IMPERVIOUS COVER THRESHOLDS OF THE NORTH CAROLINA PIEDMONT FISH ASSEMBLAGE

by

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ABSTRACT

PATRICK COLE WEBSTER. Impervious Cover Thresholds of the North Carolina Piedmont Fish Assemblage. (Under the direction of DR. SANDRA CLINTON)

Researchers have placed emphasis on quantifying and identifying ecological thresholds to study biological responses to urbanization. As watersheds become urbanized, they exhibit a systemic pattern of degradation that disrupts the natural biogeochemical and geomorphologic processes, ultimately leading to a decline in freshwater biodiversity. In North Carolina, an increase in population is leading to an aquatic biodiversity crisis which can be observed in declining freshwater fish abundance and diversity. Although studies in the Eastern Piedmont and specifically North Carolina have quantified the relationship between aquatic biotic communities and urbanization, they have fallen short of identifying individual tolerances. This study uses land cover data from the National Land Cover Database and biomonitoring datasets from the North Carolina Department of Environmental Quality and Mecklenburg County Stormwater Services with the Threshold Indicator Taxa Analysis (TITAN) to quantify percent impervious cover (IC) thresholds and change points at the community and individual level to identify biological indicators and conservation priorities for watershed health. Non-parametric and pairwise testing was used to identify IC tolerance trends among ecological functional groups and pollution tolerance designations. Results of the land cover analysis reveal that IC increased by 1.73% throughout the North Carolina Piedmont in the 16-year period, but watersheds with < 15% IC decreased by 9.7% and watersheds between 45-60% increased 329%. TITAN revealed that Z- taxa experience the greatest change in frequency and abundance, also known as change point, at 6.10% IC and have an aggregate threshold of ~ 7% IC (5.79-12.78%); Z+ taxon have a change point of 16.59% and an aggregate threshold of 41.30% IC (16.07-57.37%). Kruskal-Wallis results demonstrated IC tolerance among pollution tolerance classifications and trophic guilds thresholds were significant but insignificant for spawning guilds. TITAN also revealed several taxa whose IC tolerances differentiated from their respected pollution tolerance. Overall, this study revealed that with the current NC state watershed development regulations, ~88% of the state's watersheds could exceed IC thresholds of ~75% of taxa within the NCP fish assemblage.

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NCP	North Carolina Piedmont
IC	Impervious Cover
LULC	Land Use/Land Cover
TITAN	Threshold Indicator Taxa Analysis
CFD	Cumulative Frequency Distribution

CHAPTER 1: INTRODUCTION

1.1 Aquatic Biodiversity and Urbanization in the Southeastern United States

Over the last few decades, aquatic biodiversity in North American has been on a rapid decline. Species listed as imperiled (i.e. endangered, threatened, or species of concern) have increased by 92% in the United States alone and freshwater taxa are disappearing up to 5 times faster than land animals (Helfrich et al. 2009, Jelks et al. 2011). Loss of aquatic biodiversity is largely attributed to the destruction, degradation, and/or fragmentation of habitat and the introduction of invasive/exotic species and diseases, which often are exacerbated by urbanization (Wilcove et al. 1998). As watersheds become urbanized, they exhibit a systemic pattern of degradation known as the Urban Stream Syndrome (Walsh et al. 2005). Urban streams tend to have flashier hydrographs, elevated concentration of nutrients and contaminants, altered channel morphology and stability, reduced summer baseflow, and increases in suspended solids (Barnum et al. 2017, Smith and Lamp 2008, Utz et al. 2009). These patterns disrupt the natural geophysical and biogeochemical processes and functions that sustain aquatic biodiversity richness and ultimately stream health.

Symptoms associated with the Urban Stream Syndrome can have a widespread impact on fish. Directly, higher flows associated with flashier hydrographs cause downstream displacement in juvenile fish (Harvey 1987) and scouring of the stream bed which leads to increased suspended solids, sedimentation, and destruction of vital habitat. In turn, habitat loss can decrease refugia causing increased predation (Lonzarich and Quinn 1995), delay spawning, and reduce reproductive and foraging success (Jelks 2001, Zamor and Grossman 2007). Low summer baseflow conditions mimic those of drought with increased water temperatures and altered water chemistry (Keaton et al. 2005). Low baseflows can cause habitat fragmentation (Knouft and Chu 2015) that can limit habitat resources and fish mobility (Lohr and Fausch 1997). Because fish are ectothermic, increased water temperatures can cause an increase in metabolism (Johnston and Dunn 1987), and effect reproduction and juvenile recruitment (Freeman et al. 1988, Schlosser et al. 2000). Lastly, increased nutrients and contaminants can cause several physiological issues and disrupt reproductive and immune systems (Jobling et al. 2003, Tyler et al. 1998).

Past studies that have looked to quantify the impact of urbanization on aquatic biodiversity have found that watersheds between 0-10% imperviousness generally have good water quality, and diverse biological communities; 10-20% imperviousness show clear signs of stream health degradation; and watersheds with > 25% imperviousness only support streams with pollutant intolerant fish and insects (Karr and Chu 2000, Miltner et al. 2004, Morgan and Cushman 2005, Schueler 1994, Yoder and Smith 1999). Regardless, these thresholds are species and geographically specific. And because some regions may be more sensitive to urbanization than others due watershed, habitat, and hydrological characteristics and/or the species composition that inhabit them, the effects of urbanization on aquatic biota can vary across and within ecoregions (Brown et al. 2009, Morgan and Cushman 2005).

In the Southeastern United States there is an urgent need to understand the relationship between urbanization and stream health. In 2008, the South Atlantic ecoregion, which encompasses North and South Carolina, Southern Virginia, and the eastern half of Georgia, were 1 of 6 ecoregions throughout North America that were classified as having the greatest amount of imperiled aquatic taxa (31-58 taxa; Jelks et al. 2011). And with urban land covers projected to increase by 27.5 million acres by 2050 (Wear 2011), we can expect the decline in biodiversity to continue. North Carolina is a prime example; as of 2018, there were 162 state designated aquatic taxa listed as imperiled (NCDNR 2018). This decline in North Carolina's aquatic biodiversity has coincided with a steady increase in population and land cover/land use (LULC) change over that last decade; the population has increased by 8.9% and building permits for private housing have doubled (2010-2018; census.gov). Across the North Carolina Piedmont (NCP), IC has increase by an average of 2%. In areas surrounding the urban center of like Charlotte, NC, IC has increased by 127% over the last 30 years (mecknc.gov). Researchers have recently placed an emphasis on the identification and quantification of ecological thresholds as method to study these responses of aquatic taxa to urbanization (King et al. 2011, King et al. 2007, Peng et al. 2017, Utz et al. 2009).

1.2 Ecological Thresholds

Ecological thresholds have a long history in ecological research and more recently are being considered in adaptive management and conservation. The concept of ecological thresholds were developed in the 1970's based on the principles that ecosystems do not maintain a stable state; as Holling (1973) stated, "the behavior of ecological systems is profoundly affected by random events (climatic, anthropogenic, inter- and intra-species dynamics)." Because the environment does not maintain a homogenous state, populations flux, causing a shift in community dynamics due to species-specific tolerances (Fig. 1). Ecological thresholds have been defined as "the point at which a driver may produce large responses in the ecosystem (Groffman et al.

2006)." However, for the purpose of this study, ecological threshold is defined as the range of adverse reaction to an environmental gradient.

In its simplest form, ecological thresholds are best observed in the succession of phytoplankton populations in response to fluctuations in nutrient levels due to variations in species-specific nutrient uptake rates (Lassiter and Kearns 1973). For instance, uptake rates can vary among species due to the variations in cell physiology; number of nutrient uptake sites, ion handling times (the time interval where uptake of an ion at an uptake site is prevented because of handling of another ion), and number of encountered ions (Aksnes and Egge 1991). Cells that have large numbers of uptake sites can quickly gather ions making them more competitive in an oligotrophic environment. But in eutrophic environments where ions are more abundant, uptake sites might not be as important as ion handling time. Furthermore, in environments that have constant spatial shifts in nutrients levels, cells that have better locomotive abilities increase their chances of having more ion encounters. Therefore, depending on the concentration of ions, phytoplankton populations will flux accordingly.

Thresholds and change points, or the point of maximum change in frequency and abundance, are calculated using a variety of methods. Andersen et al. (2009) identified a number of methods such as Principle Component Analysis (PCA), Average Standard Deviates (ASD), Artificial Neural Network (ANN) based-approach, Intervention Analysis, and Threshold Autoregressive (TAR) used in threshold and change point calculations and the faults/limitations associated with each method. These faults/limitations range from being prone to false positives (ANN and ASD), the inability to capture relationships that are non-linear (PCA), and complications by missing values and measurement errors (TAR; Anderson et al. 2009).

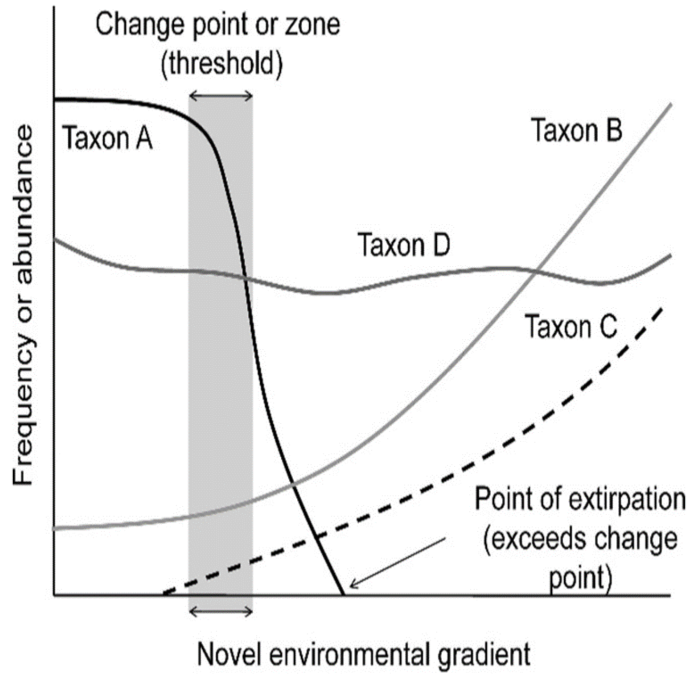


Figure 1. Conceptual diagram from Baker et al. 2010. This figure shows the response of different taxa to an increase in a novel gradient. Taxon A is a native species that is intolerant to an increase in a novel environmental gradient. As the environmental gradient increases past Taxon A's threshold, its frequency or abundance begins to decline to the point of extirpation. Taxon B is a native species that is tolerant of the increase in the novel and with the decrease in Taxon A, can thrive. Taxon C is a non-native species that before the increase in the environmental gradient was not able to establish its population. Taxon D is a native species that the increase in novel gradient has no effect on its frequency or abundance.

