RESPONSE OF BROOK TROUT (*SALVELINUS FONTINALIS)* POPULATIONS TO HABITAT CONDITIONS AND COMPETITION IN SOUTHERN APPALACHIAN STREAMS

A Thesis
by
AMBER OLSON

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Abstract

RESPONSE OF BROOK TROUT (*SALVELINUS FONTINALIS)* POPULATIONS TO HABITAT CONDITIONS AND COMPETITION IN SOUTHERN APPALACHIAN STREAMS

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Brook Trout (*Salvelinus fontinalis*) are the only freshwater salmonid native to Southern Appalachian streams but were largely extirpated from this region in the late 19th and early 20th centuries. This extirpation was largely caused by wide-scale logging of Appalachian forests and resulting stream habitat degradation. Following the collapse of native Brook Trout populations, non-native *S. fontinalis* from northern hatcheries were stocked along with Rainbow (*Oncorhynchus mykiss)* and Brown Trout (*Salmo trutta*). Studies on Brook Trout growth and abundance have shown that they are influenced by the presence of non-native competitors as well as by abiotic factors including stream elevation and land use. Currently, native Brook Trout in North Carolina are largely relegated to headwater streams, presumably as a result of historical habitat alteration and contemporary competition with non-native salmonids. I conducted a study comparing the growth and age-class structures of 13 Brook Trout populations across western North Carolina in the presence or absence of non-native salmonids along with environmental co-variates such as pH and elevation. I found that the average size and age of Brook Trout was higher in sympatric streams compared to populations in allopatric streams, but that L_{∞}. was higher in allopatric populations. Populations occurring in sympatry with non-native salmonids exhibited increased age and length variability when compared to allopatric stream populations. The average size and age of Brook Trout in both allopatric and sympatric populations appeared to increase with elevation. Habitat variables (PCA scores) were used in Generalized Linear and Generalized Linear Mixed models to examine the effects of environmental variables on mean age and length, as well as age-class structure of sympatric and allopatric Brook Trout populations. AIC and AICc scores revealed that no models were more informative than the null. Despite the lack of significant results in explaining what factors affect native Brook Trout growth and age-structure the most, the results of this study suggest that environmental factors including elevation and water chemistry may be more important to Brook Trout growth than competition with non-native salmonids. Future studies should re-visit these questions using more focused studies designed to address questions related to understanding how native and introduced fishes interact across communities in this region.

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I would first like to thank the North Carolina Wildlife Commission and Auburn University for generously providing the otoliths for this project, and to the Office of Student Research for funding the supplies needed to complete it. I would next like to thank my advisor, Dr. Michael Gangloff, for both his willingness to take me on as a graduate student and for his patience and encouragement in helping me get through my degree, especially during the unprecedented time of Covid-19. I would also like to thank my committee members, Dr. Robert Creed and Jacob Rash, for both their support and suggestions to make my analyses stronger. I want to extend a special thanks to Samuel Fritz, without whom my statistical analyses would not be possible. A large thanks goes to the numerous people across multiple departments and agencies who patiently answered the many questions I had regarding otolith processing and analyses, including in no particular order and not limited to: Jason Selong, Kevin Hining, Derek Ogle, Brian Ertel, Keith Wellstone and Philip Doepke. A massive thanks goes to Anthony Love, for both entrusting me with access to the isomet saw necessary for my research, and for the expertise that allowed my otolith sections to come out as good as possible. I would like to thank all of the members of my lab who supported me throughout the process of completing my thesis. In no particular order, Freddy Ortega, Brandon Williams, Henry Gates, Vincent Santini, Elijah Thompson and Locke Revels were instrumental in the completion of my thesis, through both helping with my data collection and analyses and in motivating me to complete this project. Finally, I would like to thank my parents, Christopher Olson and Sarah Williams, for their unrelenting support and encouragement in getting through the (long) process of completing my Masters.

Dedication

I dedicate this thesis to all of the trout included in this study who sacrificed their lives in the name of science

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Foreword

The research detailed in this thesis will be submitted to the peer-reviewed journal Transactions of the American Fisheries Society. This thesis has been formatted according to the style requirements for publication in this journal.

Introduction

Invasive species (i.e., introduced, non-native species that readily spread within or among ecosystems) have been listed as one of the main drivers of global biodiversity loss, and many are also a major economic and human health concerns (USDA 1999; US NRC 2002). Invasive species are also the second most commonly cited cause of species extinction and endangerment behind human development (Crowl et al. 2008). Anthropogenic activities, including land use change and increased global trade and travel, are among the main drivers of the spread of invasive species. Climate change may also facilitate the spread of invasive species and it is predicted that warming of the southeastern US will make this region more suitable to potential invaders (Dix et al. 2009; Mainka and Howard 2010).

Freshwater ecosystems across the globe are especially vulnerable to invasions and many freshwaters support some of Earth's most at-risk species (Dextrase and Mandrak 2006; Hermoso et al. 2011). In North American freshwaters, native species are disproportionately impacted by invasive species compared to their terrestrial counterparts (Dextrase and Mandrak 2006; Moorhouse and Macdonald 2014). Invasive species that have been problematic in North American freshwater systems are taxonomically diverse and include aquatic macrophytes (e.g., water milfoils, *Myriophyllum* spp.), crustaceans (e.g., Rusty Crayfish, *Orconectes rusticus*), mollusks (e.g., Zebra Mussels, *Dreissena polymorpha*) and fishes (e.g., Silver Carp, *Ctenopharyngodon Idella*), (Macisaac 1996; Creed 1998, 2000; Strayer 2008; USDA 1999).

Although many invasive species were introduced accidentally into freshwaters, some were intentionally introduced for aesthetic, commercial, or recreational purposes (U.S. Fish and Wildlife Service 2012). This is the case for two popular game fish that have been introduced globally: Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) (Myers et al. 2014; Budy and Gaeta 2018). Rainbow Trout are native to Pacific Coast drainages in western North America, and Brown Trout were introduced from Europe in the late 18th century (Jenkins and Burkhead 1994). In many areas where they have been introduced, these exotic salmonids often outcompete and subsequently replace native fishes, including Brook Trout (*Salvelinus fontinalis*) in eastern North America (Myers et al. 2014; Kanno et al. 2016; Budy and Gaeta 2018).

Brook Trout (*Salvelinus fontinalis*) are the only salmonid native to the southeastern United States, with a historical range in North America that extended from southern end of Hudson Bay to northwestern Georgia (Jenkins and Burkhead 1994). Populations in the southern Appalachian Mountains represent the southernmost extent of *Salvelinus* spp. in the Northern Hemisphere (Jenkins and Burkhead 1994; Behnke 2002; Bugas et al. 2019). A primarily insectivorous fish, Brook Trout are like other *Salvelinus* in that they are able to tolerate colder temperatures than many other salmonid species and prefer colder waters with an optimal temperature range of roughly 13 – 16°C (Wesner et al. 2011; Chadwick and McCormick 2017). In the Southern Appalachians, Meisner (1990) hypothesized that a minimum elevation of 614 m is required to maintain optimum water temperatures for Brook Trout, notably higher than the minimum elevations needed at more northern latitudes. In western North Carolina many Brook Trout populations occur in cold, clear, high-elevation streams characterized by both low dissolved ion concentrations and low benthic productivity (Hurley et al. 1989; Stranko et al. 2011; Wesner et al. 2011).

In the late 19th and early 20th centuries, anthropogenic activities including widespread clear-cut logging of steep slopes largely extirpated native Brook Trout from across much of their historical range in the Southern Appalachians (Habera and Moore 2005; Davis 2007; Kazyak et al. 2018). Many streams were subsequently restocked with both non-native salmonids and Brook Trout from northern hatcheries (Habera and Moore 2005; Davis 2007; Kazyak et al. 2018). Hudy et al. (2008) found that native Brook Trout distributions were reduced in at least 116 sub-watersheds out of 119 sampled in North Carolina, and Brook Trout are currently listed by the North Carolina Wildlife Resources Commission (NCWRC) as a "Species of Greatest Conservation Need" (SGCN) (NCWRC 2020). Presently, southeastern Brook Trout populations are mostly relegated to headwater streams, often above barriers that exclude non-native salmonids (Hudy et al. 2008; Myers et al. 2014). This alienation has led to concerns about genetic isolation in remaining native Brook Trout populations, and many impacted populations in streams that were re-stocked with hatchery Brook Trout have been found to have high levels of genetic introgression with introduced fish (Hudy et al. 2008; Wesner et al. 2011; Kazyak et al. 2018; Weathers et al. 2018).

