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A Study of Salmonid Growth in Two Southern Appalachian Headwater Streams

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by

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ABSTRACT

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This study sampled salmonid populations in two headwater streams in East Tennessee, Briar Creek and Left Prong Hampton Creek. Length and weight data were used to calculate the growth of these populations to determine if significant variation exists between isolated brook trout populations. Slope comparisons concluded that there was a difference in growth between brook trout populations of these streams ($p<0.001$), but none between rainbow trout populations ($p=0.655$). Coincidently, this study was conducted during a drought, which previous studies have shown to negatively influence higher age classes of high-elevation salmonids. Comparison of Fulton's Condition Factor indicated that older age classes of brook trout were influenced more than younger classes. Brook trout exhibited significant difference in condition factor between Age 0 and Age 1+ classes in Briar Creek ($p<0.001$) and in Left Prong Hampton Creek between Age 0 and Age 1+, Age 3+ classes, with p-values of 0.002 and 0.010, respectively.

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CHAPTER 1

INTRODUCTION

Range, Distribution, and Habitat

The brook trout, *Salvelinus fontinalis*, is a salmonid commonly found throughout the eastern United States, and the only salmonid native to the southern Appalachians. The species' native distribution ranges throughout much of eastern Canada, The Great Lakes region, New England, and the higher elevations of the southern Appalachian Mountain range. However, the current native range has been significantly reduced over the past century when compared to its historical range (Hudy et al. 2008). In a survey of subwatersheds in Tennessee, *S. fontinalis* was either extirpated or was predicted to be extirpated in 41 of 81 subwatersheds where brook trout had been previously recorded (Hudy et al. 2008). Of the remaining 40 subwatersheds, 37 were either reduced or predicted to be reduced meaning that 50-99% of that habitat is predicted to no longer sustain brook trout populations. This trend seems to continue throughout most of the brook trout's range in Appalachia, from Georgia to Maine (Hudy et al. 2005; Hudy et al. 2008).

Both biotic and abiotic factors play a role in the distribution of brook trout and the success of those populations (Mitro et al. 2014). One of the primary abiotic factors that determines the success of *S. fontinalis* populations is a stream's pH. Studies have suggested that although brook trout can establish populations and compete intraspecifically at neutral pH levels, they gain a significant advantage at slightly lower pH levels ranging from 4.6 to a neutral pH of 7 (Kocovsky and Carline 2005). This is not to say that a stream with lower pH is considered optimal for brook trout, only that they seem to outcompete other salmonids in such a condition. Other studies have concluded that while at a slightly acidic pH of 5.5, brook trout growth was not influenced. However, unhatched eggs at pH 5.5 suffered from increased mortality, and growth rate of newly-hatched fry was reduced in a pH of 4.5 (Kwain and Rose 2011). Brook trout may have adapted their tolerance to lower pH because of the chemistry of the headwaters in which they live, giving them a sort of home-field advantage. Headwater streams are generally more acidic than waters downstream (Kocovsky and Carline 2005). As water flows from headwaters to larger stream orders, it interacts with acid-neutralizing compounds, like calcium carbonite, found within the bedrock of the stream. The pH will begin to neutralize as the stream drops in elevation, displaying a gradually increasing pH gradient between the higher and lower elevations (Kocovsky and Carline 2005).

Another pivotal abiotic variable in trout stream management is temperature. Even though all salmonids are referred to as cold water fishes, there is some difference in the temperature tolerances among species. *S. fontinalis* are known to prefer streams with a temperature range of 14-16°C with a maximum stream temperature of 22.4°C (Etnier and Starnes 1993). These values are more variable in rainbow trout *Oncorhynchus mykiss* with an average stream temperature range of 12-19°C and a maximum of 24°C (Myers et al. 2014). Increased solar energy into a stream from open forest canopy has been shown to not only raise stream temperatures but also increases the density of macroinvertebrates, a vital food source for salmonids (Nislow and Lowe 2006).

Southern Appalachian brook trout have adapted to living in cold, fast-flowing mountain streams that are rich in dissolved oxygen (Raleigh 1982). In addition to riffle and cobble runs, these streams are characterized by plunge pools, deep basins beneath small waterfalls that provide cover for both salmonids and other species. Evidence suggests that plunge pools are vital in brook trout habitat and while smaller salmonid individuals tend to forage in riffles, larger individuals primarily reside within deeper pools (Ecret and Mihuc 2013). In addition, plunge pools play an important role in the more extreme months, providing refuge for fishes from summer heat and from stream freezing in winter. The importance of these pools was observed first hand during this study as a severe drought effected Briar Creek. As portions of the stream failed to retain water throughout the study, the majority of sampled trout were collected within these pools.

Life History of Brook Trout

The life of a brook trout begins during spawning events which take place between September and November, depending on water temperature and latitude (Etnier and Starnes 1993). In the northern portions of the *S. fontinalis* range, spawning occurs earlier in late summer, but for the mountain streams of East Tennessee in the southern range, the spawning timeframe is generally October to early November (Etnier and Starnes 1993).

During this event, females venture to gravel stream beds where they hollow out a bowl-shaped indention into the gravel, called a redd. Redds serve as a sort of nursery for the eggs until they hatch approximately one hundred days after spawning. Once a redd is constructed, female brook trout will swim alongside a male over the gravel indention. The male and female will deposit their gametes simultaneously into the redd. The newly fertilized eggs will then settle into the redd where they wedge between the crevices of gravel, effectively holding them in place until they hatch. The female will then move on to build another redd and deposit more eggs, and this continues until she has depleted her egg stores. Emergence of sac fry occurs roughly in late-January to February in Tennessee. However, if the winter months are harsh and anchor ice accumulates in the stream, salmonid eggs are in danger of freezing within the redds (Lennon 1967). Spawning and hatching time frames are different for rainbow trout in Tennessee, as spawning occurs between February and April with fry emerging in late spring/early summer (Etnier and Starnes 1993).

Sac fry are the earliest emergent form of the salmonid life cycle. After emerging from the egg, fry, now referred to as yolkfry, are still attached to a nutrient-rich yolk sac but are still largely immobile (Teears et al. 2016). The fish will obtain sustenance from the yolk sac until it is fully depleted, at which time the young fish will then be free to roam from the gravel bed to forage primarily on small invertebrates, with approximately 90% of the prey including Ephemeroptera, chironomids (larvae and pupae), and Simuliidae (Miller 1974). By the summer months, small fry will have grown into parr, also referred to as fingerlings because they are roughly the size of an adult human’s finger (Teears et al. 2016). Parr have easily identifiable vertical markings along their flanks called parr marks that help to camouflage the juveniles fishes among the rocks and gravel of the stream bed. Evidence suggest that the majority of average yearly growth for southern-strain brook trout adults occurs between May-July (Utz and Hartman 2009). Sexual maturity occurs in some males by the end of their first year, but the majority of individuals reach sexual maturity by Age 2. Although some *S. fontinalis* populations contain individuals of 5+ years of age, very few southern-strain brook trout survive past age 3 possibly due to resource constrains in these small headwater streams.