In 2010, Baker and King introduced a new analytic approach, referred to as the Threshold Indicator Taxa Analysis (TITAN). TITAN combines a modified change point analysis which incorporates multi regression tree analysis and bootstrap resampling, with an Indicator Species Analysis (Baker and King 2010). The model assesses when a threshold response occurs in response to an environmental stressor based on the individual species/taxa responses of the community. Compared to other change point analyses, TITAN is more accurate, more sensitive, and defines change points for species based on their individual response to a novel environmental gradient (Baker and King 2010). TITAN quantifies a z-score for each individual species/taxon that provides an indication of how that species/taxa responds across the environmental gradient. The model however, is susceptible to z-score bias and extreme sample skews when using small datasets, disturbance extremes, or the occasional ubiquitous taxa (Baker and King 2013, Cuffney and Qian 2013). Thus, if the sample is skewed towards IC tolerant fish, IC tolerant change points would be biased towards even more sensitive change points.

1.3 Research Objectives and Questions

Although studies specific to the Piedmont region have identified relationships between urbanization and stream health (Kennen et al. 2005, Morgan and Cushman 2005, Utz et al. 2009, Walters et al. 2005), they have fallen short of identifying IC thresholds for fish at both the community and taxa level. My ***overall purpose*** is to quantify the relationship between urbanization and the NCP fish assemblage. Using TITAN, I specifically quantify the degree of urbanization tolerance at both the community and individual level, and further analyze how tolerances differentiate among ecological functional groups and pollution tolerance classifications. The results will be used identify conservation priorities and biological indicators of watershed health.

Q1: How do IC tolerances differentiate amongst spawning guilds?

H1.1: Polyphilic taxa will have a higher tolerance to IC than taxa that belong to specialized spawning strategy guilds due to the lack of the biological and geologic material required for most specialized spawners like Lithophil (rock and gravel spawners), Phytophil and Phytolithophil (plant spawners), and Speleophil (cave or crevice spawners).

H1.2: Impervious cover tolerance will not differ among spawning strategy guilds.

Q2. How do IC tolerances differentiate amongst trophic guilds?

H2.1: Insectivores will have lower IC thresholds and change points than omnivores and piscivores due to the decreases in foraging success caused by habitat degradation and increased suspended solids.

H2.2: IC threshold and change points do not vary between trophic guilds.

Q3. How do species IC tolerances compare to their pollution tolerances classifications?

H3.1: Pollution intolerant taxa will have lower IC thresholds and change points than both Pollution Intermediate and Pollution Tolerant taxa; due to the similarity in variables that are the basis of both species pollution designations of the NCP fish and increased urbanization (habitat, water quality, pollution concentrations, flow regimes, etc.).

H3.2: Species IC tolerance designations will not differ from their state designated tolerance designations.

CHAPTER 2: METHODS

2.1 Site description

Given its origin, IC is often synonymous with urbanization and commonly used as a metric/measurement when looking at the effects of urbanization on the natural environment (Finkenbine et al. 2000, Wang et al. 2001). Coupled with TITAN, IC change points and thresholds can be a useful tool in identifying community and individual responses across an urban gradient (King and Baker 2010). To identify the community and individual change points and thresholds of the NCP fish assemblage, species abundance data and land cover data were sampled from 2001-2016 throughout NCP (Figure 2). The NCP lies within the greater eastern Piedmont which stretches from New Jersey to central Alabama, from the coastal plains to the Blue Ridge Mountains. The NCP is nearly 300 miles wide and its area is approximately 19,000 square miles. Elevation in NCP ranges from 90 to 340 m. Average annual precipitation in the NCP is 106 cm and temperatures range from 10.4 degrees Celsius in Jan to 31.7 degrees Celsius in July (NCEI 2019). There are 14 different types of soil found throughout the NCP with Cecil found on nearly one third of the piedmont plateau (NRCS 2019). In genereal, all the soils throughout the piedmont are well drained and moderately permeable, with the exception of the Helena series which are slow permeable soils.

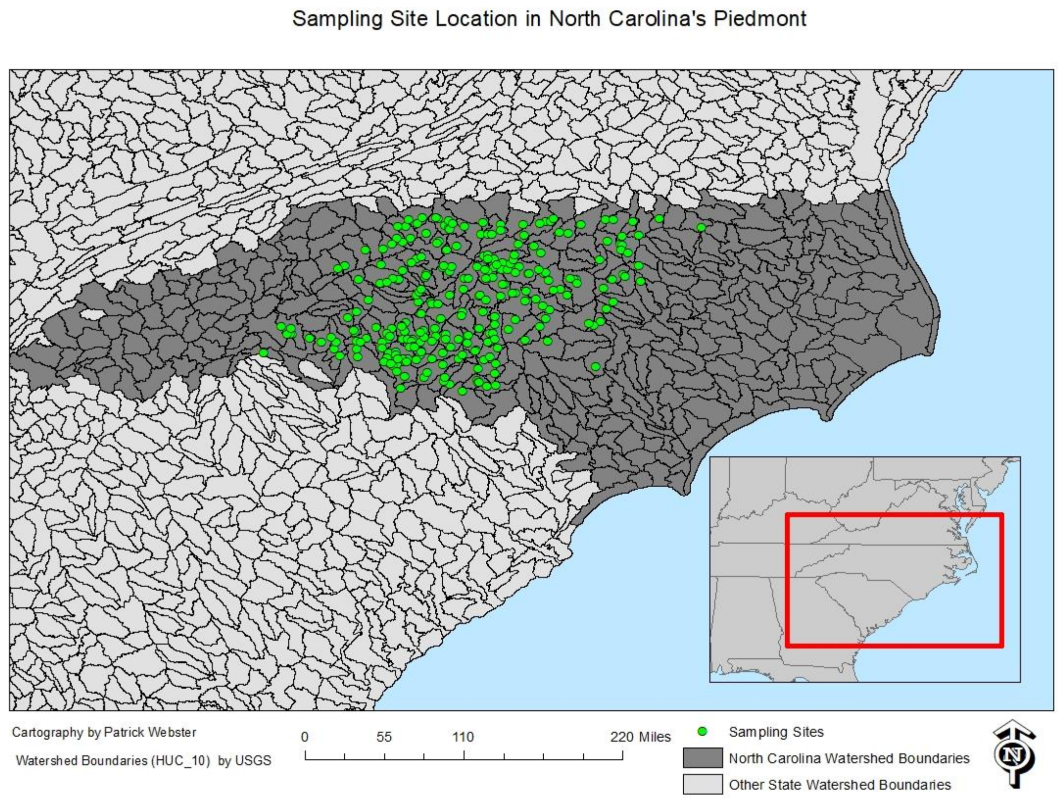


Figure 2. Sampling site locations.

Biologically, the NCP harbors a wide diversity of freshwater fish taxa. Seasonal sampling efforts from 2001-2016 identified a total of 161 taxa. This is consistent with the abundance of biodiversity richness across the rest of the Southeastern US; Tennessee (320 species), Kentucky (246 species), and Virginia (226 species; Helfrich et al. 2019). Of these taxa, only 10 (*Cottus bairdii, Dorosoma petenense, Enneacanthus chaetodon, Etheostoma chlorobranch, Hybopsis amblops, Hypentelium nigricans, Luxilus chrysocephalus, Micropterus punctulatus, Nocomis platyrhynchus, Nocomis raneyi, and Pimpephales promelas*) were rare or occurred < 3 times within the datasets. Taxa were relatively homogenously distributed throughout the NCP and the majority (n=150) of all species accumulated throughout the span of this study, occurred within < 50% of the sample sites (Figure 3A). Species diversity scores across sampling sites were normally distributed, while the distribution of species evenness scores was positively skewed (Figure 3B & 3C).

