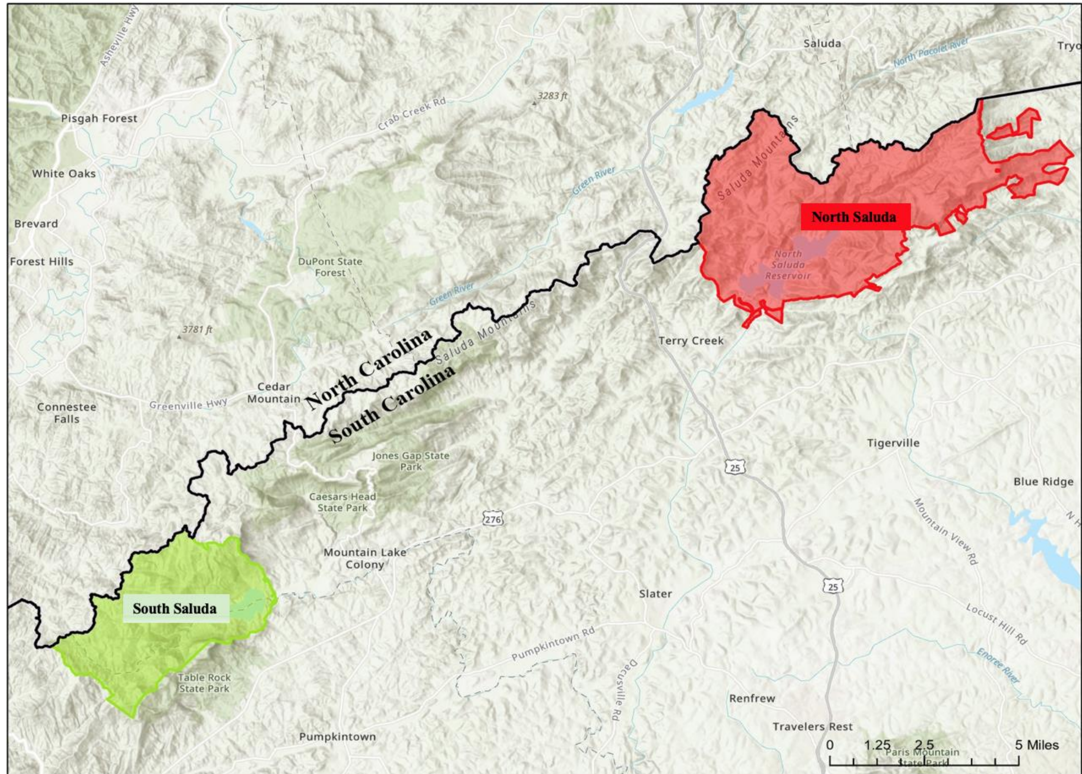

Figure 1: Map of South Saluda and North Saluda sub-watersheds. The green area indicates Southern Appalachian Brook Trout (SABT) presence in South Saluda, and the red area indicates SABT absence in the North Saluda.

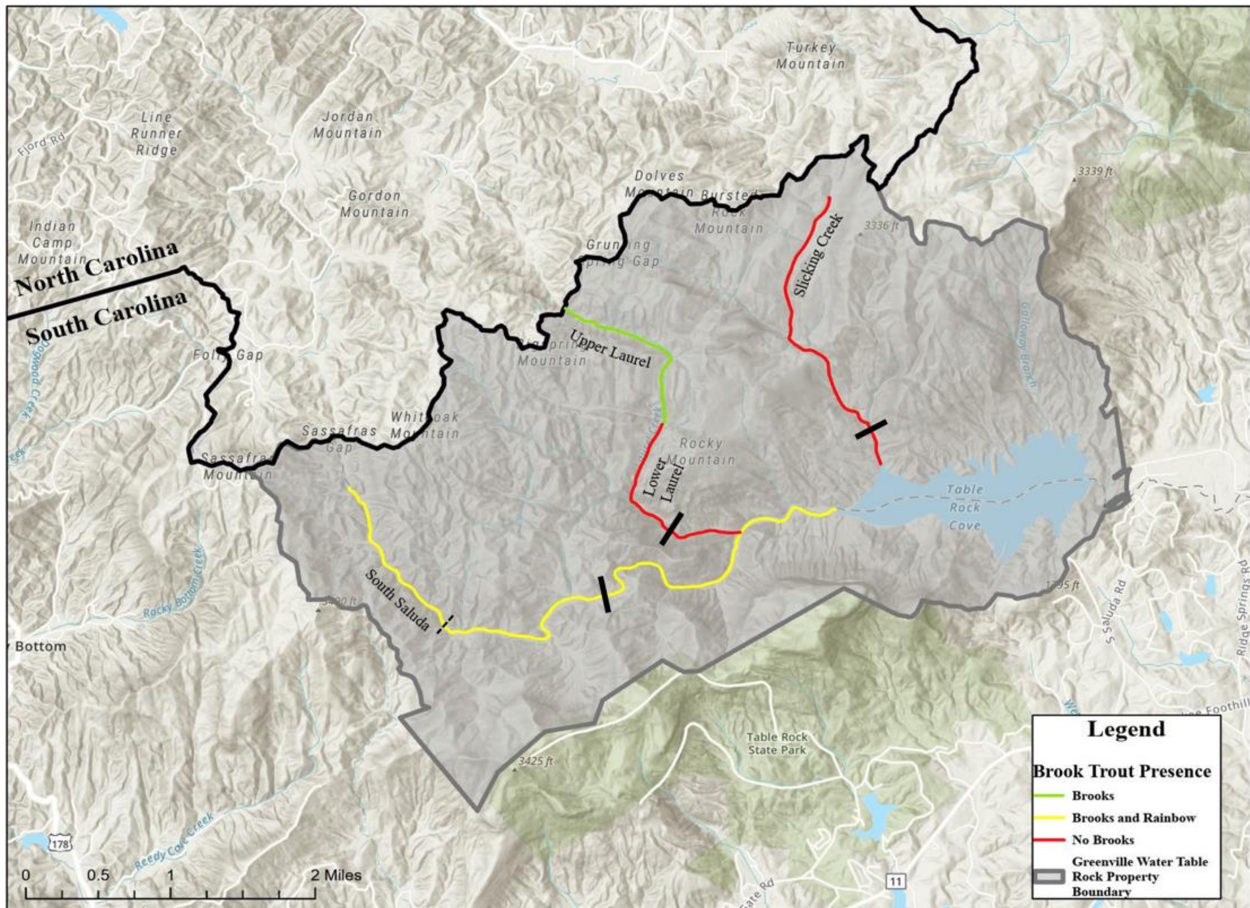

Figure 2: Map of the South Saluda sub-watershed with three target streams: South Saluda River, Laurel Creek, and Slicking Creek. The bold black lines indicate barrier locations, and the thin dashed black line indicates a potential barrier.

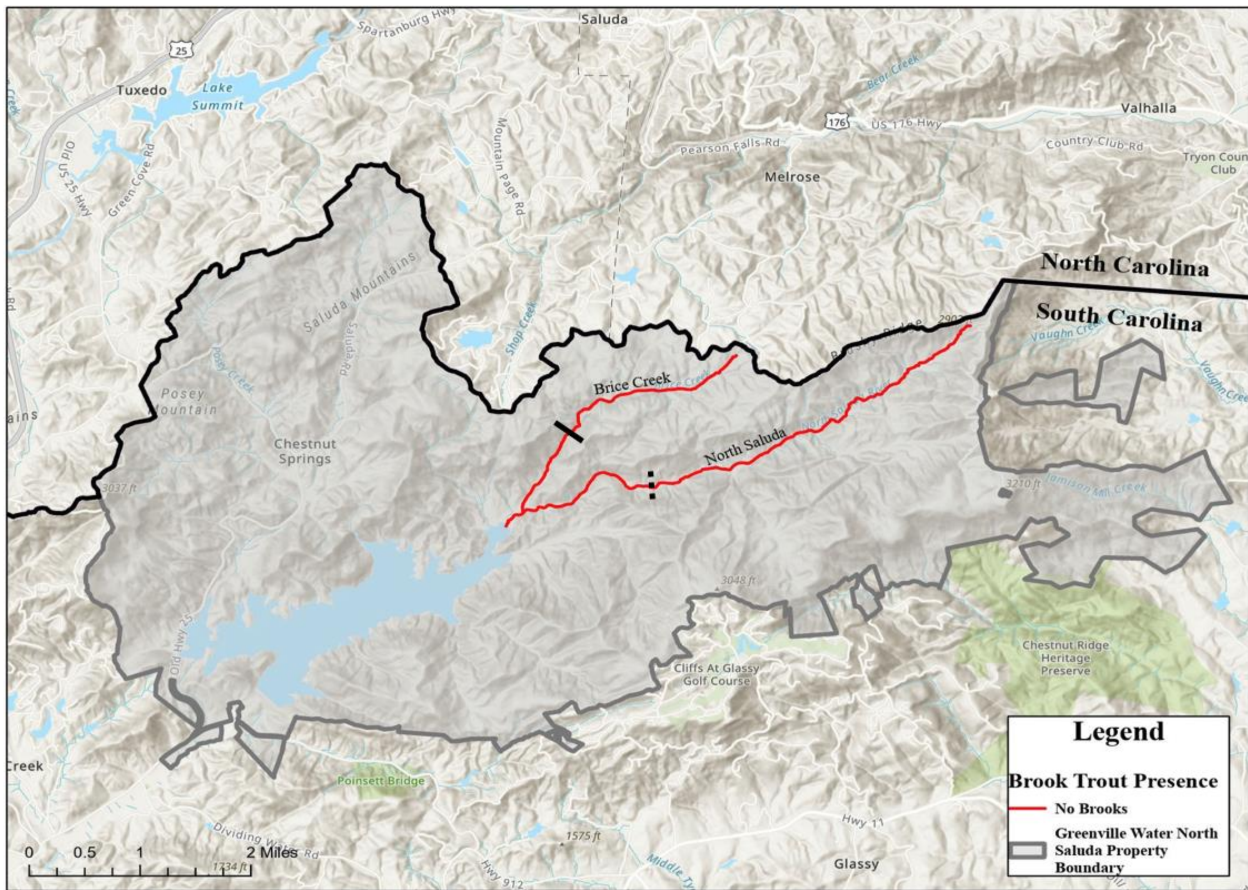

Figure 3: Map of the North Saluda sub-watershed with two target streams Brice Creek and North Saluda. The bold black line indicates a barrier location, and the thin dashed black line indicates a potential barrier.