Genetic Distinction Between Northern and Southern Brook Trout

Geographically, northern-strain brook trout (NBKT) are populations of *S. fontinalis* found north of the New River drainage in Virginia, while southern-brook trout (SBKT) are those found in and south of the New River Watershed (Wesner et al. 2011). In the southern ranges, SBKT are confined primarily to small headwater streams while NBKT can be found in lakes and rivers in the northern range. Genetic analysis of brook trout from 47 streams across the Great Smoky Mountain National Park concluded that the northern and southern strains of *S. fontinalis* are genetically distinct (McCracken 1993; Moore et al. 2005), with other findings (Danzmann et al. 1998) supporting this conclusion with significant phylogenetic differences shown between these two regional strains. Due to this genetic distinction and the limited habitat available to these populations, it is important to preserve the southern Appalachian brook trout and the streams that they inhabit as they continue to lose habitat across their native range (Hudy et al. 2005)

In addition, not only are SBKT genetically distinct from NBKT, but there is also significant evidence that genetic diversity exists between individual SBKT populations (Kriegler et al. 1995). Kriegler et al. hypothesized that this diversity could be the result of genetic drift and population isolation caused by the extirpation of *S. fontinalis* from larger-order streams. With no downstream population(s) to connect the headwaters, the small-order stream populations would have the potential to become more genetically distinct based on locally adapted gene pools. However, such divergence could also be attributed to genetic bottlenecks (Kriegler et al. 1995) caused by a catastrophic abiotic event (Phillips et al. 1987; Nagel 1991). With potential genetic diversity present between individual populations, it is likely that each headwater population has its own management concerns and should be treated as such. This study examines the growth of two isolated brook trout population in northern East Tennessee to determine if significant differences in growth persist among populations.

Brook Trout Competition with Rainbow Trout

It was originally hypothesized that competitive exclusion of native *S. fontinalis* by exotic *O. mykiss* was the major contributing factor to the decline of brook trout populations in southern Appalachia (Nagel and Deaton 1989). In a study by Rose (1986), brook trout and rainbow trout diets for young-of-the-year (YOY) were analyzed. Brook trout YOY grew at a rate of 0.51 mm/day until rainbow trout YOY emerged in the same area. After rainbow trout emergence, brook trout YOY growth declined to 0.05 mm/day as diets were restricted to fewer, large prey items including Ephemeroptera, Tricoptera, and Diptera while rainbow trout diets covered a spectrum of prey sizes, including Ostracoda, Collembola, and Arachnida in addition to those previously mentions for brook trout. This competition occurs for approximately 1-2 months in the summer before rainbow trout YOY move to swifter waters, after which brook trout growth rebounds slightly to 0.12 mm/day (Rose 1986). It was hypothesized that this restricted diet for young brook trout could possibly reduce YOY brook trout growth significantly enough to increase winter mortality, and this could account for the rainbow trout's competitive edge over brook trout (Rose 1986).

However, further studies indicated that is not entirely the case. Despite the presence of migratory barriers (man-made or otherwise) to restrict the upstream movement of rainbow trout, the decline of native brook trout populations persists, which suggests that habitat loss and stochastic events (Phillips et al. 1987) may play a role in brook trout population declines (MacArthur and Wilson 1967; Nagel 1991).

Threats to Trout and Streams

Habitat loss is thought to be the primary contributing factor to the decline of native brook trout populations in North America (Hudy et al. 2005; Hudy et al. 2008). Deforestation removes vital shade cover from stream banks, allowing for increased levels of solar energy to reach the stream. This influences stream warming, making the waters less suitable for these cold-water fishes. Nislow and Lowe (2006) suggested that logging may cause an increase in macroinvertebrate abundance, and that brook trout abundance then increased due to higher foraging success. Results from a prior study show that brook trout density is inversely proportionate to the length of time that has passed since a logging event due to the increase in prey availability caused by the canopy opening (Nislow and Lowe 2006). It has been stated that although food availability for salmonids increases after logging, consequences like sedimentation have the potential to negatively impact the overall health of a stream. In laboratory settings, fine sediment deposits of 0.43-0.85mm in diameter have reduced dissolved oxygen availability to brook trout eggs which could increase the egg mortality of wild populations, and that egg survival was inversely proportionate to the amount of sediment present (Argent and Flebbe 1998). In another multi-stream survey, it was concluded that *S. fontinalis* abundance and overall stream health was negatively impacted by logging events in line with Nislow and Lowe's concerns with factors like sedimentation (VanDusen et al. 2005). Therefore, even if macroinvertebrate density increases post-logging, the benefits observed may not compensate for the degree of habitat degradation that occurs. Anthropogenic deforestation is perhaps the most widespread source of habitat loss.

Previous Works on Briar Creek

Briar Creek is a small, second order stream located on Buffalo Mountain, Washington County, TN. Former ETSU biology faculty member Dr. Jerry Nagel began monitoring this stream in 1979 and concluded that although exotic rainbow trout, *Oncorhynchus mykiss,* were present, no native brook trout, *S. fontinalis,* population existed there (Nagel and Deaton 1989; Nagel 1991). The rainbow trout population was decreased to open stream resources for southern-strain brook trout that were released into the stream between 1983-1987. The introduced brook trout population overtook the higher elevations of this stream from rainbow trout with 66% of individuals being brook trout by the end of the study, and Nagel (1991) concluded that *S. fontinalis* can successfully compete with exotic salmonids of similar size in headwater streams. However, it was also noted that rainbow trout are highly competitive and have the potential to retake brook trout habitat after a catastrophic event such as flooding or drought.

Southern-strain brook trout are restricted primarily to headwater streams and are susceptible to catastrophic abiotic events. A computer model was formulated by Phillips et al. (1987) that used an 11-year data set of brook trout population values from Lawrence Creek in Wisconsin (Hunt 1974) to assess the threat of catastrophic events (specifically flooding and drought) on salmonid populations based upon the length of inhabited stream. The Lawrence Creek data were constrained to fit the population parameters found in Briar Creek (Lambert 1998). Variables for this model included fecundity, mortality, stream length, and frequency of reproductive failure over a set number of years. The model suggested that inhabited stream length and probability of salmonid extirpation are inversely proportionate, with the chance of extirpation due to catastrophic events increasing as inhabited stream length decreases (Phillips et al. 1987). This trend is likely due to the isolated nature of southern brook trout headwater populations, with few (if any) downstream individuals available to repopulate areas effected by drought or flooding. If an inhabited area is shorter, there is a higher probability that the entirety of that area will be effected by a stochastic event, leaving fewer individuals to re-establish the population, which could result in extirpation.

Lambert (1998) observed the distribution of the brook trout population and noted the density and age class structure of each salmonid species at monitoring stations spanning a 2.8km stretch of stream. Lambert's conclusion was similar to Nagel's (1991) in that brook trout and rainbow trout can co-exist sympatrically within a stream without extirpation by competitive exclusion alone with 88% of individuals in the upper headwaters being brook trout (Lambert 1998), and while eradication of an exotic species may be a useful method of native salmonid reintroduction (Kanno et al. 2016), it may not always be necessary.