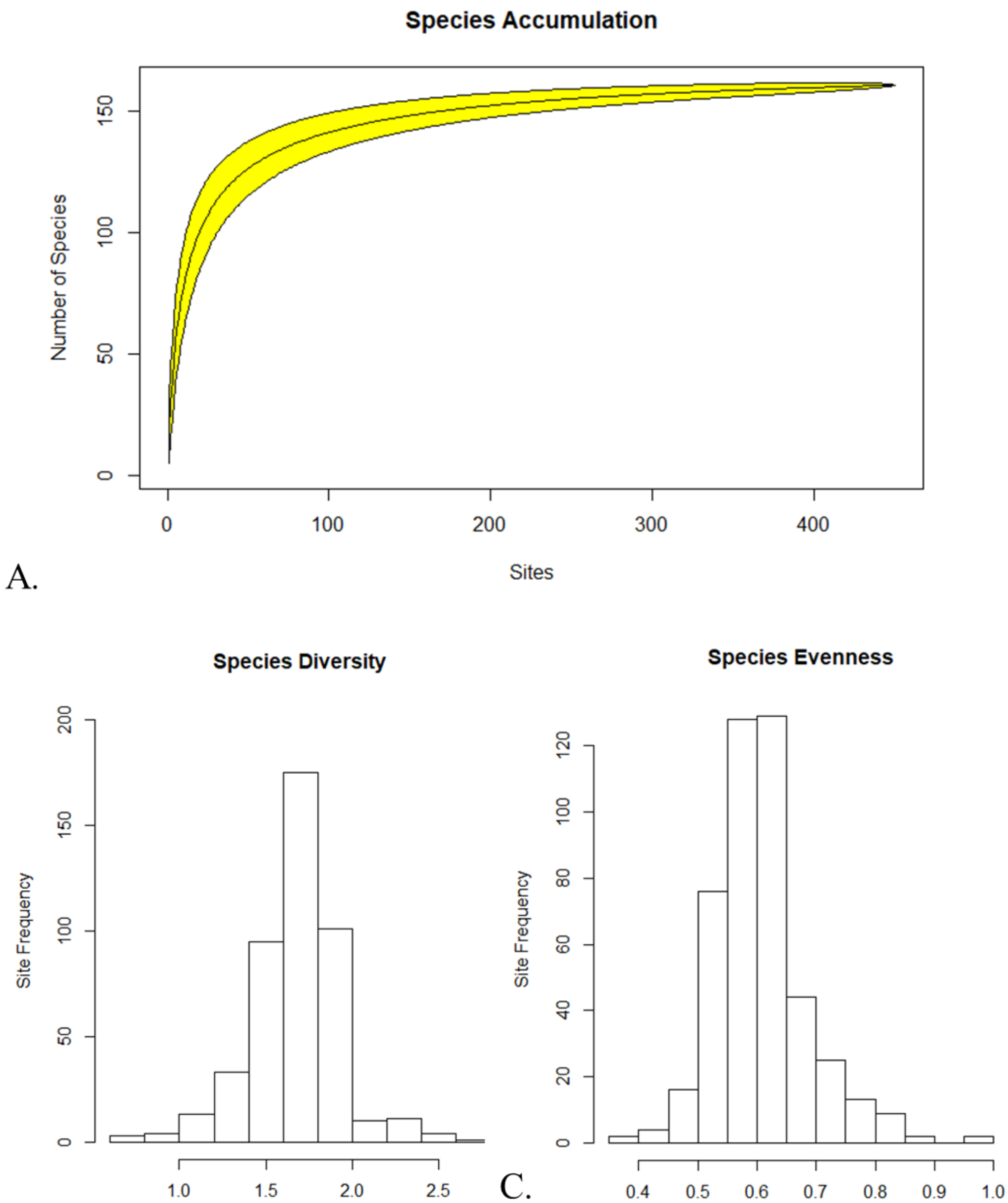


Figure 3. Community ecology metrics for the 450 sampling sites over the duration of the study. A) Species accumulation curve. B) Species diversity using Shannon-Weiner diversity index. C) Species evenness using Pielou's evenness

2.2 Data

Seasonal fish sampling data were acquired from North Carolina’s Department of Environmental Quality (NCDEQ) and Charlotte-Mecklenburg Storm Water Services (CMSWS). Data for both agencies were collected between April and October using methods presented in the NCDEQ (2013) Standard Operating Procedure Biological Monitoring Stream Fish Community Fish Assessment Program. Pollution tolerance and trophic guild classification were attained from NCDEQ (2013) Standard Operating Procedures Biological Monitoring and spawning guild classifications were based on several taxonomic guides and Virginia Tech Fish Traits database (Appendix B; Frimpong and Angermeier 2009)

To quantify watershed IC geospatial data were acquired from multiple sources. Watershed boundary data were acquired from the USGS National Watershed Boundary Dataset (NWBD). This dataset includes hierarchical hydrologic unit data based on topographic and hydrological features at a HUC 10-digit scale. Analyzing percent IC at the HUC-10 watershed scale offers a balance between stream order and percent IC variability. In addition, sub-watersheds (HUC-12), based on their location withing the greater watershed (HUC-10), may be impacted by the amount imperviousness of surrounding sub-watersheds, ultimately running the risk of assigning a false biological state to a percent of IC. Analyzing percent IC at the sub-basin (HUC-8) scale or greater, assigns an overall average value to all watersheds within the respected hydrological unit even though they may have little to no environmental impacts on watersheds throughout the hydrological unit. In addition, adverse effects of IC on the biological community are diminished at the larger HUC scale (Schiff and Benoit 2007) and the arrangement of IC within these large scale HUCs would cause increased uncertainty surrounding the results. In both cases, this could misrepresent taxa IC tolerances (higher IC tolerance for intolerant taxa and/or lower IC tolerances for tolerant taxa) and inhibit the ability to capture change for critical areas at the forefront of urbanization like the edges of urban sprawl. Watersheds had a mean area of 480 ± 163km^2. Land cover (LC) data were acquired for the years 2001, 2004, 2006, 2008, 2011, 2013, and 2016 from the National Land Cover Database (Helms et al. 2005). All LC data are 30 m resolution and had high user accuracies for both high and low intensity developed area (≥70%; Yang et al. 2018). I assumed that Landsat images used in the NLCD were taken prior to the first sampling date of that given year and LC remained constant throughout the year. Each dataset is a mosaic of Landsat images that met a predefined threshold of cloud cover (<20%) during a period of leaf-on (Yang et al. 2018); NCP "leaf-on" period can begin as early as late March and extend until November.

2.3 Data Analysis

Each watershed was analyzed using a geoprocessing model created using the Model Builder tool in ArcPro®. This geoprocessing model, which will be referred to as Watershed Land Cover Delineation Model (WLCD) for now on, uses of a series of sequential ArcGIS geoprocessing tools that quantifies the area of IC polygons within isolated sampling-specific watersheds (Figure 4). Attribute tables for each year were exported into SQLite and percent impervious cover was calculated by HUC10. Percent IC was calculated using the sum area of the four "Developed" land cover classifications (open space, low, medium, and high intensity) divided by the total watershed area. Developed open spaced areas are categorized as a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Low intensity developed area IC accounts for 20% to 49% mostly single-family housing, Medium IC accounts for 50-79% single family housing, High Intensity developed areas 80 to 100% and include apartment complexes, row homes and commercial/industrial (Yang et al. 2018).

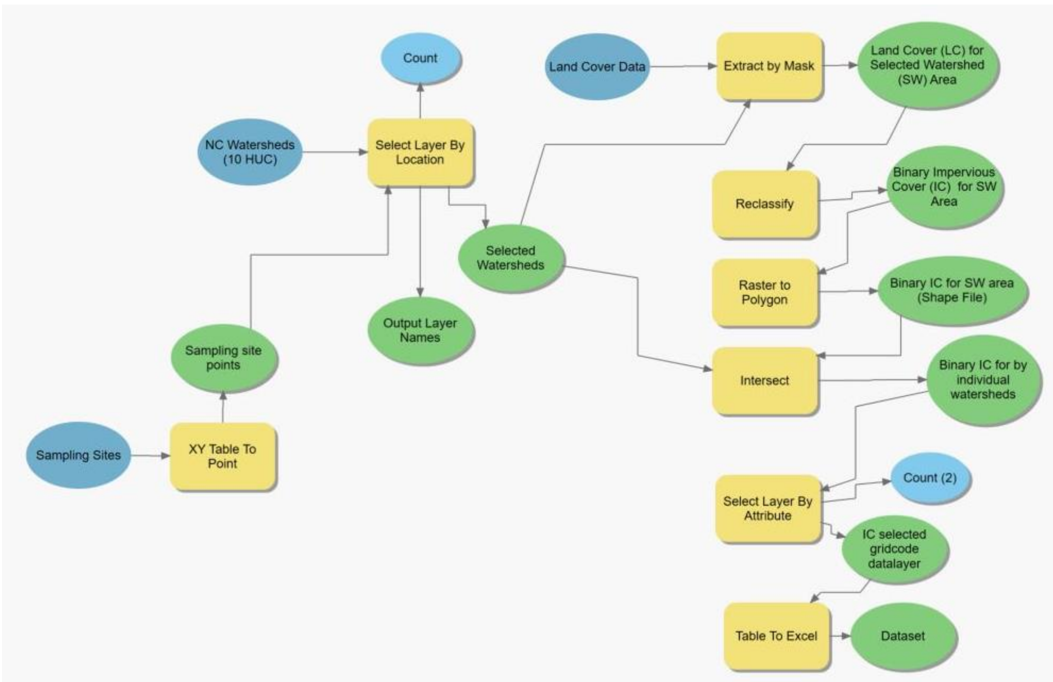


Figure 4. Schematic model for delineating IC (%) by watershed. Blue ovals represent external data inputs, yellow rectangles represent ArcGIS tools, and green internal created data sets.

To find an environmental change point value, TITAN uses binary partitioning and indicator species scores (Dufrêne and Legendre 1997) to locate taxon-specific change and bootstrap resampling to quantify change point quantiles and statistical significance (purity and reliability; see Appendix C) surrounding taxa-specific change point (see Baker and King 2010 for further explanation of TITAN). TITAN also partitions taxa into two response groups based on the direction of response in relation to its change point;

individuals increasing at change point are classified as having a positive response (Z+) to the increase in the environmental gradient and taxa decreasing at the change point are classified as having a negative response (Z-) to the increase in the environmental gradient. Individual z scores are aggregated by response group and summed across each percent IC partition. The value resulting in the largest cumulative change in response group frequency and abundance determines each response group's percent IC change point.

Prior to performing TITAN, environmental and species abundance datasets were reconciled for data alignment and taxa with < 3 occurrences were discarded from the analysis. This brought the number of taxa used in the analysis from 161to 95. Species abundance data was $\log_{10}(x+1)$ transformed to mitigate the influence of highly variable abundances on taxa indicator score calculations (King et al. 2011). TITAN was performed using TITAN2 package in R (Baker and King 2010). Taxa that met both purity and reliability (values < = 0.05) were grouped by ecological guilds and pollution tolerance (Table 1).   Group means among IC Tolerance metrics were evaluated with the Kruskal-Wallis test, a non-parametric analysis of variance, with Tukey distance and the "random" method to break ties (if existed; Lenat and Crawford 1994). Where the Kruskal-Wallis test indicated a statistically significant difference (p-value <0.05) a posthoc pairwise testing using a Nemenyi test was used to identify specific differences within each group. All hypothesis testing was performed using PMCMR and PMCMR plus packages in R.

Table 1. Independent and Dependent Variables used in this study. Dependent variable metrics are calculated using TITAN2 based on the IC gradient. Definitions of the dependent variable metrics are summarized in Appendix B.