Although isolation is a concern for the genetic integrity of native Brook Trout populations, barriers including small dams and waterfalls may provide refuge from non-native Brown and Rainbow trout (Kirk et al. 2018). In streams where they have been introduced, Brown and Rainbow trout have been shown to have a number of negative effects on Brook Trout (Fausch and White 1981; Myers et al. 2014; Hoxmeier and Dieterman 2019; Hitt et al. 2017; Budy and Gaeta 2018). Fausch and White (1981) and Hitt et al. (2017) found that introduced Brown Trout may limit both Brook Trout movements and access to thermal refugia, and adult Brown Trout prey upon Brook Trout juveniles. Carlson et al. (2007) conducted a study in Massachusetts that found that when the two species are in sympatry, Brown Trout tended to grow larger and faster than Brook Trout. Rainbow trout will also tend to grow larger and displace native Brook Trout when in they are in sympatry, and the removal of non-native Rainbow Trout has been shown to allow Brook Trout populations to recover and reach carrying capacities more typical of allopatric populations (Whitworth and Strange 1983; Larson and Moore 1985; Myers et al. 2014; Kanno et al. 2016). Both Brown and Rainbow trout are better able to tolerate greater thermal ranges and other environmental stressors compared to Brook Trout, and it is predicted that climate change will lead to further encroachment by non-native salmonids and increased fragmentation of already isolated populations (Flebbe 1997). Non-native Brown Trout are also thought to be the original vector of introduced the Whirling Disease (*Myxobolus cerebralis*), which deforms salmonid spines and has recently been found in southeastern Brook Trout populations (Ksepka et al. 2020).

A combination of the presence of non-native trout and environmental variables may affect Brook Trout survivability and growth more than each individual factor alone. For example, a study by Hoxmeier and Dieterman (2019) found that in streams where environmental variables were favorable, Brook Trout were able to resist invasion from non-native Brown Trout. Petty et al. (2014) suggests that the interactions between environmental variables and the presence of potential competitors may be critical in explaining growth trends of Brook Trout Populations sympatric with non-native salmonids.

Analyses of growth and age classes can provide critical insight into the recruitment, survival and overall health of a population, and have been used extensively in fisheries management (Maceina et al. 2007; Quist et al. 2017; Kerns and Lombardi-Carlson 2017; Paukert and Spurgeon 2017). Although there are a variety of structures that can be used to age fish (e.g., scales, fin rays, spines) otoliths are considered to be most accurate for aging in most freshwater species, including salmonids (Konopacky and Estes 1986; Hining et al. 2000; Whitledge 2017). In this study, I compared the growth and age/size-class structures of 13 populations of Brook Trout across western North Carolina using otoliths collected by NCWRC biologists during a recent survey of the distribution of Whirling Disease in western North Carolina salmonid populations (Ksepka et al. 2020). I predicted that Brook Trout populations in the presence of non-native Brown and/or Rainbow Trout would exhibit decreased growth compared to allopatric populations. I also predicted that environmental and land-use variables such as temperature, pH and forest cover would have a significant impact on the size and age of Brook Trout.

Methods

Fish Collection

Between June 2017 and October 2019, biologists with the NCWRC collected wild Brown Trout, Rainbow Trout and Brook Trout across 113 localities in 7 river basins to examine the distribution of *Myxobolus cerebralis* in western North Carolina (Ksepka et al. 2020). Per Ksepka et al. (2020), “State agencies selected sites that had high recreational value, sustained natural reproduction of trouts, or were in under sampled systems, when possible trout of a size likely to belong to the year-1 class were prioritized for collection”. Fish were collected via electroshocking a 100 m reach in an upstream direction, until ≈ 30 fish had been collected or until the end of the reach (Jacob Rash, NCWRC pers. com). Latitude and Longitude were recorded for each site. Fish were sent to Auburn University, where standard length (SL), total length (TL) and weight (WW) were recorded for each individual (Ksepka et al. 2020). Otoliths were removed by researchers at Auburn University's Southeastern Cooperative Fish Parasite and Disease Laboratory before being stored dry in centrifuge tubes prior to processing.

Thirteen streams in North Carolina from the Ksepka et al. (2020) study sampled between April and October 2018 were chosen for this project. Eight streams containing Brook Trout (n=109), Brown Trout (n=92) and some Rainbow Trout (n=45) were designated as "sympatric", while the remaining five streams containing only Brook Trout (n=104) were designated as "allopatric". Fish that did not have otoliths, or that had otoliths that were unreadable/broken were not included in the age analyses but were included in the other statistical analyses.

Otolith Processing and Analysis

Otoliths were processed following the methods outlined in Long and Grabowski (2017) and the Idaho Fish and Game Department otolith Sectioning and Digitizing Protocol (2015). Otoliths were mounted in rubber bullet molds, in a 2:1 resin to hardener epoxy mixture (Epothin 2 resin and hardener, Buehler). Bullet molds were initially filled halfway with the 2:1 epoxy mixture, and allowed to sit until partially (≈ 4 hours) or fully hardened before the otoliths were mounted.

Unless missing or damaged, the right otolith from each fish was mounted. Otoliths were placed in the mold sulcus side up, with the rostrum positioned ≈ 1/3 of the distance of the mold away from the pointed end of the mold. Otoliths were then covered with the 2:1 epoxy mixture and left to harden for at least an additional 12 hours.

Transverse sections of the cores of the otoliths were taken with an Isomet-1000 saw to a thickness of 0.6 mm, depending on the size of the otolith. Otoliths were placed on slides in immersion oil and observed under a compound scope at 40x or 100x magnification (depending on the size of the otolith). Images of the otolith sections were captured and used for aging. Otoliths were aged independently by myself and one other observer. Discrepancies between ages were resolved with myself and the other observer after each independent analysis. Boxplots and histograms of the age and boxplots of the standard length of each Brook Trout population were created in R 4.1.0 using the package *ggplot2*.

When length-at-age data are available for a population of fish, estimated growth for said population is typically estimated through non-linear models such as the von Bertalanffy function (Ogle et al. 2017). The von Bertalanffy function is the function most commonly used to model growth in fish communities, and is typically parametrized as

$$L = f(T, L_\infty, K, t_0) = L_\infty \left[1 - e^{-K(T - t_0)} \right],$$

where L is length, T is age, and L_∞, K, and t_0 are parameters to be estimated (von Bertalanffy 1938; Ogle et al. 2017). L_∞, represents the asymptotic mean length for a population, K is a growth coefficient which describes how quickly the mean length approaches L_∞, while t_0 represents the theoretical age at length 0 (Ogle et al. 2017). I estimated L_∞, K, and t_0 and plotted the von Bertalanffy curves for the allopatric and sympatric Brook Trout populations using packages *FSA*, *MASS*, *nlstools*, *minpack.lm*, *cvTools*, *nlme,* and *lattice* in R 4.1.0 for

Windows, with code modified from Ogle et al. (2017). Due to low sample sizes and age class spread in most individual streams, all *allopatric* and *sympatric* populations were combined into singular data-sets.

Environmental Parameters

Environmental and land-use data were collected using the web-based USGS stream stats tool (www.usgs,streamstats.gov). Basins for each site were delineated using the recorded latitude and longitude from where collection reach was located. Mean elevation (m), mean slope (m), basin area (km^2), percent forest, percent impervious area, percent farmland, and percent urban area were computed using the stream stats application.

Water quality data were not collected for sites in Kspeka et al. 2020, so I took single point samples between 16 May and 4 June 2021 using a YSI Pro Series MultiParameter Meter (Yellow Springs Instruments, Yellow Springs OH) at each site sampled by the NCWRC in 2018. Water temperature (°C), atmospheric pressure (mmHg), dissolved oxygen (% saturation), specific conductance (µS/cm), conductivity(C), Salinity (ppt), pH, and nitrate concentration (NO_3^- mg/L) were recorded from each site. A subset of these variables (elevation, slope, percent forest area, temperature, DO saturation, specific conductance, pH, and nitrate) were utilized as covariates in the age and length analyses. The other environmental variables were not included in the analyses because they were auto-correlated with other environmental variables. Mean elevation, mean slope, and percent forest area were scaled into z-scores in R 4.1.0 prior to inclusion in the Principal Components Analysis

(PCA), Generalized Linear (GL) and Generalized Linear Mixed models (GLM) to help center the data for those variables.

