TEMPORAL TRENDS OF STREAM FISH COUNTS IN A SOUTHERN APPALACHIAN WATERSHED AND EVIDENCE FOR EFFECTS OF ENVIRONMENTAL VARIATION

By

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(Under the Direction of Mary C. Freeman)

ABSTRACT

The Little Tennessee River in North Carolina and Georgia is an area of high aquatic biodiversity in the Southern Appalachian Mountains. The purpose of this research was to examine changes in fish populations in the Little Tennessee River using fish count data collected over a 24-year period from 1990-2013 and to determine if these changes could be explained by variations in temperature or stream flow. The majority of the 26 selected stream fish species included in the study, showed either positive growth or no significant trends, though most non-significant results still indicated a growing population. Only 3 of the selected species showed significant declines over this 24-year time period. Decreased minimum stream flows and increased maximum temperatures seemed to have a positive effect on the counts of most species. Thus it seems that most stream fish have responded positively to observed climate changes in the Little Tennessee River watershed.

INDEX WORDS: Stream fishes, population change, temperature, discharge, long-term data, climate change, Little Tennessee River, Index of Biotic Integrity, citizen science, Land Trust for the Little Tennessee, Southern Appalachian Mountains

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CHAPTER 1

INTRODUCTION

Rivers and streams have provided water, food, energy and transportation for human civilization for millennia, yet these water bodies account for only 0.006% of the world's fresh water reserves (Gleick et al. 1993). Meanwhile, freshwater supports approximately 41% of all fish species, providing habitat for a wide array of species in a relatively small space, when compared to the oceans (Helfman et al. 2009). Since rivers and streams provide both fundamental, but limited resources and high biodiversity habitat, many people are dedicated to protecting freshwater resources and improving water quality throughout the United States. In order to help accomplish these goals many governmental, academic and non-profit organizations in the US monitor rivers and streams using various metrics and methods (Little Tennessee Watershed Association 2011, EPA 2013). This monitoring often includes collecting both physical metrics like temperature, pH, flow discharge and dissolved oxygen as well as biological metrics such as quantity of algae or diatoms (McCarthy et al. 2010, Zalack et al. 2010), number and variety of macroinvertebrates (Martin et al. 2005, Smith et al. 2011) and counts and physical characteristics of fishes (Kanno et al. 2010).

Freshwater fishes are of particular interest because they are more tangible and widely recognized by the public when compared to algae or macroinvertebrates, meaning that a larger proportion of people may show increased interest in a decline in fish populations compared to algae or diatoms, despite the ecological importance of those photosynthetic organisms (Karr 1981). As a result, a wide array of biomonitoring programs have been developed throughout the

US that collect large amounts of data about fish populations (EPA 2006, 2013). Participants in these programs often survey fish populations using backpack electro-shockers, which temporarily stuns the fish, which are then collected in a large seine net and/or smaller dip nets. Once collected, they are identified, sometimes measured for length and/or weight and often released back into the stream.

Dr. William McLarney (ANAI, Inc. San Jose CR and Franklin, NC) established one such fish biomonitoring program in 1988 to survey reaches on the Upper Little Tennessee River (LTR) main stem and its tributaries in southwestern North Carolina and northeastern Georgia (Little Tennessee Watershed Association 2011). With the 1993 founding of the non-profit Little Tennessee Watershed Association (LTWA), Dr. McLarney partnered with the LTWA to make fish biomonitoring part of the LTWA's work. More recently, in 2011, the LTWA merged with the Land Trust for the Little Tennessee (LTLT), another local non-profit, to become the aquatics division of the organization, which previously did not have a focus on water resources. Thus through this biomonitoring program, in its many iterations, Dr. McLarney, employees of the LTWA/LTLT and hundreds of volunteers have helped monitor fish populations and collect data in the Upper LTR watershed for over twenty years (Little Tennessee Watershed Association 2011). As part of my research, I volunteered with Dr. McLarney and the LTLT biomonitoring team to survey fishes during the summer of 2013. In addition, I collected supplemental physical habitat data for a number of streams surveyed by the LTLT. This work with Dr. McLarney and the use of his biomonitoring database is a relatively unique collaboration, bridging the gap between the academic (University of Georgia/Coweeta LTER) and non-governmental organization (LTLT) worlds.

My overarching goal in this thesis is to determine if the LTLT biomonitoring dataset provide evidence for declining fish populations, and to assess the general condition of the watershed, using the response of fish populations as a proxy for stream condition. Thus, I use the LTLT long-term fish biomonitoring dataset to address two ecological questions of broad interest. First, I ask the question, are fish populations changing over time? This is a fundamental question in ecology, which scientists have worked to determine for almost all forms of life. In particular, many ecological studies have addressed temporal changes of fish species populations and communities (Berra and Petry 2006) in relation to changes in flow (Grossman et al. 2006, Grossman et al. 2010) including those related to dams (Alexandre et al. 2013), response to introduced species (Cobo et al. 2010) or density dependence (Grossman et al. 2006, Grossman et al. 2010), differences in life history characteristics (Johnston et al. 2012) and the variety of habitats occupied by species (Jacquemin and Doll 2013). I also evaluate evidence for an effect of stream discharge and temperature on variation of fish species counts. Understanding how aquatic biota respond to changing environmental conditions can help ecologists to better understand annual and decadal population dynamics (Mims and Olden 2013, Pool and Olden 2015) and additionally, to anticipate future fish populations changes based the predicted effects of climate change (Schindler 2001, Morrongiello et al. 2014).

The LTLT biomonitoring database contains fish counts from locations in Macon County and Swain County, North Carolina and Rabun County, Georgia, representing a variety of sampling techniques and effort. These survey sites include multiple habitat types, from headwater streams all the way to the main stem of the Upper LTR. Many of the surveys have been conducted using Dr. McLarney's index of biotic integrity (IBI) biomonitoring protocol. To minimize the effect of variation in sampling methods on observations, I have used only those surveys conducted with the intent of IBI assessment.

Gaining favor in the early 1980's, the IBI framework for surveying fishes has been in use for over three decades (Hocutt and Stauffer 1980, Karr 1981, Karr et al. 1986) in many locales worldwide, including North America (Schmitter-Soto et al. 2011), South America (Hued and Bistoni 2005), Africa (Kamdem Toham and Teugels 1999), Asia (Young et al. 2014) Europe (Mihov 2010) and Oceania (Harris and Silveira 1999, Joy and Death 2004). However, proponents of IBI assessment caution that each scientist or organization must modify this framework to suit their particular zoogeographic area, stream conditions and purpose, and that implementation must be carried out thoughtfully by biologists who understand both the capabilities and limitations of IBI assessment (Karr et al. 1986). For instance, Dr. McLarney has designed his IBI monitoring protocol to be used in wadeable streams in the Appalachian Mountains (McLarney 2013). Of the 400 sites in the LTLT biomonitoring dataset, approximately 176 have been surveyed using this IBI method. Of these 176 IBI sites, 21 sites are located on the main stem of the Upper LTR and represent a different habitat type and width as well as different surveying challenges. Therefore, count data from the main stem sites may not be sufficiently similar to be compared to those from the tributary sites. Thus all of my analyses have been performed on data from the 155 sites located on tributaries of the upper LTR.

The tributary IBI-assessment sites in the LTLT dataset also represent a range of survey frequency, providing an opportunity to compare inferences based on nearly annual surveying with those based on less-frequent assessment. Over the 24 year time period since 1990, 132 sites have been surveyed 1-5 times, 16 have been surveyed 6-11 times, and 7 sites have been surveyed 15-23 times.

My thesis has four specific objectives, outlined below.

1) Determine if fish population trends within the seven fixed sites are representative of those in the larger watershed encompassed by all tributary sites, or whether the inclusion of data from the other 148 sites provides an alternate interpretation of fish species trends in the watershed.
2) Inform our understanding of how fish populations in the Southern Appalachian Mountains may be impacted by additional future climate change with increased temperatures year-round and more variable precipitation and stream flows, causing both more severe drought years, as well as more high-intensity flows in other years.
3) Using the resulting data to justify the increased use of long-term data to study trends in populations, and for organizations that are dedicated to collection of long-term data to continue doing so.
4) Highlight the value of the LTLT stream biomonitoring database in particular, to spur others to answer questions about Southern Appalachian Mountain stream fish communities and populations using this database.

CHAPTER 2

LONG-TERM TRENDS IN COUNTS OF FISH SPECIES IN THE UPPER LITTLE TENNESSEE RIVER WATERSHED, SOUTHERN APPALACHIAN MOUNTAINS, USA

Introduction

Perhaps the most fundamental question that should be investigated when studying any population for conservation purposes is whether or not the population of the species is changing? This question has interested ecologists for decades, with a great deal of time and effort dedicated to understanding how and why populations change or remain in equilibrium (Levin 1992, Wiens et al. 1993, Meffe and Carroll 1997, Brown et al. 2004, Butchart et al. 2010). In order to study populations of stream fishes, ecologists usually survey reaches and record species-specific counts, sometimes along with other metrics such as water velocity, water temperature, stream width and turbidity.

Long-term datasets have increasingly been seen as important to understanding ecological change, as well as to establishing a baseline with which to assess future change (Turner et al. 2003, Southward et al. 2005, Magurran et al. 2010, Spencer et al. 2011). For example, Matthews et al. (2013) used a dataset collected in southern Oklahoma that spans 40 years to determine the overall trajectory of the fish community in relation to climatic disturbances (e.g. droughts). Grossman et al. (2006) used a 12-year dataset from western North Carolina to study trends in one species, *Cottus bairdii* and the relative strength of intraspecific density dependence on population trends. Jacquemin & Doll (2013) used 30 years of data to examine trends of 56 species in central Indiana comparing trends in habitat specialist and habitat generalist species. In all these cases long-term data helped to elucidate population trends that might not have been apparent otherwise.

In this chapter, I assess the long-term trends of selected fish species using 24 years of fish count data collected at 155 survey sites located on tributaries in the Upper Little Tennessee River (LTR) watershed. I use this dataset to address a fundamental ecological question of whether fish populations are changing over time. In particular, many ecological studies have addressed temporal changes of fish species populations and communities (Berra and Petry 2006) in relation to changes in flow (Grossman et al. 2006, Grossman et al. 2010) including those related to dams (Alexandre et al. 2013), response to introduced species (Cobo et al. 2010) or density dependence (Grossman et al. 2006, Grossman et al. 2010), differences in life history characteristics (Johnston et al. 2012) and variety of habitats occupied by species (Jacquemin and Doll 2013). My overarching goal is to evaluate evidence for declines of fish populations at the 155 tributary sites surveyed in the Upper LTR watershed and assess the conditions of these populations based on the occurrence of temporal trends.

One notable aspect of this dataset is that the number of times each site has been surveyed over the 24 year time period varies greatly. Since 1990, 132 sites have been surveyed 1-5 times, 16 have been surveyed 6-11 times, and 7 sites have been surveyed 15-23 times. The seven sites that were surveyed nearly every year from 1990 to 2013 are considered "fixed" sites with the LTLT biomonitoring crew specifically returning to survey these sites almost every year. Thus, an additional objective of this analysis is to compare the results from all 155 sites to the seven fixed sites. This will assess if the fixed sites are representative of population trends in the larger watershed encompassed by all tributary sites or whether the inclusion of data from the other 148 sites provides an alternate interpretation of fish species trends in the watershed.

Methods

Study Area

The Upper Little Tennessee River is located in northeastern Georgia and southwestern North Carolina in the southern Appalachian Mountain range and is a tributary of the Tennessee River. Originating in Georgia, the Little Tennessee River flows northward and through the town of Franklin, NC. The river is impounded north of Franklin forming the relatively small Lake Emory; farther downstream, the Upper LTR is part of the inflow to Fontana Lake reservoir. A majority of the watershed is designated by the US Forest Service as part of either the Nantahala National Forest in North Carolina or the Chattahoochee National Forest in Georgia.

The data used in these analyses were collected by the Land Trust for the Little Tennessee citizen science aquatic biomonitoring program from 155 sites in the study area using an index of biotic integrity (IBI) biomonitoring protocol. This protocol has been designed by Dr. William McLarney, a senior scientist at the non-profit conservation organization *Land Trust for the Little Tennessee*, for use in wadeable Appalachian Mountain streams that have watershed areas of 4-70 square miles (10-180 km^2). Criteria for streams surveyed also include: a mean gradient of less than a 100 foot drop per river mile (~19 m per km), an elevation less than 2800 feet (853 m), and lack of barriers inhibiting fish movement into the reach (McLarney 2013).

Of the 155 tributary sites that have been surveyed using the IBI protocol, there are seven fixed (i.e. regularly surveyed) sites located in Macon County, NC on large and small tributaries of the Upper LTR (Figure 2.1). Dr. McLarney selected these fixed sites for near continuous monitoring because of ease of access to the sites and the likelihood that the sites would remain accessible year after year. These fixed sites were likewise selected for comparison to the total 155 sites for these analyses because they have the most repeated surveys. Two sites (CARRP-087 & MIDHE-126) were surveyed 23 out of the 24 years, one site (RABRC-055) was surveyed 22 times, two sites (CULPC-075 & WAYCR-093) were surveyed 21 times, one site (SKEWC-107) was surveyed 19 times and one site (WATBM-050) was surveyed 15 times for a total of 144 surveys (Table 2.1). For comparison, the entire dataset of 155 sites contains 550 surveys over the same 24-year window.

Field Methods

Sites have been surveyed by the LTLT aquatic biomonitoring group during the months of May to August, using a modified IBI protocol. Surveys are carried out by a crew of 7-9 individuals, although adjustments are made for smaller crews and/or smaller streams (McLarney 2013). The two most experienced crew members run backpack electro-shockers and each carries a dip net. Three other crew members follow the electro-shockers to retrieve stunned fish from the water as quickly as feasible, so that the fish are not shocked multiple times. The retrieved fish are placed in buckets filled with stream water carried by two other individuals. The remaining two members of the crew maintain the seine placement at the downstream end of the survey reach, which stretches the entire width of the stream. The seine serves to capture fish that may have been missed by the rest of the crew.

This protocol is carried out in discrete subsample units that are planned out prior to the survey event. The seine is set at the downstream end of the first subsample; the rest of the crew works their way upstream from the seine, shocking and collecting fish, to the top of the first subsample, where there is usually a natural break, and then works back down to the seine. Once the crew reaches the downstream end of the subsample, the seine is quickly shocked and then immediately hauled out of the water and the fish in the seine are collected and placed in the buckets (McLarney 2013). The fish collected during each subsample are identified (usually by Dr. McLarney), checked for disease, counted and recorded. They are then released downstream of the subsample reach and the entire crew moves upstream where the seine is set at the end of the last subsample, starting the process again.