Table 1: Stream names and situation of target streams within the South Saluda sub-watershed.

Stream	SABT present/absent	RBT present/absent	Situation
South Saluda River	Present	Present	SABT were found in the upper reaches co-occurring with RBT
Slicking Creek	Absent	Absent	Historically have had SABT but have been extirpated
Laurel Creek	Present	Present	SABT in upper reaches, RBT in lower reaches

Table 2: Stream names and situations of target streams in North Saluda sub-watershed.

Stream	SABT present/absents	RBT present/absent	Situation
North Saluda River	Absent	Present	Has several large barriers and have sparse RBT populations in upper reaches
Brice Creek	Absent	Absent	Translocation opportunity for SABT if suitable

Methods:

I studied five streams located within the Saluda Basin of South Carolina. Within the greater Saluda watershed, resides the South Saluda sub-watershed which contains three of the five target streams where SABT populations currently persist in the South Saluda River (SSR) and Laurel Creek (LC). Presence of SABT in LC and SSR is supported by surveys done in 1992 (Rankin & Geddings, 1992), genetic analysis in 2018 (Pregler et al., 2018), and environmental DNA (eDNA) results supplied by Greenville Water Utility collected in 2022. Slicking Creek (SLC) has historical evidence of SABT presence (Rankin & Geddings, 1992; Pregler et al., 2018), however, the eDNA data collected in 2022 did not detect SABT in SLC. The North Saluda sub-watershed, located within the greater Saluda watershed, contains two of our five target streams, including Brice Creek (BC) and North Saluda River (NSR). Neither of these streams had a positive detection of eDNA for SABT, a result that is supported with survey results from 1992 (Rankin & Geddings, 1992). With the advancement of genetic technologies, understanding how habitat plays a role in genetic diversity is crucial for prioritizing fish conservation (Martinez et al., 2018). SABT require specific substrates for spawning and the presence of riparian vegetation for maintaining water temperature. These specific habitat requirements highlight the need for detailed habitat assessments (Ficke et al., 2009).

To further understand specific habitat requirements for SABT, I assessed the following variables: habitat unit (i.e., riffle, run, or pool) dimensions, substrate composition, large woody debris (LWD), cover count, and percent canopy cover. These habitat measurements can help quantify suitable habitat for SABT populations. Greenville Water Utility (GWU) supplied us with both eDNA results and temperature data. They collected temperature data via temperature loggers that were placed in all five target streams.

I identified potential natural geomorphic barriers before collecting SABT abundance and instream habitat data. Geomorphic barriers, such as shelves or falls, are natural and permanent obstacles that disrupt the free movement of fish through their aquatic habitat (Myers et al., 2024). Sampling above and below potential barriers is a common characteristic of streams that are analyzed for fish distribution and habitat (Kelson et al., 2015). We identified three reaches above and three reaches below potential barriers in all target streams except for SLC. We were unable to access the stream below the barrier in SLC because of steep bedrock cascades that made the stream segments too hazardous to analyze.

I sampled SABT populations in Laurel Creek and South Saluda River using single-pass electrofishing for 10 minute intervals. This non-lethal technique (Temple & Pearsons, 2007) involves swiftly removing fish from the electrical field for examination. Upon capture, we carefully inspected each individual for signs of disease and measured total length (TL) to the nearest 1 mm. We then promptly released each individual back into the stream, minimizing handling to mitigate the risk of mortality. The data collected on abundance (Catch Per Unit Effort) enabled us to assess viability of SABT populations to be source populations in Laurel Creek and South Saluda River. Additionally, we conducted electrofishing in all other targeted

streams to validate environmental DNA (eDNA) and survey findings provided by Greenville Water Utility (Fig. 4 & 5).

To initiate single-pass electrofishing methods, we extended a seine across the stream at the transition point between different habitat units, such as pools, riffles, or runs. This strategic placement ensured that our target fish remained within the intended habitat unit and prevented them from drifting downstream after being shocked. Starting at the seine, we sampled by electrofishing upstream through the targeted habitat unit, then sampling back down to the seine, then back through the habitat unit concluding upstream at the transition points between habitats to thoroughly sample each habitat unit. This involved at least one person controlling the backpack electrofisher and another person using a dip net to catch the stunned fish. In instances where additional field technicians were available, we employed two shockers and two individuals with dip nets caught the stunned fish to increase efficiency. Initially, the voltage was set at 600 volts and adjusted accordingly based on the effectiveness of capture and to minimize the risk of mortality. The collection of habitat data required us to walk through and disturb the habitat; therefore, we sampled fish beforehand to minimize disruption. The number of reaches shocked varied between streams. We shocked seven reaches in LC, six reaches in SSR, five reaches in BC, and two reaches in SLC and NSR.

The habitat sampling included the reaches electroshocked for SABT abundance and extended past the reaches electroshocked. I collected habitat data across multiple habitat units (i.e., riffle, run, pool), above and below potential barriers. The study area encompassed several 100-meter reaches across multiple streams, including six reaches in Laurel Creek, three in Slicking Creek, nine in South Saluda River, seven in North Saluda River, and six in Brice Creek. We assessed each habitat unit for habitat dimensions (i.e., length, width, and depth), substrate

composition, pebble diameter distribution, percent canopy cover, in-stream cover count, and large woody debris count.

Habitat units were identified and categorized as either riffle, pool, or run. Categorizing stream habitats into riffle, pool, or run is used often by biologists to determine sampling units (Jowett, 1993). We considered a habitat unit as a riffle if the water was quick-flowing and the majority of the water surface was broken. We considered a habitat unit as a pool if the water was deep and slow-flowing with little to no breaks on the water surface. We considered a habitat unit as a run if the water was quick-flowing with a wavy water surface with some breaks on the water surface (Jowett, 1993). SABT exhibit distinct preferences for various stream habitats, including pools, riffles, and runs, which they utilize for different activities throughout their lifecycle (Ficke et al., 2009). Pools serve as essential resting areas for SABT, providing refuge from swift currents, and during warmer months, pools offer cooler water temperatures, serving as vital thermal refuges for trout (Anglin & Grossman, 2013). Riffles play a crucial role in oxygenating the water, which is essential for maintaining a healthy environment for aquatic life. The high oxygen levels help support a diverse range of aquatic organisms upon which Brook Trout feed (Fausch et al., 1995). Runs, which connect riffles to pools, facilitate efficient movement within the stream network, allowing Brook Trout and other aquatic species to navigate and use different habitat zones effectively. This connectivity is vital for the lifecycle of Brook Trout, especially for spawning and accessing feeding areas (Schlosser, 1995).

We took width measurements at 25%, 50%, and 75% of the habitat unit's total length, creating three transects (Fig. 7). We measured depth across the three width measurements at 25%, 50%, and 75% of each transect, totaling nine depth measurements in each identified habitat unit.

We visually evaluated dominant and subdominant substrate composition and measured pebble diameter at nine locations (three samples per transect) that corresponded with depth measurements in each habitat unit (Fig.

6). We measured pebble diameter (in millimeters) by reaching beneath the depth stick and haphazardly selecting a pebble at each depth measurement location and measuring the pebble along its intermediate axis. We used pebble diameter to further characterize substrate compositions. SABT need varying substrate compositions depending on what stage of the life cycle. For example, during the spawning season, SABT preferentially select gravel-sized substrates, ranging from 4mm to 64mm, for constructing their spawning beds, or redds (Curry & Noakes, 1995). SABT during this time tend to avoid finer substrates smaller than 4mm due to the associated reduction in embryo survival (Alexander & Hansen, 1986).

We determined percent canopy cover using “Canopeo” (Patrignani & Ochsner, 2015), at the center of each habitat unit to quantify light exposure at the water surface (Fig. 6). Percent canopy cover refers to the percentage of the water surface that is covered by shade from overhanging vegetation. Canopy cover serves as a fundamental component of SABT habitat and survival. As cold-water fish, SABT are highly sensitive to changes in water temperature, and canopy cover plays a role in regulating thermal conditions within their aquatic environment (Petty et al., 2012). The shade provided by overhanging vegetation helps to mitigate temperature fluctuations by reducing solar radiation exposure, thus maintaining cooler water temperatures essential for cold water fish, such as SABT (Beechie et al., 2003). Canopy cover also contributes to the preservation of dissolved oxygen levels in the stream, as cooler water holds more oxygen than warmer water. This oxygenation is vital for Brook Trout respiration and overall metabolic functions (Chicoine, 2017). Moreover, the structural complexity created by canopy cover offers

essential habitat features for SABT, providing refuge from predators and facilitating foraging opportunities. Fallen branches, submerged roots, and overhanging vegetation create diverse microhabitats within the stream, enhancing SABT ability to feed, rest, and spawn (Hilderbrand et al., 1997).

I identified in-stream cover as any feature, such as boulders, woody debris, or undercut banks, that could potentially provide refuge for Brook Trout from predators or high flow events (Kozel & Hubert, 1989). I counted woody debris as LWD if it exceeded 10 cm in diameter and 1 m in length within the stream channel. We separated large woody debris (LWD) from counts of other cover types because it is more beneficial than large boulders and undercut banks for Brook Trout (Faustini & Jones, 2003), and the presence of LWD enhances stream morphology (Bilby & Ward, 1991).

Our statistical objective was to: (1) determine Catch Per Unit Effort (fish/minute) for SABT populations and (2) describe the measured habitat characteristics in streams where SABT were present and correlate them with habitat characteristics in streams where SABT were absent. Upon completion of data collection and statistical analysis in Laurel Creek, comparisons across sites were facilitated, enabling the examination of habitat characteristics in Slicking Creek, South Saluda River, North Saluda River, and Brice Creek, where SABT populations were either absent or low. This comparative analysis aimed to identify stream reaches exhibiting habitat characteristics similar to Laurel Creek for potential reintroduction.

All multivariate computations were done using PRIMER-e software (PRIMER-e, Innovation Complex – Level 3.64, Massey University, 5 University Avenue, Gate 1, Albany Expressway, Albany, Auckland 0632, New Zealand). I used principal component analysis (PCA) to further visualize patterns of habitat characteristics among our five target streams. Analysis of

similarities (ANOSIM) was used to determine if present, absent, and extirpated sites varied significantly based on their habitat characteristics. I used Similarity Percentage (SIMPER), to identify what variables contributed most to differences among streams. I created box plots to display the distribution of my data, including habitat characteristics such as percent canopy cover. Such comparisons enabled the identification of potential translocation opportunities to reestablish SABT populations throughout the South and North Saluda sub-watersheds.