Principal Components Analysis

Principal Components Analysis is a method of dimension reduction that compresses a large set of variables into a set of reduced uncorrelated variables for easier data analyses, and has been utilized in other studies analyzing the impact of environmental gradients and competition on Brook Trout age structure (Kirk et al 2018). I conducted a correlation-based PCA using environmental data from focal streams in R 4.1.0. PC scores with Eigen values >1 were used as covariates in subsequent GL and GLM analyses.

Growth Analyses

Average age, length, length-at-age, and the number of age classes of each Brook Trout population were chosen as response variables for the GL and GLM analyses. Average age and length were determined to be normally distributed, (Shapiro test: W=0.915, p-value=0.217; W=0.960, p-value=0.760) and were used as response variables for GL in the function (lm) in base R 4.1.0. Length-at-age and number of age classes were non-normal, (Shapiro test: W=0.861, p-value=0.04; W=0.567, p-value=$3.477e^{-05}$), and could not be log transformed. Generalized Linear Mixed models were created in the (glm) function in R4.1.0 for the length-at-age and age class response variables with Gamma and Poisson family distributions respectively. A Poisson distribution was chosen for the number of age classes response variable as it is count data. "Competition" (whether Brook Trout were in allopatry or sympatry) was used as a factor for all analyses other than the null.

Generalized Linear and Generalized Linear Mixed models analyzed the effects of the PCA axes or individual environmental variables on average age, length, length-at-age and number of age classes for sympatric and allopatric Brook Trout. The *AICmodavg* package was used to calculate second order Akakie's Information Criterion (AICc) to account for the small sample size, with models being considered competitive if they had a $\triangle$AICc of < 2.0. The top three models from each GL or GLM output was recorded. A model was considered a top model if it was significant, or was the best model behind the null.

Results

Age and Growth

Analysis of von Bertalanffy curves shows that Brook Trout L_∞ is greater for the allopatric populations (Quasi-R^2: 0.817) than for sympatric populations (Quasi-R^2: 0.495). However, K and t_0 were both higher in the sympatric Brook Trout populations (Figure 8). The lower K value in the allopatric curve indicates that allopatric Brook Trout reach L_∞ more slowly than do sympatric fish, as an increase in K results in a decreased time to L_∞. Growth curves also exhibited greater variability in the age and size of sympatric Brook Trout populations when compared to allopatric populations. This was most notable in age-2 individuals (Figure 8). No fish in the allopatric Brook Trout populations were older than age-3, whereas sympatric populations had multiple age 3+ individuals (Figure 6, Figure 7, Figure 8). Both models converged in R 4.1.0, though there was indication that the sympatric model may be influenced by the high degree of growth rate variability among these populations.

Sympatric Brown Trout growth was also plotted on a curve (Quasi-R^2: 0.862) and had both a higher L_∞ and lower, K than either allopatric or sympatric Brook Trout populations, indicating that Brown Trout grew larger but slower than either Brook Trout population (Figure 9). Rainbow Trout growth could not be plotted due to lack of convergence in R, likely due to the low sample size compared to the Brook Trout and Brown Trout populations.

Environmental Parameters

PCA analyses of environmental variables revealed 3 PC axes with Eigenvalues >1 (Table 4). The total variation explained by the first 3 axes is 76.1% (PC1= 32.8%, PC2=27.3%, PC3=16.0%). None of the loadings for the 3 axes were particularly significant, but area, zelevation, zforest, zslope, and temperature had loadings ≥ 0.4/-0.4 on PC1. A plot of PC scores for the 13 Brook Trout streams shows some clustering of the 5 allopatric streams along the PC1 axis, as well as separation from sympatric streams (Figure 10). PC scores from sympatric streams exhibited limited clustering in ordination space around PC axis 1.

General Linear and General Linear Mixed Models

The top Generalized Linear models (LMs) selected for Brook Trout population average age and length indicate that the presence of non-native trout, elevation and NO_3^- may best explain the data for each response variable. However, only two of the models with a ΔAICc < 2 were ranked higher than the null models for each LM and had ΔAICc values of 1.58 and 1.4 respectively (Table 5, Table 6). None of the top models that were ranked higher than the null for either linear response variable was significantly different than the null model (i.e., the ΔAICc between the top models and the null was never > 2). The top models for the average age response that were ranked by AICc as being better than the null included both "competition" and elevation. The top models that were ranked above the null for the average length response included both competition and NO_3^- (mg/L).

The top GLMs for Brook Trout length-at-age and number of age classes also generally indicate that competition, elevation and NO_3^- many be important predictors of growth and age class structure. However, among top models with a ΔAICc < 2 only one model (competition alone, ΔAICc=0.88) provided robust predictive power (Table 7, Table 8). No GLM models (as ranked by AICc) were ranked higher than the null in contrast to the LM models.

Models generally became less parsimonious as more environmental variables were added, and none of the "top" models with a PC axis included were more significant than the null or than the models including only competition, elevation, and NO_3^-, or a combination of those three variables (Table 7, Table 8). Boxplots of Brook Trout and non-native trout standard length and age indicate that as elevation increases, so too does the average length and age of both allopatric and sympatric Brook Trout populations. A similar trend was observed for both Brown and Rainbow trout (Figure 2-Figure 5).

Discussion

Brook Trout Growth, Length and Age Structure

Results of the von Bertalanffy curves for the allopatric and sympatric Brook Trout populations indicate that the predicted L_∞ was higher in allopatric populations, though sympatric populations had a higher K value, meaning that L_∞ was approached faster. Sympatric Brook trout appeared to have generally larger and older aged fish, as indicated by both the box-plots and histograms, but this was not reflected by the growth model. Brown Trout had a larger L_∞ than either Brook Trout population, though a lower K as well, which does not fully support the results found in Carlson et al. (2007) who observed that Brown Trout grew both larger *and* faster in sympatry with Brook Trout.

Although I observed that sympatric Brook Trout populations had fish that grew older than conspecifics in allopatric streams, there was greater variation among individual sympatric populations compared with allopatric populations, and the sympatric Brook Trout model did not fit the data as well as did the allopatric Brook Trout or Brown Trout models (as indicated by the Quasi-R^2 values). This high degree of variation among sympatric populations likely led to the Bertalanffy model parameters being skewed towards lower growth rates despite generally having larger and older aged fish than the allopatric populations (Ogle et al. 2017).

Due to limitations in the sample size of trout and limited age class spread, the results of the three growth curves should be treated as somewhat artificial as each curve represents a composite of multiple populations. A better indicator of differences in the growth models between the populations would have been a comparison between the 3 estimated parameters

(L_∞, K, t_0) for each individual population, but this was not possible with the available data (Ogle et al. 2017).

Another limitation of the data that likely led to skewed results for the growth curves was the overall study design of Ksepka et al. (2020). That study sought to map the distribution of Whirling Disease, and examination of trout age and growth was done as something of an afterthought. In Ksepka et al. (2020), age-1 trout were prioritized for collection for all fish populations, and growth rates are generally higher in younger fish compared with older individuals (Ogle et al. 2017). Greater numbers of age-1 fish and lack of collection priority for older fish likely means that many of the fish sampled from each stream do not represent the true distribution of ages and sizes of the populations they were sampled from.

Despite the limitations of the growth analyses, it is interesting to note the high degree of variability of size and age seen in the sympatric Brook Trout populations compared to the allopatric Brook Trout and Brown Trout populations. It is likely that the differing environmental variables and interactions with non-native salmonids between individual sympatric populations led to the variation seen in the curve, and that favorable environmental conditions in some of the streams possibly helped to mitigate the impact of the presence of non-native salmonids. As noted previously in Petty et al. (2014) and Hoxmeier and Dieterman (2019) environmental conditions including temperatures and food availability may help Brook Trout persist in the presence of non-native salmonids. In some cases, Brook Trout appear to grow faster than would be expected without the presence of non-native salmonids. It is possible that the presence of non-native salmonids can potentially release Brook Trout from intraspecific competition, which in some populations may be stronger than interspecific competition between salmonids (Newman 1956; Petty et al. 2014; Kanno et al. 2016). Intraspecific competition between Brook Trout may also explain why the allopatric Brook Trout populations had reduced sizes and ages compared to the sympatric populations.