A complete survey usually consists of 7-14 subsample units, which is determined by both the length of each subsample and the average stream width of the survey site. The length of a complete survey reach is usually equal to or greater than 15 times the average width of the stream. Each subsample is usually 10-15 meters in stream length and units are chosen to represent the variety of habitat types present in the vicinity of the survey reach. These units must include riffle, run and pool subsamples (McLarney 2013). At sites that are surveyed year after year, measures are taken to ensure that nearly the same subsample units are surveyed each year.

There are other factors that Dr. McLarney also takes into account during the planning stages and sampling process. The survey reach usually includes at least 2 bends and at least 2 full riffle/pool combinations and he recommends approximately 20 minutes of elapsed electro-shock time. In addition, a minimum of 200 fish should be counted and all "expected" species should be found in the survey reach (McLarney 2013). On the rare occasion when all expected species are not found in the predetermined survey reach, an auxiliary subsample may be added upstream to target a missing species.

Once the survey at a site is complete, the species counts are totaled and Dr. McLarney computes an IBI score which helps to communicate the general integrity of the fish community at that site. Later, these total counts are entered into the LTLT fish survey database. Dr.

McLarney has made this dataset publically available for download from the Coweeta Long Term Ecological Research data catalog (http://coweeta.uga.edu/dbpublic/dataset_details.asp?accession=LTWA_2010_06_01).

Statistical Analysis

In this analysis I modeled trends of the 26 fish species that were caught in the greatest numbers across 176 sites over the 24-year study period. To evaluate trends in annual counts of these species, I used a linear mixed-effects regression model. Each species' count data were modeled separately. The regression model treated counts as though they were drawn from a Poisson distribution and included a random effect to account for overdispersion in the count data. Specifically, the linear regression model analyzed species-specific counts for each site and year combination as a function of a single intercept, year, site watershed area, an interaction between watershed area and year, a random effect of site for each year and a random effect of watershed group. Thus, for a given species:

$$\mathrm{Count}_{year,site} \sim \mathrm{Poisson}(l_{year,site})$$

$$\mathrm{Ln}(l_{year,site}) = \alpha + \beta_{year} * \text{year} + \beta_{wsa} * \text{watershed area} + \beta_{int} * \text{watershed area} * \text{year} + \varepsilon_{year,site} + \varepsilon_{ws.group}$$

The alpha term was single grand mean intercept for all sites. The beta year term was the effect of year for all data, which indicated the 24-year trend of the population. A fixed effect of site watershed area was included to account for the effect of stream size on species-specific counts. An interaction between watershed area and year was included to test if temporal trends differ in counts as a function of stream size. For example, I would expect the interaction to be non-zero if counts were increasing more at larger sites compared to smaller sites, or vice versa. If instead, counts have similar trends (positive, negative or neutral), regardless of watershed area, I would expect to find no interaction. To account for overdispersion in the data, a random effect of site in each year was added, which allowed the model to accommodate greater variability than would be expected from a Poisson distribution. Finally, a random effect of watershed group was added to account for the possibility that sites in the same tributary network (i.e. watershed group), are more similar to each other than to sites in other watershed groups.

Models were evaluated using the counts for each species from all 155 sites. The seven fixed sites were analyzed using a similar model, but with the effect of watershed area, the interaction of watershed area and year, and the random effect of watershed group all removed. The effect of watershed area was removed due to the small number of sites; the effect of watershed group was removed because all seven of the fixed sites were in a different watershed group. The year effects from these models were then compared to determine if the seven fixed sites were representative of population trends in the larger watershed encompassed by all 155 sites, or whether the inclusion of data from the other 148 sites provided an alternate interpretation of fish species trends in the watershed.

An additional analysis was performed on Yellowfin Shiner (*Notropis lutipinnis*) data to test a hypothesis that this species was acting as a "native invader" starting in the main stem and moving into the tributaries. The Yellowfin Shiner was the one possibly introduced non-game species represented in the database. For this additional analysis, I added the 21 sites located on the main stem of the Upper LTR and reran the model with all main stem and tributary data instead of using only the tributary survey data.

All models were fit with a Bayesian framework using R (R Core Development Team 2014), R package R2jags, and JAGS (Plummer 2013). I used non-informative priors for regression parameters (Appendix A). For the tributary-sites data, I used the following JAGS

Markov Chain Monte Carlo settings: 3 chains, 15,000 total iterations, 2000 iteration burn-in, 3 iteration thinning and uninformative prior distributions. For the fixed-sites data, I used the same JAGS setting, except 10,000 total iterations. Both the year and watershed area covariates were scaled using the "scale" function in R (R Core Development Team 2014). Model fit was assessed using Bayesian p-values and model convergence was assessed by the value of R-hat (Kery and Schaub 2012).

Species were classified as highland endemic, cosmopolitan or non-native, following the classification system of Scott (2006). I added this categorization to assess, post hoc, if fish with a particular distribution, especially highland endemics, had differing trends from either cosmopolitan or non-native species.

Results

Seventy fish species were encountered in 24 years of data collection at 176 sites including main stem sites. The 26 most abundant species represented over 97 percent of all the individual fishes identified and counted during the surveys. Of the 26 species, all but one (*Etheostoma gutselli*) totaled over 1,000 individuals counted over 24 years (Table 2.2). Of the remaining 44 species, 32 were represented by fewer than 200 individuals, and the next most commonly captured species after *Etheostoma gutselli* had a total (740) that was at least 24% smaller than more frequently detected species. These 26 species were deemed an appropriate representation of the core fish community in the Upper LTR watershed.

The number and location of sites surveyed each year by the LTLT aquatic biomonitoring program varied somewhat based on weather, stream flows, circumstances in the watershed and availability of volunteers. The range of sites sampled each year spanned 9-50, but those extremes only occurred once. Usually between 24 and 30 sites (1^{st} & 3^{rd} quartiles) were surveyed annually with a median of 27 (Figure 2.2).

Analysis of counts using all tributary sites

Models using the data from all sites had an adequate fit, with Bayesian p-values between 0.77 and 0.21, and indicated a mixture of species that were significantly increasing, decreasing or that had a year effect indistinguishable from zero over the 24-year period (Figure 2.3). Twelve (46%) of the 26 species appeared to have populations that increased, with a similar number of species (11) having year effect estimates that included zero in the 95% credible interval (Figure 2.2) indicating weak evidence for a change in counts. Only three species (12%) showed evidence of declining populations.

Watershed area had a minimal effect on species counts, but some effects were significant (Figure 2.4). The watershed area of all sites ranged from 1-234 km^2 and had a mean of 26 km^2, a median of 12 km^2 and a standard deviation of 42 km^2. The majority of watersheds (135 sites) had an area of less than 50 km^2 (Figure 2.5); five sites did not have an estimated watershed area available in the LTLT database. Thirteen species had significant positive coefficients for watershed area. Because watershed area is a scaled value in this model, a coefficient value of 0.4 would indicate an approximate 50% increase (i.e., $e^{0.4} = 1.49$) in fish counts per 42 km^2 increased in watershed area (i.e., an increase by one standard deviation of watershed area). Two species (Creek Chub and Smokey Dace) had significant negative watershed area effects, with the largest effect being for Smokey Dace. (Figure 2.4)

The interaction between year and watershed area for nearly all species was either not significantly different from zero (i.e., 95% credible intervals included 0), or was small (Figure 2.4). Those species that did have small but significant interactions all had negative coefficient values. The interpretation of the interaction depends on the value of the year effect (Figure 2.3). For species with positive year effects, the negative interaction means that the positive trend was greater in smaller streams than larger ones. For species with negative year effects, the negative interaction means that the negative trend in smaller streams was slight compared to the steeper decline in larger streams. The Smokey Dace had the most extreme watershed and interaction effects; this species had a slight negative trend in smaller streams compared to larger streams.

The ecological classification of species as highland endemic, cosmopolitan or non-native did not seem to correspond with any of the estimated year effects. For instance, of the highland endemic species, three (Greenfin Darter, Warpaint Shiner and Tennessee Shiner) showed strong or weak growth, one (Mirror Shiner) had weak evidence for any change and two (Smokey Dace and Tuckasegee Darter) indicated declines.

Analysis of counts at fixed sites

Model results using counts from the fixed sites were similar to the tributary-sites results, with year effect estimates for 18 of the 26 species indicating similar positive or negative growth, or a non-trending population, as inferred from the analyses using all sites. However, there were some notable exceptions. The fixed sites provided evidence that five species had positive growth, while the credible interval for the effect of year based on analysis of all tributary sites included zero. These five species were two highland endemics (Tennessee Shiner and Mottled Scuplin), and three cosmopolitan species (Redbreast Sunfish, Golden Redhorse and Creek Chub). On the other end of the spectrum, one species, Smokey Dace (*Clinostomus* sp.), showed a declining population based on data using either data set, although the year effect estimate for the fixed sites has a 95% credible interval that included zero (Figure 2.3). One species, Telescope Shiner did not have enough data from the fixed sites to calculate a meaningful year-effect estimate and credible interval.

An additional analysis was conducted for Yellowfin Shiner by adding data from 21 IBI sites located on the main stem of the Upper LTR to the tributary sites and rerunning the tributary-sites model. This species was detected at 88 of the 176 total sites (71 tributary sites and 17 main stem sites), thus the tributary-sites model used data from 88 sites. It was found that Yellowfin Shiner showed weak evidence for growth when the main stem sites were included, while there was significant positive growth at the tributary sites (Figure 2.6). The fixed sites indicated significant positive growth with a year effect estimate that was much great than either of the other two estimates. Thus the inclusion of the main stem sites decreased the year effect estimate for Yellowfin Shiner.

Discussion

Despite a few declining species, results presented here support a hypothesis that most species' populations have either weak non-significant trends or are increasing at these sites. Thus, the long-term biomonitoring data collected in the Upper LTR watershed have been useful for evaluating evidence of temporal trends across multiple stream fish species. The 26 species analyzed show a range of values for temporal change, including increasing and decreasing trends for both cosmopolitan and highland endemic fishes. Most species in the Upper LTR watershed have either positive growth or weak evidence for changing populations, indicating that the population trends for at least these 23 species appear favorable. Three species (Smokey Dace, Longnose Dace and Tuckasegee Darter), however, show population declines.

Other long-term datasets have also been used to address trends in fish populations and these studies have linked those trends to other metrics. For instance, Matthews et al. (2013)

showed that climatic disturbances could significantly shift the community away from its original composition; however, they also revealed the value of long-term datasets because the last few surveys conducted indicated that the community was returning to a state similar to that found prior to the disturbances. Grossman et al. (2006) used a 12-year dataset from western North Carolina to study trends in one species, *Cottus bairdii* (Mottled Sculpin). They found that *C. bairdii* populations were "highly stable" and that a stable habitat usually indicated a stable population. In addition, Grossman et al. indicated that intraspecific density-dependence was the main component driving population trends. These authors also expounded on the importance of long-term data. Jacquemin & Doll (2013) used 30 years of data to examine trends of 56 species in central Indiana. They found that niche breadth helped to explain the growth rate of species and that habitat specialists had a greater increase in abundance when compared to habitat generalists.

While most species show evidence of increase or little change based on counts from the biomonitoring surveys, the three species that show evidence of decline share some characteristics that may help explain their apparent decrease. Smokey Dace is considered either a subspecies of Rosyside Dace (*Clinostomus funduloides*) (Etnier and Starnes 1993) or is recognized as a distinct, undescribed, but closely related species to Rosyside Dace (North Carolina Administrative Code 2008) and is additionally noted as a species of "special concern" in North Carolina (North Carolina Administrative Code 2008). Either way it is categorized (subspecies or species), Smokey Dace is labeled a highland endemic species with a range restricted almost exclusively to headwater streams of the Little Tennessee River watershed (Etnier and Starnes 1993). Tuckasegee Darter (*Etheostoma gutselli*) is similarly a highland endemic species with a range restricted to streams and small rivers in the Little Tennessee River and Pigeon River watersheds in North Carolina and Tennessee (Etnier and Starnes 1993). The Longnose Dace (*Rhinichthys cataractae*) has a very different distribution; it is a widespread species found mainly in the northern United States and Canada. The Upper LTR represents the southernmost extent of the Longnose Dace in the eastern US and is thus at the edge of the species' range. It may be that these three species that prefer smaller, cool, rocky streams are being affected by the warming trends observed in the Southern Appalachian Mountains, however, this is a hypothesis based on published life history traits and a definitive mechanism for their decline is not currently known. Therefore, these species may warrant additional, finer scale study and more attention now that they have been identified as potentially in decline.

An additional question I was able to address using this dataset regards the usefulness of monitoring a small number of sites. To assess this, I asked if the seven fixed sites, which have been surveyed nearly every year, were good indicators of broader trends in the Upper LTR watershed. The 7 fixed-site dataset did predict trends in the greater watershed for many species, as indicated by similar year-effect estimates and 95% credible intervals between the fixed and tributary sites analyses. Results from both analyses showed similar positive, negative or neutral trends for most species. However, there were some notable exceptions, the most prominent being analyses for the Yellowfin Shiner. While both the fixed-site analysis and tributary-site analysis indicated a significant positive trend for the Yellowfin Shiner, these estimates had the most separation of any species between their year-effect estimates. In fact, the Yellowfin Shiner was estimated to have the fastest growing population at the 7 fixed sites. However, the analysis using data from all tributary sites showed a smaller positive trend with a confidence interval that almost included zero. When data from the 21 main stem sites were included in the tributary dataset and analyzed, this result showed only weak positive growth that was non-significant.