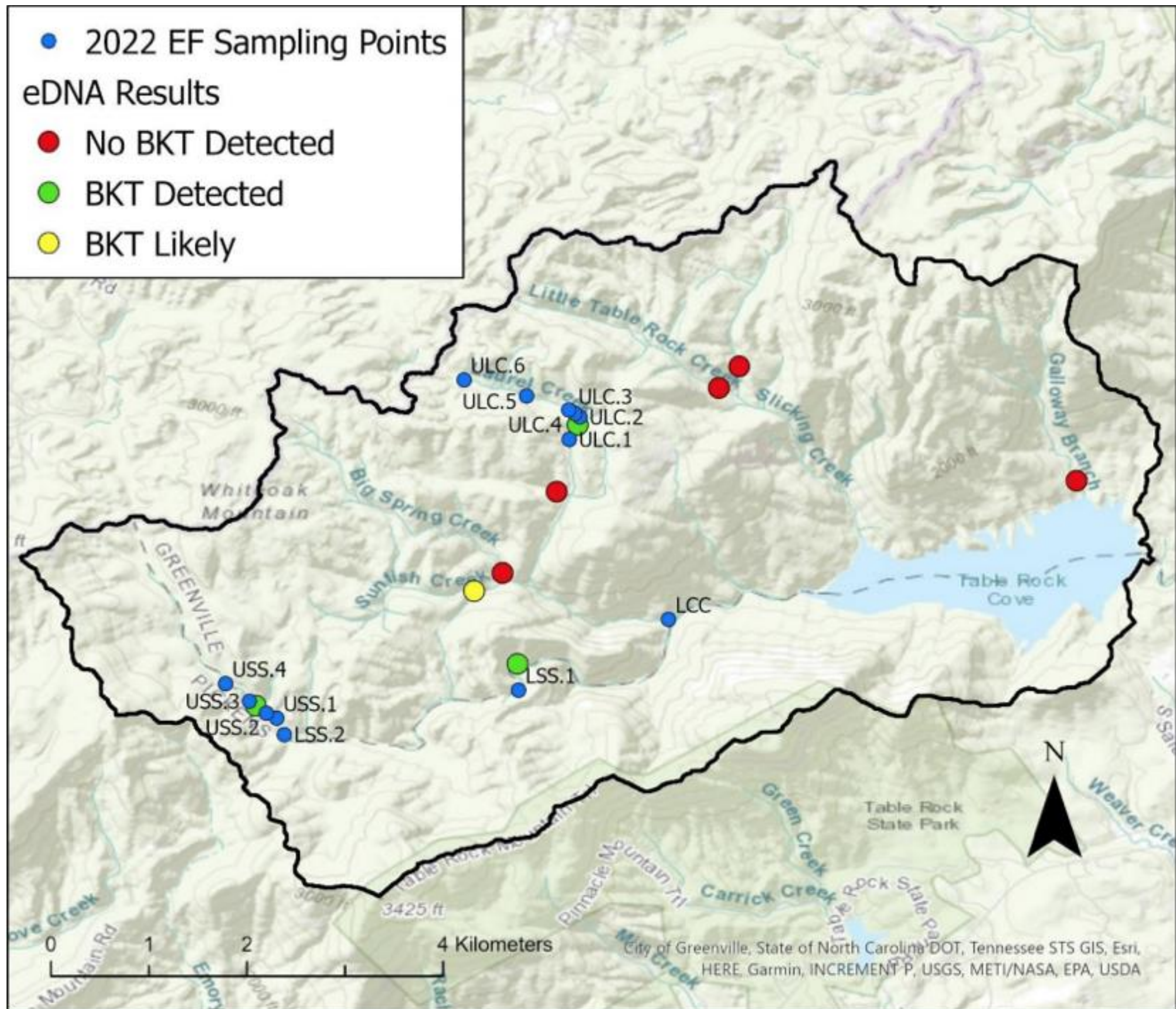

Figure 4: Map of environmental DNA sampling points, electrofishing sampling points (EF) and results for streams in the South Saluda sub-watershed provided by Greenville Water Utility.

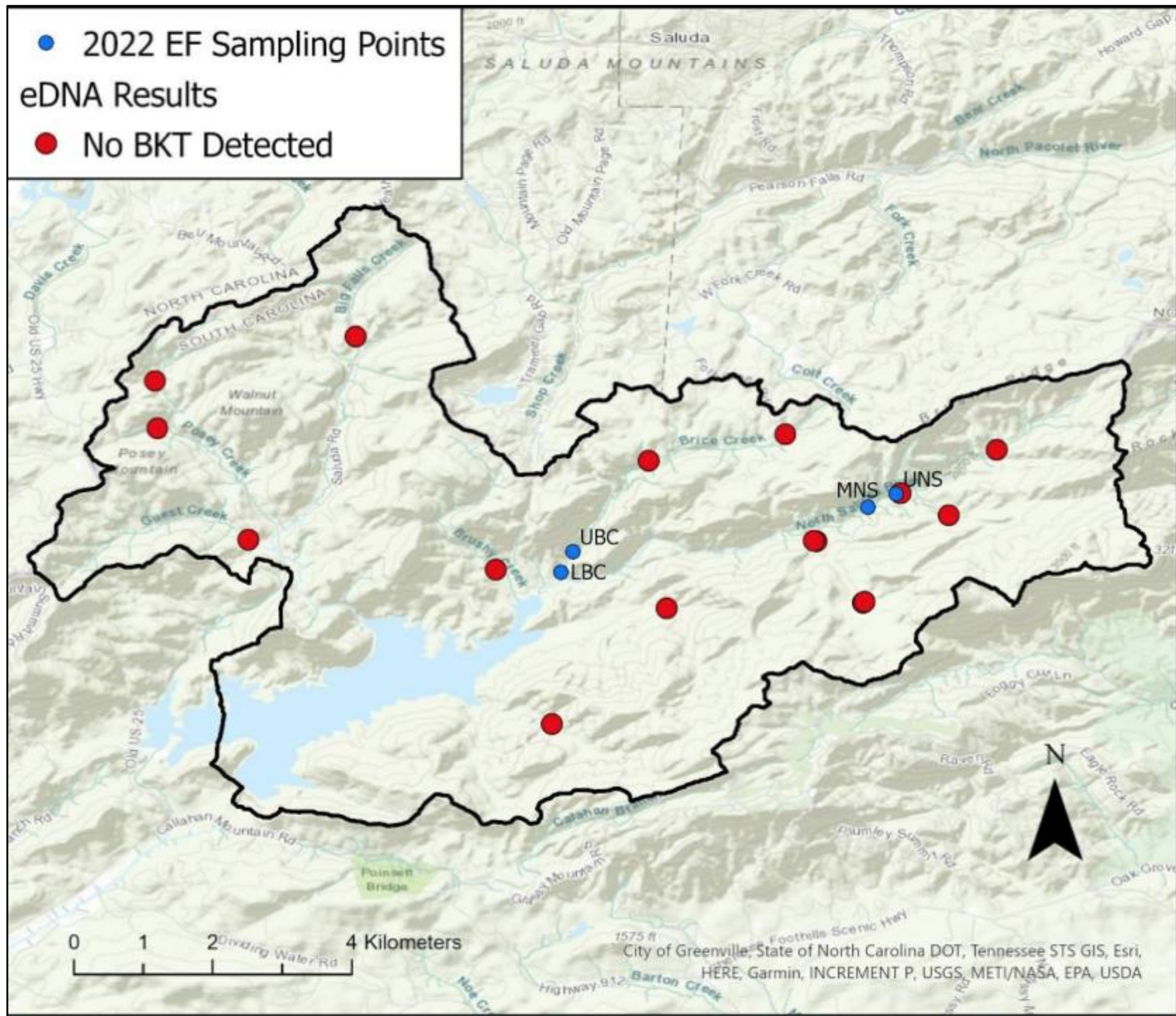

Figure 5: Map of environmental DNA sampling points and results for streams in the North Saluda sub-watershed provided by Greenville Water Utility.