Environmental analyses

Because the data had limitations, the impacts of the environmental variables on trout growth could not be properly applied to the growth models, and so average age, length, length-at-age, and the number of age classes of each Brook Trout population were chosen as response variables for GL and GLM analyses instead. Though none of the LMs or GLMs were significantly better than the null models, results indicate that competition, elevation and NO_3^- are potentially important explanatory variables for the overall size and age class structure of Brook Trout populations. This is further supported by comparisons of the standard lengths and ages of allopatric and sympatric populations. These indicate a general increase in overall Brook Trout size and age as elevation increases. This is consistent with prior studies that indicate Brook Trout exhibit higher growth rates and larger populations in higher-elevation streams, especially in systems with non-native salmonids (Meisner 1990; Carlson et al. 2007).

Interestingly, elevational segregation between Brook Trout and non-native salmonids did not appear to occur in focal streams. Previous studies found that allopatric populations of Brook Trout tend to be found at higher elevations and upstream from natural barriers that prevent non-native salmonids from accessing headwaters (Kirk et al 2018). As indicated by the box plot in Figure 4, the five highest-elevation Brook Trout populations were all sympatric and all allopatric Brook Trout populations were located at elevations ≤ 1200 m. However, this is likely an artifact of the method of sampling from Ksepka et al. (2020). As fish in Ksepka et al. (2020) were not collected with a focus on growth and Brook Trout, they likely greatly underrepresent the true elevational distribution of all Brook Trout populations across western North Carolina.

Excess NO_3^- concentrations have been shown to be negatively associated with growth and survival of Brook Trout, but are also indicators of primary productivity in streams (Vannote et al. 1980; Wallace et al. 1999; Hudy et al 2008). Although nitrate concentrations were low in most of the study streams, two allopatric streams had higher NO_3^- concentrations than did the two sympatric streams with the highest NO_3^- concentrations. It is possible that increased NO_3^-in those streams contributed to decreased size and survivability but this was not specifically indicated by any models. It is also possible that the variation of NO_3 between individual streams can be attributed to differences in benthic productivity (Vannote et al. 1980; Wallace et al. 1999; Romaniszyn et al. 2006). Food availability and abundance is critical to Brook Trout growth and survivability, and it is likely that differences in both the growth models and GL and GLM models can be attributed to food availability in addition to the other environmental variables and non-native salmonid presence (Wallace et al. 1999; Romaniszyn et al. 2006; Petty et al. 2014). Benthic production was not measured however, and it can not be determined from the models whether or not the NO_3^- concentrations can be attributed solely to benthic production.

Implications

Though the results of this study were not significant in explaining important variables to native Brook Trout growth, they do suggest areas to explore in future native Brook Trout studies. The von Bertalanffy curves indicate that there may be a potentially significant difference between the growth of allopatric and sympatric Brook Trout populations, and that these differences may be due to a combination of biotic and abiotic environmental variables as well as inter and/or intraspecific competition. Models indicate that the presence of non-native salmonids, elevation, and NO_3^- are potentially important variables to Brook Trout growth, and should be prioritized when planning studies. Managers interested in future growth and conservation studies for native Brook Trout should be sure to carefully select stream reaches and prioritize collecting population samples that accurately represents the spread of sizes and ages within them. Long term monitoring of a multitude of environmental variables, including benthic productivity, pH, and temperature, should also be implemented into growth studies in order to better explain their effects on growth. Native Brook Trout are a species many are interested in conserving, and robust studies modeling its growth are critical to ensure it has a future in the southern Appalachian Mountains.

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Figures and Tables

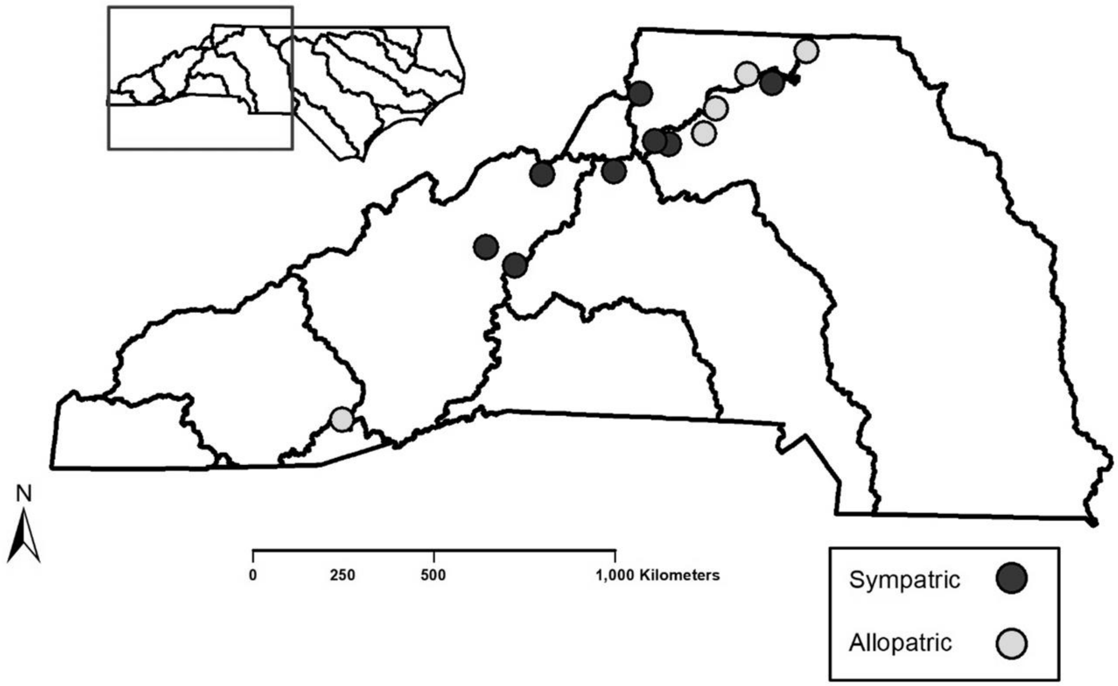

Figure 1. Map of the 13 focal sites in western North Carolina sampled for fish between May and October 2018 and for water quality between May and June 2021. Map credit Vincent Santini.