These results for Yellowfin Shiners is particularly interesting in light of anecdotal observations shared by Dr. McLarney from his twenty plus years of experience in the Upper LTR. He asserts that during the early years of regular sampling in the Upper LTR watershed in the 1990's, few Yellowfin Shiners were detected and those that were observed occurred in the main stem headwaters. Dr. McLarney maintains that over the past two decades the Yellowfin Shiner has dispersed down the main stem of the Upper LTR and colonized new habitats in tributaries, as proposed by the "Native Invasion" hypothesis describe by Scott and Helfman (2001). The question of whether the Yellowfin Shiner is a native species and thus could even be a "native invader" in the Upper LTR is still unclear. Scott et al. (2009) collected the Yellowfin Shiner from parts of its known native range in North Carolina, South Carolina and Georgia and from Coweeta Creek, a tributary of the Upper LTR and compared two DNA loci to identify the likely source of the Upper LTR population. However, they were unable to definitively determine the source due to high heterogeneity at the two loci in the Upper LTR population, and thus could not rule out the possibly that the Yellowfin Shiner is native to the Upper LTR watershed. The results presented here support Dr. McLarney's hypothesis that the species is dispersing through the Upper LTR system by moving from the main stem into tributaries, since analyses using tributary sites showed evidence of significant population growth whereas the analyses including the main stem sites did not. While this is an interesting initial result, I believe this information warrants further spatial analysis to better understand the movements and population growth of the Yellowfin Shiner in the Upper LTR basin and to evaluate whether this is truly a case of native invasion (Scott and Helfman 2001).

The other three species that have larger differences between the two analyses are Black Redhorse, Golden Redhorse and Creek Chub. For these three species, the fixed sites indicate that their populations are growing at a significant positive rate, while the tributary-sites analysis indicates a population with less change. These differences could result from two possible, but not mutually exclusive scenarios. One, these fixed sites could truly have a different growth regime from the larger watershed, which could stem from any number of reasons, from better habitat to increased food availability. Two, since the data are limited to seven fixed sites, the model may be more sensitive to a few aberrant large count values that may have occurred when the survey crew captured a school or a spawning aggregation. This second scenario seems to be likely for the Redhorse species, which are strong swimmers and particularly difficult to capture. Most of the Golden Redhorse counts are in the low single digits, however, there seem to be two outliers in 2007, where at two sites 9 and 12 Golden Redhorse were detected. The counts at those same two sites in the next year, 2008, were 2 and 3, respectively. The model may be sensitive to these two outliers. I tested this hypothesis by replacing the two outliers with data that more closely matched the rest of the data (a count of 2 and 4). This substitution decreased the estimated year effect only slightly. I conclude that when counts are especially small, the model is very sensitive to an increase of even 1 or 2 individuals detected during a given survey, and that the outliers with 4 or 5 additional individuals had less of an impact than expected.

The seven fixed sites seem to do an adequate job of estimating the trends in the larger watershed for most species. Other than the four species discussed above, most species have estimates based on the seven fixed-sites that are similar to analyses based on data from all tributary sites. However, the year effect estimates for the fixed sites are almost always greater than the estimates for all sites. This difference seems to demonstrate that including more data, which encompasses a wider spatial range, tend to result in more conservative estimates of the effects. However, the 95 percent credible intervals for these effects also tend to shrink, such that

I am more confident in the results found from analyzing all tributary sites compared to the fixed sites only.

One caveat to these results is that because these sites were selected non-randomly, they may not be representative of conditions in the entire Upper LTR watershed. Keeping that in mind, all data analyzed were collected using an IBI methodology, which is designed to assess the general status (excellent, good, fair, poor) of each stream. My results may provide additional insights into the combined status of the sites surveyed. While IBI methods have been used for over three decades (Hocutt and Stauffer 1980, Karr 1981, Karr et al. 1986) in many locales worldwide including North America (Schmitter-Soto et al. 2011), South America (Hued and Bistoni 2005), Africa (Kamdem Toham and Teugels 1999), Asia (Young et al. 2014) Europe (Mihov 2010) and Oceania (Harris and Silveira 1999, Joy and Death 2004), these methods are only one possible way of measuring stream health and water quality. In fact, proponents of IBI assessment caution that its implementation must be carried out thoughtfully by biologists who understand both the capabilities and limitations of IBI assessment (Karr et al. 1986). This caution is certainly warranted. However, because fishes occupy multiple trophic levels and live for multiple years, integrating variable stream conditions over their lifetimes, they may be as good an indicator as any one other metric by which to assess stream condition. Thus, based on the number and variety of species counts that were either increasing or showing no trends, we might conclude that the general condition of these sites as a whole over the 24-year survey period appears to be good. However, it is also plausible that increasing counts in fact reflect increasing capture efficiency through time rather than increasing population abundances. In order to better support the conclusion that species abundances are increasing, sites in the Upper LTR watershed should continue to be surveyed using the same IBI biomonitoring protocol to augment this long-term dataset. Additionally, government agencies and non-profit organizations might consider adding other survey methods to evaluate variation in fish capture efficiency in these streams, to better assess fish communities and stream conditions in the Upper LTR system.

Table 2.1. Table of the 7 fixed fish survey sites. The table includes the Land Trust for the Little Tennessee unique site identifier, the stream where the site is located, geographic coordinates, watershed area, elevation and total number of years surveyed.

Site ID	**Stream Name**	**Latitude (DD)**	**Longitude (DD)**	**WS area (km^2)**	**Elevation (m)**	**Surveys**
CARRP-087	Cartoogechaye Creek	35.15674 N	83.38679 W	148.0	614	23
MIDHE-126	Middle Creek	35.04068 N	83.36140 W	28.7	643	23
RABRC-055	Rabbit Creek	35.20849 N	83.35198 W	22.9	617	22
CULPC-075	Cullasaja River	35.14194 N	83.29412 W	146.6	633	21
WAYCR-093	Wayah Creek	35.15409 N	83.48787 W	36.0	660	21
SKEWC-107	Skeenah Creek	35.11182 N	83.39021 W	17.1	623	19
WATBM-050	Watauga Creek	35.22535 N	83.36575 W	19.9	608	15

Table 2.2. Species analyzed for temporal trends in counts at tributary sites in the Upper Little Tennessee River system. Total is the summed catch over all IBI sites and years; # of sites is occurrence out of 155 tributary sites, WSA Range (km^2) is the range of watershed area of the species occurrence, Elevation Range (m) is the range of elevation of the species occurrence.

Species	**Common Name**	**Total**	**# of sites**	**Status**	**WSA Range**	**Elevation Range**
Cottus bairdii	Mottled Sculpin	97035	143	Highland Endemic	1-234	524-1137
Campostoma anomalum	Central Stoneroller	21074	124	Cosmopolitan	1-234	524- 715
Nocomis micropogon	River Chub	20573	120	Cosmopolitan	2-234	524- 826
Luxilus coccogenis	Warpaint Shiner	18900	114	Highland Endemic	1-234	524- 819
Notropis leuciodus	Tennessee Shiner	17463	94	Highland Endemic	1-234	524- 700
Notropis lutipinnis	Yellowfin Shiner	10392	71	Possibly Introduced*	1-234	572- 700
Rhinichthys atratulus	Blacknose Dace	7625	94	Cosmopolitan	1-230	524-1152
Percina evides	Gilt Darter	7584	66	Highland Endemic	3-234	524- 703
Hypentelium nigricans	Northern Hogsucker	6797	119	Cosmopolitan	2-234	524-1020
Clinostomus sp.	Smokey Dace	6528	96	Highland Endemic	1-230	547- 715
Semotilus atromaculatus	Creek Chub	6113	132	Cosmopolitan	1-234	524-1137
Lepomis auritus	Redbreast Sunfish	5466	87	Cosmopolitan	1-234	547-1152
Cyprinella galactura	Whitetail Shiner	4836	57	Cosmopolitan	2-234	524- 679
Ichthyomyzon greeleyi	Mountain Brook	4296	77	Cosmopolitan	1-234	547- 715
Etheostoma chlorobranchium	Greenfin Darter	4295	59	Highland Endemic	3-234	524- 700
Ambloplites rupestris	Rock Bass	3987	101	Cosmopolitan	1-234	547-1020
Notropis spectrunculus	Mirror Shiner	3332	50	Highland Endemic	1-234	547-1020
Rhinichthys cataractae	Longnose Dace	2758	77	Cosmopolitan	3-147	572-1152
Oncorhynchus mykiss	Rainbow Trout	2397	90	Non-native	2-234	524-1009
Lepomis cyanellus	Green Sunfish	1342	74	Cosmopolitan	1-234	547-1137
Moxostoma erythrurum	Golden Redhorse	1292	45	Cosmopolitan	2-234	524- 700
Moxostoma duquesni	Black Redhorse	1217	38	Cosmopolitan	2-234	524- 700
Lepomis macrochirus	Bluegill	1178	65	Cosmopolitan	2-234	524-1152
Notropis telescopus	Telescope Shiner	1013	32	Cosmopolitan	2-234	524- 646
Salmo trutta	Brown Trout	1001	67	Non-native	3-148	572-1152
Etheostoma gutselli	Tuckasegee Darter	976	63	Highland Endemic	3-234	555- 703

* It is unclear whether *N. lutipinnis* is an introduced or native species in the Upper LTR based on DNA analysis (Scott et al. 2009).

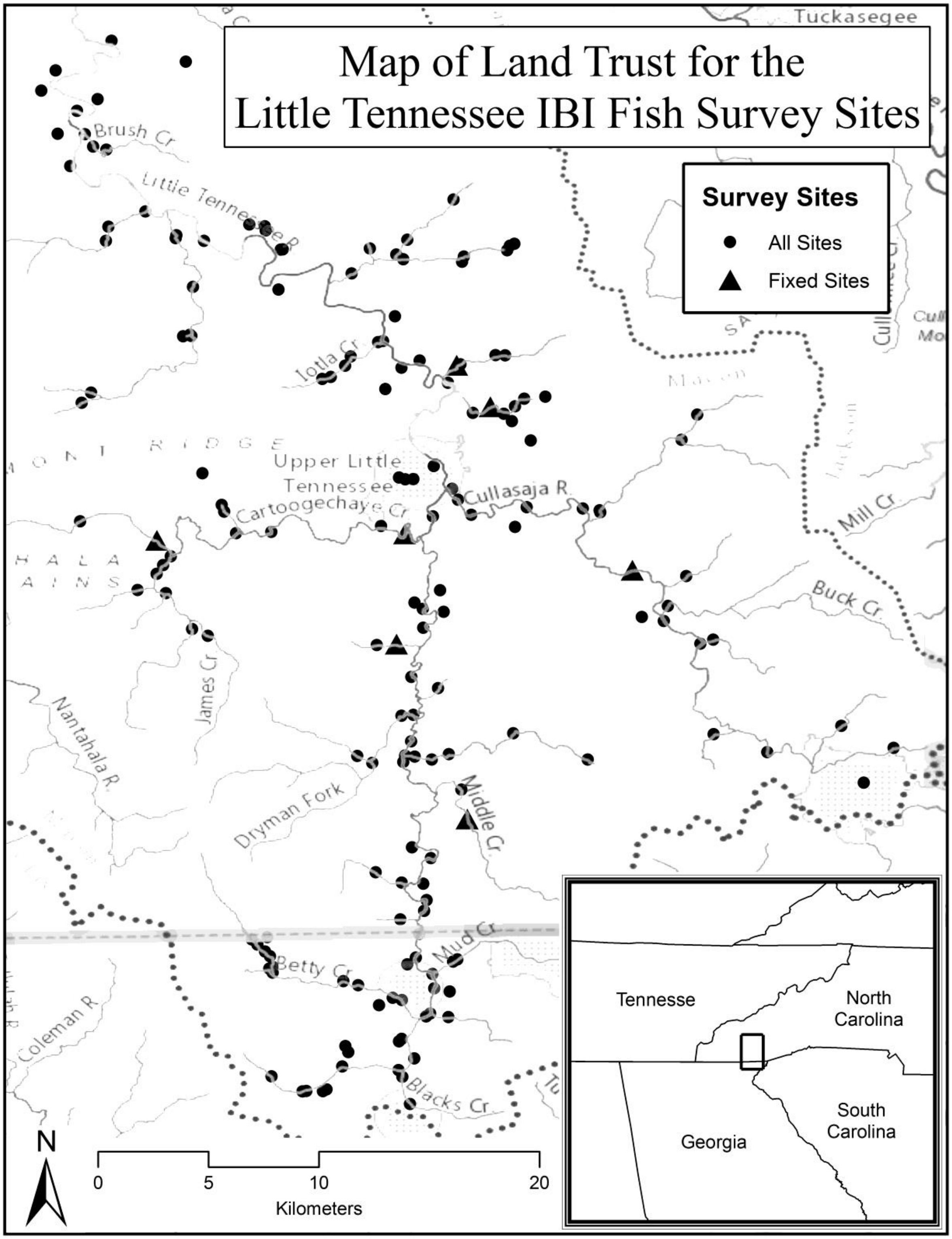

Figure 2.1. Fish survey sites in the Upper Little Tennessee River watershed. The 176 sites indicated include the seven fixed sites (triangle icons) as well as sites on the main stem.