Independent Variable		**Dependent Variables Metrics %(IC)**
Group	**Sub-Groups**	
Spawning Guild	Lithophil Phytolithophil Phytophil Polyphil Speleophil	Change point
Trophic Guild	Insectivore Piscivore Omnivore	Change Point Quantiles (5%, 10%, 50%, 90%, 95%)
Pollution Tolerance	Intolerant Intermediate Tolerant	Threshold (5-95%)

CHAPTER 3: RESULTS

3.1 Land cover analysis

Land cover analysis revealed that at the broadest scale, the NCP experienced relatively little change in %IC from 2001 to 2016 with an average increased IC of 1.73%. At the individual watershed level, most watersheds (n=60) experienced an increase in IC < 1.00%. Five of these watersheds experiencing the least increase in IC occurred within Cumberland, Montgomery, Richmond, Wilkes, and counties bordering Wilkes county. Since 2010, Montgomery Wilkes, and Richmond Counties all experienced population declines (Census.gov 2020), while Cumberland only saw a modest population increase of 1.5%. Combined these counties saw an average population flux of -1.2%. The NCP also had 5 watersheds that had increases > 9.00% IC. These watersheds occurred in the two most populated counties in North Carolina, Wake and Mecklenburg County and bordering counties (Cabarrus, Irdel, Rowan, Union, and Johnson) which the watershed extended into. Together, these counties experienced an average growth rate of 15.32% since 2010(Census.gov 2020). Overall, the number of watersheds < 15% IC decreased from 165 to 149, watersheds with 16-30% IC increased from 36 to 46, watersheds with 30-45% IC decreased from 45 to 28, 45-60% IC increased from 7 to 30, and watersheds > 60% IC (n=16) had no increase (Figure 5).

Changes in Watershed Impervious Cover (%) in the NCP, 2001-2001

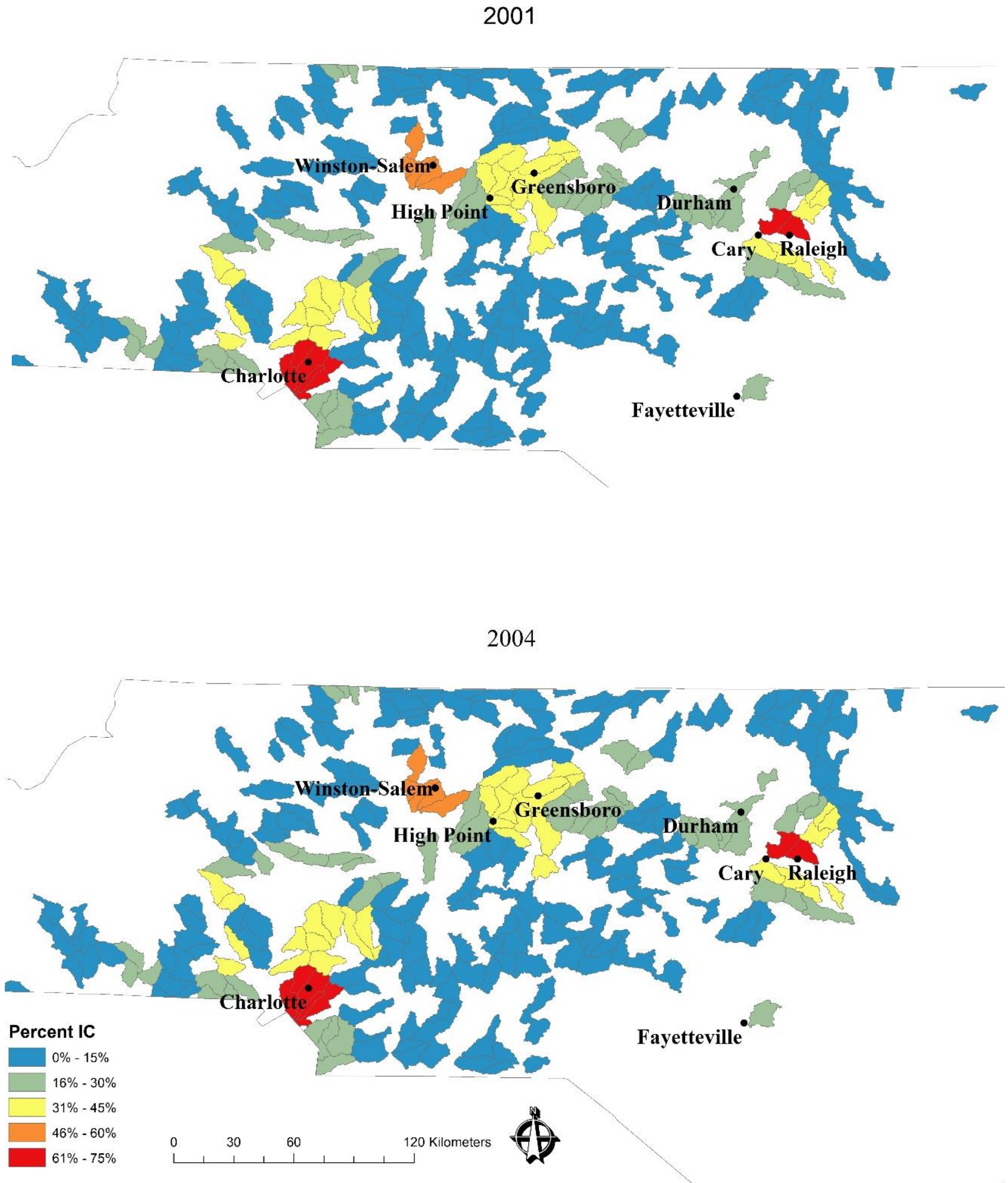
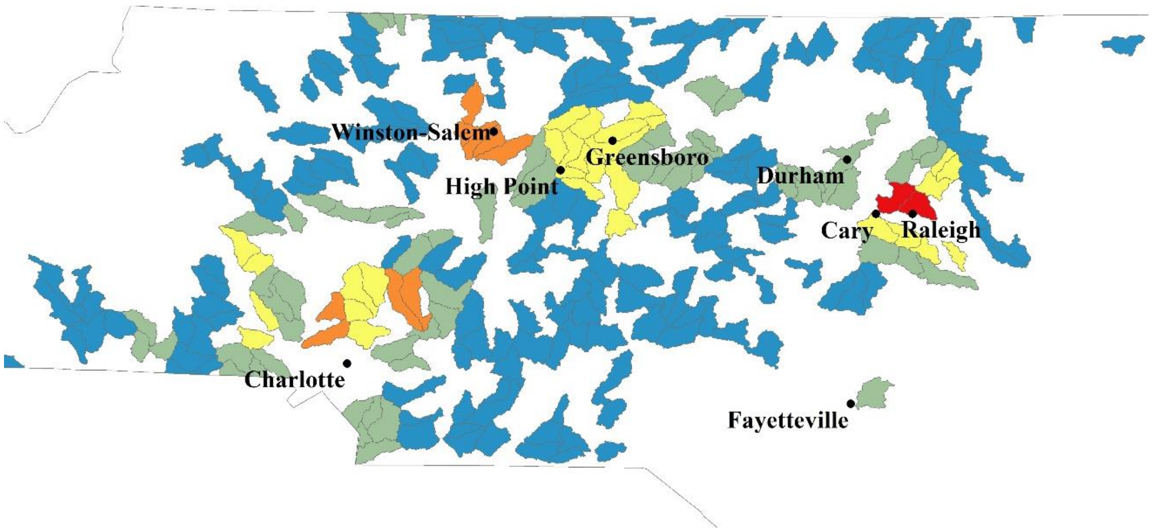


Figure 5 NCP watersheds change in percent IC from 2001-2016.

2006



2008

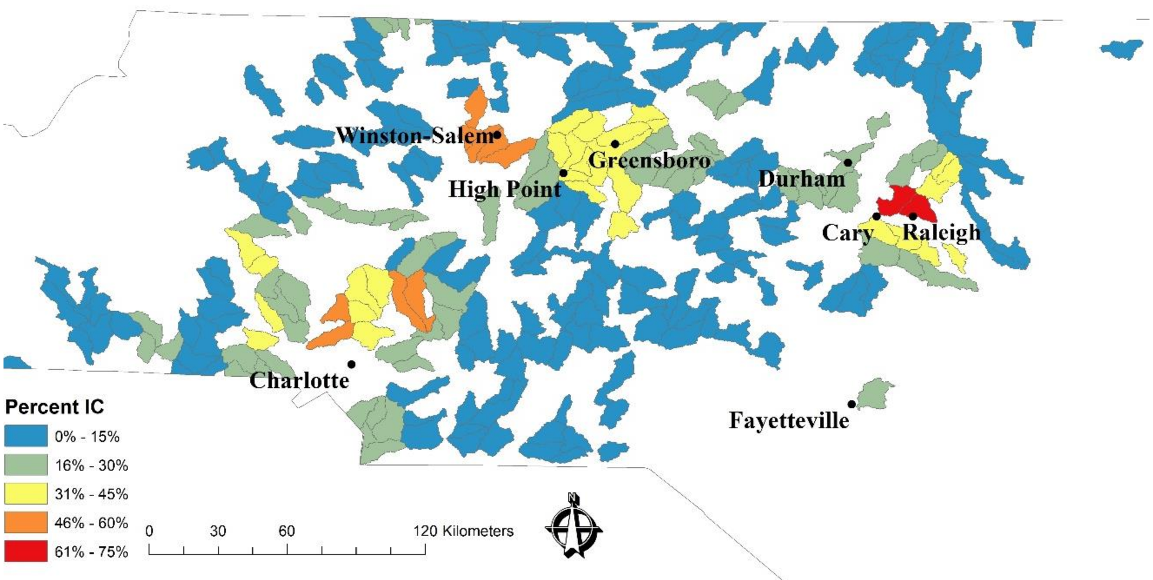
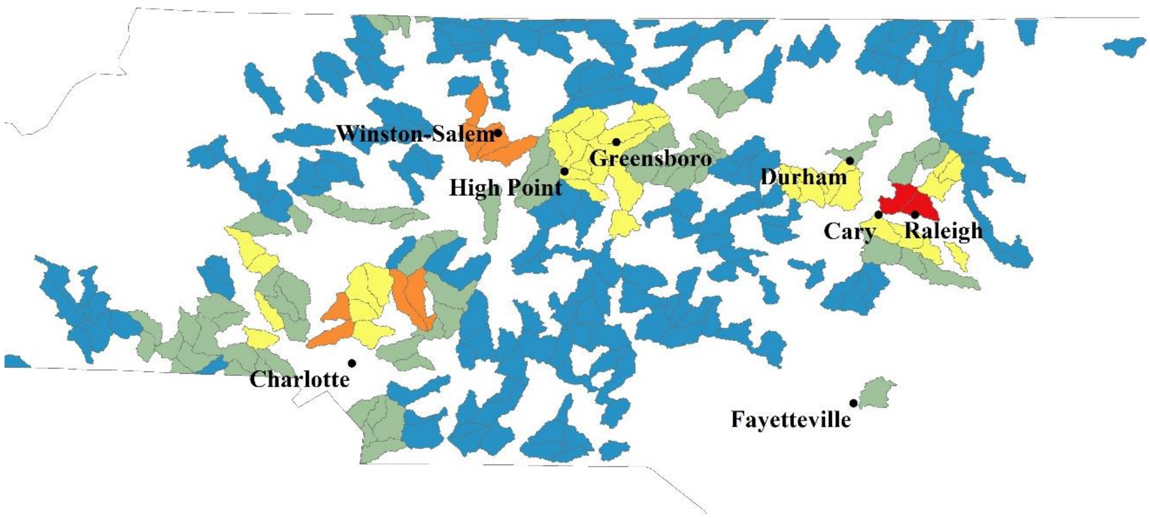


Figure 5. NCP watersheds change in percent IC from 2001-2016 (Continued).