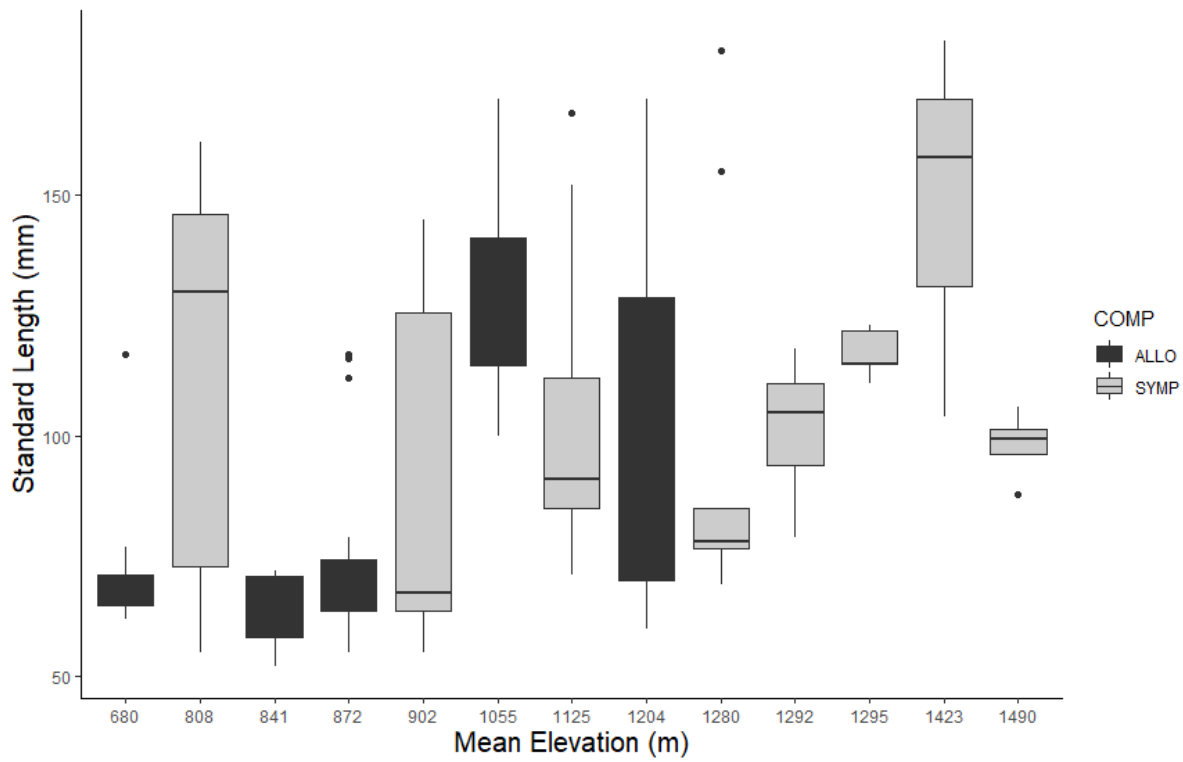

Figure 2- Box plots of the Standard Length (mm) of the 13 Brook Trout populations by Competition (allopatric or sympatric) and Elevation (m). ALLO= allopatric, SYMP=sympatric.

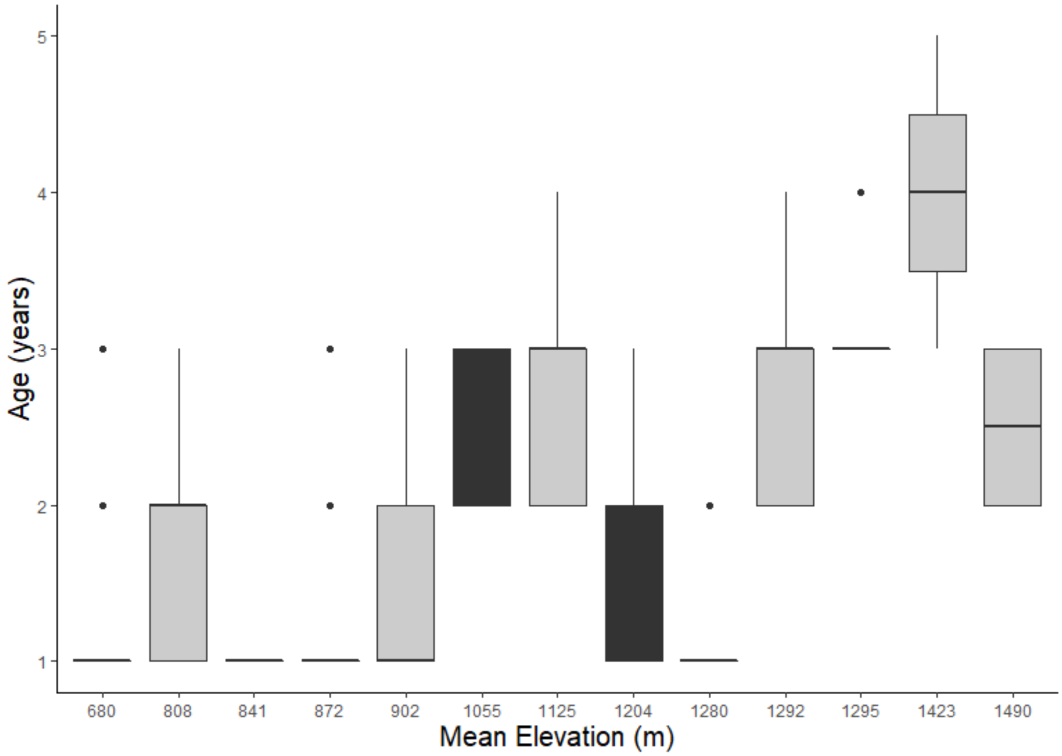

Figure 3.- Box plots of the Age (years) of the 13 Brook Trout populations by Competition (allopatric or sympatric) and Elevation (m). ALLO= allopatric, SYMP=sympatric.

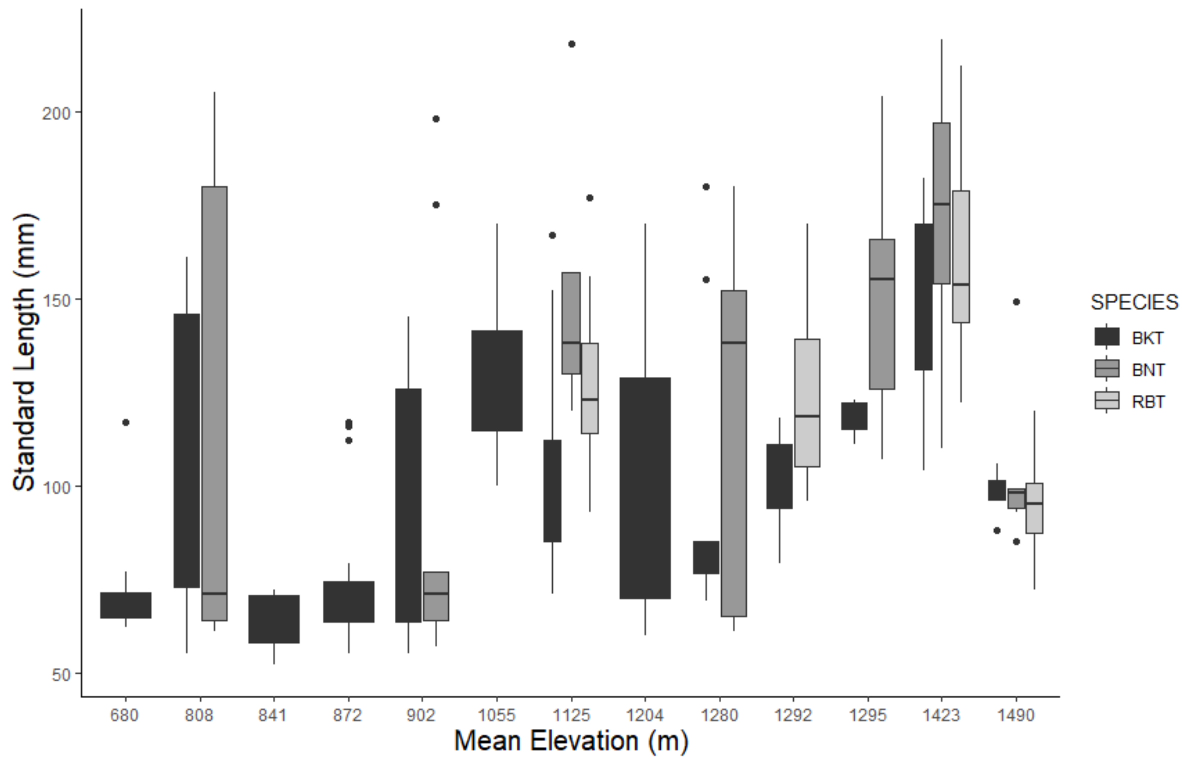

Figure 4.-Box plots of the Standard Length (mm) of all Fish by Species and Elevation (m). Brook Trout=BKT, Brown Trout=BNT, Rainbow Trout=RBT.