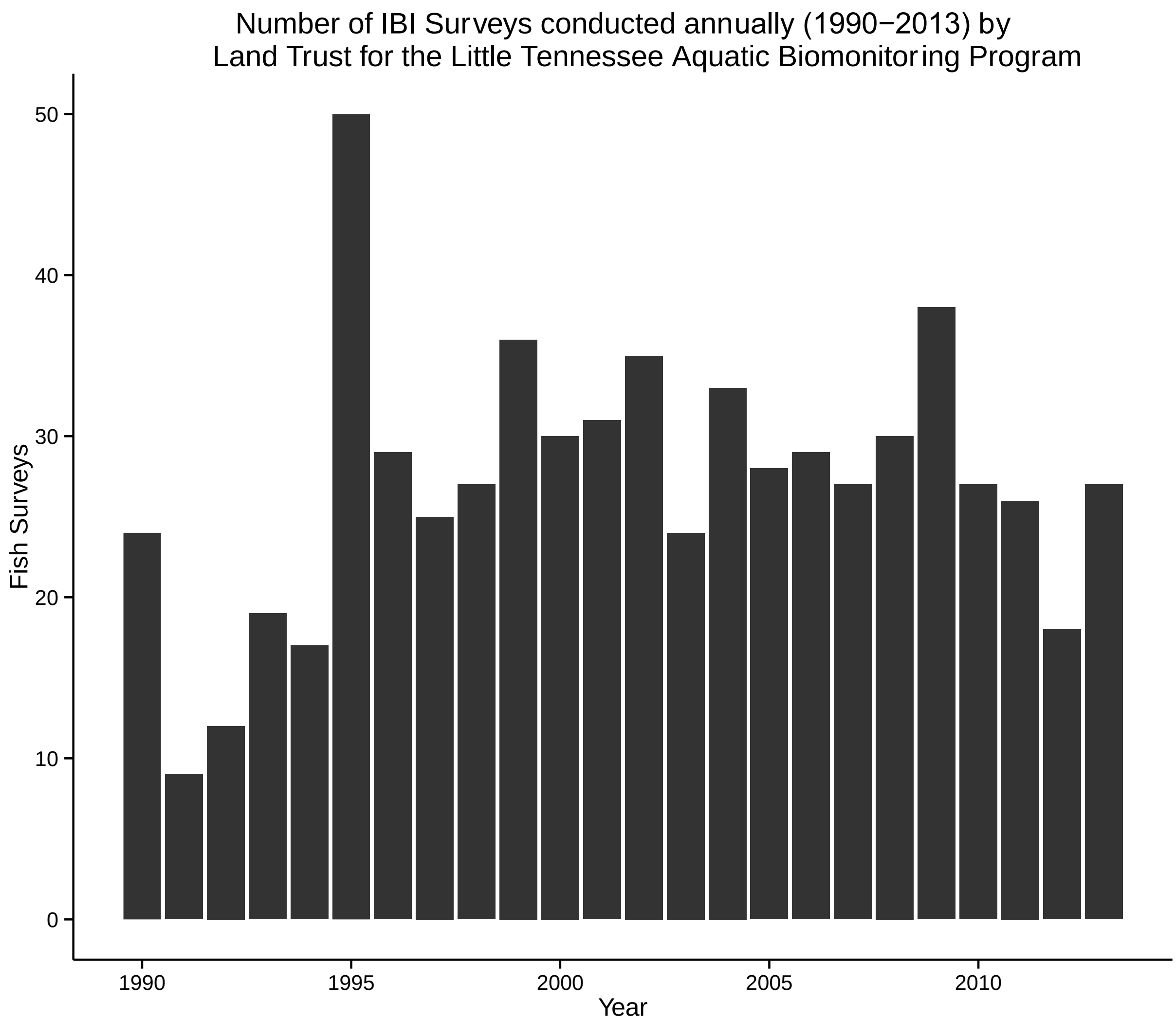

Figure 2.2. Total sites surveyed annually by Land Trust for the Little Tennessee Aquatics Biomonitoring Program. Number of sites surveyed per year ranged from 9 to 50 with a median of 27.

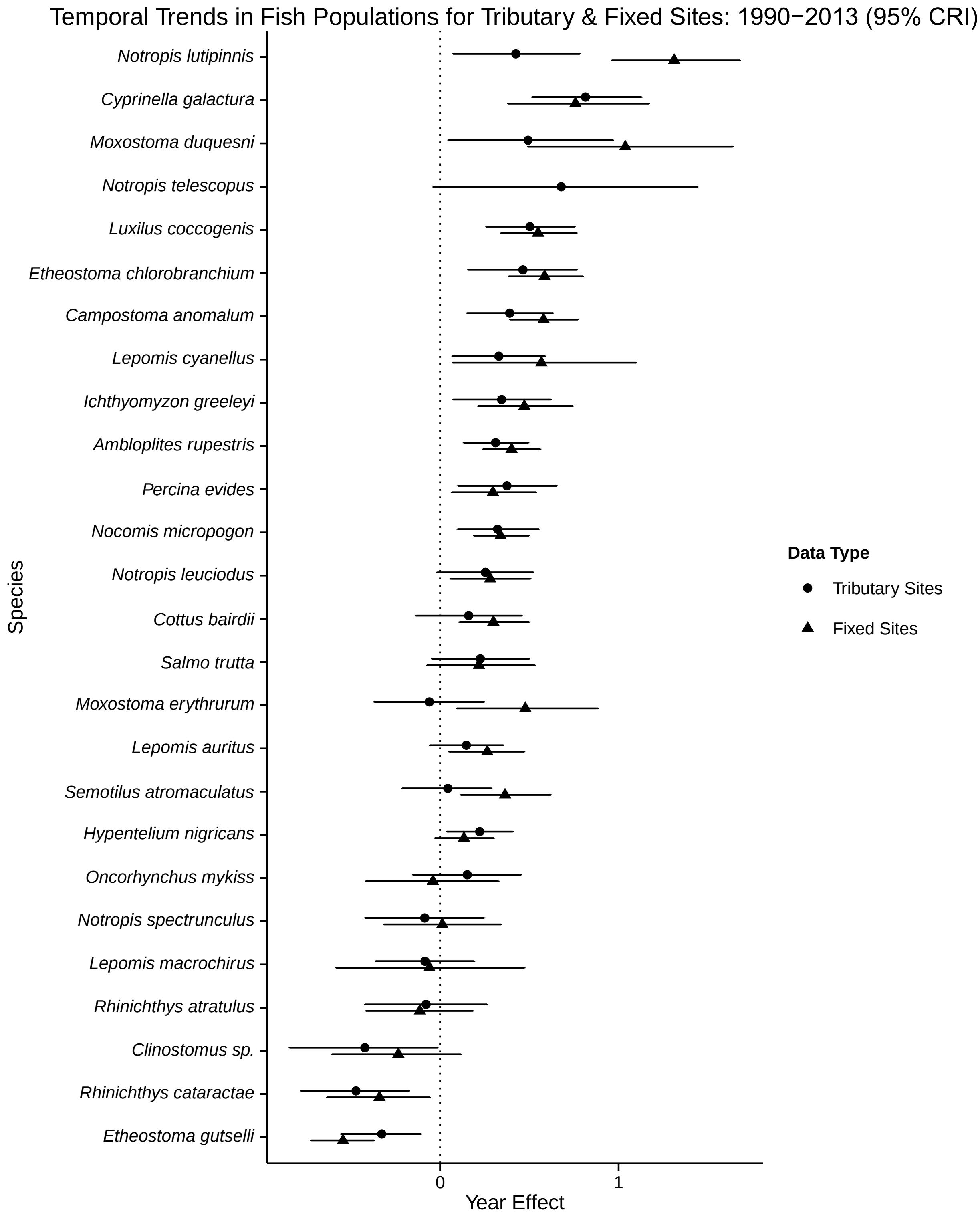

Figure 2.3. Overall temporal trends in fish species counts indicated by the year-effect coefficient; mean and 95% credible intervals. Credible intervals that includes zero indicate weak temporal trends. Year is scaled for analysis; a year-effect coefficient of 0.4 indicates an approximate 50% increase in fish counts for an increase of 7 years (i.e. one standard deviation of year)

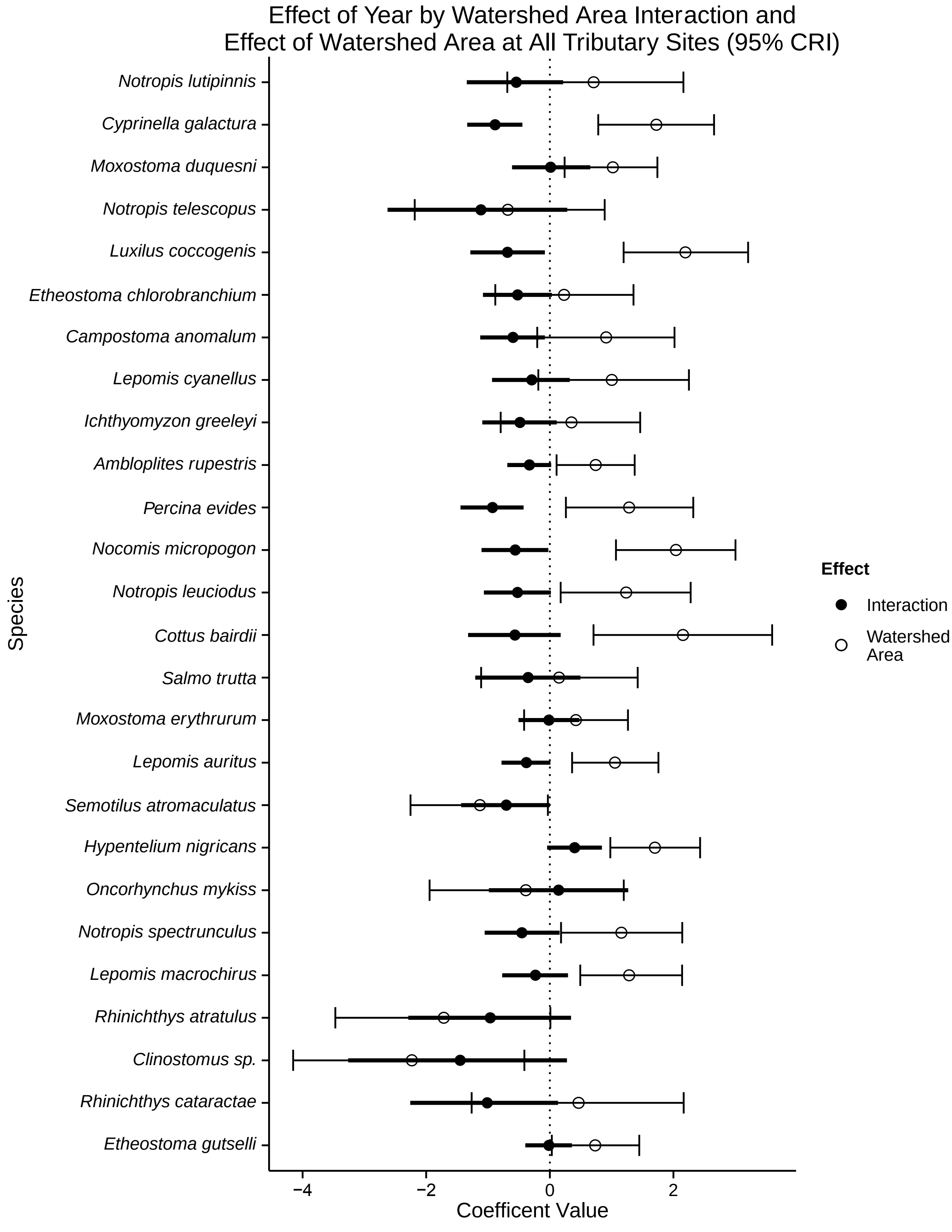

Figure 2.4. Effect of year by watershed area interaction and effect of watershed area on species- and survey-specific counts, indicated by coefficient values with 95% credible intervals. Because watershed area is a scaled value in the model, a coefficient value of 0.4 indicates an approximate 50% increase in fish counts per watershed area standard deviation. The standard deviation of watershed area is 42 km^2. Species are ordered as in Figure 2.3.

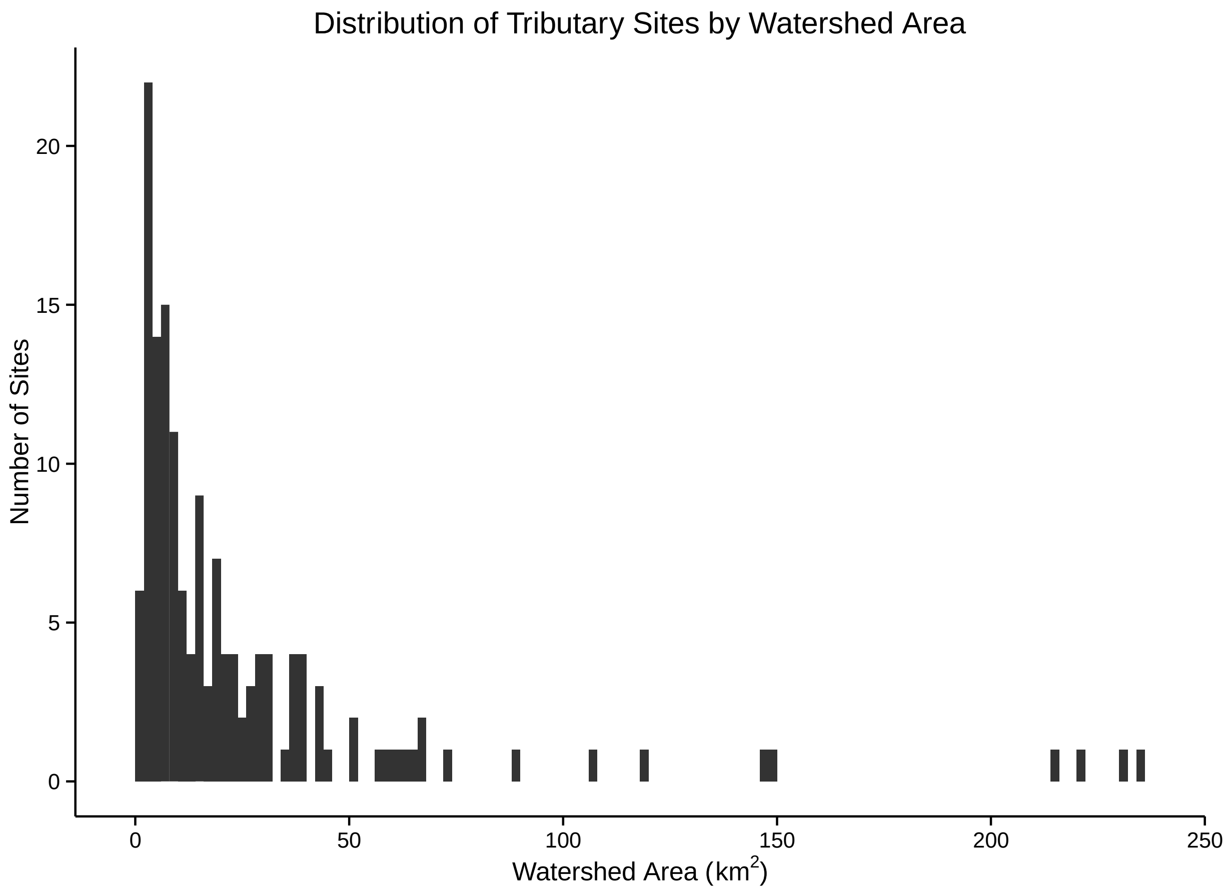

Figure 2.5. Distribution of tributary sites by watershed area (km^2). The majority of sites have a watershed area less than 50 km^2.