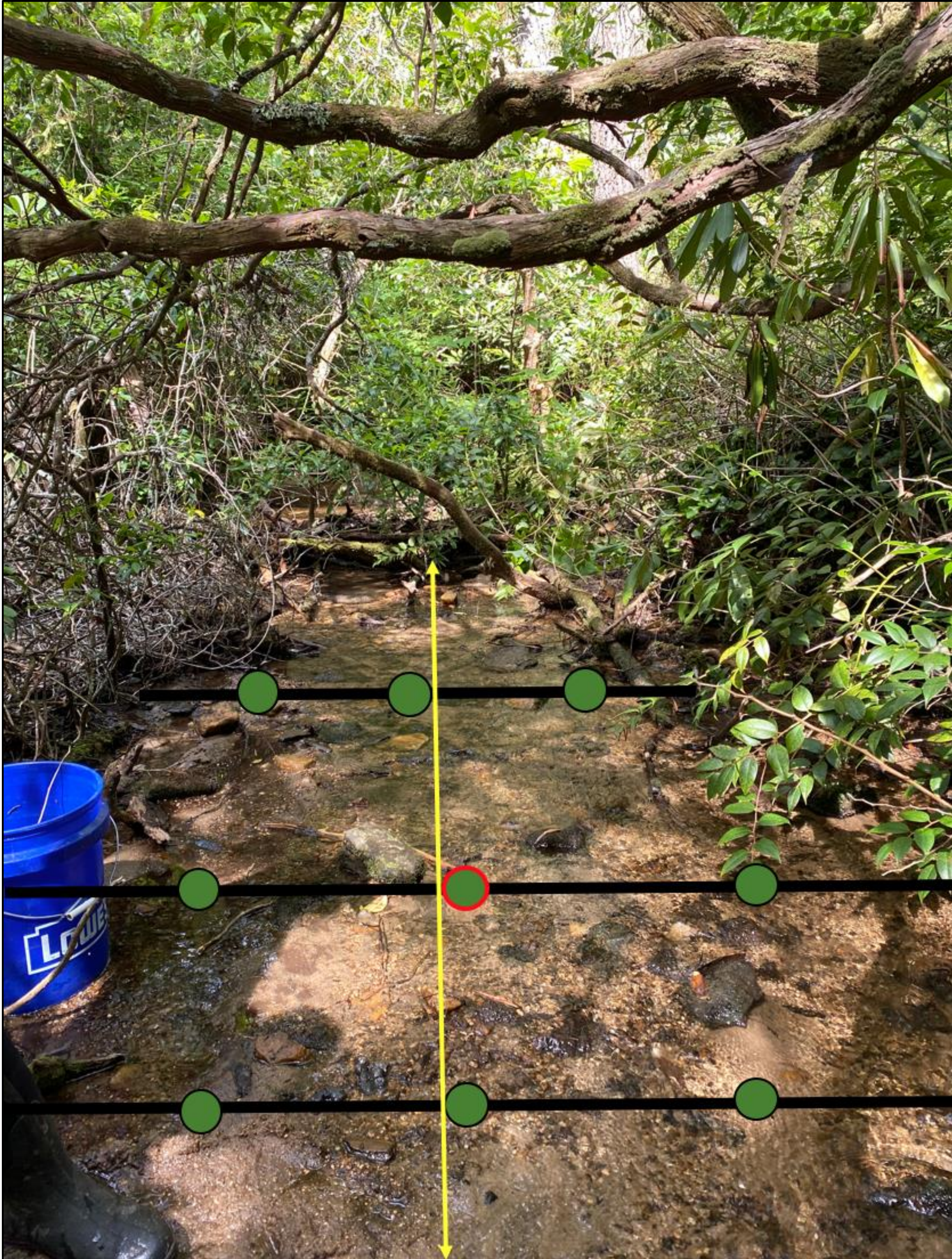

Figure 6: Habitat unit example: The yellow vertical line indicates the measurement taken for total length of habitat unit, the three-black lines represent width measurements at 25%, 50%, and 75% of total length, the nine green points are sampling points for depth, pebble diameter, and dominant and sub-dominant substrate, and the green point outlined in red is where percent canopy cover was collected.

Results:

I found potential barriers, of varying makeup and size, in all five target streams. In the South Saluda sub-watershed, Laurel Creek (LC) had a potential barrier consisting of a series of plunge pools spanning for ~400 meters (Fig. 2). Below the barrier there was no detection of SABT, however, Rainbow Trout (RBT) catch per unit effort (CPUE) was 3.1 fish per minute (f/m) (Fig. 7). Above the barrier, we exclusively detected SABT at an average CPUE of 2.06 f/m.

The South Saluda River had two potential barriers (Fig. 2); the downstream barrier was a waterfall with an approximate height of 6 m. Below the small cascade, we exclusively detected RBT with a CPUE of 1.1 f/m (Fig. 8). Above the small cascade, we detected SABT at an average CPUE of 0.12 f/m and RBT at an average CPUE of 1.6 f/m (Fig. 8). The large cascade (upstream of the first barrier, Fig. 2), was steep with low water depth and approximately 46 m long. We observed significant differences in CPUE above and below this large cascade barrier (Fig. 8). Upstream of the barrier, RBT's average CPUE decreased to 0.3 f/m, while SABT's average CPUE increased to 0.96 f/m. Slicking Creek had a significant barrier; however, downstream reaches were not safely accessible and we did not detect any fish species above the barrier.

In the North Saluda sub-watershed, where no current populations of SABT persist, we identified barriers in Brice Creek (BC) and potential barriers in the North Saluda River (NSR) (Fig. 3). The potential barrier in BC consisted of a waterfall with an approximate height of 9 m. Below this barrier, we detected RBT at a CPUE of 4.3 f/m and did not detect any RBT above the barrier. We did detect the Creek Chub (*Semotilus atromaculatus*) with a CPUE of 1.43 f/m.

The potential barrier in the North Saluda River was a waterfall that is approximately 3 m in height. We detected RBT with a CPUE of 1.0 f/m below the barrier and detected RBT with a CPUE of 0.1 f/m above the barrier.

The analysis of SABT populations in the South Saluda sub-watershed revealed distinct catch per unit effort (CPUE) variations among different streams. Laurel Creek exhibited the highest average CPUE, while South Saluda River demonstrated a comparatively lower average CPUE. The eDNA results provided by Greenville Water Utility were corroborated through electrofishing. SABT were not detected via electrofishing in Slicking Creek, Brice Creek, and North Saluda River.

ANOSIM analyses of principal components indicated that the habitat characteristics of South Saluda River, Slicking Creek, Brice Creek, and North Saluda River did not differ significantly from those of Laurel Creek ($p=0.64$). The first two axes of the PCA accounted for approximately 49% of total variation. There was no obvious clustering of SABT presence or absence sites (Fig. 9). However, the extirpated sites tended to cluster toward lower canopy cover and smaller pebble sizes (Fig. 9). I used ANOSIM to compare present to extirpated sites, and we did see significant dissimilarities ($p= 0.005$), with percent canopy cover making up 77.5% of the variation between present and extirpated sites (Fig. 10). Temperature loggers recorded the highest average monthly temperature in Slicking Creek, followed by North Saluda River, Brice Creek, Laurel Creek, and South Saluda River (Fig. 11). These comparisons offer valuable insights into potential translocation opportunities for reestablishing SABT populations throughout the South Saluda and North Saluda sub-watersheds.

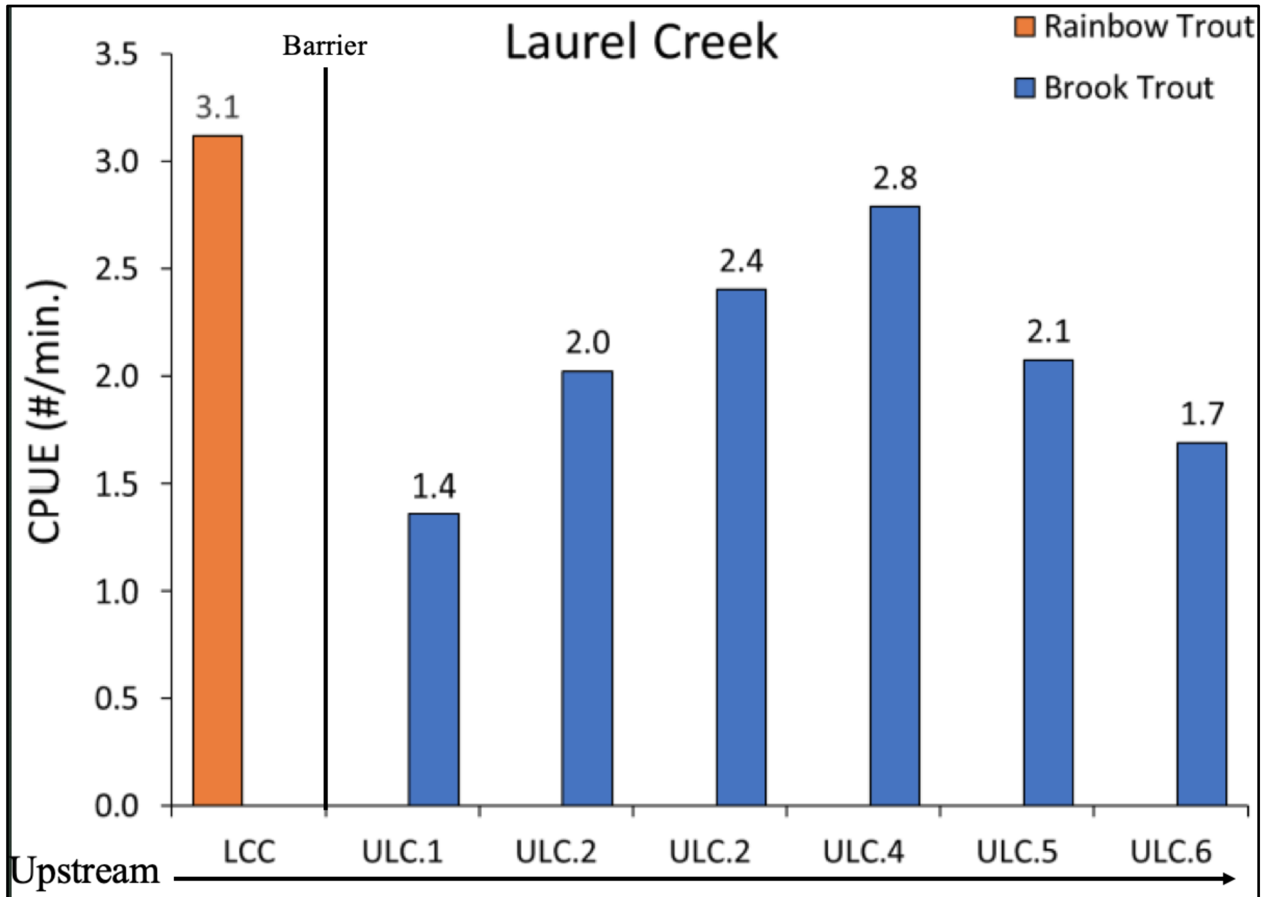

Figure 7: Catch per unit effort (CPUE) for Laurel Creek. Blue bars represent CPUE for SABT and orange bars represent CPUE for RBT. Showing Lower sections of Laurel Creek (LCC) and upper Laurel Creek (ULC) CPUE.