Previous Works on Left Prong Hampton Creek

Left Prong Hampton Creek is located in the Hampton Creek State Natural Area near Roan Mountain State Park in Carter County, TN. The stretch of stream has been under observation by the TWRA since at least 1994 when the first permanent monitoring station was established, followed by two more stations in 1996. Since then, these stations have been monitored annually to survey the current population density of wild salmonids that reside within the streams (Habera et al. 2017). Restoration of native brook trout habitat and the introduction of *S. fontinalis* was successful at this site. In 1997, a log-crib rainbow trout barrier was built along the stream to isolate downstream rainbow trout from the brook trout populations in the headwater reaches of the stream. However, severe flooding destroyed the original barrier, and it was replaced with a temporary structure until the current barrier, a 3-meter waterfall, was constructed in 2007 with the aid of the Natural Resource Conservation Service (Habera et al. 2009). Efforts were made the following year to remove the remaining *O. mykiss* from upstream of the structure, and the *S. fontinalis* restoration area has been free of rainbow trout presence since 2008, the year after the waterfall was constructed (Habera et al. 2009). Annual sampling of the stream suggests that Hampton Creek supports healthy populations of both rainbow and brook trout, with the mean biomass of brook trout at the headwater sampling station being four times larger than the statewide average brook trout biomass in Tennessee (Habera et al. 2017).

As part of this brook trout restoration initiative, any rainbow trout found above the barrier were removed, and anglers visiting the area have been encouraged to do the same. Hampton Creek Cove is open to anglers, but fishing presence there is reportedly low and is unlikely a significant factor that negatively influences the brook trout population (Jim Habera, TWRA, personal communication).

Length-Weight Regression and Condition Factor in Relation to Fish Health

The length-weight relationship is a commonly used method of analysis that calculates how much mass individual fishes gain as they grow in relation to their length, using the formula, W=aL^b, where W=weight in grams, *a*=intercept, L=length in millimeters, and *b*=slope of the log-log regression. The slope value (b) is an indication of growth. As individuals grow in three dimensions, the slope would ideally be three, a concept known as cube law (Le Cren 1951), but this is rarely true in sample populations as the growth coefficient generally lies between 2.5-3.5 and suggests either positive or negative allometric growth depending whether the value lies above or below three, respectively (Le Cren 1951; Froese 2006). These data are obtained by taking sample measurements of individual fishes within a population and are used to plot a log-log length-weight linear regression. This regression describes the average mass of an individual within that population at a given age. Length measurements are more easily obtained compared to mass, so once this regression is calculated, average mass can be inferred from the regression by the calculating weight-at-length (Le Cren 1951). Even though much work has been conducted on brook trout in the southern Appalachians, calculated length-weight regression is population-specific for each stream, so this study could provide useful comparative data for future conservation efforts in these areas.

Fulton's Condition Factor is a calculation of the relative health of individual fishes within a population, and like length-weight regression, uses length and mass measurements to do so. In that regard, both length-weight regression and condition factor estimates can infer not only the size of individuals within a population, but also the presence of factors that are potentially influencing the health of those fishes (Le Cren 1951; Jin et al. 2015). A fish's condition factor can indicate the well-being of fishes or the presence of stress, both biotic and abiotic, that could potentially be negatively impacting those individuals (Datta et al. 2013). Individuals gain mass as they grow in length, and that relation of mass-to-length is represented by the condition factor value, C.

Brook trout are the subject of wide-spread re-introduction and rehabilitative efforts across the eastern United States as their populations have been in decline (Hudy et al. 2005), yet with potential variability between populations (Danzmann 1998; Hudy et al. 2008), it is important to quantify the current growth and condition factor of those populations to determine their current health and status. Such information could be utilized by local wildlife authorities, such as the Tennessee Wildlife Resources Agency, to manage these streams and salmonid populations.

Goal of the Study

This study uses length and weight data from two streams in northeast Tennessee to calculate the length-weight relationship between headwater brook trout populations to determine if there is a significant difference in growth between these populations for use in future fisheries management practices. Although the TWRA has extensive records of salmonid populations in East Tennessee streams, those reports can be somewhat constrained by time and scale, as only so many resources can be allocated to each stream survey. This study sampled many sites (28 in Briar Creek and 14 in Left Prong Hampton Creek) to determine an accurate estimate of the growth of individual salmonids in the populations within these streams. It is hypothesized that there will be variability in brook trout growth among isolated brook trout populations, as previous work suggests that variability could exist amongst individual southern brook trout populations (Danzmann et al. 1998).

In addition to the primary hypothesis of this study, East Tennessee experienced a period of drought in 2016 in the duration of this study (Habera et al. 2017) which provided the opportunity to examine the potential influence that catastrophic events have on brook trout, as well. With evidence suggesting that older age classes of salmonids are more heavily impacted by drought (Elliot 1987), condition factor analysis was conducted to assess the relative health of Left Prong Hampton Creek's and Briar Creek's native salmonids. It was hypothesized that older age classes of brook trout would have lower condition factors than younger age classes, which would support results from previous studies (Elliot 1987; Hakala and Hartman 2004).

CHAPTER 2

MATERIALS, METHODS, AND SAMPLING SITE OVERVIEW

Collection and Identification

Identification of all fish was with Peterson's Field Guide to Freshwater Fishes, Second Edition (Page and Burr 2011). All methods and materials used for the capture and sedation of specimens were reviewed and approved by the university's Animal Care and Use Committee (file code: P160801). Two streams were sampled in East Tennessee: Briar Creek on Buffalo Mountain in Washington County and Hampton Creek on Roan Mountain in Carter County. For each site, the required sampling permits were obtained from Tennessee Wildlife Resource Agency (TWRA permit: 1493), USDA Forest Service (file code: 2670), and the Tennessee Department of Environmental Conservation (TDEC permit: 2016-050).

Sampling was conducted by the use of a direct-current an LR-24 backpack electrofisher from Smith-Root, Inc. out of Vancouver, Washington. The voltage level used during sampling varied between 550-750V. Sampling sites were marked with a flag and site number along the streams at intervals of 100-meters. Each collection site was approximately a 30-meter stretch, centered on the site flag. A single-pass sampling method was used in the study, by working upstream. The site was sampled until no additional individuals were seen or collected. A single-pass method was preferred over three-pass depletion due to health concerns for both target and non-target species. Studies have suggested that organisms who are subjected to excess shocking could experience elevated levels of physiological and behavioral stress (Nagel 1989; Panek and Densmore 2011). With a multiple-pass method, target species are removed from the stream once netted, which excludes them from further shocking stress. However, non-target organisms and uncaptured specimens experience multiple shocking events over a short timeframe, possibly contributing to a decline in health (Panek and Densmore 2011). Therefore, a single-pass method (Bertrand et al. 2006) was preferred to reduce potential physiological stress on non-target species due to repeated shocking.

The trout were measured for standard length to the nearest millimeter. In addition to length measurements, mass was also recorded for every individual to the nearest tenth of a gram. Brook trout age classes were determined using results from a previous study that examined scale and otolith measurements in relation to salmonid age from fishes in The Great Smoky Mountain National Park (Kulp 1994). From this work, age class estimates for Briar Creek and Left Prong Hampton Creek were assigned at Age 0 (<112mm), Age 1 (113-142mm), Age 2 (143-171mm), and Age 3 (>173mm).