2011



2013

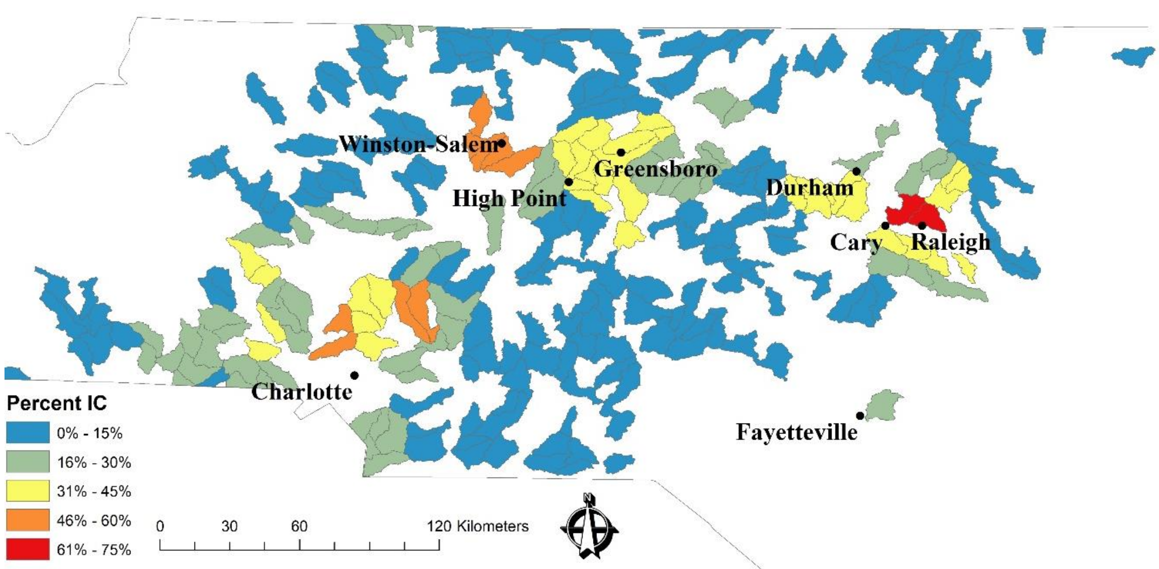


Figure 5 NCP watersheds change in percent IC from 2001-2016 (Continued).

2016

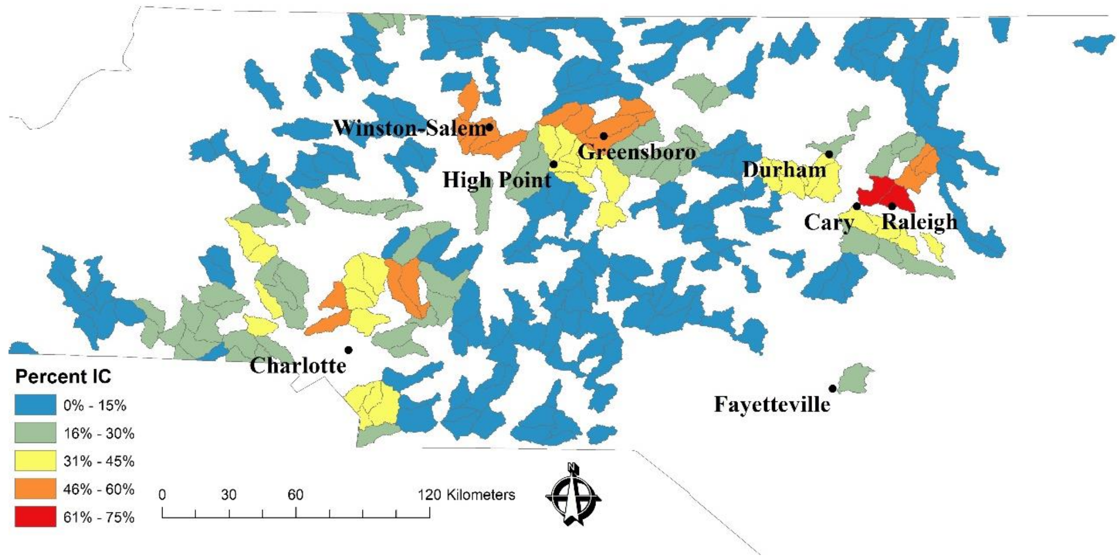


Figure 5. NCP watersheds change in percent IC from 2001-2016 (Continued).

3.2 Individual IC Thresholds

TITAN identified 33 taxa as Z- and 17 as Z+. *Etheostoma nigrim* (johnny darter) had the lowest change point of 3.90% IC and along with five other Z- taxa, *Luxilus coccogenis* (warpaint shiner), *Hypentelium roanokense* (Roanoke hogsucker), *Percina roanoka* (Roanoke darter), *Etheostoma vitreum* (Glassy darter), and *Etheostoma podostemone* (Riverweed darter) had a change point of < 6.00 % IC. *Notropis scepticus* (sandbar shiner) had the lowest change point (8.30% IC) of Z+ taxa; 1 of 8 Z+ taxa that had a change point < 20.00% IC. *Notropis procne* (swallowtail shiner), *Notropis hudsonius* (spottail shiner), *Cyprinella chloristia* (greenfin shiner), *Ameiurus catus* (White bullhead) and *Micropterus punctulatus* (Spotted bass) had change points > than 50.00% IC.

TITAN also revealed a large disparity in IC threshold (5-95% change point quantiles) among response groups. Twenty-three Z- taxa had a threshold < 10.00% IC (Figure 6). Of those taxa, *Petromyzon marinus* (Sea lamprey), *Esox niger* (Chain pickerel), Riverweed Darter, *Notropis rostrata* (Pugnose shiner), and *Aphredoderus sayanus* (Pirate perch) all had thresholds < 1.00% IC. Narrow IC thresholds were not uniform among all Z- taxa. *Nocomis leptocephalus* (Bluehead chub) had an IC threshold of 45.52% *and Semotilus atromaculatus* (Creek chub) had an IC threshold of 56.80%; among the top ten largest thresholds within the NCP fish assemblage. In contrast, 14 Z+ taxa had a threshold > than 20.00% IC. The Spottail shiner, *Dorosoma cepedianum* (American shad), *Lepomis cyanellus* (Green sunfish), and Sandbar shiner all had IC thresholds >60.00% (Figure 6). Although the majority of Z+ designated taxa were on the larger end of the IC threshold spectrum, *Lepomis macrochirus* (Bluegill) and had a 4.00% IC threshold.

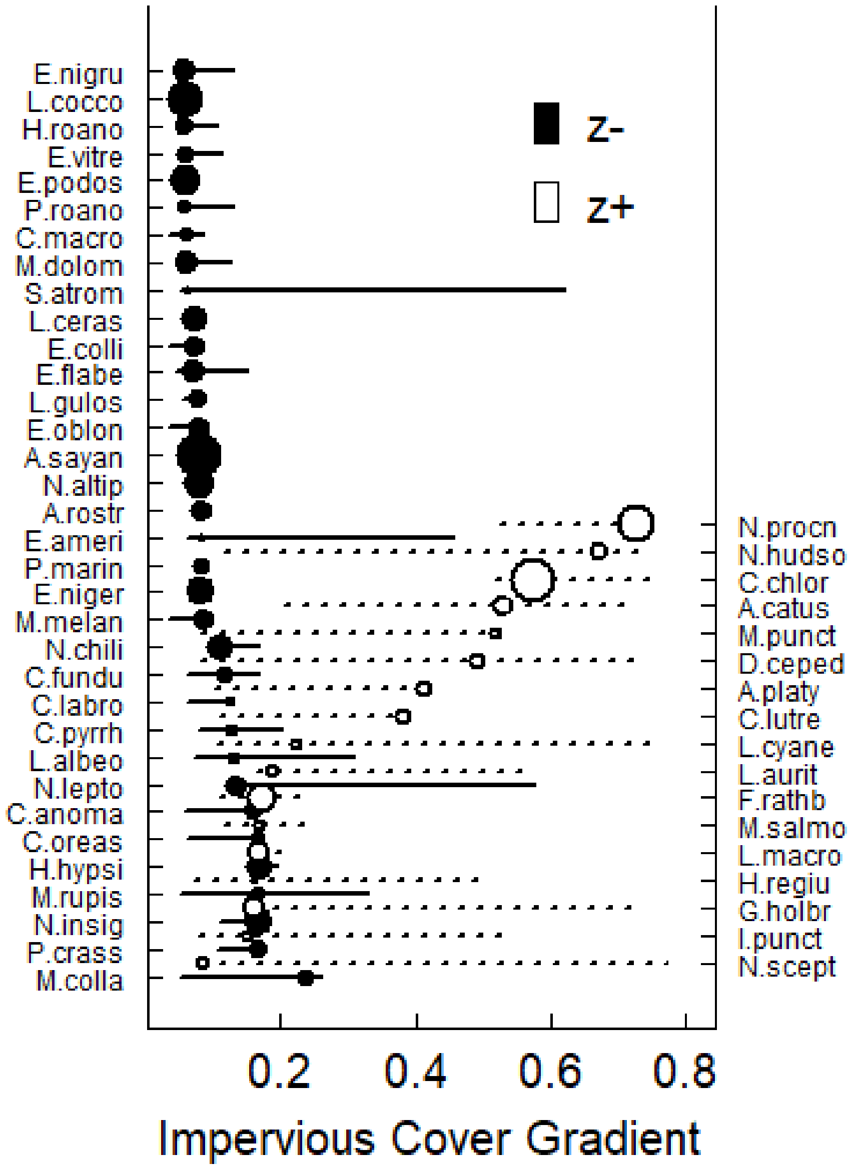


Figure 6 Taxon-specific IC change points and thresholds. Taxa represented meet both purity and reliability (p-value < 0.05). Each plot includes Z- taxon on the left axis and Z+ taxon on the right. Change points are indicated by circular symbols. The horizontal lines suggest the thresholds or 5-95% quantile change points. Taxa codes are explained in Appendix E.