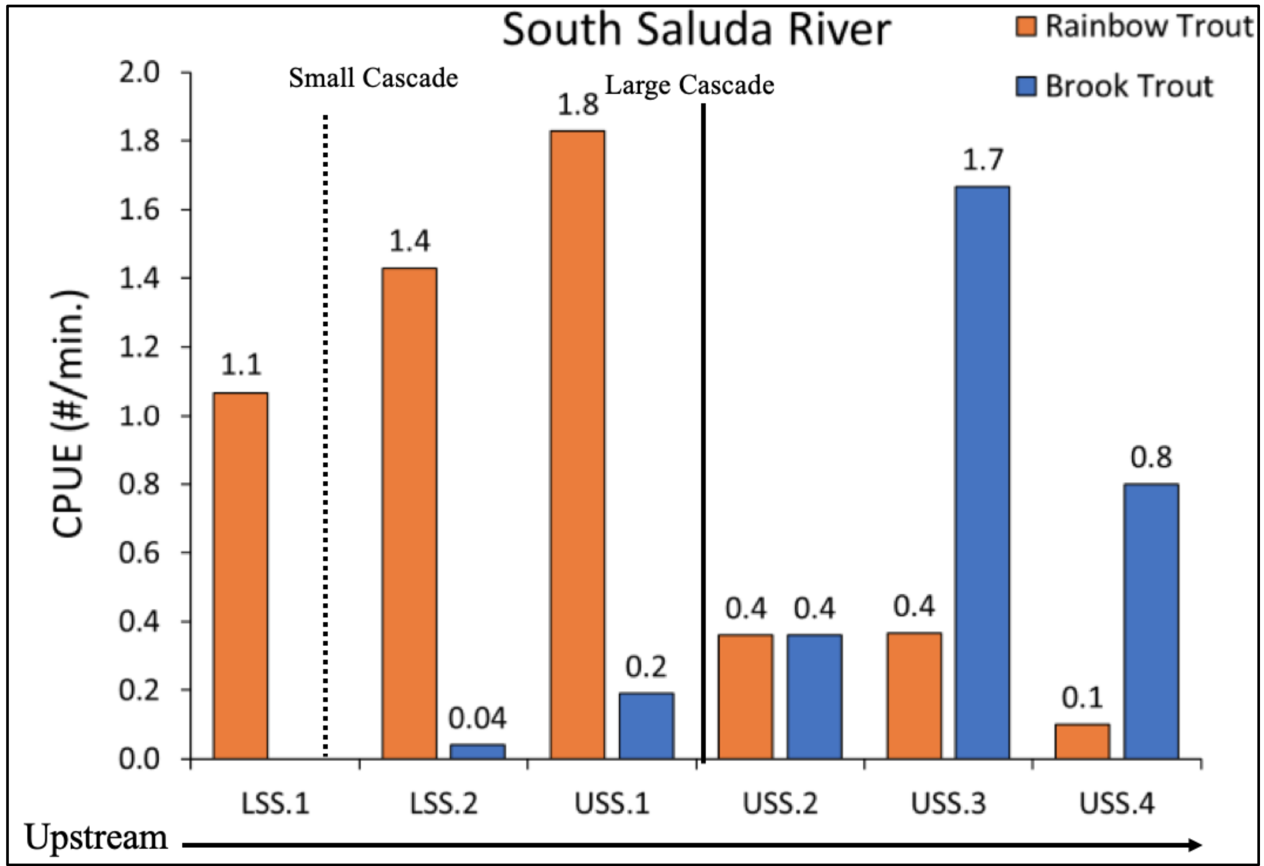

Figure 8: Catch per unit effort (CPUE) for SABT and RBT. Blue bars represent CPUE for SABT and orange bars represent CPUE for RBT.

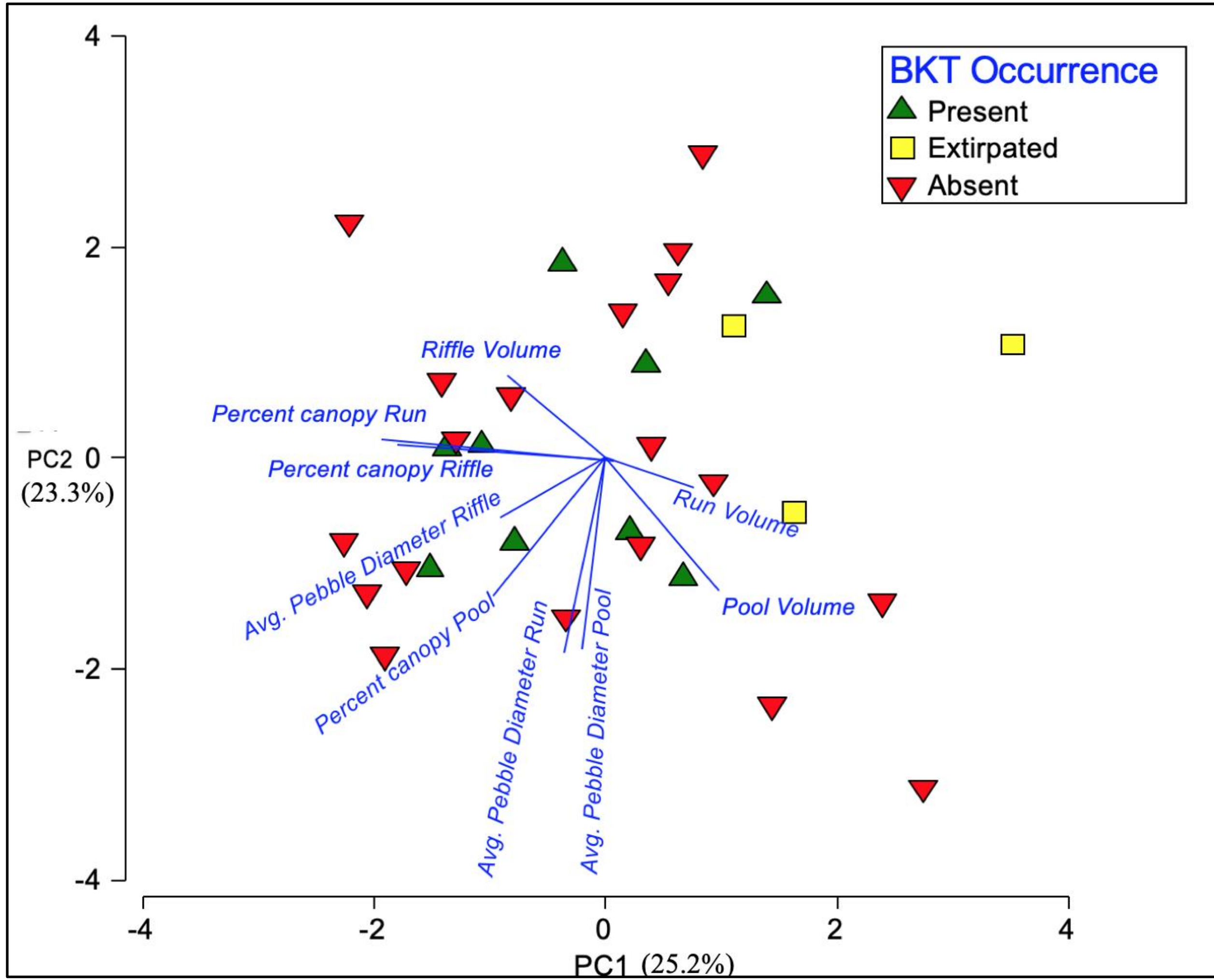

Figure 9: Principal Component Analysis (PCA) results. Habitat characteristics cluster between present (green), extirpated (yellow), and absent (red) sites.

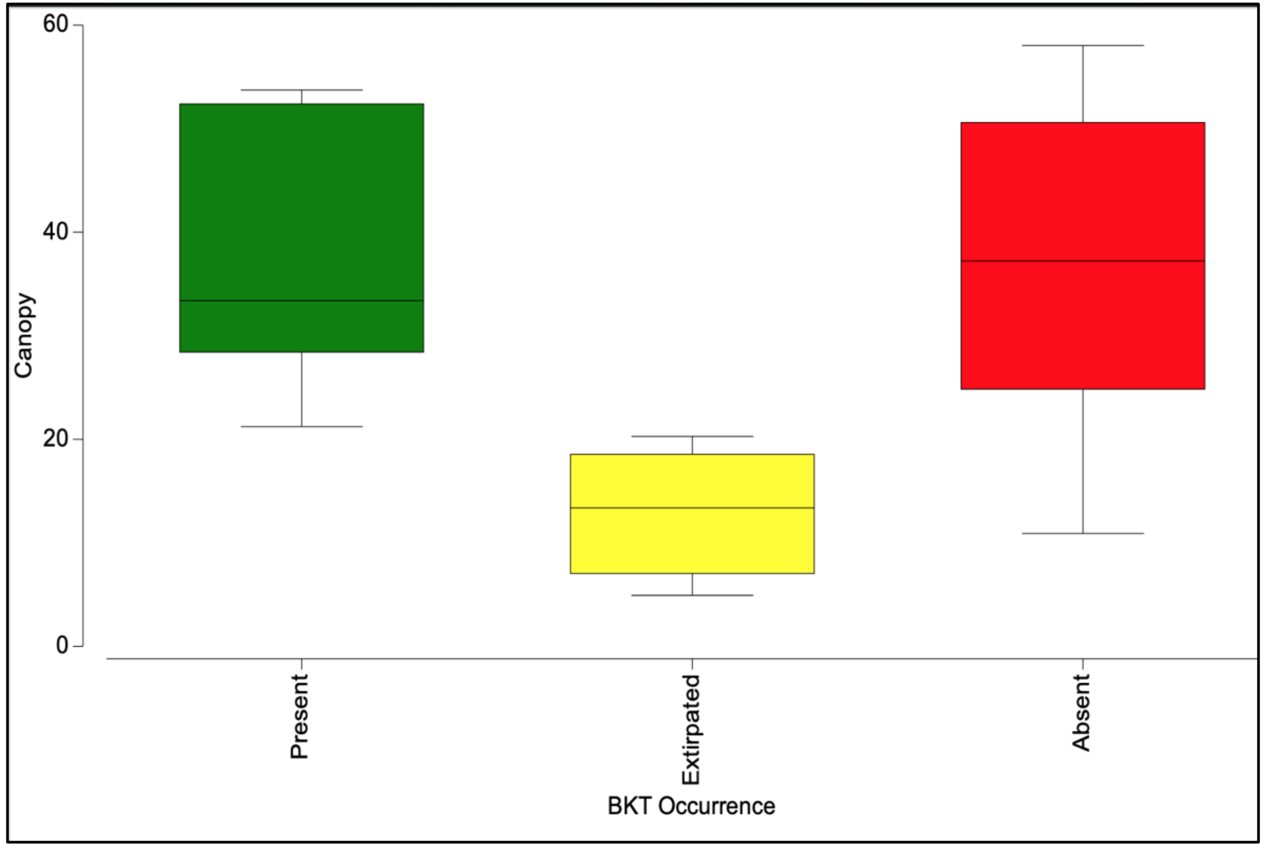

Figure 10: Differences in percent canopy cover between present (green), extirpated (yellow), and Absent (red) sites.