To prevent harming the specimens during measurements and the tagging process, they were immobilized by use of an anesthetic, Tricaine-S (MS-222) from Syndel USA, formerly known as Western Chemical, based in Ferndale, Washington. MS-222 is a pharmaceutical-grade, FDA approved anesthetic for cold-blooded vertebrates and was used to immobilize captured fish. The anesthetic was administered to a container of stream water at a concentration of 0.1g/L (with five liters used) as stated by the Institutional Animal Care and Use Committee protocol guidelines. The solution was buffered for neutral pH by adding food-grade baking soda to the MS-222 solution at a ratio of 1:1. A holding container with captured fish was strained through a net, and fish were then placed into the anesthetic bucket. Fish would begin losing equilibrium after approximately one minute, going from an upright swimming position to lying on their sides on the bottom of the container. Once fish seemed completely immobilized, mass and length measurements were taken for each individual. After measurements, the trout were returned to a bucket of clean, fresh water to recuperate from the anesthetic, a process which occurs usually in less than two minutes. Once fish were upright and swimming, they were returned to the stream. This process was performed as quickly as possible to reduce the fishes' exposure to the anesthetic, and the entire process from the administering of MS-222 to measurements/tagging and recovery took approximately five minutes per fish.

Because sampling took place over many weeks, every individual was marked to ensure the most accurate estimation of the populations. Each collection site was designated a unique marking configuration using Visible Implant Elastomer (VIE) from Northwest Marine Technology, Inc. based in Shaw Island, Washington. The elastomer comes in many different colors that are florescent under ultraviolet light. If kept cold, the elastomer will stay in a liquid form for hours which allows for easy transport. This florescent liquid is administered subcutaneously by carefully penetrating the skin of the fish with the needle of the VIE syringe and slowly injecting the solution. This was performed in multiple anatomical sites on the sample population, each one unique for the stream site in which they were sampled. Areas of injection included the caudal peduncle (left or right flank), behind the post-anal fin, left or right of the dorsal fin, and between the eye and operculum with colors including red, blue, green, yellow, and pink. This array of marking sites and colors allowed for many tagging combinations, ensuring each stream sampling site was properly represented. Once injected into the fish, the liquid hardens into a pliable solid. An ultraviolet light was used to ensure the tag was clearly visible and would shine brightly when the VIE was illuminated. VIE tagging was chosen over external tagging methods or clipping due to a lower risk of infection, predation, and hydrodynamic drag to reduce overall stress on the individuals (Josephson and Robinson 2008).

The sampling method was as follows: all sampling was conducted between the hours of 9AM-3PM. Starting from downstream, electrofishing collection was conducted moving upstream at each flagged stream site, with one to two assistants working dipnets to catch any stunned salmonids. The dipnets were 16”x 9” with 1/4” netting. These fishes were placed into a holding container of fresh stream water and were retained until the sampling at this site was completed. There was no bias toward fish length or weight, and all captured individuals were measured. Once the single-pass had concluded, another container was filled with the MS-222 and water solution to immobilize the fishes for measurement. After approximately one minute when the individuals were adequately anesthetized, each individual was measured for mass and length, and the VIE tag was administered. After the first day of sampling, a UV light was used to locate a potential VIE tag in individuals before they were anesthetized. If a tag was present, that individual had already been processed on a previous sampling date, and the fish was returned to the stream immediately. When measurements and tagging had been performed, the fishes were placed into a container of fresh water to recuperate and regain mobility, and when all fishes were measured and mobile, they were returned to the section of the stream from whence they were sampled.

Population Analysis

Data were analyzed using the von Bertalanffy Growth Function, VBGF (Bertalanffy 1957; Lorenzen 1995). This is one of the most widely-used models in fisheries studies because of its relative simplicity, requiring only the data for size (length or mass) and time/age. Below are the VBGF models for length and weight:

$$L_t = L_\infty(1 - e^{-K(t-t_0)}) \quad (1)$$

$$W_t = (W_\infty(1 - e^{-K(t-t_0)}))^3 \quad (2)$$

L_t/W_t = length or mass at time, t
L_∞/W_∞ = asymptotic length or mass
t_0 = time at length zero
t = time/age in years
K = growth coefficient

These two models are very similar in that they both predict the theoretical length-at-age for an individual within a specific population when appropriate data are available. The asymptotic length or weight (L_∞ and W_∞, respectively) represents the largest individual that a population could produce over an infinite amount of time based upon the current population sample estimates. The variable K represents the growth coefficient of the population. Time, t (in years), is the relative age of the individual based upon the average size-at-age for that species while t_0 represents the time at which the individuals in the population's size was approximately zero. Since all individuals are larger than size zero at the time of hatching, t_0 is a negative value. However, estimates using von Bertalanffy growth models require at least four age classes. The values for L_∞, K, and t_0 were estimated using Fisheries Analysis and Modeling Software (FAMS 1.64, acquired via the American Fisheries Society, Bethesda, Maryland) for each population, as each population differs in these estimates. FAMS was also used to calculate the weight-length regression of each population.

Each population of salmonids in this study was subjected to length-weight regression analysis, W=aL^b. The log-transformed equations for this relationship is Log(W)=Log(a)+bLog(L). Slope (b) is representative of the rate at which individuals grow, and larger slope values suggest fishes that put on more weight-at-length (Froese 2006). This value is approximately 3 (isometric growth), as fishes grow in three dimensions, but it can range in value above or below this standard, ranging from 2.5-3.5 (Froese 2006; Datta et al. 2013) which suggests positive or negative allometric growth, respectively. The variation among these data sets was then compared using an independent t-test in IBM SPSS Statistics Version 23. Kolmolgorov-Smirnov test of normality was used to assess the assumption of normality, and the data were normally distributed so the independent t-test was a viable method of analysis.

Fulton's Condition Factor (C) is a method of analysis that indicates the relative health of fishes using the equation $C=100(W/L^3)$. The calculated value is an estimate of the nutritional and physiological health of individual fishes (Jin et al. 2015). A condition value >1 generally indicates good health in individuals, while a value <1 indicates poor health (Datta et al. 2013). These values were compared across age classes of brook trout from both streams using one-way ANOVA in SPSS with a Tukey post-hoc analysis.

Briar Creek Site

Briar Creek is a second-order stream where Nagel (1991) and Lambert (1998) completed previous studies. Briar Creek is located between Johnson City and Erwin off Dry Creek Road (State Highway 2587) on Buffalo Mountain in Washington County, TN and is within The Cherokee National Forest. The stream is approximately 2100-2400 feet above sea level (US Geological Survey, 2003, Erwin quadrangle, Tennessee). The entrance to the site is on Forest Service Road 188 (also known as Briar Creek Road, though no street signs mark the road) at 36° 13' 41"N, 82° 24' 06"W. Following the gravel road will lead to a crossing of the stream over a concrete culvert.

The study area was a 2.8 km portion of the stream, stretching from 0.7 km upstream of the culvert to 2.1 km downstream of the culvert. Sampling stations were marked with flags every 100 meters making a total of 28 sampling sites. This site ends downstream at a large waterfall of approximately 10-meters. These sections were divided into the same zones used by Nagel and Deaton (1989) and Lambert (1998) in the previous studies. The Upper Invasion Zone comprised 0.7 km of the reach upstream of the culvert, and was primarily brook trout habitat. The lowest 0.7 km reach upstream of the waterfall was The Lower Invasion Zone and was primarily rainbow trout habitat. The 1.4 km stretch between these two zones is the Introduction Zone, where the introduced *S. fontinalis* population was released between 1983-1987 (Nagel and Deaton 1989), and where both species were present.