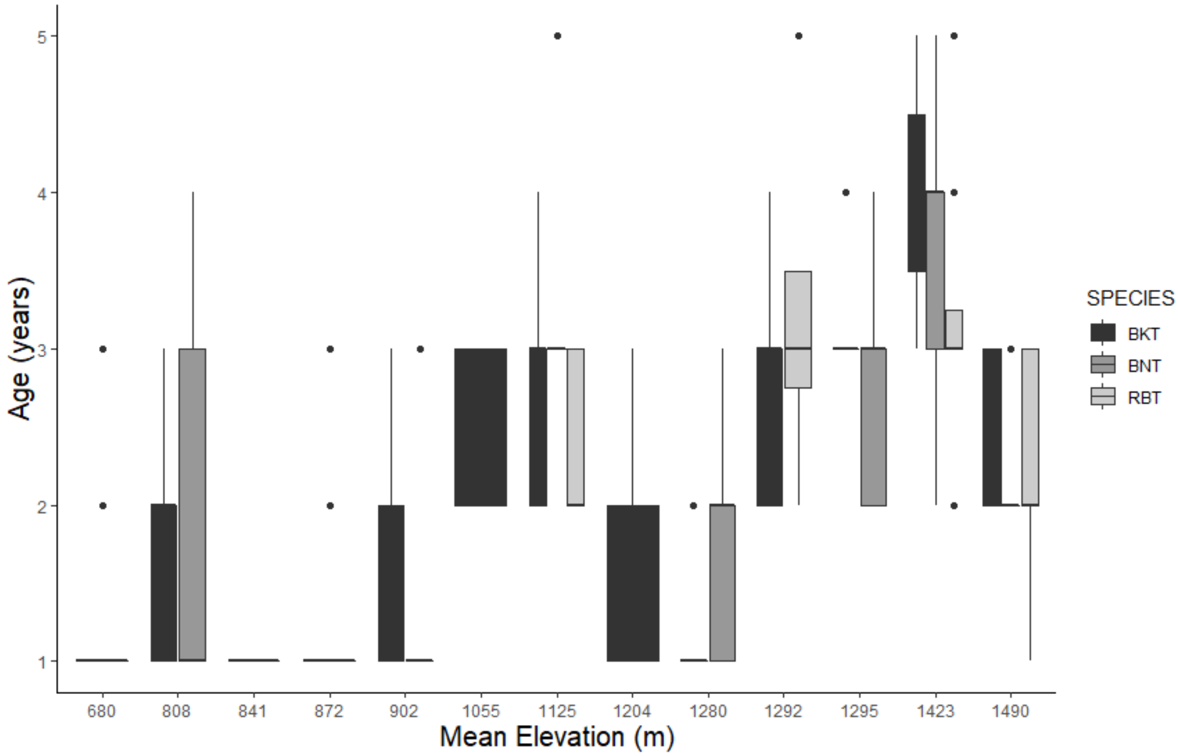

Figure 5.- Box plots of the Age (years) of all Fish by Species and Elevation (m). Brook Trout=BKT, Brown Trout=BNT, Rainbow Trout=RBT.

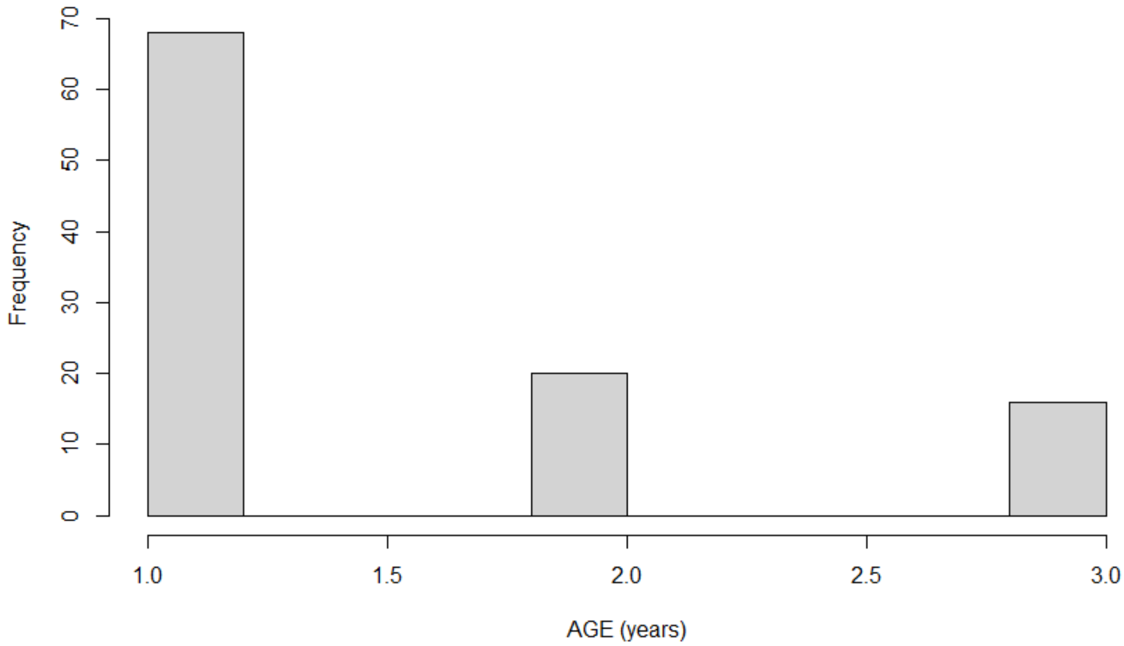

Figure 6.-Histogram of the frequency of age classes for all 5 allopatric Brook Trout populations (n=104).

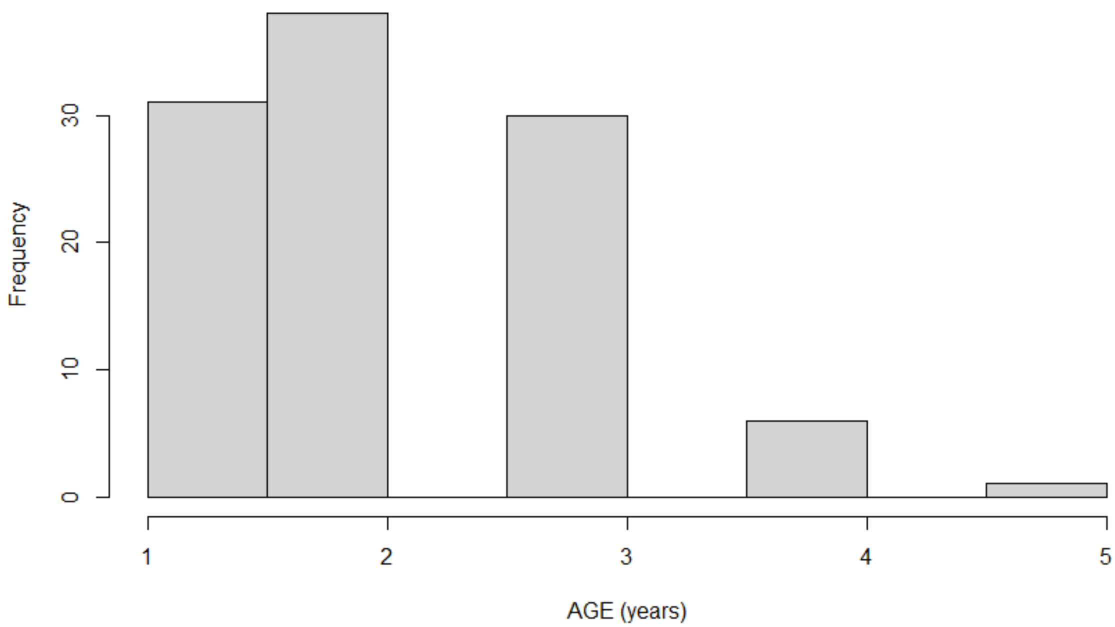

Figure 7.- Histogram of the frequency of age classes for all 8 sympatric Brook Trout populations (n =109).