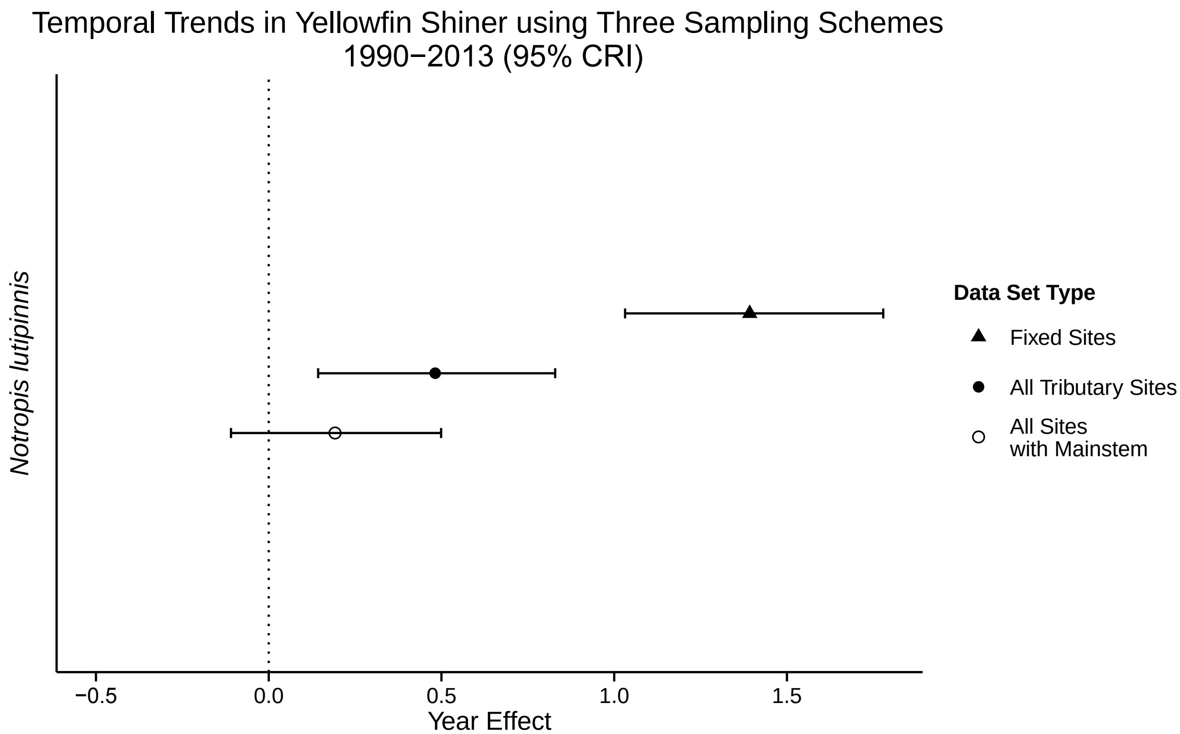

Figure 2.6. Temporal trends of Yellowfin Shiner indicated by year effect using three different sampling schemes with 95% credible intervals. These analyses include: all 155 tributary sites in the watershed, only the seven fixed sites and a third analysis with the tributary sites plus 17 main stem sites.

CHAPTER 3

RELATIONS BETWEEN TEMPERATURE AND STREAM DISCHARGE AND TEMPORAL VARIATION IN COUNTS OF STREAM FISHES IN THE UPPER LITTLE TENNESSEE RIVER WATERSHED

Introduction

Researchers have been working to understand the effects of environmental variables on stream fish populations for close to a century (Muttkowski 1929, Ward 1930). In particular, environmental variables such as temperature have been shown to affect survival (Beitinger et al. 2000, Grenouillet et al. 2001), and stream flow has often been shown to affect juvenile recruitment and survival (Bradford 1994, Nunn et al. 2003). Both effects are of interest in the context of increasing temperatures and greater variability in stream flows currently observed as well as forecasted under climate change scenarios (Xenopoulos and Lodge 2006).

Although temperature and flow are known to influence fish population dynamics, few studies allow testing for hypothesized effects, especially in the species-rich assemblages of the southeastern United States. In addition, it would be expected that species responses to changes in climate would not be uniform. For instance, warmwater stream fishes may not be as sensitive to temperature increases as species adapted to cooler climates. Also, lower stream flows could have either negative or positive effects on populations, e.g. by reducing habitat availability (Mulholland et al. 1997, Hodges and Magoulick 2011) or by increasing juvenile production because of increased temperatures associated with low flows (Nunn et al. 2003). Similarly, high flows can be beneficial or detrimental to reproduction by stream fishes depending on their particular life history traits (Craven et al. 2010).

This analysis uses a long-term dataset comprising stream fish surveys collected using a biomonitoring protocol in a Southern Appalachian watershed to test hypothesized effects of temperature and flow on fish populations. An earlier analysis (Chapter 2) has shown that about 46% of the most commonly captured fish species in these surveys had evidence of increasing counts over time, whereas fewer species showed temporal declines. Conversely, counts for many species (42%) displayed weak evidence of temporal trends. Moreover, counts for all species varied through time. Here, I evaluate evidence that variation in temperature or flow corresponds to changes in species-specific counts.

The fish counts analyzed here have been collected during stream surveys in a Southern Appalachian Mountain river system and provide a good opportunity for comparing evidence for temperature and flow effects on stream fishes. Climate data collected at the US Forest Service Coweeta Hydrologic Laboratory in Otto, NC since 1934 show trends of increasing air temperature and increased frequency of both high and low precipitation extremes (Ford et al. 2011, Laseter et al. 2012). In particular, data collection for this analysis has spanned a time period with a demonstrated trend of increasing air temperature (Laseter et al. 2012) and a period of drought and exceptionally low flows (Laseter et al. 2012), thus providing an opportunity to test for effects of temperature and flow on stream fishes.

These analyses also investigate the usefulness of indirect measures of climate-driven stream conditions. Water temperature and stream discharge data generally are not available for even a fraction of the sites where stream fishes have been monitored. Thus, I have used variation in air temperature data collected at the Coweeta facility as a proxy for variation in water temperature at fish monitoring sites. Similarly, I have used stream discharge data collected at a USGS gage station just outside of Franklin, NC on Cartoogechaye Creek; a major tributary of the Upper Little Tennessee River. As to be expected, the trends observed in precipitation data are also observed in the stream discharge data. However, using these stream discharge data as a proxy for variation in flows at fish survey sites provides an opportunity to model changes in fish populations more directly than relying on precipitation data. My question is thus, whether stream discharge data from Cartoogechaye Creek and temperatures measured in the Coweeta basin are correlated with variation observed in fish counts in diverse tributary streams of the Upper Little Tennessee River.

By testing for effects of environmental variables, I hope to explain some of the temporal variations observed in the fish count data. If certain environmental variables correlate strongly with variations of fish populations, then researchers and managers may be able to use these correlations to predict future population changes under multiple climate change scenarios as well as to plan and implement mitigation strategies in order to maintain a diverse fish community.

Methods

Study area

The Upper Little Tennessee River (LTR) is located in northeastern Georgia and southwestern North Carolina in the Southern Appalachian Mountain range and is a tributary of the Tennessee River. Originating in Georgia, the Little Tennessee River flows northward through the town of Franklin, NC. The river is impounded north of Franklin forming the relatively small Lake Emory; farther downstream, the Upper LTR is part of the inflow to Fontana Lake reservoir. A majority of the watershed is also designated by the US Forest Service as part of either the Nantahala National Forest in North Carolina or the Chattahoochee National Forest in Georgia.

The 155 sites used in these analyses have been surveyed using an index of biotic integrity (IBI) biomonitoring protocol. This protocol has been designed for use in wadeable Appalachian Mountain streams that have watershed areas between 4 to 70 square miles (10 to 180 km^2). Streams surveyed must also have an average gradient of less than a 100 foot drop per river mile (~19m per km), an elevation less than 2800 feet (853 m) and should not be located above a natural fish movement barrier (McLarney 2013).

Field Methods

Field methods for this chapter are as described in Chapter 2. In brief, a field crew of 7-9 individuals conduct fish surveys using a standardized protocol, which calls for a backpack electro-shockers, dip nets and a seine net. The survey reach is defined prior to surveying and the crew starts at the downstream end of the reach, setting the seine then shocking roughly a 10 meter subsample length, collecting fish along the way. After the subsample is complete the seine is hauled out of the water, the remaining fish are collected and all fish are identified to species, counted and recorded. This process continues 7 to 14 times until the end of the survey reach is attained. Counts from each subsample are totaled to get a complete survey count for each species. These totals are the counts used for the following analyses.

Data selection – fish counts

Fish count data analyzed in this chapter are the same data that were analyzed in the previous chapter (see Chapter 2 for complete data selection methods). In brief, the Land Trust for the Little Tennessee citizen science aquatic biomonitoring program has conducted surveys over 24 years at 176 IBI sites under the direction of Dr. William McLarney. Most of the 176 sites have been sampled on one to five occasions over the entire study period; however, seven sites on tributaries of the Upper LTR ("fixed sites") have been sampled nearly every year. For these analyses, I have analyzed data from the 155 sites located only on tributaries of the Upper LTR because the main stem survey site counts may not be accurately compared to the tributary sites because of differences in stream habitat, stream width and survey techniques.

Data selection – environmental variables

The environmental variables used to model fish counts included four discharge metrics and one temperature metric, chosen based on life history traits of the selected fish species. Stream discharge data were collected at the USGS gage station on Cartoogechaye Creek (Gage 03500250) just outside of Franklin, NC. Cartoogechaye Creek is a major tributary of the Upper LTR and may be a better indicator of stream flows in Upper LTR tributaries, than data for nearby gages on the main stem.

Flow metrics were calculated using daily mean discharge data from the USGS Cartoogechaye Creek gage station. The four flow metrics calculated were: spring (March-May) 10-day minimum discharge, spring 10-day maximum discharge, summer (June-August) 10-day minimum discharge and summer 10-day maximum discharge. The 10-day minimum and maximum discharges were calculated by finding the lowest and highest 10-day running average discharge for each season in every year. These four metrics represented key periods for spawning (spring) and juvenile rearing (summer) for most fish species examined (Etnier and Starnes 1993).

Climate data have been collected since 1934 at Coweeta Hydrologic Laboratory Climate Station 01, resulting in one of the most extensive climate datasets for the area. Maximum, minimum and mean temperature are collected each day at this station. For this analysis, I have used the mean of the daily maximum temperatures to calculate an average annual maximum temperature for each year in the study period.

Statistical Analysis

In these analyses, I used fish counts, stream discharge and temperature data to assess whether these five environmental metrics are correlated with the number of fish counted each year. I modeled trends of the 26 fish species that were caught in the greatest numbers across the 176 sites over the 24-year study period. In order to evaluate the effect of environmental variables on annual counts of these species, I modified the linear mixed-effects regression model used to assess temporal trends (Chapter 2). This model treated counts as though they were drawn from a Poisson distribution and included a random effect to account for overdispersion in the count data. Each species' count data were modeled separately. The linear regression model analyzed species-specific counts for each site and year combination as a function of a single grand mean intercept, an environmental variable (i.e. temperature or one of the four flow variables), site watershed area, an interaction between watershed area and the environmental variable, a random effect of site for each year and a random effect of watershed group. Thus, for a given species:

$$\text{Count}_{year,site} \sim \text{Poisson}(l_{year,site})$$

$$\text{Ln}(l_{year,site}) = \alpha + \beta_{env.var} * \text{environmental variable} + \beta_{wsa} * \text{watershed area} + \beta_{int} * \text{watershed area} * \text{environmental variable} + \varepsilon_{year,site} + \varepsilon_{ws.group}$$

The α parameter was a single grand mean intercept for all sites. The $\beta_{env.var}$ parameter represented the effect of the environmental variable on the site- and year-specific counts, and estimated the strength of association between each environmental variable and variation in species-specific counts. A fixed effect of site watershed area (β_{wsa}) was included to account for an influence of stream size on species-specific counts. An interaction between watershed area and the environmental variable was included to test if environmental effects on counts differed in relation to stream size. For example, I would expect a non-zero interaction if counts were more influenced by temperature or flow in larger streams compared to smaller streams, or vice versa. To account for overdispersion in the data, a random effect of site in each year was added, which allowed the model to accommodate greater variability than would be expected from a Poisson distribution. Finally, a random effect of watershed group was added to account for the possibility that counts at sites in the same tributary network (i.e. watershed group), were more similar to each other than to counts at sites in other watershed groups.

Because counts made using the LTLT biomonitoring program do not include young-of-year fish, I have modeled counts of the current year with environmental variables from the previous year (i.e. the environmental data was "lagged"). Specifically, this tests if temperature or flow variables have an effect on reproductive success and survival of individuals in early life stages (i.e. young-of-year). This is because the young-of-year cohort, which may be most strongly affected by environmental variables, would only be included in counts in the next year's survey. Species-specific models are evaluated using counts from all 155 tributary sites that were surveyed using the LTLT IBI biomonitoring protocol.

All models were fit with a Bayesian framework using R (R Core Development Team 2014), R package R2jags and JAGS (Plummer 2013). I used the following JAGS Markov Chain Monte Carlo settings: 3 chains, 15,000 total iterations, 2000 iteration burn-in, and 3 iteration thinning. All priors used in these models were selected from uninformative distributions (Appendix A). Year, watershed area and all environmental variable data were scaled using the "scale" function in R (R Core Development Team 2014) so that effect sizes could be compared directly. Model fit was assessed using Bayesian p-values and model convergence was assess by the value of R-hat (Kery and Schaub 2012).

Results

Climate and discharge data

Annual maximum average temperature increased over the study period (Figure 3.1) showing a positive trend that was significant ($r = 0.48$, $p < 0.009$, $n = 24$). Yearly precipitation totals and median yearly discharge at the Cartoogechaye Creek gage from 1962 to 2012 (the years where these datasets overlap) were highly correlated ($r = 0.86$, $p < 0.001$, $n = 51$). All four of the selected discharge variables, spring and summer 10-day minimum and maximum discharge, had decreasing trends from 1989-2012 (i.e. the data used in lagged year analyses; $n = 24$). The correlations between year and summer 10-day maximum discharge ($r = -0.31$, $p = 0.079$) was not statistically significant. The other sets of discharge data all had significant negative trends: spring 10-day minimum discharge ($r = -0.54$, $p < 0.004$), spring 10-day maximum discharge ($r = -0.56$, $p < 0.003$), summer 10-day minimum discharge ($r = -0.37$, $p < 0.041$). However, trends in discharge variables were less obvious than for temperature (Figure 3.2), and standardized values for all environmental variables displayed substantial among-year variation across the study period (Figure 3.3)

Effects of discharge and temperature on counts

Model results using data from all tributary sites show significant evidence of temperature or discharge effects for 11 of the 26 species examined. For illustration, the effects of discharge and temperature are plotted by species and separated into three groups as defined by their temporal trends in counts (i.e., positive year effect, weak effect of year, or a negative year effect) as reported in Chapter 2. Six of the 12 species with evidence for increasing counts through time

(Figure 3.4) have positive associations between counts and annual maximum temperature. Five out of the 12 species in this group have one or more significant negative discharge effects. In all cases, counts increase in relation to lower spring and (in 3 cases) summer 10-d minimum flows. Species with weaker evidence for population trends (Figures 3.5 & 3.6) have mostly weak evidence for effects of environmental variables, either discharge or temperature. Only two of these 11 species, Tennessee Shiner and Telescope Shiner, have increased counts in relation to lower spring 10-d minimum flows and increased annual maximum temperature. Counts of the three species with evidence of declining populations are positively related to spring 10-d maximum discharge (Figure 3.7), although the 95% credible interval for Tuckasegee Darter counts included zero. Smokey Dace and Longnose Dace show evidence of negative temperature effects, although the credible intervals again include zero.