Left Prong Hampton Creek

This stream is within the Hampton Creek Cove State Natural Area. The parking area for Hampton Creek Cove is at approximately 36° 9’ 9”N, 82° 3’ 21”W adjacent to Hampton Creek Road near Roan Mountain State Park in Carter County, TN. The state recreation area is 693 acres, and the sample sites for this study were situated approximately 3000-3500 feet in elevation (US Geological Survey, 2003, White Rock quadrangle, Tennessee). Left Prong Hampton Creek’s native brook trout population has been described as one of the best in the state of Tennessee with a biomass of approximately four times the state average (Habera et al. 2017), and serves as a good comparison for the Briar Creek population. Habitat quality assessments were also performed at these sites as they were at Briar Creek, and the survey suggested this was also a sub-optimal stream due to sediment deposits and erosion of stream banks.

This site is split into two sampling portions: above the waterfall and below it with each reach being 0.7 km long. Unlike Briar Creek, this stream does not have an intermediate stretch of sympatric mingling, so it was assumed that all salmonids above the waterfall would be *S. fontinalis* while most, if not all, downstream salmonids would be *O. mykiss*. I say “most” because it is possible for upstream salmonids to traverse the waterfall, but it is very much a one-way trip. It seems unlikely that any salmonid would cross this barrier.

CHAPTER 3

RESULTS

The von Bertalanffy growth function was performed for the brook trout population at Left Prong Hampton Creek. This analysis determined that the population (n=101) had a growth coefficient (K) of 0.419, a t0 value of -0.0149 years, an asymptotic length of 218mm, and an R^2 value of 0.9985 (Figure 1). With these estimates, it would be possible to project the theoretical length-at-age for any point in a brook trout's life from this stream. Unfortunately, drought conditions at Briar Creek may have caused age class failures at Briar Creek, as no Age 2+ individuals were present (Figure 2). For von Bertalanffy models, at least four age classes must be present, so it could not be used for Briar Creek.

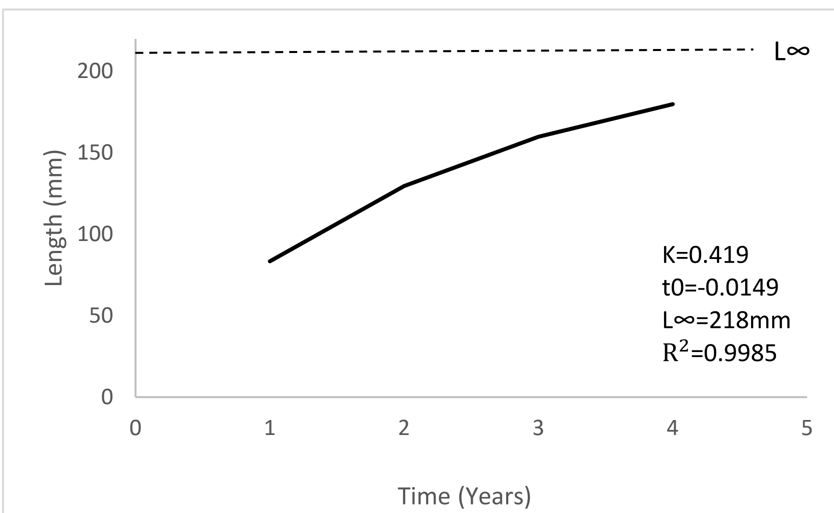

Figure 1. Von Bertalanffy growth model for brook trout in Left Prong Hampton Creek. The curve represents the average length-at-age for individual brook trout within this stream, with the dotted line representing asymptotic length. Produced using FAMS 1.64

Fulton's Condition Factor ($C=10^5 \text{x } W/L^3$) was calculated for each brook trout individual in both populations to determine the individual health of those relative to their weight-at-length.

For Briar Creek (n=81), the average condition value was C=1.36±0.22SD (Table 4) while Left Prong Hampton Creek (n=101) had an average of C=1.33±0.13SD (Table 3).

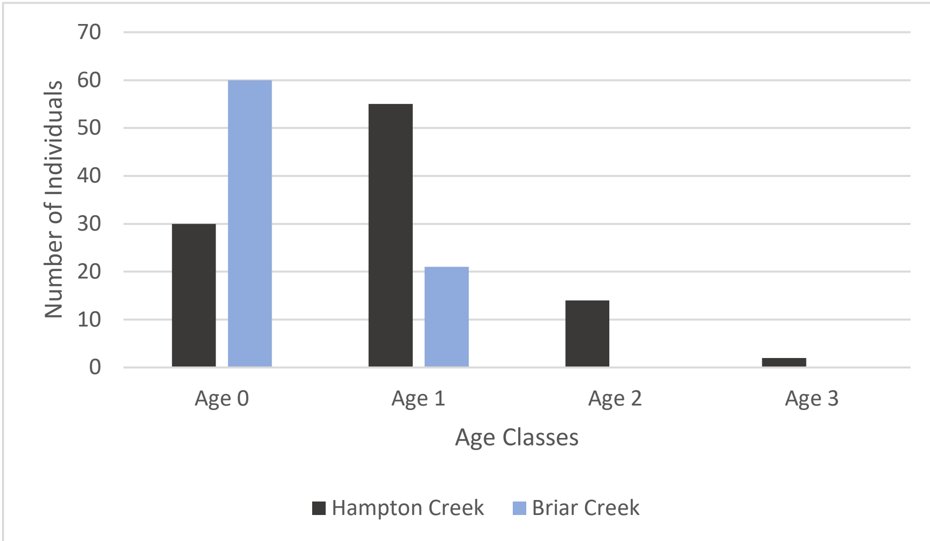

Figure 2. Frequency of brook trout individuals per age class in Briar Creek and Left Prong Hampton Creek populations. It is important to note that Briar Creek is absent of higher age classes of Age 2+ (Kulp 1994).