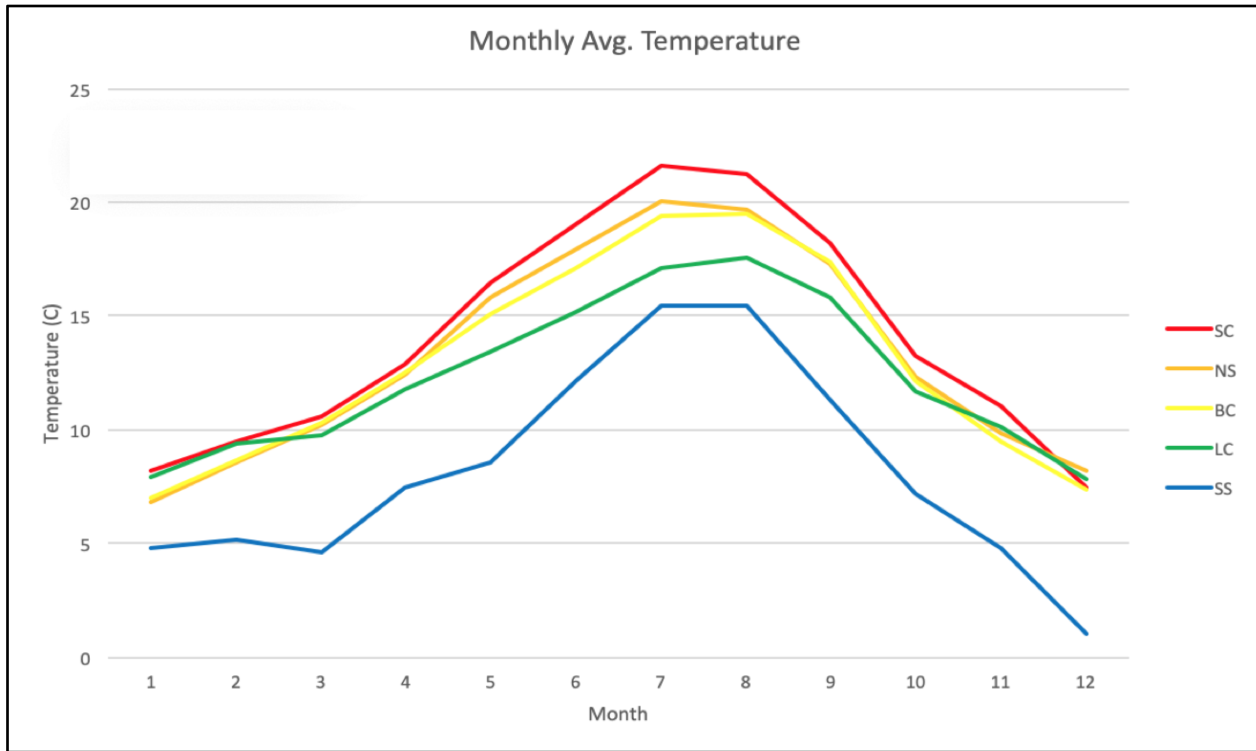

Figure 11: Average monthly temperatures of all five target streams, Slicking Creek (red), North Saluda (orange), Brice Creek (yellow), Laurel Creek (green), and South Saluda River (blue).

Discussion:

Potential barriers affecting fish distribution were identified across five target streams. In the South Saluda sub-watershed, a series of plunge pools in Laurel Creek (LC) acted as a barrier with a complete absence of Rainbow Trout (RBT) below the barrier and only SABT above the barrier. I identified two potential barriers in South Saluda River (SSR), but captured RBT above both cascades which indicated they were not effective at preventing their upstream movement. However, SABT were still more abundant above the largest cascade. I also identified potential barriers in Brice Creek (BC) and North Saluda River (NSR) that may provide suitable habitat for SABT reintroductions provided competing species (i.e., RBT and creek chub) can be removed from upstream reaches. Electrofishing efforts confirmed SABT extirpation from Slicking Creek

(SLC) following the 2016 wildfires. The resulting loss of canopy cover is still affecting SLC water temperatures and may prevent SABT reintroductions until the canopy is fully recovered.

Identification of a source population within the South Saluda sub-watershed will facilitate potential reintroduction of SABT across the two sub-watersheds. Using a source population of SABT found within the same watershed as the potential reintroduction sites is recommended and scientifically supported (Pregler et al., 2018; Smith et al., 2024). Two out of the three candidate source streams, LC and SSR, harbor detectable populations of the SABT. LC demonstrates a significantly higher average catch rate, with an average CPUE of 2.06 fish per minute, than the SSR's average CPUE of 0.63 fish per minute. Within LC, we captured 96 SABT over approximately 0.3 km, and in SSR, we captured 35 SABT across a similar distance of about 0.3 km. There is not a fixed source population size applicable to all situations. Nonetheless, a study working on SABT restoration in the Great Smoky Mountain Park (GRSM) targeted streams with 125 SABT per kilometer to avoid over-depletion of source populations (Smith et al., 2024). We were able to collect 96 SABT with approximately 0.4 km electroshocked in LC, meeting GRSM requirements for source populations. However, SSR does not meet this requirement with 35 SABT collected over 0.3 km electroshocked. This suggests that Laurel Creek hosts a more robust SABT population, making it a preferable candidate for sourcing future conservation initiatives. I would not recommend using SSR as a source population until the competing RBT populations have been either eradicated or greatly reduced (Kulp et al., 2017).

Laurel Creek is initially more suitable for sourcing conservation projects due to its higher SABT population density, any conservation plans must consider the unique ecological factors and conservation status at each site. To further understand current SABT conservation practices, we pursued discussion with Tennessee Aquarium Conservation Institute (TNACI) personnel.

When collecting broodstock for propagation, they only collected 20% (8 of 40 individuals) from a single source stream. In terms of number of individual SABT sampled from Laurel Creek and SSR, both can be considered source populations. However, SSR SABT exhibit interspecific competition with RBT, until RBT populations have been either eradicated or greatly reduced I would not recommend using SSR as a source population (Kulp et al., 2017). Conservation efforts are highly site-specific and require careful evaluation of local conditions.

Given the success the National Park Service has had in reintroductions of SABT in GRSM streams (Kulp & Moore, 2000), using their restoration criteria for reintroduction sites is advisable. The criteria are as follows: (1) non-native species are removed, (2) a sufficient barrier is present to prevent reinvasion of non-natives, and (3) there is historical evidence of SABT (Lingerfelt, 2015; Kanno et al., 2016). Greenville Water Utility is not legally obligated by the criteria listed above, however, following the criteria should increase success for reintroductions.

Slicking Creek is a potential reintroduction site for SABT based on restoration criteria used by GRSM. However, I documented a significant decrease in canopy cover and increased stream temperatures in comparison to the other target streams in this study. The increased stream temperature can be mitigated by reestablishing canopy cover and this should be done before the reintroduction of SABT in Slicking Creek. Both the North Saluda River and Brice Creek meet two of the three requirements, having sufficient barriers and having historical evidence for SABT. The historical evidence of SABT presence in these streams is anecdotal from Greenville Water Utility employees (A. Williams, pers. comm.). Non-native Rainbow Trout above identified barriers in NSR need to be removed before reintroduction of SABT. Conversely, we did not detect non-native species in BC but we did detect Creek Chub (*Semotilus atromaculatus*) at a CPUE of 1.43 f/m. In the presence of Creek Chub, SABT aggression is reduced as water

temperatures increase (Colby et al., 2022). I suggest reducing the population of Creek Chub before reintroduction of SABT.

Conservation efforts for the SABT in the Southern Appalachian Mountains of South Carolina should encompass a multi-faceted approach that includes non-native species removal, enhancement of genetic viability, and vegetation restoration. Strategies such as targeted fishing, electrofishing (Moore et al., 1986), and antimycin (Fintrol; Gibbs et al., 2016) are all useful methods for the removal of non-native species such as RBT. Success of these techniques are dependent on the use of barriers to prevent upstream migration of non-native species (Webb & Kynard, 2013; Moore et al., 1986; West, 1990). Improving genetic viability is another cornerstone of SABT conservation. Efforts focus on increasing genetic diversity through the careful selection of genetically distinct and resilient Brook Trout from multiple source populations for reintroduction projects (Smith et al., 2024). This strategy helps to enhance the adaptability of the populations to changing environmental conditions and reduces the risk of inbreeding (King et al., 2012). Riparian vegetation replacement plays a vital role in stabilizing stream banks, reducing sediment runoff, and improving water quality, all of which are beneficial for Brook Trout habitats (Sweeney et al., 2004). The reintroduction of native plant species along stream corridors also helps to recreate the shade necessary for maintaining the cool water temperatures that SABT require (Petty et al., 2012). Such ecological restoration not only supports the direct habitat needs of SABT but also enhances the overall ecosystem health, providing benefits to a wider range of aquatic and terrestrial species (Johnson et al., 2014).

Future Directions and Management Recommendations:

Northern-Strain Brook Trout (NSBT) and SABT exhibit distinct habitat preferences associated with the differing environmental conditions of their respective geographic ranges (Rahel & Nibbelink, 1999). Northern Brook Trout are typically found in the cooler, well-oxygenated waters of northeastern America, thriving in large streams and rivers with stable flow, abundant riparian cover, and substrates rich in gravel and cobble which are essential for spawning (Rahel & Nibbelink, 1999). In contrast, Southern Appalachian Brook Trout, adapted to the warmer climates of the southern Appalachian Mountains, are predominantly found in smaller, higher elevation streams (Petty et al., 2005). These streams, though cooler than the lower elevation waters, often experience more variable flow rates and temperatures. SABT require shaded streams with dense canopy cover to maintain the cooler water temperatures necessary for their survival (Curry and Noakes, 1995). They are also more isolated which influences their genetic diversity and vulnerability to environmental changes (Petty et al., 2005). Since the environments between NSBT and SABT vary, their use of habitat will also vary. This study focused on abiotic habitat characteristics, and future studies should consider biotic factors such as vegetation in the riparian zones, macroinvertebrates, and biomass of SABT.

Specific conservation plans should be systematically and logically implemented. My recommendation is to first focus on the reestablishment of canopy cover in Slicking Creek. Canopy cover reestablishment will take several years, but promoting quick canopy growth will allow Slicking Creek to be considered for reintroduction of SABT sooner. The Eastern Hemlock (*Tsuga canadensis*) has historically been concentrated in riparian zones within southern Appalachian Mountains creating a dense evergreen canopy decreasing stream temperature year-round (Brantley et al., 2013). Unfortunately, since the introduction of the Hemlock Woolly

Adelgid *(Adelges tsugae)*, the Eastern Hemlock is no longer a suitable species for canopy cover reestablishment. Instead of finding a single species of tree to replace Eastern Hemlocks, we should instead reestablish hardwood-dominant stands as quickly as possible in riparian zones of Slicking Creek (Roberts et al., 2009). This could be done by disturbing the riparian zones during the transition to hardwood-dominant stands, thus, preventing non-hardwood vegetation from establishing populations, streamlining secondary succession to hardwood-dominant stands (Roberts et al., 2009).