3.3 Community IC Tolerances

TITAN's community level metrics revealed a clear distinction between taxa having a negative response (Z-) or a positive response (Z+) to the increase in the IC gradient. Decline in aggregate frequency and abundance for Z-taxa occurred at much lower levels of percent IC for all change point quantiles (5%, 10%, 50%, 90%, and 95%) compared to Z+ taxa (Figure 7). On average, Z+ taxa change point quantiles were 3.27 (± 1) times higher than Z- taxa (Table 2). Z- taxa also had a narrower percent IC threshold of 6.99% in respects to Z+ taxa threshold of 41.30%. Among aggregate response metrics, the least disparity was between change points. The aggregate change point for Z- tax was 6.10% and Z+ taxon had an aggregate change point of 16.59% IC.

Table 2. Community percent IC thresholds for TITAN identified response taxa.

Response Taxa	Change Point	Change Point Quantiles					Threshold
		5%	10%	50%	90%	95%	
Z-	6.10%	5.79%	5.91%	7.77%	12.69%	12.78%	6.99%
Z+	16.59%	16.07%	16.49%	16.59%	52.85%	57.37%	41.30%

TITAN's community results also revealed an unequal response in communal decline to increases along the IC gradient among response groups. In Figure 7, the cumulative frequency distribution (CFD) shows Z- taxon exhibit a relatively sharp, linear decline with little uncertainty surrounding where maximum change occurs. The decline in CFD of Z- taxa from 5-50% occurred over a 2.00% increase in IC and CFD from 50-95% declined, at an only 5.00% increase in IC. In contrast, Z+ taxon exhibited more of an exponential decline with broad uncertainty regarding locations of maximum change. Z+ taxon saw a significant decline in CFD (2-85%), over a 1.00% increase of IC (16-17%), while the remaining 15% of CFD declined over a much larger gradient (36.00% IC;

Figure 7). However, the broad uncertainty surrounding points of change for Z+ may be attributed to the lack of datapoints associated with these areas. In Figure 7, areas of uncertainty are depicted by plateaus which occur between 50%-60% and 60-70% IC. Sampling sites occurring within this range of percent IC account for only 2% of the total abundance. In contrast, in areas of narrow uncertainty (0-20% IC), sampling sites account for 66% of the total site abundance.

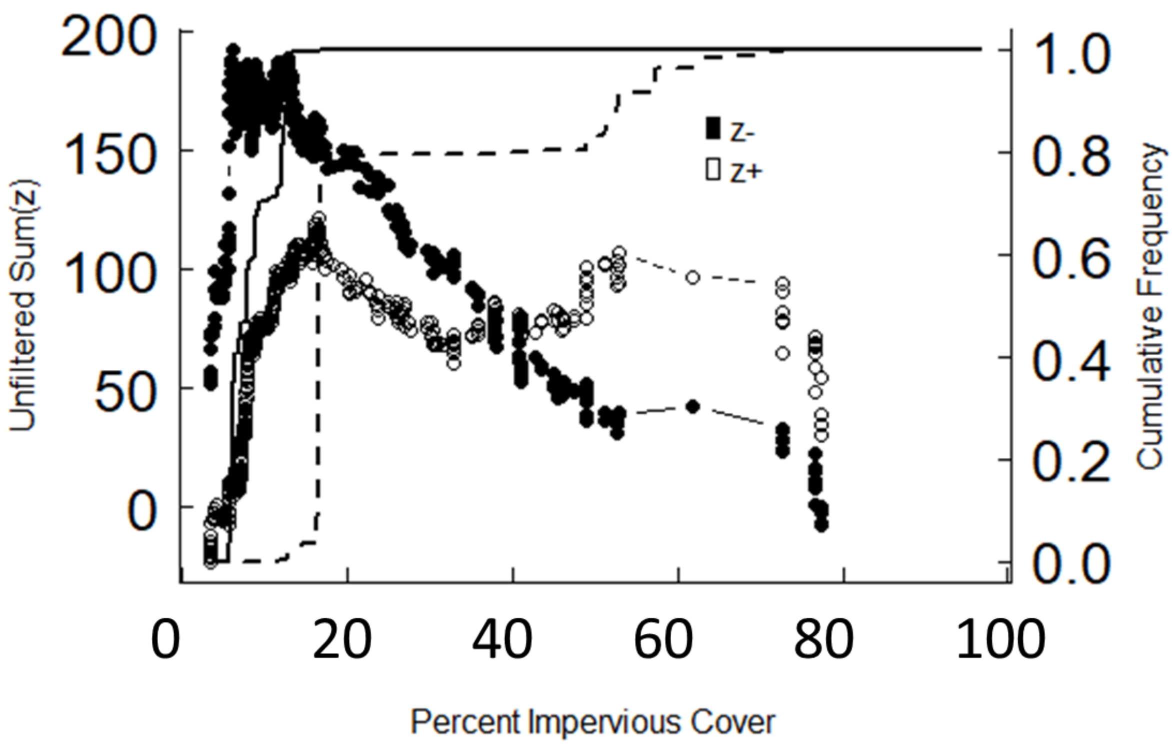


Figure 7. Aggregate IC tolerances by response groups. The filled and hollow circular symbols denote the magnitude of summed Zz scores for Z- and Z+ taxa, respectively. Peaks in values indicates areas of large change in community frequency (CFD) and abundance. Plateaus indicate regions of change. Solid and dashed lines are cumulative frequency distributions response group sum(z) maxima. Vertical CFD denote areas of narrow uncertainty while horizontal or stair stepped CFD’s indicate broad areas of uncertainty regarding the location of maximum change.

3.4 Ecological functions and Impervious Cover

Results for IC tolerances among ecological functional groups varied. Non-parametric testing showed that mean IC tolerances (represented by the change point quantiles) among spawning and trophic guilds were insignificant across all IC categories (Table 3); however, IC thresholds among trophic guilds were significant (p-value < 0.05). Furthermore, posthoc Nemenyi test revealed that mean IC thresholds between Omnivores and Insectivores were significantly distinct (Figure 8). Insectivores had a median IC threshold of 7.00% which was close the 9.00% median IC threshold for Piscivores. As expected, Omnivores had a median IC threshold of 49.00% (Figure 8).

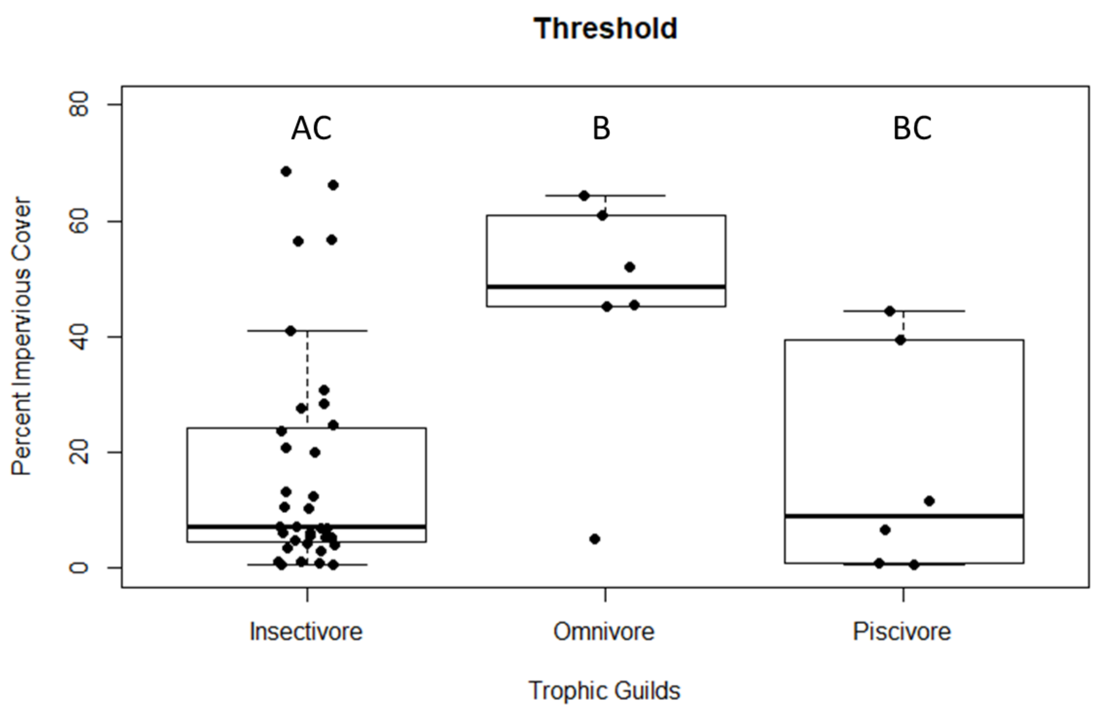


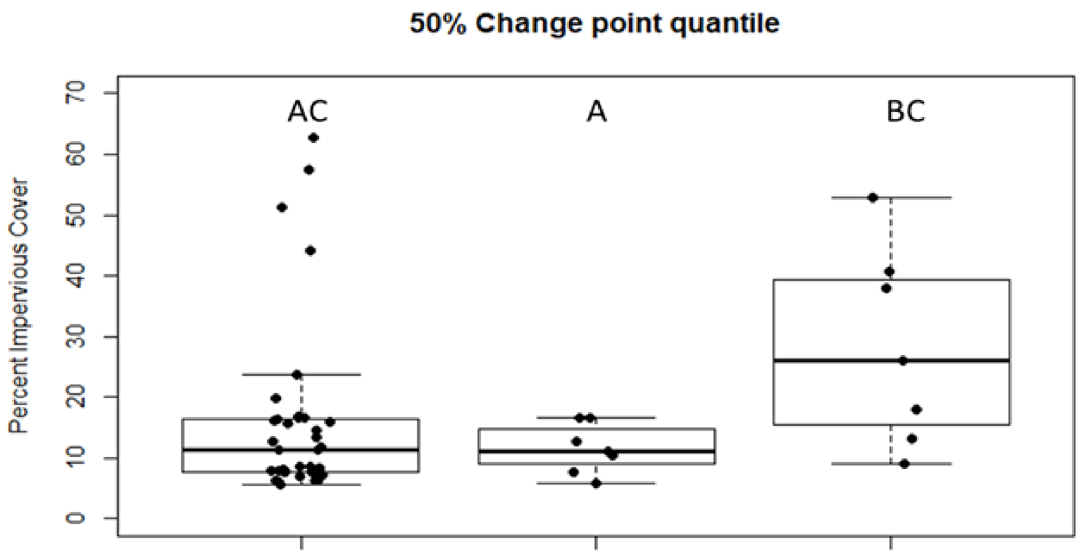
Figure 8. Percent IC thresholds among the North Carolina's piedmont fish assemblage trophic guilds. The IC threshold is represented by the difference of the 95% and 5% change point quantiles. Letters above trophic guilds indicate the results of pairwise testing in which insectivores and omnivores are the only functional feeding groups that are statistically different (p-value < 0.05).