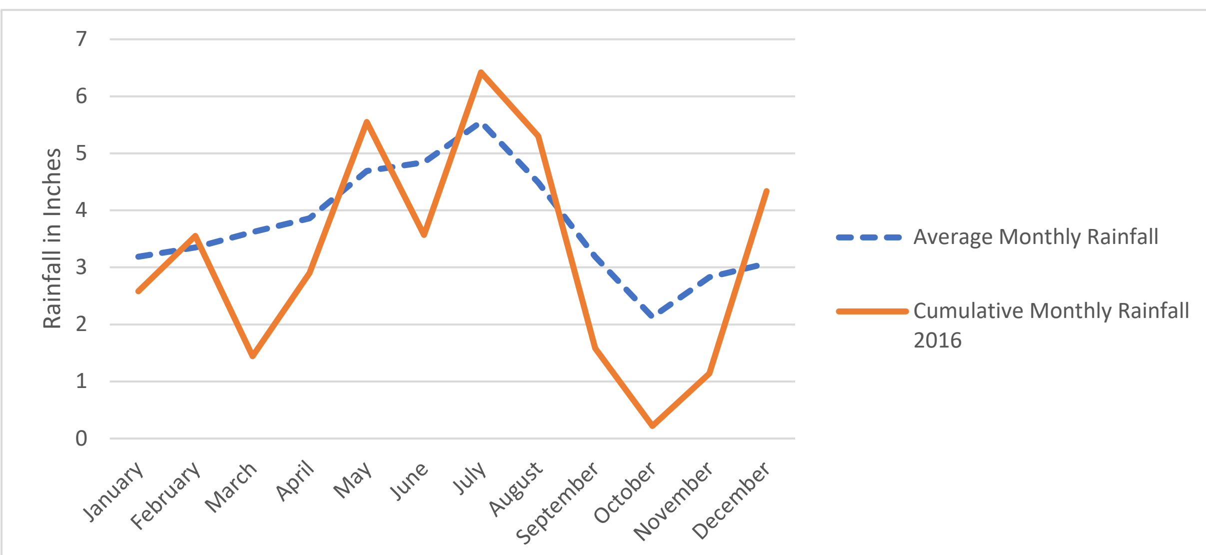

Figure 3. Mean average rainfall (in inches) per month for Erwin, TN compared to the cumulative monthly rainfall for 2016. (Source: National Oceanic and Atmospheric Administration).

Northeast Tennessee experienced severe-to-extreme drought conditions during this study with precipitation measuring 35% below average for March-May and 50% below average for September-November (Habera, TWRA, personal communication) as displayed in Figure 3. Previous evidence suggests that during drought conditions, older trout are influenced more so than younger, smaller individuals (Elliot 1987). To examine this affect in these brook trout populations, ANOVA was performed between age classes. The means of condition factor results can be seen in Figure 4 while the results of statistical analysis are found in Tables 1 and 2 (ANOVA for significance between age classes for each stream) and Tables 3 and 4 (descriptives of individual age classes).

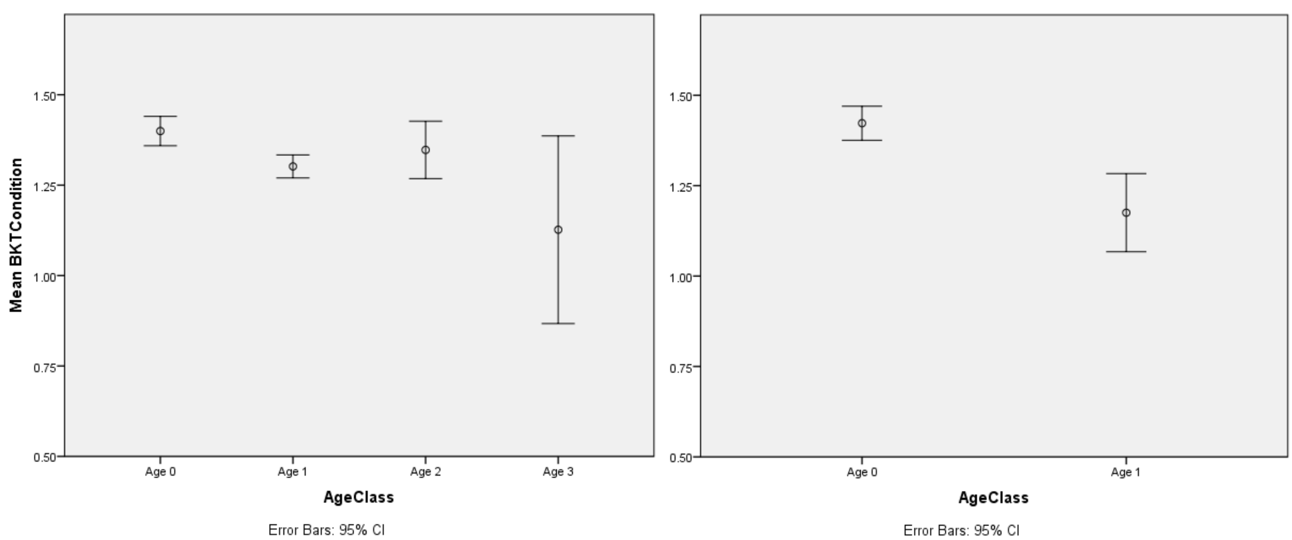

Figure 4. Mean Fulton's Condition Factor values compared between the four age classes of brook trout in Left Prong Hampton Creek (left) and the two age classes at Briar Creek (right). Error bars represent 95% confidence intervals.

With the Age 2+ classes absent from Briar Creek (Figure 2), it was expected that the population could have been in decline and that the remaining individuals in the stream were unhealthy. Condition factor results indicate that this may not be the case. Briar Creek condition for age classes (Ages 0 and 1+) were significantly different, with a p-value of <0.001 (Table 1).

Even though the Age 1+ group sustained a mean condition value of 1.18±0.24SD (Table 4), which is still considered as healthy by Fulton's Condition standards (Datta et al. 2013), the value was significantly lower than the Age 0 class's mean of 1.42±0.18SD, which could suggest that the older age class was less successful in allocating resources during this time period. The Age 1+ and 3+ classes differed significantly from Age 0 in Left Prong Hampton Creek, with p-values of 0.002 and 0.010, respectively. The Age 1+ and Age 3+ classes showed no significant difference in Fulton's Condition Factor (p=0.169), and the Age 2+ class did not show a significant difference from Age 0 (p=0.518), Age 1+ (p=0.568), or Age 3+ class (p=0.068) with 95% confidence. These relationships are represented graphically in Figure 4. Notice that the error bars for the Age 3+ brook trout in Left Prong Hampton Creek are quite large, and this could be due to the low number of individuals being within this age class (two brook trout) which has the potential to skew these estimates.

Table 1. ANOVA for condition factor between Briar Creek brook trout age classes

BriarBKT	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.953	1	.953	24.350	.000
Within Groups	3.090	79	.039		
Total	4.043	80			

Table 2. ANOVA for condition factor between Left Prong Hampton Creek brook trout age classes

HamptonBKT	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.275	3	.092	6.643	.000
Within Groups	1.336	97	.014		
Total	1.611	100			

ANOVA results from both Briar Creek (Table 1) and Left Prong Hampton Creek (Table 2) suggest that there was a significant difference of condition factor between individuals of those respective populations, both with significance of $p<0.001$. There were only two age classes compared in Briar Creek, but four age classes were compared in Left Prong Hampton Creek. The analysis of condition factor between individual age classes are displayed in Tables 3 & 4 with a graphic representation for each stream in Figure 4.