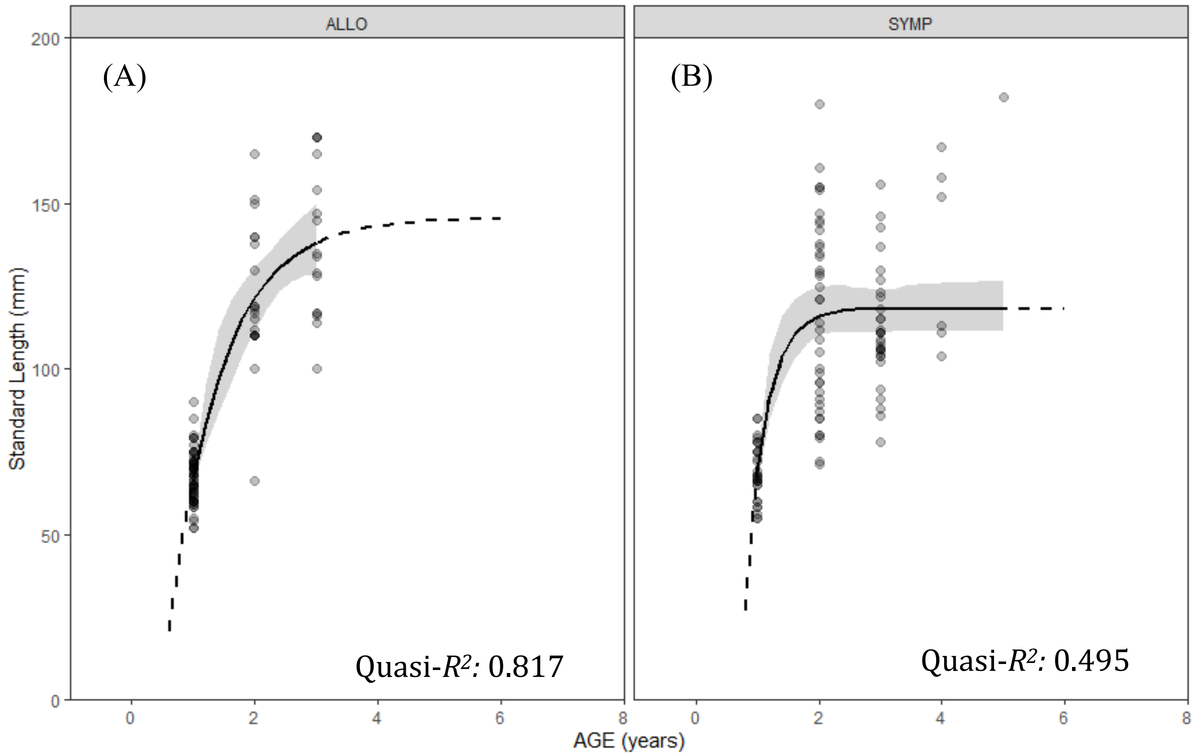

Figure 8.- Estimated Bertalanffy curves of all allopatric (A) and sympatric (B) Brook Trout populations. Allopatric estimated parameters: L_∞= 145.64 (mm), K= 1.17, t_0=0.47. Sympatric estimated parameters: L_∞= 118.27 (mm), K=3.09, t_0=0.72. Confidence intervals are indicated by grey.

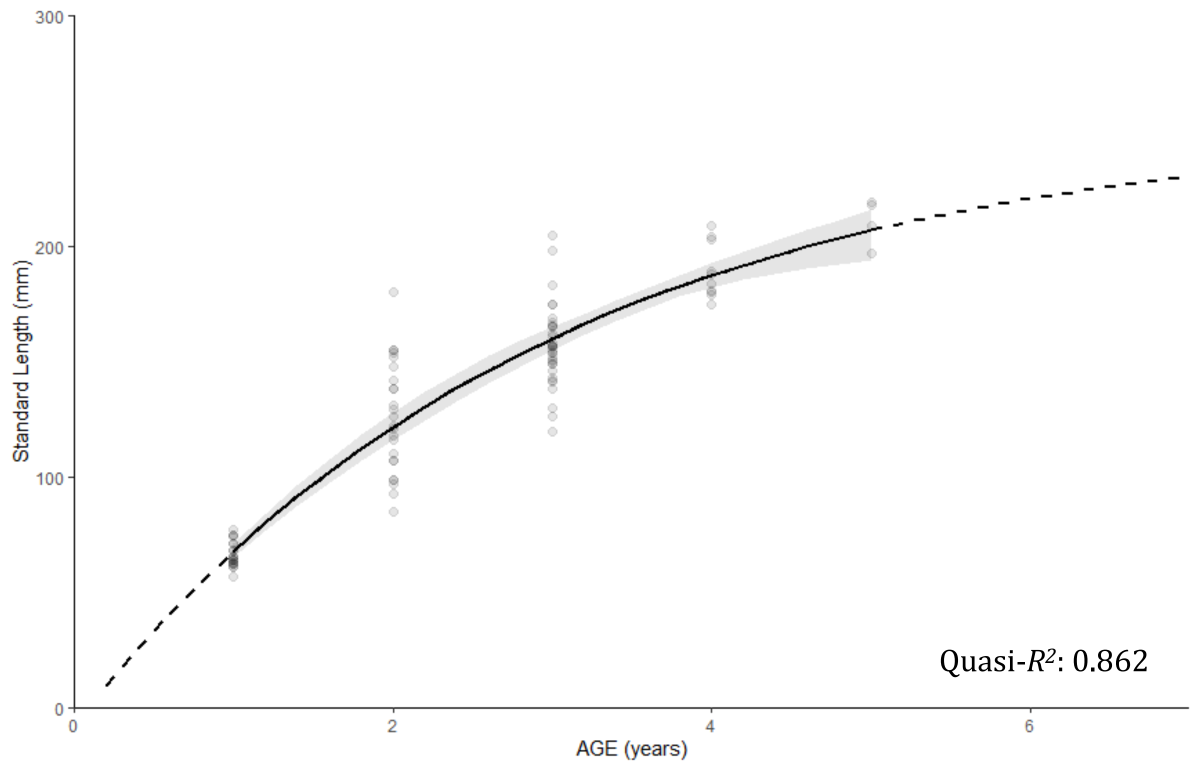

Figure 9.-Estimated Bertalanffy curve of all Brown Trout Populations. Estimated parameters: L_∞= 254.86 (mm), K= 0.34, t_0=0.09. Confidence intervals are indicated in grey.

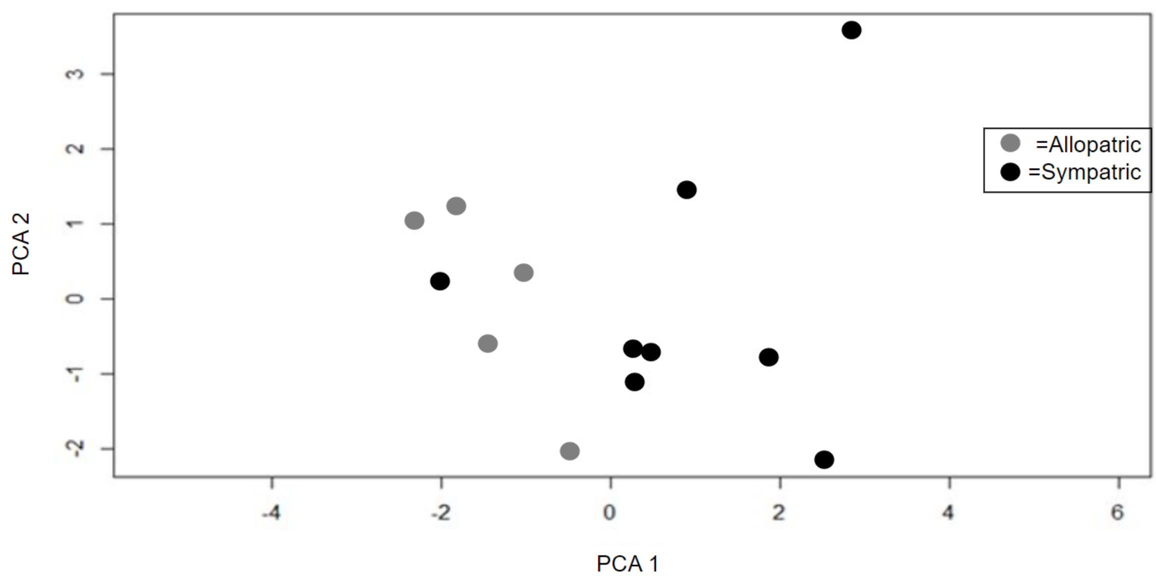

Figure 10.- Principal components plot of the 13 allopatric and sympatric Brook Trout populations. Loadings include: Area-drainage basin area (km^2), zelevation, zforest, zslope, temperature (°C), dissolved oxygen (% saturation), specific conductivity (µS/cm), pH and nitrate ($NO3^-$). Light gray points represent allopatric streams, while black represent sympatric.

Table 1. Stream ID, Stream Name, Date of Collection, Latitude and Longitude of Sites, and total number of Brook Trout (BKT), Brown Trout (BNT), and Rainbow Trout (RBT) collected between May and October of 2018.