Discussion

An analysis of correlations between long-term variation in species-specific counts at diverse sites and environmental variables has shown predominantly positive correlations with higher annual maximum temperatures and with lower minimum flows. Counts for 8 of the 26 species examined increase in relation to higher average annual maximum temperature, compared to no species having significantly lower counts in relation to higher temperatures. Lower 10-day minimum flows also are associated with higher counts for 7 of the 26 species, whereas two species have higher counts in association with higher 10-day maximum flows in the spring. These trends are not unexpected, given that maximum annual temperature is positively correlated with year, and minimum flows are negatively correlated with year, across the study period, and over 40% of the examined species also show evidence of temporal increase in counts (Chapter 2). However, two species (Telescope Shiner and Tennessee Shiner) with weak evidence of temporal trends have increased counts in association with higher temperatures and lower minimum flows. Overall, only two species (Smokey Dace and Longnose Dace) have shown specific evidence of negative effects of rising temperatures or decreasing flows.

The two species that appear to respond negatively to rising temperatures and decreasing flows are Longnose Dace and Smokey Dace, however, there is only weak evidence for the effect of rising temperatures since the effect estimates include zero in their 95% credible intervals. Both of these species prefer cool to cold rocky streams (Etnier and Starnes 1993). Longnose Dace is also a high flow-velocity specialist, living and spawning in swift riffle habitat. Spawning usually occurs from April to early June, thus warmer, reduced spring flows may not provide suitable spawning habitat. Less information is published regarding the life history traits of Smokey Dace, however, it is a highland endemic with a limited range found in upland streams in the Little Tennessee River system. Most notably Smokey Dace have a brief spawning window that lasts from late May to early June (Etnier and Starnes 1993). Craven et al. (2010) provide evidence that high flows during the spawning period increase young of year production, particularly for species with shorter spawning periods..

Other than the two species discussed above, this study primarily provided evidence for a positive effect on fish counts of reduced flows during spring and summer, even though high or low stream flows can have both positive and negative effects on stream fishes. This positive effect of low flows may be due to decreased energy expenditure (Nunn et al. 2003), or beneficial effects on reproduction success (Craven et al. 2010). Most of the species that have higher counts in years with lower flows spawn in the spring or summer. Increases in flows during this window can have detrimental impacts on young-of-year (Nunn et al. 2003), therefore decreasing the recruitment and survival of this cohort which would then be observed in the next year’s survey.

I anticipated that variation in temperature would be associated with fish counts because temperature often cues spawning behavior in these species (Etnier and Starnes 1993). Higher temperatures also can lead to heat stress in fishes and lower oxygen saturation, compromising survival (Mulholland et al. 1997, Beitinger et al. 2000). Thus an increase in high temperatures may cause fish to spawn earlier than normal and there may be an increase in mortality resulting from high summer temperatures. On the other hand, higher water temperatures can lead to increased productivity by lengthening the growing season and increasing growth rates (Mulholland et al. 1997, Nunn et al. 2003). I used maximum annual average temperature in these analyses because it integrates the warmest temperatures throughout the year and also because warming trends have been observed over the 24-year survey period (Laseter et al. 2012). Taking into account the additional predicted warming (Kirtman et al. 2013), a maximum temperature metric may provide the most insight into current and possible future effects of temperature trends on fish counts. However, my results do not show evidence for significant negative effects of increasing maximum annual temperature on most of the species examined.

By modeling the environmental variable metrics of stream discharge and average maximum temperature versus the counts of fish species over time, I have been able to evaluate the question of whether fish counts are correlated with observed variations in the climate. This question is especially interesting due to the fact that the climate trends of increasing temperatures and heavier rains along with more severe droughts have already been observed in the Upper LTR watershed (Laseter et al. 2012). In fact, three record years of precipitation have been set in the last eight years including the driest year since record keeping began in 1934 (2007), and the two wettest years (2009 and 2013). In addition, these trends of increased warming and extreme swings in precipitation (and thus stream flow) are expected to continue regionally and globally through the end of this century (Muller et al. 2011, Kirtman et al. 2013). It has already been established that stream discharge and temperature can impact fish populations in general (Etnier and Starnes 1993, Bradford 1994, Hodges and Magoulick 2011), and specifically these environmental variables have shown a correlation with some of the changes in fish counts seen in the Upper LTR watershed. Now that 24 years of data have been collected, baseline counts and trends for these species' populations have been established. Continued annual monitoring of the sites will augment an already extensive dataset and will provide additional information regarding the impacts of environmental variables. This study has only begun to reveal the possible applications of this dataset, and since it is now publicly available, hopefully many more researchers will take the opportunity to unlock its potential. Long-term data collections such as this biomonitoring program may ultimately be one of the best ways to detect systemic changes in streams and rivers.

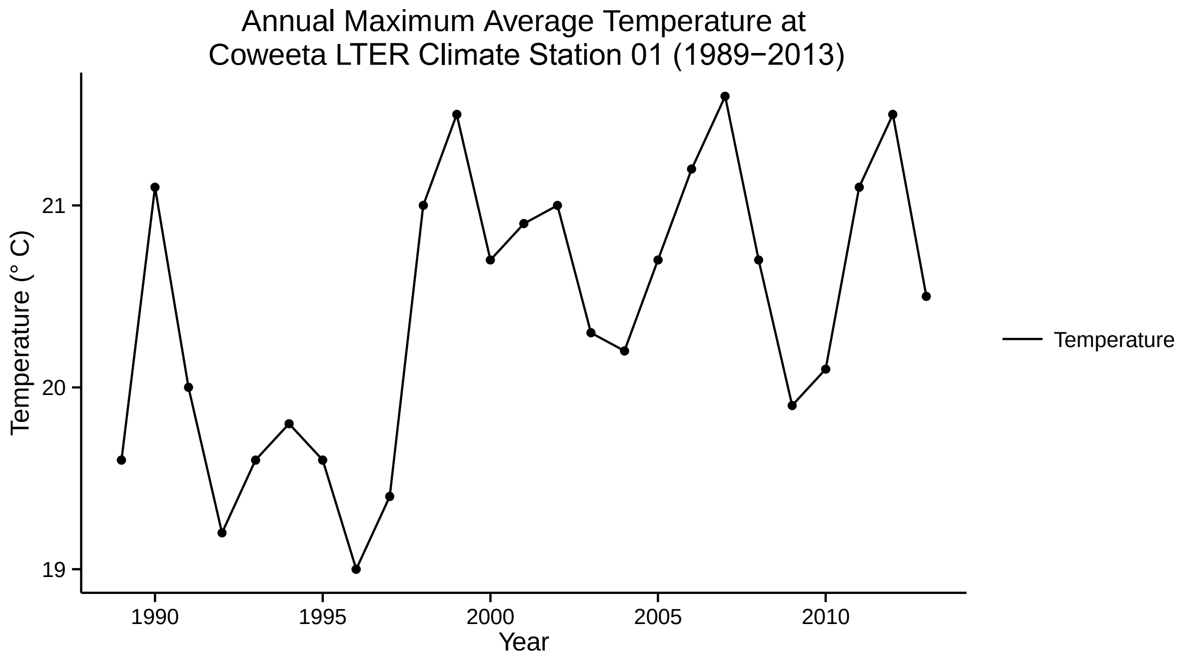

Figure 3.1. Annual maximum average temperature at Coweeta LTER Climate Station 01 from 1989-2013.

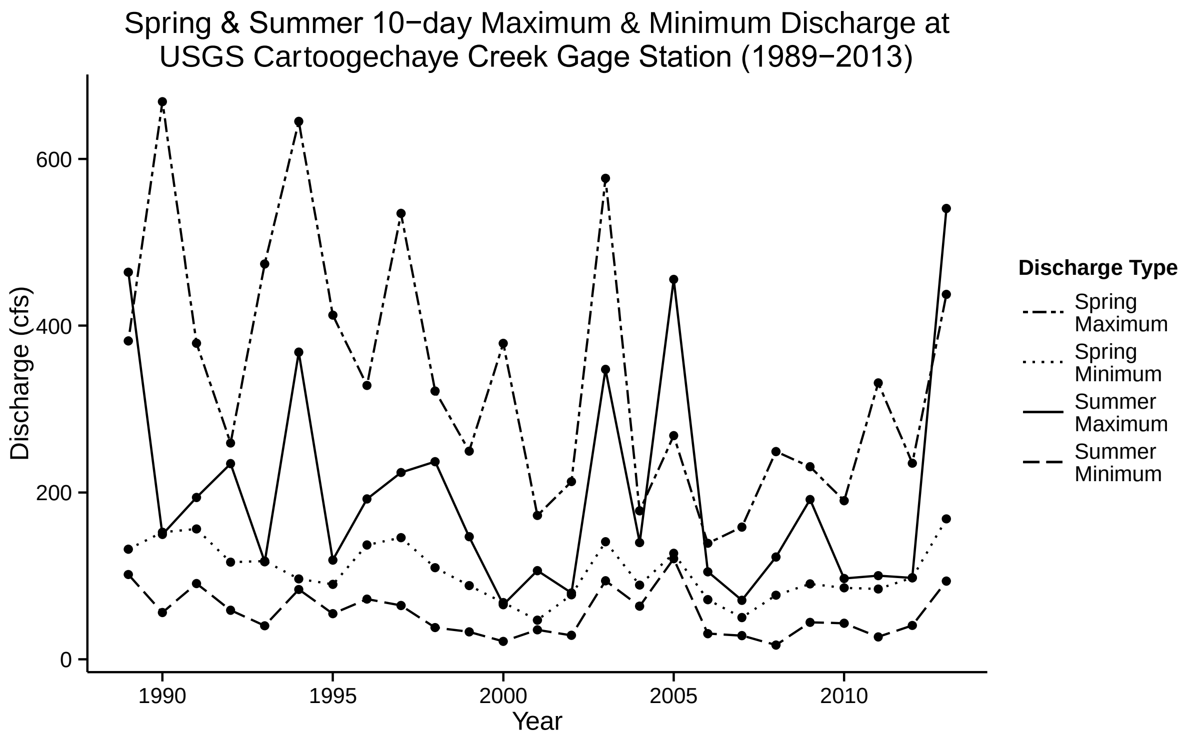

Figure 3.2. Spring and summer 10-day average maximum and maximum discharge (in cubic feet per second) at USGS Cartoogechaye Creek Gage Station (03500240) from 1989-2013

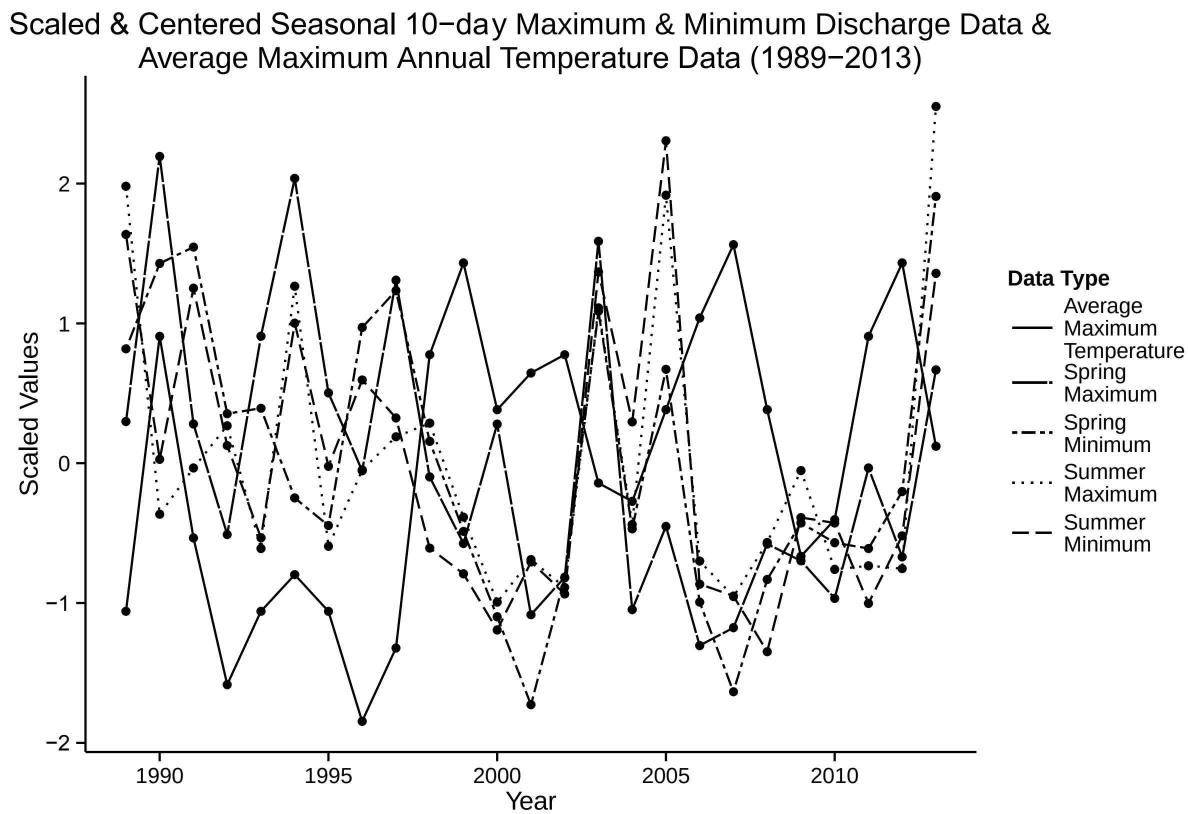

Figure 3.3. Scaled and centered spring and summer 10-day maximum and minimum discharge data and average maximum annual temperature data from 1989-2013. Data were scaled using the "scale" function in R, which scales by the mean and standard deviation, centering on zero. Each data type is scaled to its own mean and standard deviation.