Table 3. Descriptive statistics of Fulton's Condition Factor for each brook trout age class in Left Prong Hampton Creek

				95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Lower Bound	Upper Bound	Minimum	Maximum
Age 0	30	1.3996	.10850	1.3591	1.4402	1.01	1.57
Age 1	55	1.3018	.11774	1.2700	1.3337	.96	1.52
Age 2	14	1.3474	.13747	1.2680	1.4268	1.09	1.62
Age 3	2	1.1267	.02888	.8673	1.3862	1.11	1.15
Total	101	1.3337	.12693	1.3087	1.3588	.96	1.62

Table 4. Descriptive statistics of Fulton's Condition Factor for each brook trout age class in Briar Creek

				95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Lower Bound	Upper Bound	Minimum	Maximum
Age 0	60	1.4227	.18240	1.3756	1.4698	.93	1.82
Age 1	21	1.1752	.23742	1.0671	1.2833	.74	1.55
Total	81	1.3585	.22480	1.3088	1.4082	.74	1.82

Habitat quality assessments from the Division of Water Pollution Control for moderate-to-high gradient streams were completed at each site before sampling began, and were averaged for both streams. The results indicated both streams were of sub-optimal condition, primarily due to large deposits of sediment in some places and erosion of the stream banks. Sediment can accumulate and impede stream flow, filling vital pools and crevices that salmonids use for cover and reproduction (Argent and Flebbe 1998). Another factor was low stream depth, which was impacted by substantial drought in the region in 2016. The drought was so severe that one 15-meter stretch of stream in the Introduction Zone was almost completely void of water. Stream temperatures were recorded at an average of 18°C in Briar Creek, and the temperature may have been influenced by drought conditions.

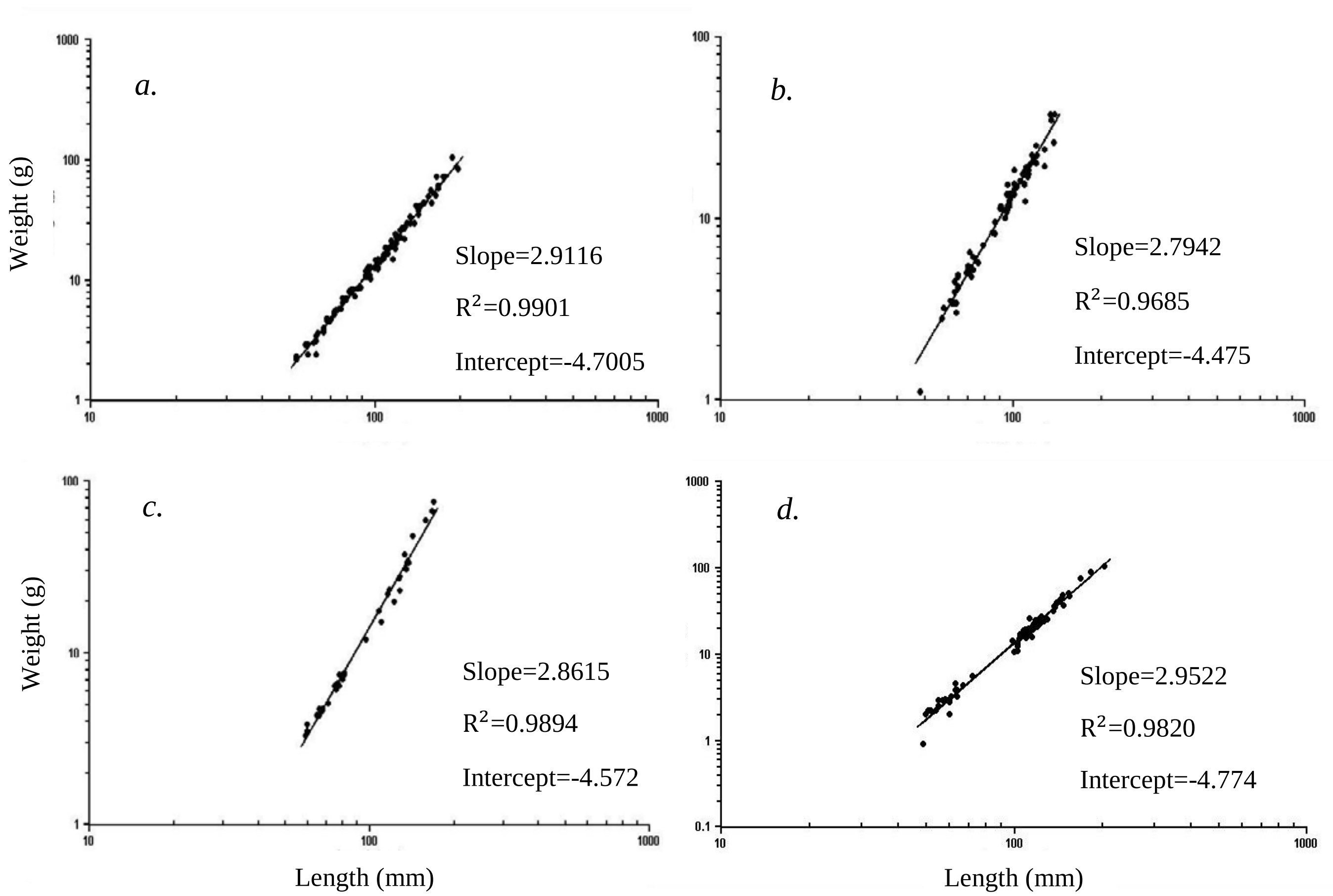

Figure 5. A side-by-side comparison of the log-log length-weight regression of brook trout populations in Left Prong Hampton Creek (*a*, n=101) and Briar Creek (*b*, n=81). The same analysis was conducted for rainbow trout from Left Prong Hampton Creek (*c*, n=36) and Briar Creek (*d*, n=71).

Log-log length-weight regression was performed for *S. fontinalis* populations in both streams (Figure 5). R-squared values for Hampton Creek (0.9901) and Briar Creek (0.9509) suggest that both samples fit their calculated regressions well. This method of analysis was conducted for the *O. mykiss* populations of both streams, as well. Slope, intercept, R-squared values are displayed on their respective regressions in Figure 5. The slopes of the length-weight regression (Figure 5) were analyzed via an independent t-test to compare the growth rates of both salmonid species between streams. The results suggest that the growth of brook trout in Briar Creek (slope=2.7942) is significantly lower than those in Left Prong Hampton Creek (slope=2.9116) where $p<0.001$, but there was no significant difference between the rainbow trout populations ($p=0.655$), which suggests that there is significant difference in growth of the brook trout populations, but not the rainbow trout. Residual analysis of the logarithmic values indicated that the data were evenly and randomly distributed around the mean with no trends present for either stream, suggesting no other factors are likely to be influencing these results (Figure 6).