Once reestablishment of canopy cover in Slicking Creek is underway, non-native and competitive species of fish need to be removed from other target streams (SSR, NSR, and BC). In small mountainous streams, fish removal is possible via electrofishing techniques. I recommend using two removal events in the summer, beginning upstream between two boundaries before moving downstream (Kulp and Moore, 2000). Since SSR has both SABT and RBT inhabiting the same stream reaches, removing RBT will promote population growth of SABT. Once RBT are removed from SSR, we can then mix the source populations (LC and SSR) as a useful method of translocations to propagate genetic diversity of SABT within the two sub-watersheds (Smith et al., 2024). I would then begin non-native removal in NSR, since NSR has similar temperatures to BC and we recorded RBT CPUE of 0.1 (f/m) above the barrier. Prepping NSR for the reintroduction of SABT takes priority over BC because BC has a much higher CPUE for Creek Chub (1.43 f/m) making species removal more attainable in NSR. Lastly, reducing the population of Creek Chub (Colby et al., 2022) above the barrier in BC is recommended. I recommend one removal during the spawning season, between February and May, to disrupt their courtship during spawning season, then another removal between June and

July. I only recommend this for NSR and BC since neither have extant populations of SABT and we do not have to worry about negatively impacting SABT fingerlings.

This research increases our understanding of habitat characteristics and distribution of SABT in southern Appalachia (SC), specifically at the southern extent of their distribution. This study provides a detailed examination of in-stream habitat characteristics and identifies suitable source populations for conservation efforts, potential barriers to fish movements, and potential reintroduction sites. This comprehensive study guides the conservation and management of SABT populations within the Upper Saluda River basin and helps fill the knowledge gap between SABT's optimal habitat and Northern Brook Trout optimal habitat. By balancing robust source populations with the strategic selection of individuals, and addressing the multifaceted challenges posed by barriers and stochastic events, we can support the resilience and longevity of SABT populations in their native habitats.

References:

Alexander, G. R., & Hansen, E. A. (1986). Sand bed load in a Brook TroutTrout stream. *North American Journal of Fisheries Management*, *6*(1):9-23.

Anglin, Z. W., & Grossman, G. D. (2013). Microhabitat use by southern Brook TroutTrout (*Salvelinus fontinalis*) in a headwater North Carolina stream. Ecology of Freshwater Fish, 22(4):567-577.

Austin, K., & Krueger, E. (2014, January). Natural Resource Management Plan for the Greenville Watersheds Table Rock and North Saluda. Natural Resource Management Plan for the Greenville Watersheds Table Rock North Saluda.

Baker, W. L. (1995). Longterm response of disturbance landscapes to human intervention and global change. Landscape Ecology, 10, 143-159.

Behnke, R. J. (2002). Trout and Salmon of North America. The Free Press.

Beechie, T. J., Pess, G., Beamer, E., Lucchetti, G., & Bilby, R. E. (2003). Role of watershed assessments in recovery planning for salmon. *Restoration of Puget Sound Rivers*, 194-225.

Bennett, M. (2019). Wild salmon: protecting the icon of the Pacific Northwest. DU Quark, 4(1), 26-32.

Bennie, J., Huntley, B., Wiltshire, A., Hill, M. O., & Baxter, R. (2008). Slope, aspect and climate: Spatially explicit and implicit models of topographic microclimate in chalk grassland. Ecological modelling, 216(1), 47-59.

Bilby, R. E., & Ward, J. W. (1991). Characteristics and function of large woody debris in streams draining old-growth, clear-cut, and second-growth forests in southwestern Washington. Canadian Journal of Fisheries and Aquatic Sciences, 48(12), 2499-2508.

Brantley, S., Ford, C. R., & Vose, J. M. (2013). Future species composition will affect forest water use after loss of eastern hemlock from southern Appalachian forests. Ecological Applications, 23(4), 777-790.

Greenville Water (2023). Celebrating 30 years of protecting our watersheds. Greenville Water. (2023, May 25). https://www.greenvillewater.com/news/celebrating-30-years-of-protecting-our-watersheds

Chicoine, S. (2017). Effects of winter and summer thermal variability regimes on growth and metabolism in brook trout (Salvelinus fontinalis).

Clark, M. E., Rose, K. A., Levine, D. A., & Hargrove, W. W. (2001). Predicting climate change effects on Appalachian trout: Combining GIS and individual-based modeling. Ecological Applications, 11(1), 161-178.

Clark, S., Back, J., Tubiolo, A., & Romanaux, E. (2001, October). The Southern Appalachians: A Changing World. In US Geological Survey Appalachian Region Integrated Science Workshop Proceedings, Gatlinburg, Tennessee, October 22-26, 2001 (p. 3).

Colby, B. R., Niles, J. M., Persons, M. H., & Wilson, M. J. (2022). Shifting thermal regimes influence competitive feeding and aggression dynamics of Brook Trout (*Salvelinus fontinalis*) and Creek Chub (*Semotilus atromaculatus*). Ecology and evolution, 12(7), e9056.

Curry, R.A., & Noakes, D.L.G. (1995). Groundwater and the selection of spawning sites by Brook Trout (*Salvelinus fontinalis*). Journal of Fish Biology, 47, 763-771.

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. Science, 292(5517), 673-679.

Dunham, J. B., Pilliod, D. S., & Young, M. K. (2004). Assessing the consequences of nonnative trout in headwater ecosystems in western North America. Fisheries, 29(6), 18-26.

Ekka, A., Pande, S., Jiang, Y., & der Zaag, P. V. (2020). Anthropogenic modifications and river ecosystem services: a landscape perspective. Water, 12(10), 2706.

Ewing, R. (2023, September 25). Restoring Southern Appalachian Brook Trout: U.S. Fish & Wildlife Service. FWS.gov. https://www.fws.gov/story/2023-09/restoring-southern-appalachian-brook-trout

Fausch, K. D., Torgersen, C. E., Baxter, C. V., & Li, H. W. (2002). Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes: a continuous view of the river is needed to understand how processes interacting among scales set the context for stream fishes and their habitat. BioScience, 52(6), 483-498.

Faustini, J. M., & Jones, J. A. (2003). Influence of large woody debris on channel morphology and dynamics in steep, boulder-rich mountain streams, western Cascades, Oregon. Geomorphology, 51(1-3), 187-205.

Ficke, A.D., D.P. Peterson, and W.A. Janowsky (2009, June 26). Brook Trout (Salvelinus fontinalis): a technical conservation assessment. [Online]. USDA Forest Service, Rocky Mountain Region.

Flebbe, P.A., Roghair, C.N., & Bruggink, J.L. (2006). Spatial modeling to project southern Appalachian trout distribution in a warmer climate. Transactions of the American Fisheries Society, 135(5), 1371-1382.

Gall, B. G., & Mathis, A. (2010). Innate predator recognition and the problem of introduced trout. Ethology, 116(1), 47-58.

Gibbs, W. K., Kulp, M. A., Moore, S. E., & Cook, S. B. (2015). Effects of antimycin and its neutralizing agent, potassium permanganate, on aquatic macroinvertebrates in a Great Smoky Mountains National Park watershed. North American Journal of Fisheries Management, 35(6), 1239-1251.

Graham, L. K. (1997). American Paddlefish: Biology and Aquaculture of *Polyodon spathula*. Krieger Publishing Company.

Greenville Water (2023). Celebrating 30 years of protecting our watersheds. Greenville Water. (2023, May 25). https://www.greenvillewater.com/news/celebrating-30-years-of-protecting-our-watersheds

Habera, J., & Moore, S. (2005). Managing southern Appalachian Brook Trout: a position statement. Fisheries, 30(7), 10-20.

Hampe, A., & Petit, R. J. (2005). Conserving biodiversity under climate change: the rear edge matters. Ecology letters, 8(5), 461-467.

Hayes, J. P., Guffey, S. Z., Kriegler, F. J., McCracken, G. F., & Parker, C. R. (1996). The genetic diversity of native, stocked, and hybrid populations of Brook Trout in the southern Appalachians. Conservation Biology, 10(5), 1403-1412.

Hilderbrand, R. H., Watts, A. C., & Randle, T. J. (1997). The myths of restoration ecology. Ecology and Society, 1(1), 1-6

Hoekstra, J. M., Bartz, K. K., Ruckelshaus, M. H., Moslemi, J. M., & Harms, T. K. (2007). Quantitative threat analysis for management of an imperiled species: Chinook salmon (Oncorhynchus tshawytscha). Ecological Applications, 17(7), 2061-2073.

Hudy, M., Thieling, T. M., Gillespie, N., & Smith, E. P. (2008). Distribution, status, and land use characteristics of subwatersheds within the native range of Brook Trout in the eastern United States. North American Journal of Fisheries Management, 28(4), 1069-1085.

Januchowski-Hartley, S. R., McIntyre, P. B., Diebel, M., Doran, P. J., Infante, D. M., Joseph, C., & Allan, J. D. (2013). Restoring aquatic ecosystem connectivity requires expanding inventories of both dams and road crossings. Frontiers in Ecology and the Environment, 11(4), 211-217.

Johnson, S. L., Jones, K. A., & Arismendi, I. (2014). Stream restoration in Brook Trout habitat: A review. Restoration Ecology, 22(5), 539-548.

Jowett, I. G. (1993). A method for objectively identifying pool, run, and riffle habitats from physical measurements. New Zealand journal of marine and freshwater research, 27(2), 241-248.

Kanno, Y., Kulp, M. A., & Moore, S. E. (2016). Recovery of native Brook Trout populations following the eradication of nonnative Rainbow Trout in southern Appalachian Mountains streams. North American Journal of Fisheries Management, 36(6), 1325-1335.

Kazyak, D. C., Hilderbrand, R. H., Keller, S. R., Colaw, M. C., Holloway, A. E., Morgan, R. P., & King, T. L. (2015). Spatial Structure of Morphological and Neutral Genetic Variation in Brook Trout. Transactions of the American Fisheries Society, 144(3), 480–490.