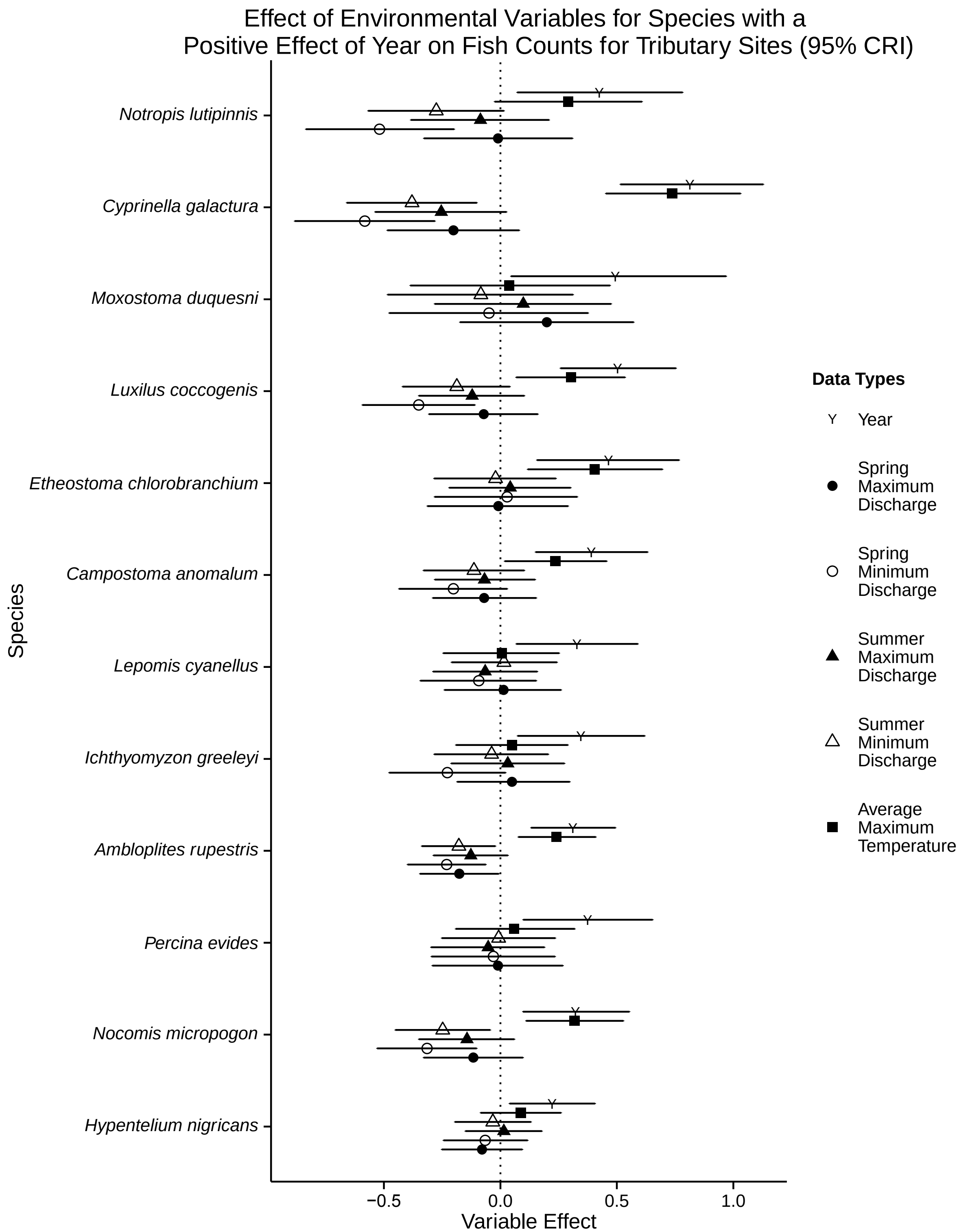

Figure 3.4. Comparison of all discharge effects and temperature effect on counts for species with a positive year effect. Discharge data are spring and summer 10-day average maximum and maximum discharge and temperature data are average maximum annual temperature data, using environmental variables lagged by one year for all tributary sites.

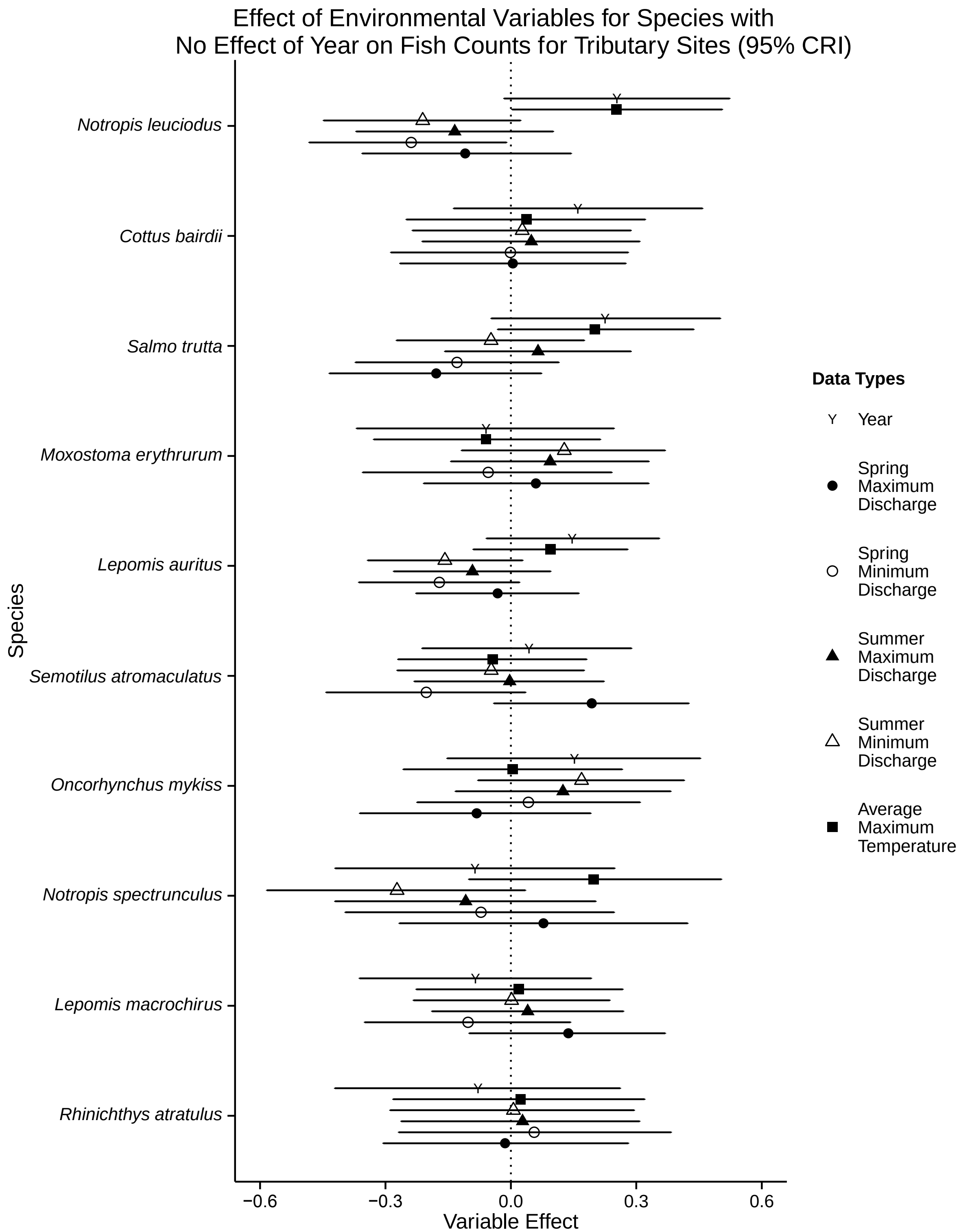

Figure 3.5. Comparison of all discharge effects and temperature effect on counts for species with no year Discharge data are spring and summer 10-day average maximum and maximum discharge and temperature data are average maximum annual temperature data, using environmental variables lagged by one year for all tributary sites.

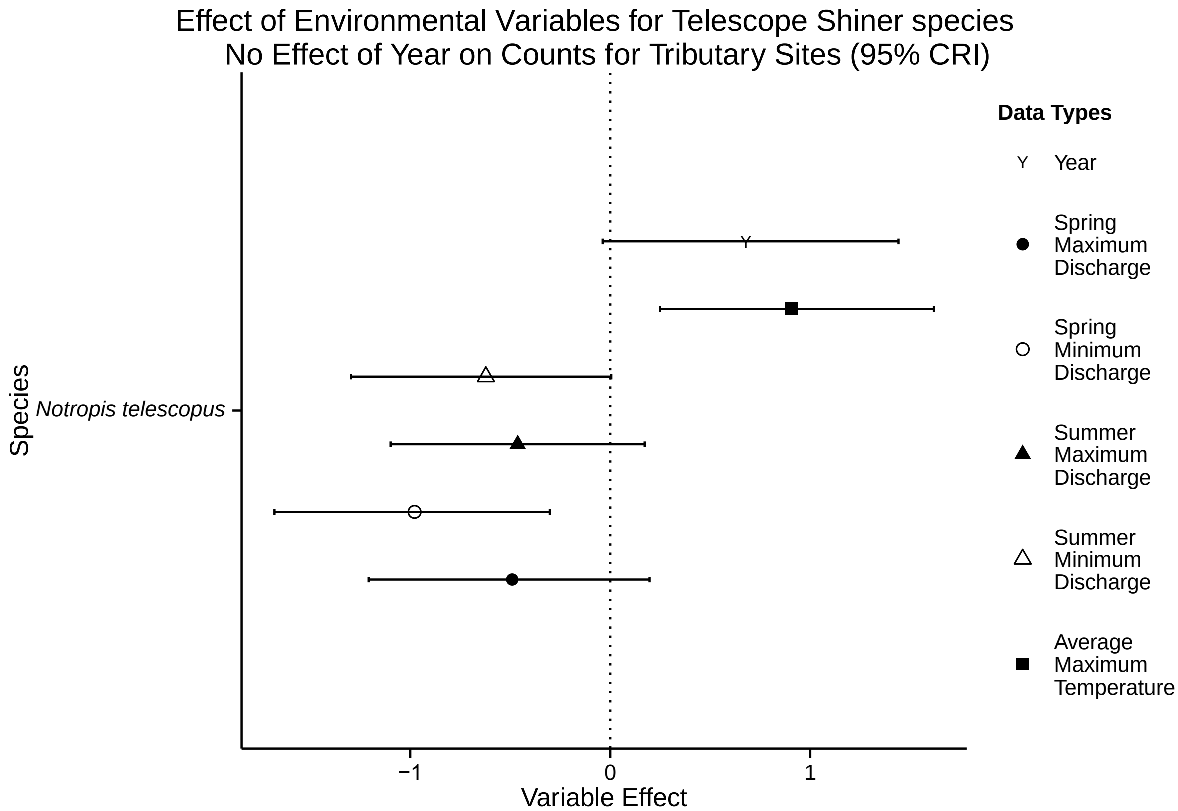

Figure 3.6. Comparison of all discharge effects and temperature effect on counts for Telescope Shiner, which had no effect of year. Discharge data are spring and summer 10-day average maximum and maximum discharge and temperature data are average maximum annual temperature data, using environmental variables lagged by one year at all tributary sites. Telescope Shiner results were displayed separately from the rest of species with no effect of year due to the larger effect and credible interval estimates compared to other species in this group.

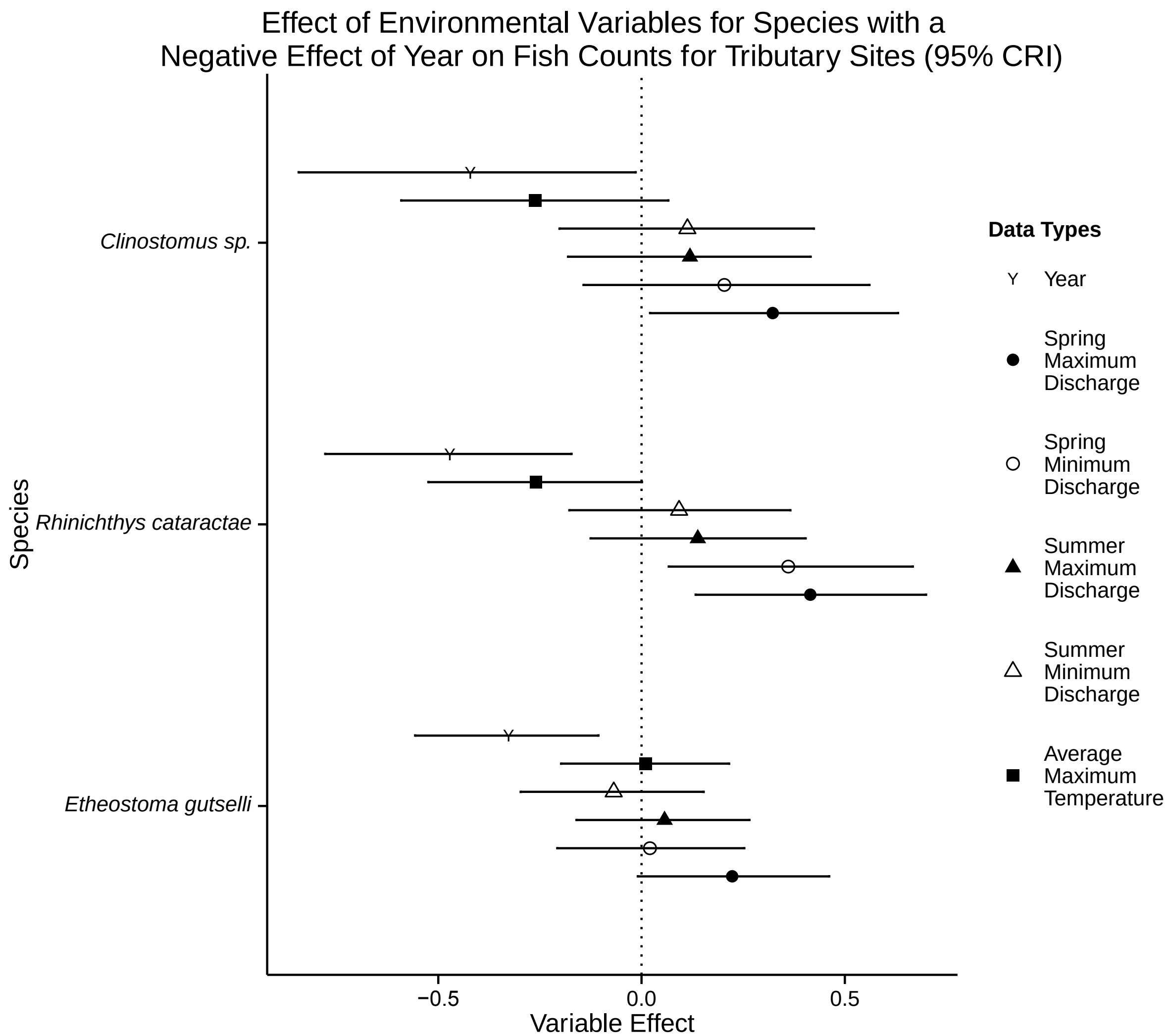

Figure 3.7. Comparison of all discharge effects and temperature effect on counts for species with a negative year effect. Discharge data are spring and summer 10-day average maximum and maximum discharge and temperature data are average maximum annual temperature data, using environmental variables lagged by one year at all tributary sites.