Stream	**Stream**	**Date**	**Latitude**	**Longitude**	**BKT**	**BNT**	**RBT**
1	Wilson Creek	2-May-18	36.07449	-81.7955	5	25	-
2	Upper Creek	13-Apr-18	35.73139	-82.2389	4	6	20
3	N Fork New River	19-Jul-18	36.35606	-81.6775	11	21	-
4	N Fork Ivy Creek	27-Apr-18	35.79803	-82.3702	22	-	8
5	Little Rock Creek	30-Apr-18	36.06206	-82.1161	5	17	8
6	Laurel Creek	19-Jul-18	36.17185	-81.5467	24	9	-
7	Garden Creek	3-Jul-18	36.39271	-81.0855	21	9	-
8	E Fork New River	11-Apr-18	36.18344	-81.6107	17	5	9
9	Ramey Creek	16-Aug-18	36.50967	-80.9297	29	-	-
10	Pumpkin Run	9-Aug-18	36.21152	-81.3894	18	-	-
11	Panthertown Creek	2-Oct-18	35.16632	-83.017	18	-	-
12	Meadow Fork	23-May-18	36.42611	-81.1961	25	-	-
13	Clear Branch	9-Aug-18	36.30107	-81.338	14	-	-
Total N					213	92	45

Table 2. Average age (years), length (mm), mean length-at-age (LOA) (mm) and number of age classes for Brook Trout in each stream used as response variables in GLM and GLMM analyses. Sympatric = StreamID 1-8. Allopatric = StreamID 9-13.

Stream	Species	Mean Age (y)	Length (mm)	LOA	N Age Classes
1	BNT	2.72	149.4	55.76	3
1	BKT	3.20	117.2	37.22	2
2	RBT	2.26	95	44.32	3
2	BNT	2.17	103.7	47.69	2
2	BKT	2.50	98.3	41.04	3
3	BKT	1.18	94.4	79.14	3
3	BNT	1.81	117.4	66.39	3
4	BKT	2.67	101.1	39.62	3
4	RBT	3.25	125.4	40.72	3
5	BNT	3.65	172	47.93	4
5	RBT	3.25	162.9	51.41	4
5	BKT	4.00	140.6	36.86	3
6	BNT	1.44	94	66.37	2
6	BKT	1.46	90.3	62.51	3
7	BNT	1.89	70	62.48	3
7	BKT	1.76	114.9	67.11	3
8	BKT	2.76	102.8	37.44	3
8	BNT	3.40	152.6	45.05	2
8	RBT	2.44	129	54.89	2
9	BKT	1.13	72	66.04	3
10	BKT	1.19	71.2	64.00	3
11	BKT	1.61	100.4	66.27	3
12	BKT	2.42	113.2	55.25	2
13	BKT	1.00	63.1	63.14	1

Table 3. Environmental covariates used in the PCA for each of the 13 study sites (Table 5; Figure 9).

STREAM	AREA	ELEV	FOREST	SLOPE	TEMP	DO	SPC	SAL	pH	NO_3^-
1	5.4	1295.4	93.1	38.9	14.9	96.7	24.8	0.01	6.88	0.09
2	6.6	1490.5	98.0	50.3	10.7	98.6	9.3	0.00	6.48	0.04
3	12.0	1280.2	84.7	35.8	15.0	98.4	42.3	0.02	7.31	0.04
4	6.2	1292.3	97.9	43.9	11.4	96.9	17.2	0.01	7.23	0.03
5	18.2	1423.4	85.6	38.9	10.4	106.3	44.4	0.02	7.30	0.25
6	7.2	902.2	96.4	47.7	15.9	97.2	20.9	0.01	7.03	0.17
7	7.0	807.7	97.5	49.0	17.1	99.9	21.2	0.01	6.96	0.07
8	6.1	1124.7	67.1	27.3	16.1	89.5	38.1	0.02	6.73	0.12
9	2.8	871.7	70.7	22.2	15.6	99.8	26.4	0.01	6.83	0.28
10	2.9	679.7	86.6	29.4	17.0	99.9	16.7	0.01	7.15	0.05
11	9.8	1204.0	92.5	26.9	16.2	92.1	10.0	0.00	6.52	0.07
12	2.2	1054.6	68.6	29.9	15.0	96.7	26.3	0.01	7.09	0.31
13	2.1	841.2	80.0	28.5	14.6	100.6	29.2	0.01	7.16	0.03

AREA- drainage basin area (km^2), ELEV- mean elevation (m), FOREST- % watershed forested, SLOPE- Channel slope (m/km), TEMP- temperature (°C), DO- dissolved oxygen (% saturation) SPC- specific conductivity (μS/cm), SAL- salinity (ppt), pH, Nitrate (NO_3^-) mg/L

Table 4. Loading factors for principal component analysis for Environmental variables (Table 3). Loading factors above >0.4 are underlined, bolded values indicate the variance explained by the PC axes.

Environmental Variable	PC1	PC2	PC3
Area (km^2)	<u>0.40</u>	0.26	0.17
zElevation	<u>0.41</u>	0.05	<u>0.55</u>
zForest	<u>0.42</u>	-0.36	-0.27
zSlope	<u>0.45</u>	-0.20	-0.22
Temperature (°C)	<u>-0.45</u>	-0.15	-0.22
DO%	0.25	0.33	<u>-0.46</u>
SPC (µS/cm)	-0.01	<u>0.56</u>	0.07
pH	0.08	<u>0.42</u>	<u>-0.50</u>
$NO3^-$	-0.16	0.39	0.18
%Variation Explained	**32.80**	**27.30**	**16.00**

Area- drainage basin area (km2), zElevation- z-transformed mean elevation (m), zForest- z-transformed % watershed forested, zslope- z-transformed Channel slope (m/km), DO%- dissolved oxygen (% saturation) SPC- specific conductivity (µS/cm), pH, Nitrate-(NO3-)

Table 5. LM model AICc values for the top models describing the average age of Brook Trout in the 13 streams. Competition was included in all models as a factor.

Model	Ka	ΔAICc[b]	AICcWt[c]
Average Age~ Elevation+ Competition	4	0.00	0.36
Average Age~ Competition	3	0.78	0.25
Null model: Average Age	2	1.58	0.17
Average Age~ Elevation+ $NO3^-$+ Competition	5	1.81	0.15

Table 6. LM model AICc values for the top models describing the average length of Brook Trout in the 13 streams. Competition was included in all models as a factor.

Model	Ka	ΔAICc[b]	AICcWt[c]
Average Length~ Competition	3	0.00	0.32
Average Age~ $NO3^-$ +Competition	4	0.23	0.29
Null model: Average Length	2	1.40	0.16
Average Age~ Elevation+ Competition	4	1.49	0.15

Table 7.- GLM model AICc values for the top models describing the average length-at-age of Brook Trout in the 13 streams. Competition was included in all models as a factor with a Gamma distribution.

Model	Ka	ΔAICc[b]	AICcWt[c]
Null model: Average Length-at-age	2	0.00	0.47
Length-at-age~ Competition	3	0.88	0.30
Length-at-age~ Elevation+ Competition	4	2.50	0.13
Length-at-age~ $NO3^-$+ Competition	4	4.66	0.05
Length-at-age~ PC1+Competition	4	5.02	0.04

Table 8.- GLM model AICc values for the top models describing the number of age classes of Brook Trout in the 13 streams. Competition was included in all models as a factor with a Poisson distribution.

Model	Ka	ΔAICc[b]	AICcWt[c]
Null model: Average Class	1	0.00	0.7
Age Class~ Competition	2	2.57	0.19
Age Class~ $NO3^-$+ Competition	3	6.02	0.03
Age Class~ Elevation + Competition	3	6.04	0.03
Age Class~ Elevation+ PC1	3	6.04	0.03

Vita

Amber Nicole Olson was born in Salt Lake City, Utah, to Christopher Olson and Amie Jensen in 1996. She graduated from Appalachian State University in December 2017 with a B.S in Biology. In the August of 2018, she re-entered Appalachian State to pursue a Master of Science degree in biology, and was awarded the degree in August 2021. Amber is a member of both the national American Fisheries Society and the North Carolina chapter of the Fisheries Society, as well as the Southeastern Fishes Council. She currently resides in Boone with her two dogs, and can be found hiking or reading a good book next to a creek.