Kazyak, D.C., Lubinski, B.A., Kulp, M.A., Pregler, K.C., Whiteley, A.R., Hallerman, E., Coombs, J.A., Kanno, Y., Rash, J.M., Morgan, R.P. and Habera, J., 2022. Population genetics of Brook Trout in the southern Appalachian Mountains. Transactions of the American Fisheries Society, 151(2):127-149.Brook Trout

Kelson, S. J., Kapuscinski, A. R., Timmins, D., & Ardren, W. R. (2015). Fine-scale genetic structure of Brook Trout in a dendritic stream network. Conservation genetics, 16, 31-42.

King, T. L., Lubinski, B. A., & Spidle, A. P. (2012). Genetic management of Brook Trout populations in the southern Appalachian Mountains. North American Journal of Fisheries Management, 32(3), 515-529.

Kozel, S. J., & Hubert, W. A. (1989). Testing of habitat assessment models for small trout streams in the Medicine Bow National Forest, Wyoming. North American Journal of Fisheries Management, 9(4), 458-464.

Kulp, M. A., & Moore, S. E. (2000). Multiple electrofishing removals for eliminating Rainbow Trout in a small southern Appalachian stream. North American Journal of Fisheries Management, 20(1), 259-266.

Kulp, M. A., Mitchell, S., Kazyak, D., Kuhajda, B., Henegar, J., Weathers, C., ... & King, T. (2017). The southern Appalachian Brook Trout management conundrum: what should restoration look like in the 21st century. Wild Trout XII: science, politics, and wild trout management: who's driving and where are we going, 65-75.

Larson, G. L., & Moore, S. E. (1985). Encroachment of exotic Rainbow Trout into stream populations of native Brook Trout in the southern Appalachian Mountains. Transactions of the American Fisheries Society, 114(2), 195-203.

Lingerfelt , K. (2015, August 7). Southern Appalachian Brook Trout Restoration. National Parks Service. https://www.nps.gov/grsm/learn/nature/lynn-camp-prong.htm

Martinez, A. S., Willoughby, J. R., & Christie, M. R. (2018). Genetic diversity in fishes is influenced by habitat type and life-history variation. Ecology and Evolution, 8(23), 12022-12031.

Meisner, J.D. (1990). Effect of climatic warming on the southern margins of the native range of Brook Trout, Salvelinus fontinalis. Canadian Journal of Fisheries and Aquatic Sciences, 47(6), 1065-1070.

Mitchell, J.C., Pauley, T.K., Withers, D.I., Roble, S.M., Miller, B.T., Braswell, A.L., Cupp Jr, P.V. and Hobson, C.S., 1999. Conservation status of the southern Appalachian herpetofauna. *Virginia Journal of Science*, *50*(1):2.

Moore, S. E., Larson, G. L., & Ridley, B. (1986). Population control of exotic Rainbow Trout in streams of a natural area park. Environmental Management, 10, 215-219.

Morse, J. C., Stark, B. P., & McCafferty, W. P. (1993). Southern Appalachian streams at risk: implications for mayflies, stoneflies, caddisflies, and other aquatic biota. Aquatic Conservation: Marine and Freshwater Ecosystems, 3(4), 293-303.

Mulholland, P. J., Roberts, B. J., Hill, W. R., & Smith, J. G. (2009). Stream ecosystem responses to the 2007 spring freeze in the southeastern United States: unexpected effects of climate change. Global Change Biology, 15(7), 1767-1776.

Myers, M. R., Hunkins, T. E., Bossenbroek, J. M., Mayer, C. M., & Weimer, E. J. (2024). Assessing potential barriers to migratory Walleye in the Sandusky River, Ohio. Transactions of the American Fisheries Society.

National Fish Habitat Partnership. (2021). Through a Fish's Eye: The Status of Fish Habitats in the United States 2020. Retrieved from https://www.fishhabitat.org/files/uploads/fishhabitatreport.pdf

Neff, K. J., Schwartz, J. S., Henry, T. B., Bruce Robinson, R., Moore, S. E., & Kulp, M. A. (2009). Physiological stress in native southern brook trout during episodic stream acidification in the Great Smoky Mountains National Park. *Archives of Environmental Contamination and Toxicology*.

Patrignani, A., & Ochsner, T. E. (2015). Canopeo: A powerful new tool for measuring fractional green canopy cover. *Agronomy journal*, *107*(6), 2312-2320.

Pess, G. R., Quinn, T. P., Gephard, S. R., & Saunders, R. (2008). Reconnecting rivers: Natural channel design in dam removals and fish passage. Aquatic Conservation: Marine and Freshwater Ecosystems, 18(5), 611-618.

Petty, J. T., Hansbarger, J. L., Huntsman, B. M., & Mazik, P. M. (2005). "Brook Trout Displacement by Brown Trout in Three Appalachian Streams". American Fisheries Society Symposium, 45, 203-215.

Petty, J. T., Hansbarger, J. L., Huntsman, B. M., & Mazik, P. M. (2012). Brook Trout movement in response to temperature, flow, and thermal refugia within a complex Appalachian riverscape. Transactions of the American Fisheries Society, 141(4), 1060-1073.

Pregler, K. C., Kanno, Y., Rankin, D., Coombs, J. A., & Whiteley, A. R. (2018). Characterizing genetic integrity of rear-edge trout populations in the southern Appalachians. Conservation genetics, 19, 1487-1503.

Rahel, F.J., & Nibbelink, N.P. (1999). Spatial patterns in relations among stream fish assemblages, geology, and landscape structure. Landscape Ecology, 14(2), 113-124.

Rankin, D., & Geddings, R. (1992). (rep.). Fisheries Survey Results on the Greenville Water System Properties.

Roberts, S. W., Tankersley, R., & Orvis, K. H. (2009). Assessing the potential impacts to riparian ecosystems resulting from hemlock mortality in Great Smoky Mountains National Park. Environmental management, 44, 335-345.

Sagoff, M. (2005). Do non-native species threaten the natural environment?. Journal of Agricultural and Environmental Ethics, 18, 215-236.

Schlosser, I. J. (1995). Critical landscape attributes that influence fish population dynamics in headwater streams. Hydrobiologia, 303, 71-81.

Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., ... & Pyšek, P. (2013). Impacts of biological invasions: what's what and the way forward. Trends in Ecology & Evolution, 28(1), 58-66.

Solomon, S., Plattner, G. K., Knutti, R., & Friedlingstein, P. (2009). Irreversible climate change due to carbon dioxide emissions. Proceedings of the National Academy of Sciences, 106(6), 1704-1709.

South Carolina Forestry Commission. (2021, July 12). Wildfire history. South Carolina Forestry Commission. https://www.scfc.gov/protection/fire-burning/fire-resources/wildfire-history/#:~:text=The%20largest%20mountain%20wildfire%20on,was%20controlled%20on%20December%2016.

Smith, P. W. (1985). The Inland Fishes of New York State. New York State Department of Environmental Conservation

Smith, R. J., Kazyak, D. C., Kulp, M. A., Lubinski, B. A., & Fitzpatrick, B. M. (2024). Genetic structure of restored Brook Trout populations in the Southern Appalachian Mountains indicates successful reintroductions. Conservation Genetics, 1-14.

Stephenson, S. L., & Adams, H. S. (1998). The Southern Appalachians: A History of the Landscape. USDA Forest Service, Southern Research Station. General Technical Report SRS-18.

Stoneking, M., Wagner, D. J., & Hildebrand, A. C. (1981). Genetic evidence suggesting subspecific differences between northern and southern populations of Brook Trout (Salvelinus fontinalis). Copeia *1981*(4):810–819. https://doi.org/10.2307/1444182

Sweka, J.A., & Hartman, K.J. (2001). Influence of turbidity on Brook Trout reactive distance and foraging success. Transactions of the American Fisheries Society, 130(1), 138-146.

Sweeney, B. W., & Newbold, J. D. (2004). Streamside Forest Buffer Width Needed to Protect Stream Water Quality, Habitat, and Organisms: A Literature Review. Journal of the American Water Resources Association, 40(3), 353-365.

Temple, G. M., & Pearsons, T. N. (2007). Electrofishing: backpack and drift boat. *Salmonid field protocols handbook: techniques for assessing status and trends in salmon and trout populations. American Fisheries Society, Bethesda, Maryland*, 95-132.

Walburg, C. H., & Nichols, P. R. (1967). "Biology and management of the American shad and status of the fisheries, Atlantic coast of the United States, 1960". Special Scientific Report -- Fisheries.

Walsh, S. J., Haney, D. C., & Timmerman, C. M. (1997). Variation in thermal tolerance and routine metabolism among spring-and stream dwelling freshwater sculpins (Teleostei: Cottidae) of the southeastern United States. Ecology of Freshwater Fish, 6(2), 84-94.

Warren, M. L., Jr., Burr, B. M., Walsh, S. J., Bart, H. L., Jr., Cashner, R. C., Etnier, D. A., ... & Starnes, L. B. (2000). "Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States". Fisheries, 25(10), 7-31.

Weathers, T. C., Kazyak, D. C., Stauffer Jr, J. R., Kulp, M. A., Moore, S. E., King, T. L., & Carlson, J. E. (2019). Neutral genetic and phenotypic variation within and among isolated headwater populations of Brook Trout. *Transactions of the American Fisheries Society*, *148*(1), 58-72.

Webb, P. W., & Kynard, B. (2013). Effects of non-native species on native Brook Trout populations. Journal of Fish Biology, 83(3), 1004-1013.

West, J. L. (1990). Evaluation of electrofishing as a management technique for restoring Brook Trout in Great Smoky Mountains National Park. US Department of the Interior, National Park Service, Southeast Regional Office, Science and Natural Resources Division.

Whiteley, A. R., Coombs, J. A., Hudy, M., Robinson, Z., Colton, A. R., Nislow, K. H., & Letcher, B. H. (2013). Fragmentation and patch size shape genetic structure of brook trout populations. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(5), 678-688.

Xu, M., Li, S. P., Liu, C., Tedesco, P. A., Dick, J. T., Fang, M., ... & Mu, X. (2024). Global freshwater fish invasion linked to the presence of closely related species. Nature Communications, 15(1), 1411.

Zastavniouk, C., Weir, L. K., & Fraser, D. J. (2017). The evolutionary consequences of habitat fragmentation: Body morphology and coloration differentiation among brook trout populations of varying size. *Ecology and Evolution*, *7*(17), 6850-6862.