CHAPTER 4

SUMMARY AND RECOMMENDATIONS

Models results for 26 fish species from a long-term fish dataset collected by Dr. McLarney at the Land Trust for the Little Tennessee indicated trends of either significant population growth or weak growth that was not statistically significant. Counts for only three of these species (Smokey Dace, Longnose Dace and Tuckasegee Darter) showed significant population declines. In addition, I modeled fish count data against stream flow and temperature metrics, because these metrics are known to impact fish recruitment and survival. Over the 24-year study period, precipitation (and thus stream flow) has generally declined, while temperature has increased (Laseter et al. 2012). I found that, for most species, the observed decline of stream flows over the past 24 years has actually had a positive association with fish counts. Meanwhile, there were only two species to show declines with decreased flows. Inverse results were found for temperature. Specifically, the abundance of most species was positively associated with temperature, but a few species showed declines as temperatures increased. Therefore, it seems that these fish communities have thus far shown resilience to the observed climate trends of increased droughts and warmer temperatures.

Hopefully my analyses will bring more attention to the value of this fish count dataset and perhaps even spur others to support continuing the valuable biomonitoring work of the Land Trust for the Little Tennessee, as well as similar organizations. I also hope to encourage other researchers to explore this information-rich dataset because there are many possible avenues of additional research.

Of the 26 species that I analyzed, I found that three, Smokey Dace, Longnose Dace and Tuckasegee Darter, were being negatively impacted by climate trends. Thus an important step would be to determine if other species not analyzed here but in the dataset also are being negatively impacted by climate changes and then to elucidate trends of all declining species further using temperature, discharge or other metrics. One metric that could be used in future analyses is land use change. Landcover raster data for the Southern Appalachians are available for download from the Coweeta LTER GIS catalog in 5-year intervals from 1986-2006. Although landcover data for 2011 have not yet been uploaded to the Coweeta LTER GIS catalog, they are available by request from Dr. Jeff Hepinstall-Cymerman at the University of Georgia. Using landcover data as covariates in these analyses may help to explain differences in fish count among sites and may weaken or strengthen estimated impacts of the environmental variables.

Another area of inquiry, which would greatly benefit from spatial analysis, is the possible "invasion" of the Yellowfin Shiner. According to Dr. McLarney, the Yellowfin Shiner has been moving from the headwaters of the Upper Little Tennessee River in Georgia, down the main stem into North Carolina and into tributaries farther downstream. These count data, combined with GIS data, may help to further support this hypothesis.

An additional area of research might be to use the long-term fish dataset for a community analysis. While my analyses examined trends of individual species, it could be interesting to look at overall community dynamics. This would also involve using all, or most of, the species in the database, as opposed to the 26 selected species used in my analyses.

Finally, there is one possible research goal that is not currently attainable using the dataset, but may lead to additional insights into the fishes and biomonitoring techniques. This involves using what I call the "subcount" data. The LTLT biomonitoring protocol involves setting a seine, collecting and counting fishes and repeating this process 7 to 15 times in separate, short, adjacent sections of stream. The count of each fish species is recorded independently for each seine set (i.e. a subcount). Subcounts are then totaled to attain an entire site count, which is the only number recorded in the database. However, most field datasheets are still extant in Dr. McLarney's collection and I have scanned field datasheets and digitized these subcount data for all fixed sites from 2004-2013, as well as some other sites during this time period. These datasheets also contain additional data such as subsample habitat type, subsample dimensions, water temperature, start and stop time, presence of disease as well as other data. While some of these data are recorded in the database, most are not. Thus it seems that there is a large amount of additional information and knowledge available for access if the remaining datasheets were scanned and digitized. This information could then be added to the current database and made accessible for further studies.

While the inclusion of the subcount data may be one way to augment the usefulness of the LTLT fish dataset, there are also other possible changes that may make the dataset more useful for scientific research. This might include a standardized protocol that involves stratified randomization of the sites selected for surveying each year. While the fixed sites should continue to be surveyed every year, it is recommended that an additional set of rotating sites be selected and surveyed on a regular multi-year schedule.

In order to enhance the replicability of the biomonitoring process, it may help to log GPS coordinates at the beginning and end of the survey reach, as well as the breaks between all subsamples. Recording the air temperature, water temperature and flow velocity at the time of each survey in the database may provide important covariates when trying to answer additional questions using these data. Finally, it would be helpful for Dr. McLarney and LTLT to include an introduction to the dataset, in addition to the one I include in Appendix B, so that people not familiar with the dataset can better understand the value and structure of the data presented.

Fortunately, the Land Trust for the Little Tennessee continues to conduct biomonitoring surveys and collect fish count data, the Coweeta climate station continues to collect climate data and the USGS gages continue to collect discharge data. These datasets will hopefully continue to grow and provide opportunities to answer more questions about fish populations and community trends and how they are linked to environmental changes. The fish community trends uncovered by my research may provide a starting point for the continued assessment of stream health and water quality in this aquatic biodiversity hotspot of the Southern Appalachian Mountains.

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APPENDIX A – JAGS MODEL CODE

```
model{
    b.var ~ dnorm(0, 0.0001)   #Prior for b.var is drawn from a normal distribution
    b.wsa ~ dnorm(0, 0.0001)  #Prior for b.wsa is drawn from a normal distribution
    b.int ~ dnorm(0, 0.0001)    #Prior for b.int is drawn from a normal distribution

    alpha ~ dnorm(mu, tau.eps.site) #Prior for alpha is drawn from a normal distribution

    tau.eps.site <- pow(sigma.eps.site, -2) #Defines precision for alpha normal distribution
    sigma.eps.site ~ dunif(0,100)       #Prior for standard deviation to convert to precision
    mu ~ dnorm(0, 0.0001)               #Prior for mean of alpha normal distribution

      for (t in 1:nyears){              #eps.year term adds extra variation to the model
      for (i in 1:nsites){              #eps.year accounts for overdispersion in the data
        eps.year[t,i]~dnorm(0, tau.eps.year)
        }}
    tau.eps.year<-pow(sigma.eps.year, -2)            #Defines precision for eps.year normal
                                                     #distribution
    sigma.eps.year~dunif(0,100)               #Prior for standard deviation to convert to
                                              #precision in eps.year normal distribution
```

```
for (j in 1:nws){
        eps.ws[j]~dnorm(0,tau.eps.ws)             #Prior for random effect of watershed group
        }
      tau.eps.ws<-pow(sigma.eps.ws,-2)          #Defines precision for eps.ws normal #distribution
      sigma.eps.ws~dunif(0,100)                 #Prior for standard deviation to convert to
                                                #precision in eps.year normal distribution

      for (t in 1:nyears){                #Linear regression model loops over fish counts for
      for (i in 1:nsites){                #defined number of years and sites using the log-link
                                          #function
      count[t,i]~dpois(N[t,i])
log(N[t,i]) <-  alpha + b.var*z[t]  + b.wsa*wsa[i] + b.int*z[t]*wsa[i]+
              eps.year[t,i] + eps.ws[ws[i]]

              d[t,i]~dpois(N[t,i])        #Draws an estimated count from the model to compared
                                          #with the actual count from the data
 resid[t,i] <- pow((count[t,i]-N[t,i]),2)/N[t,i]  #Computes the residuals for actual counts
 resid.new[t,i] <- pow((d[t,i]-N[t,i]),2)/N[t,i]           #Computes the residuals for estimated
}}                                                         #counts
           fit<-sum(resid[,])                   #Sums all actual residuals based on data
           fit.new<-sum(resid.new[,])           #Sums all estimated residuals
           b_pvalue <- step(fit.new-fit)        #Compares actual to estimated residuals
      }                                         #This comparison is called a Bayesian p-value
```

APPENDIX B – METADATA FOR LTLT FISH COUNTS DATABASE

A "metadata" document created to introduce the Fish Counts Database of the Land Trust for the Little Tennessee (LTLT) for people interested in using the database for research

The LTLT Fish Counts Database is a unique long-term dataset that contains fish count data by species from over 400 survey sites and over a quarter century. This document serves as introduction to the dataset and explains how to begin to understand and negotiate the structure of the database, including challenges that I encountered when using the database and suggestions for how one can start an analysis of the data.

1) The LTLT database is a record of fish count biomonitoring surveys conducted by: a) the Land Trust for the Little Tennessee (2012-Present); b) the Little Tennessee Watershed Association (LTWA) (1993-2011); and c) Dr. William McLarney (1988-1992).
    a. Dr. McLarney oversaw the collection of all data from 1988 to the present (2015).
2) For people unfamiliar with Microsoft Access, I suggest the following to begin to appreciate the complexity of this database:
    a. Open the file and select "Database Tools" from the ribbon.
    b. Click the "Relationships" button in Database Tools .
    c. Clicking this button will open a window with all the connections among the approximately 25 different tables that make up the database.
    d. The key icons denote the fields on which the tables in the database are connected

e. Additionally, I would recommend watching a tutorial video on MS Access before exploring the database in depth.

f. Final note about MS Access: Learn how to perform “Queries” via a tutorial

   i. Queries are used to extract selected information from the database and can be very powerful when specific information is desired.

3) The LTLT fish database contains multiple survey types, e.g. Spotfin chub surveys, NC wildlife surveys, IBI survey, species presence, incomplete, etc.

   a. These survey types all have different amounts of effort expended and sometimes focus on particular habitats so their data are not directly comparable

   b. However, all IBI surveys use the same field IBI biomonitoring protocol and thus are ostensibly comparable

   c. IBI surveys in 2012 & 2013 are labeled as “ltlt” under the episode_type column

      i. The LTWA merged with LTLT in 2012 and started sampling under a new NCWRC sampling permit number, thus these samples are labeled as “ltlt” episode_type, however, the same methodologies and protocols were used and they are thus considered IBI surveys (Per Jason Meador at LTLT)

   d. The most important tables in the database for my analysis were:

      Sites, survey_episodes, ibi_survey_species_counts & auth_species

4) To retrieve just IBI counts, filter by episode_type and select “ibi” and “ltlt” (“ltlt” is the designation for 2012 & 2013 biomonitoring data)

5) There are some mistakes in the database

   a. Counts for *Cottus bairdii* (Mottled Sculpin) at WAYCR-093 are missing in years 1995 & 1997 (Episode_IDs 2309 & 2310)

        i. Those counts are 1995 = 1104 *Cottus* & 1997=1127 *Cottus*

   b. Four sites have data entry errors where the same counts are repeated in two different episode_ID's (i.e. – the same data is input twice, e.g. Cottus = 25, Cottus = 25), I call these "fake" repeats

        i. Site CATWA-057, Episode_ID = 3274, 3278 are "fake" repeats

        ii. Site SKEMC-108, Episode_ID = 3272, 3276 are "fake" repeats

        iii. Site YOUYC-032, Episode_ID = 3273, 3295 are "fake" repeats

        iv. Site RABRC-055, Episode_ID = 3288, 3289 are "fake" repeats

            1. RABRC-055 also has a "real" repeat; see 6.d. for explanation

   c. Some sites have only 1 count of zero fish and seem to be mistakes in data entry

        i. HODBR-712, Epi_ID=3383?; OXBRB-713, Epi_ID=3383; RABBE-711, Epi_ID=3382

   d. Site OXBGS-714, Epi_ID=3384 has a count of 1 Green Sunfish and no other fish, I deleted this count because it appears to be a data entry error, unless the site was only sampled once and only 1 fish was collected during the entire survey

   e. Counts from site PEEPC-176 should be labeled as from PEEPC-175, they are they same site

   f. Episode dates are incorrect for many samples, with June 1st of the particular year being used simply as a "place holder" when correct dates were unavailable

6) There are 5 sites that have "real" repeated IBI samples in the same year (i.e. – these sites were surveyed twice during the same summer and have two different counts of fish in the database for the same year

   a. MATSH-032, Repeat in 2008, Epi_IDs = 39, 43

b. WATAC-051, Repeat in 2008, Epi_IDs = 300, 306

c. WATBM-050, Repeat in 2008, Epi_IDs = 298, 305

d. RABRC-055, Repeat in 2009, Epi_IDs = 3288, 3347

    i. This is the “real” repeat (2 different survey occasions), as opposed to the “fake” repeats described previously in 5.B.iv.1.

e. SKEWC-107, Repeat in 2009, Epi_IDs = 3282,3346

7) Some species are identified in the database as Unspecified, or Unidentified Chub, Redhorse, etc., which is unhelpful for species specific analysis

8) Some species are identified as hybrid species (e.g. *Notropis lutipinnis* x *Notropis leuciodus*); however, there are not many of them and these data are questionable

9) While the database contains data from 1988-2011, only 1 site was surveyed in 1988 (according to the database) and 4 sites were surveyed in 1989. In contrast, there were 24 surveys in 1990 and usually between 10-30 surveys per year thereafter.

10) For my analysis of tributary sites, I deleted the following data:

    a. 1 of the 2 episodes of the “fake” repeats, one episode of “real” data was retained

    b. All data that were “unspecified” or not identified to species (“unidentified”)

    c. Species identified as hybrid species

    d. All data from 1988 and 1989; 5 sites were sampled during these two years, but these sites were never sampled again

    e. Site OXBGS-714, Epi_ID=3384 has a count of 1 Green Sunfish and no other fish

11) For years 1990-2013, there are a total of 176 sites (155 on tributaries and 21 on the main stem of the Upper LTR) sampled using the IBI biomonitoring protocol, with a total of 9924 counts (rows of data) for all species in all years.