Table 3. Results of non-parametric testing for ecological functional group percent IC thresholds. Values indicate p-value.

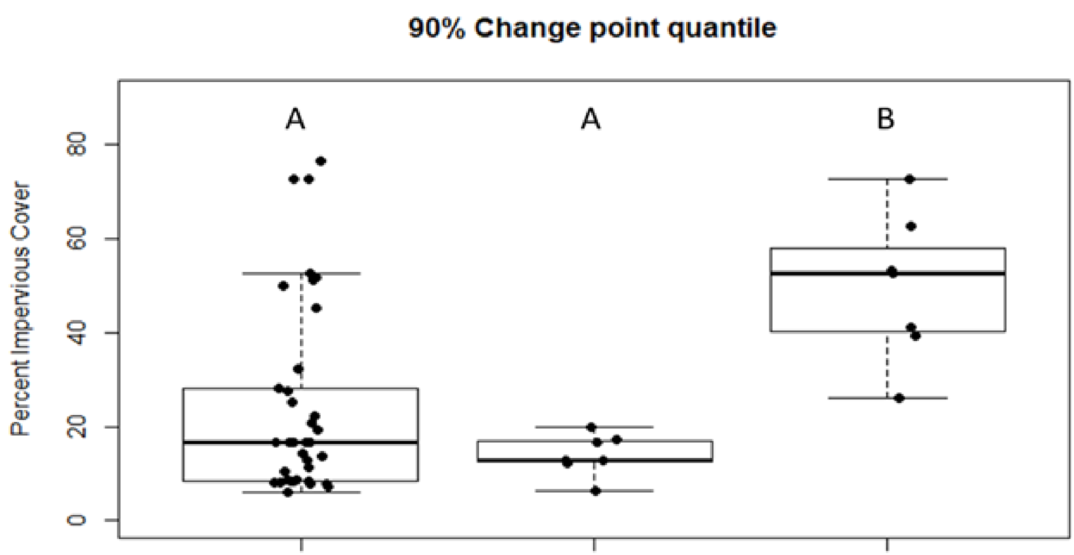
Ecological Functional Groups	Change Point	Change Point Quantiles					Threshold
		5%	10%	50%	90%	95%	
Spawning Guild	0.855	0.845	0.691	0.921	0.683	0.846	0.798
Trophic Guild	0.190	0.436	0.359	0.191	0.133	0.117	**0.044**
Pollution Tolerance	0.077	0.103	0.059	**0.038**	**0.005**	**0.006**	**0.003**

3.5 Pollution Tolerance and IC tolerance

IC tolerance among pollution tolerant were significant. Percent change point quantiles at the 50, 90, and 95%, and IC thresholds were all significant with the remaining three metrics falling relatively short of significant (Table 3). This could be attributed to the extensive research that goes into designating taxa pollution tolerances and the variables used in determining these tolerances are directly affected/connected to the amount of imperviousness in a watershed. Further analysis of IC tolerances among pollution tolerant classifications using pairwise testing revealed relatively consistent results across all significant IC tolerance metrics. IC tolerance means among intolerant and intermediate pollution tolerant taxa IC were statistically indistinguishable for all significant metrics except at the 50% change point quantile, but both were significantly distinct from taxa tolerant pollution tolerance group mean at the 90%, and 95% change point quantiles and Threshold (Figure 9). At the 50% change point quantile, pollution intolerant and tolerant taxa were statistically dissimilar.

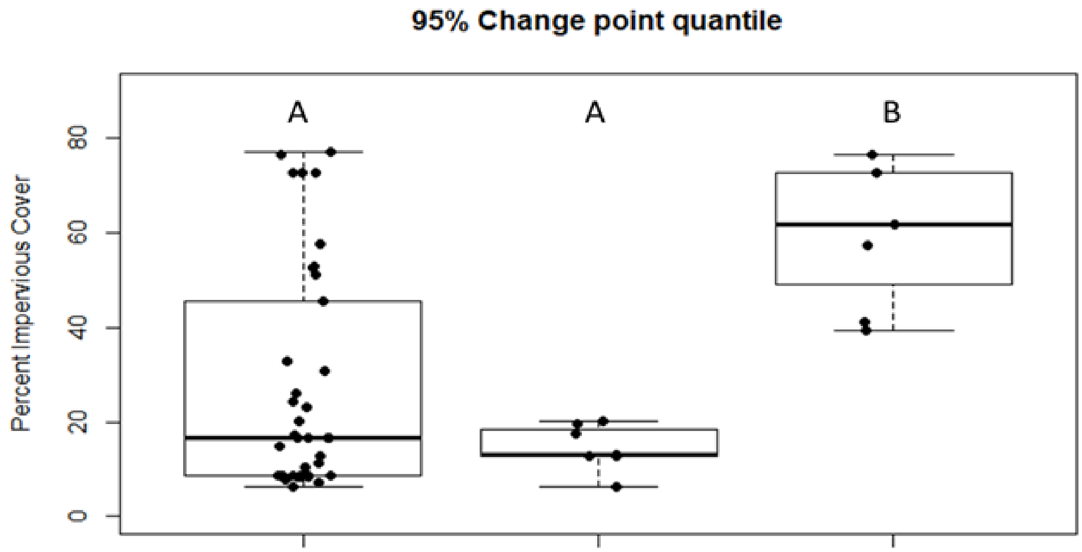


A.

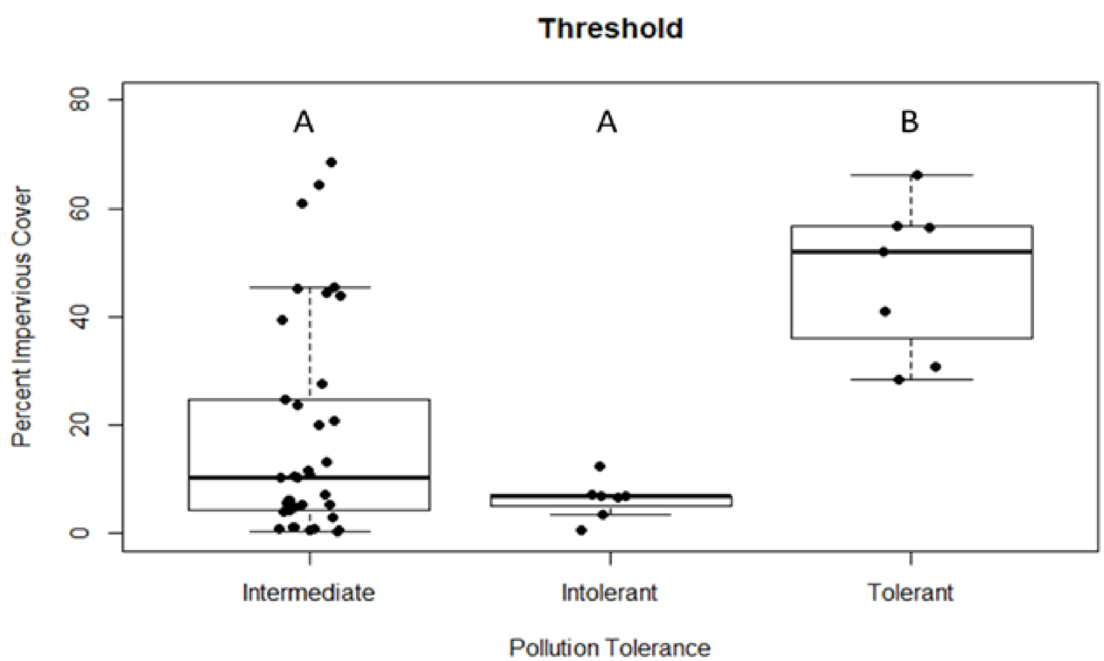


B.

Figure 9. Percent IC tolerances of NCP Fish assemblage by pollution tolerance designations. Letters above pollution tolerance classifications denoted pairwise testing results. A) The only pollution tolerant group mean that was of significance was between intolerant and tolerant. B-D) Intermediate and Intolerant pollution tolerant group means are statistically indifferent but are statically different from the pollution tolerant group.



C.



D.

Figure 9. Percent IC tolerances of NCP Fish assemblage by pollution tolerance designations. Letters above pollution tolerance classifications denoted pairwise testing results. A) The only pollution tolerant group mean that was of significance was between intolerant and tolerant. B-D) Intermediate and Intolerant pollution tolerant group means are statistically indifferent but are statically different from the pollution tolerant group (Continued).

In addition to non-parametric and pairwise testing for IC tolerance among pollution tolerant groups, taxa were identifying IC tolerant outliers within each pollution tolerant group. Taxa that fell short of the pollution tolerance median below or exceeded the pollution tolerance median above in all significant metrics were deemed outliers. The largest number (n=14) of outliers were Intermediate pollution tolerant taxa that fell below the intolerant pollution tolerance medians while only one taxon from both intolerant and intermediate pollution tolerant groups exceeded the median IC of the more pollutant tolerant adjacent group (Table 4). No pollution tolerant taxa met this criterion.

Table 4. IC tolerant outliers.