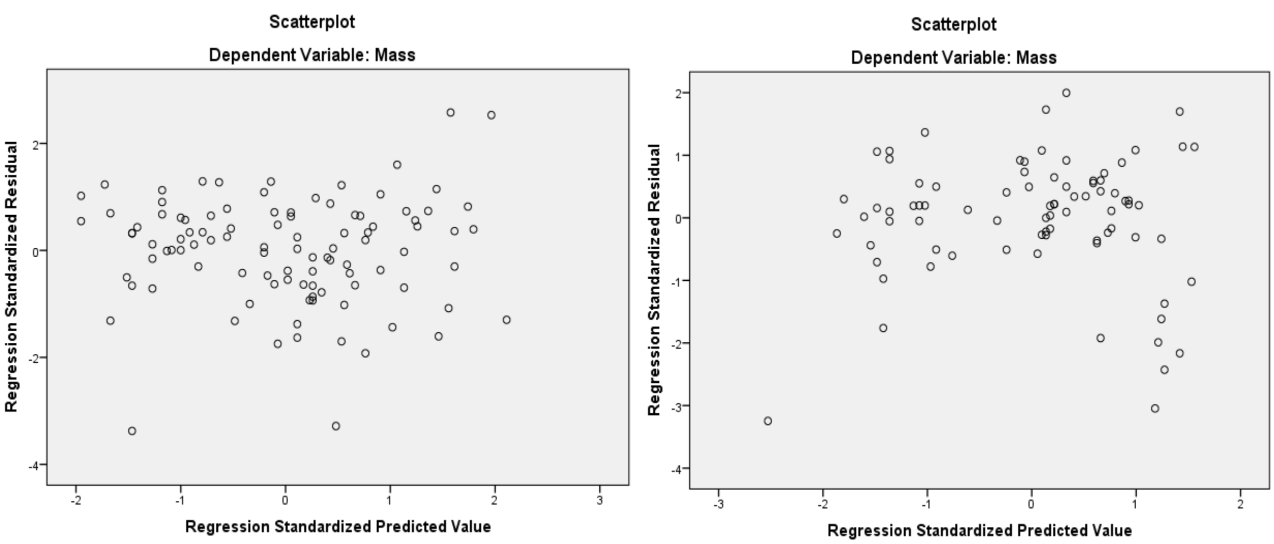

Figure 6. Residual analysis of log-log length-weight regression in brook trout populations in Left Prong Hampton Creek (left) and Briar Creek (right).

CHAPTER 4

DISCUSSION

During 2016, much of northeast Tennessee was experiencing significant drought conditions, especially in the spring and fall months, and it is possible that the salmonid populations may have been challenged during these months due to lower stream flows during a period of significant drought (Habera et al. 2017). Reduced population size and the overall absence of higher age classes could be attributed to the effects of long periods of drought (Elliot 1987; Hakala and Hartman 2004). Even after sampling nearly every weekend between August-late November, the sample size for the Briar Creek population was small and lacked the presence of higher age classes of brook trout (Age 2+).

Flood and drought have the potential to negatively impact salmonid populations in isolated headwater streams (Phillips et al. 1987; Hakala and Hartman 2004), and this may have been a contributing factor to the absence of higher brook trout age classes (Figure 2). Previous studies have shown that drought and low flow stream conditions affect older, larger salmonids more than younger age classes because of limited pool and refuge habitat because larger fishes require more spatial resources than smaller fishes (Elliot 1987). Brook trout studied under drought conditions in West Virginia suffered losses in older age classes due to drought conditions, and the young-of-the-year (YOY) abundance the following summer was only 67% of the previous year (Hakala and Hartman 2004). The older brook trout individuals that remained suffered a 10% reduction in overall condition factor. A likely factor in the reduction of YOY individuals is the loss of larger spawning adults with higher fertility (Hakala and Hartman 2004). Also, egg mortality may have increased due to lower condition factor of spawning adults, as shown in previous studies (Laine and Rajasilta 1999). It is possible that the absence of older age classes in Briar Creek could mean reduced numbers of YOY in the following summer. It would be informative to return to Briar Creek following the drought conditions to determine if the age class distribution changes significantly post-drought.

There was a complete lack of brook trout individuals older than two years of age in Briar Creek, which is likely due to habitat constraints caused by drought conditions. Larger pools and riffle runs are utilized by larger salmonids, but those refugia become less common during drought conditions as stream levels lower. One possible method of addressing the loss of pooled regions in the future would be to construct artificial dams where the water could collect and deepen, providing more habitat for larger fishes. In a stream study in Wisconsin, a 1-meter concrete wall was removed from a stream in an effort to determine if brook trout growth or condition was influenced by the removal of barriers, and to determine if salmonid stream movement or age class distribution was effected (Stanley et al. 2007). There were no significant changes in the condition of brook trout, juvenile or adult, but it was noted that there were fewer adult individuals after the removal of the structure which could have been attributed to sedimentation caused by the wall removal or by the loss of the larger pools. Movement of salmonids did not appear to differ significantly between pre- and post-removal, either, suggesting salmonids can traverse these structures (Stanley et al. 2007). By damming portions of the stream, larger pools could be created to possibly facilitate older, larger salmonids during long periods of drought.

The abiotic catastrophe model (Phillips et al. 1987) projected that headwater streams of 2.5km in length stood a 56% probability of extirpation via catastrophe, and this model was formulated specifically for Briar Creek. With the inhabited brook trout study plot at Briar Creek measuring approximately 2.8km, this a realistic concern, and the climatic conditions during the year may have eliminated the higher age classes of *S. fontinalis* within this stream. As with any field study, it is difficult to account for all variables, and the removal of larger brook trout from the stream by fishermen is a possibility. However, this is highly unlikely as this stream is small with little fishing pressure (Habera, TWRA, personal communication). While the assumption of abiotic catastrophe being the primary factor of brook trout age class loss is difficult to infer without more evidence, the results of this study add support to these claims.

Results of the logarithmic length-weight regression suggest that the brook trout population at Briar Creek exhibits the lowest rate of allometric growth out of all four salmonid populations. Lower slope values equate to slower growth rates, relative to the isometric growth rate of 3 (Froese 2006), and these results along with the significantly lower condition factor values for Briar Creek brook trout indicate there is likely some factor influencing the difference in growth observed between these populations, whether that be from genetic or abiotic influence. Regardless, it is possible to project the theoretical weight-at-length for individuals within these populations by substituting slope and intercept values from the logarithmic regression into the equations for length-weight relationship in fishes, $W=aL^b$, and this could be used in future studies as comparison for these streams.

Elevation could also play a role in the growth of salmonid populations. No previous studies of this relationship could be found specifically for southern brook trout, but growth of salmonid populations has been shown to be significantly influenced by variation in elevation (Belk et al. 2009; Warren et al. 2014). If elevation plays a role in southern Appalachian brook trout growth, it could account (at least in part) for the significance between growth and condition factor values observed in this study as Briar Creek and Left Prong Hampton Creek differ in elevation by approximately 600-800 feet. This could be a possibility, as native southern brook trout habitat starts at an elevation of approximately 2000 feet (Hudy et al. 2005), so the Briar Creek study site (2100-2400 feet) would be in the lower elevational range for these southern populations and could attribute to their lower condition factor, especially in drought conditions.

As stated previously, it is difficult to determine whether these differences of growth are attributed to genetic diversity among populations (Danzmann et al. 1998) or if it is influenced by environmental factors like drought and stream morphology. Future studies could address this question by analyzing the growth of a brook trout cohort in vitro versus the same cohort in situ by extracting eggs from brook trout in a stream population and raising them in a controlled setting over the life span of the individuals, recording growth measurements over the span of multiple years. Then the growths of the in vitro and in situ populations could be compared to determine if the potential difference in growth is due to genetic variability between populations or a result of abiotic influence.

In conclusion, it appears that condition factor in older classes of brook trout may be significantly influenced by drought conditions, and though rates of population growth seem to differ significantly in brook trout between the two streams, it is difficult to conclude if this result is primarily a product of genetic variation between isolated headwater populations or influence by abiotic factors. Further studies are warranted to make this distinction. It is suggested that Briar Creek remain monitored over the next few years to follow the status of the brook trout population there. It is possible that if normal precipitation levels return to the region, the current *S. fontinalis* population will recover as higher age classes re-emerge.

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