Intolerant taxa exceeding Intermediate IC tolerance medians	Intermediate taxa below Intolerant IC tolerance medians	Intermediate taxa exceeding Intermediate IC tolerance medians
Cyprinella pyrrhomelas (Fieryblack shiner)	*Erimyzon oblongus* (Creek chubsucker)	*Notropis hudsonius* (Spottail shiner)
	Hypentelium roanokense (Roanoke hogsucker)	
	Luxilus cerasinus (Crescent shiner)	
	Luxilus coccogenis (Warpaint shiner)	
	Notropis altipinnis (Highfin shiner)	
	Petromyzon marinus (Sea lamprey)	
	Esox niger (Chain pickerel)	
	Centrarchus macropterus (Flier)	
	Lepomis gulosus (Warmouth)	
	Aphredoderus sayanus (Pirate perch)	
	Etheostoma collis (Carolina darter)	
	Anguilla rostrata (American eel)	
	Etheostoma vitreum (Glassy darter)	
	Minytrema melanops (Spotted sucker)	

CHAPTER 4: DISCUSSION

The overall purpose of this research was to 1) gain a better understanding of the NCP fish assemblage; 2) identify conservation priorities by quantifying community and individual responses to urbanization; and 3) identify tolerance trends among ecological functional groups and pollution tolerance classifications. Species of the NCP fish assemblage showed decreases in frequency and abundance in as little as 3.30% and had terminal thresholds up to 76.60%. Change points for response groups were (Z-) 6.10% and (Z+) 16.59% IC. Unfortunately, there was little to no evidence to suggest that IC tolerances differentiated across ecological functional groups and although IC threshold amongst trophic guilds were significant, pairwise testing among classifications showed some significance, but overall insubstantial.

4.1 LULC and Individual and Community Responses

Impervious cover analysis indicated a range of watershed IC across the NCP where areas with the greatest IC were related to metropolitan regions that had the highest population growth rates. Several watersheds in rural counties had low IC (<10%) even as late as 2016 and interestingly, some of these watersheds saw little change in IC from 2001 to 2016. At the individual taxa level, several fish species responded negatively to IC as low as 6% while other taxa responded positively to IC as low as 20%. Fish abundance and diversity decreases with increasing urbanization (Morgan and Cushman 2005); however, my research has indicated which individual species are most likely to be impacted by increasing IC (e.g. Warpaint shiner, Crescent shiner, Johnny darter, Riverweed darter, Roanoke darter, Roanoke hogsucker, and Pirate perch). The NCP is an aquatic biodiverse rich ecoregion and the loss of any species would be substantial, however for species like *Nocomis leptocephalus* (Bluehead chub), it would have a cascading effect on many taxa including several *Notropis* spp., *Luxilus spp.*, and the Mountain Redbelly dace, whose reproductive success relies on the use of the spawning beds of *Nocomis spp.*

At the community level, the analysis of the cumulative individual response of the NCP fish assemblage to increasing IC, I found that as quantile change points increase, density is more distributed over the IC gradient (Figure 10). These data suggest that: 1) regardless of pollution tolerance, ecological guild, or family, all species within the NCP fish assemblage have a more synchronous response to low levels of IC and 2) terminal IC thresholds are taxa specific (Figure 10). Understanding how changes in both species and communities occur across and IC gradient is critical for targeting watersheds for conservation and management. As the NCP continues to urbanize, areas with low IC might be conserved or be targeted for novel green infrastructure to manage stormwater as IC increases. It is also important to understand the legacy impacts of LULC as fish assemblages are sensitive to whether land use prior to urbanization was forested or agriculture (Brown et al. 2009).

The accuracy of the land cover data does introduce some uncertainty surrounding the precision of community and individual IC tolerances. Although error for 30-m impervious pixels can vary between 4-12% (Goetz et al., 2004; Jantz et al., 2005; Wickham et al., 2010), they are more precise across broad areal units (Smith et al. 2010). Researchers also show that areas with <10% impervious cover (Chabaeva et al., 2009; Greenfield et al., 2009; Smith et al., 2010) have a higher level of accuracy than areas with high amounts of IC. Watersheds within this study with < 10% accounted for ~50% of the watersheds each year. Ultimately, error in LULC data would translate to greater error in Z+ taxa change points and thresholds than we would Z- taxa or taxa whose thresholds or change points that exists at higher levels of percent IC. From a conservations standpoint, the focus of these results should be directed towards the Z- taxa.

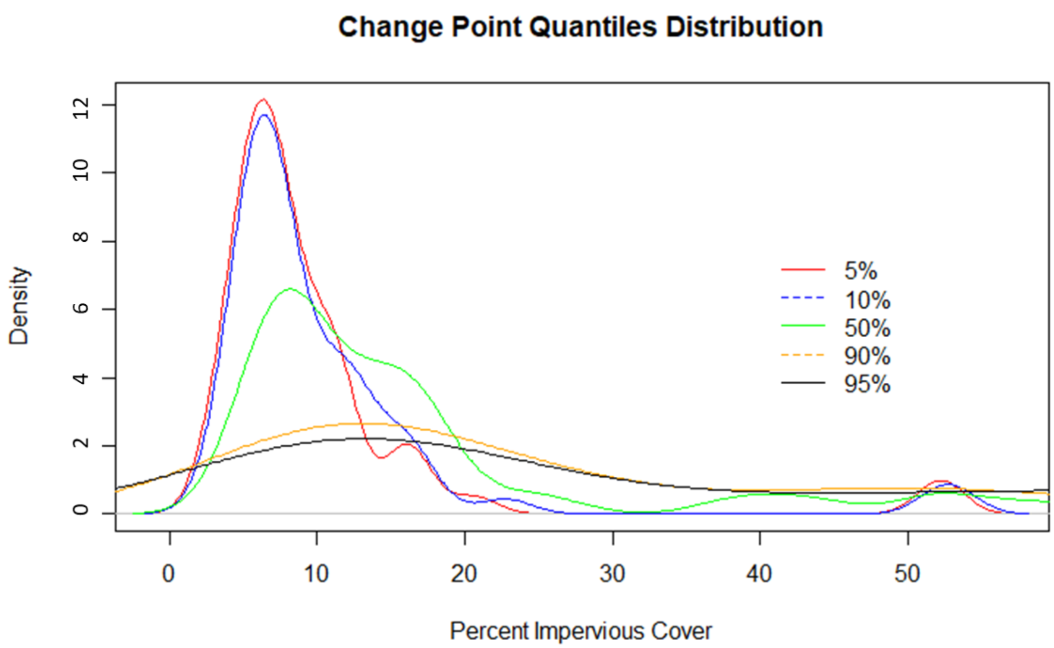


Figure 10. IC change point quantiles for the fish assemblage of NC's Piedmont.

4.2 Ecological and Pollution Tolerance Responses

Urbanization impacts both hydrology and habitat availability (Paul and Meyer 2001) which influences multiple aspects of fish life history (Freeman et al. 2001) that can either increase or decreases fish species depending on their individual thresholds. My prediction that both spawning and trophic guilds would reflect these changes in IC thresholds was not fully supported. Spawning guilds were unanimously insignificant across each IC metric and despite that urbanized watersheds should favor guild "generalists," polyphilic spawners had the lowest medians across all IC metrics. Substrate preference availability however is just one small factor in reproductive cycle and perhaps identifying trends across species early life history is, as if not, more important. For example, polyphilic spawners have free embryos and larvae which are very susceptible to displacement during periods of flashy hydrograph and/or hypoxic conditions during periods of low summer baseflow (Blaszczak et al. 2019, Shang and Wu 2004), which could explain the low thresholds to IC. Overall, the lack of synchronous IC tolerances among trophic and spawning guilds may suggest entirely something else. Fish, like benthic macroinvertebrates, are niche-specific and the expectation of a synchronous response based on broad ecological functions, implies they are equal, which undervalues their taxonomic uniqueness (Baker and King 2010, Lenat and Resh 2001).

There was evidence that pollution tolerance designation for fish within the NCP can serve as a proxy/surrogate for an IC/urbanization tolerance and although there were a significant number of taxa whom IC tolerances fell outside their designated pollution tolerance limits, there is evidence to suggest at a minimum, a re-evaluation of the pollution tolerance classification is needed. For instance, most of the intermediate pollution tolerant designated taxa that fell withing the pollution intolerant IC limits (N=10) are from four families: Catostomidae, Cyprinidae, Percidae, Centrarchidae. The North Carolina Index of Biological Integrity (NCIBI) uses taxa belonging to these families as indicators of stream health; the more species richness of each family at a location, the more healthier the site (NCDEQ 2013). Furthermore, these taxa belong to the most IC sensitive trophic and spawning guild classifications; insectivores (another indicator of stream health according to the NCIBI) and are either polyphilic or lithophilic spawners. Additional taxa not belonging to these families are semelparous and have complex life histories. *Petromyzon marinus* (Sea lamprey) are anadromous and *Anguilla rostrata* (American eel) are catadromous which makes them extremely vulnerable to anthropogenic barriers (Hard and Kynard 1997, Verreault et al. 2004). According to NCDEQ (2019), the state of NC has ~ 6,000 dams, but the current number that prevent the migration of the Sea lamprey and American eel are unknown.

4.3 Community and Individual Responses

As researchers and aquatic resource management focus on a broader spatial scale, the applicability of rapid bioassessments methods for watershed health are key in conservation and management. Another purpose of this research was to look at the relationship of the NCP fish assemblage and IC as a proxy to assess watershed health. Researchers in the past have quantified critical points in watershed health using fish and benthic assemblage data and have found that critical points exist between 5-15% IC (Stanfield and Kilgour 2006). Using TITAN's Z- and Z+ IC change points (6.10% IC) could serve as critical points for watershed health throughout NCP. Between 2001 to 2016, watersheds in the NCP exceeding Z- change points have increased 3.6% or from 83 to 86 watersheds (Figure 11). Watersheds that have exceeded Z+ have increased by 20% or from 24 to 29 watersheds over the same time period. Using response group change points allow natural resource management to assign a value with a specific interpretation of what that value represents, the greatest change in frequency and abundance for taxa that respond negative or positive to increases in IC; instead of a general ambiguous value. However, using a single metric to categorize the overall health of a watershed downplays the overall complexity and excuses the intricacies of a watershed, physical, biological, and chemical processes.

Changes in watershed impervious cover (%) in the NCP by response group change points, 2001-2016.

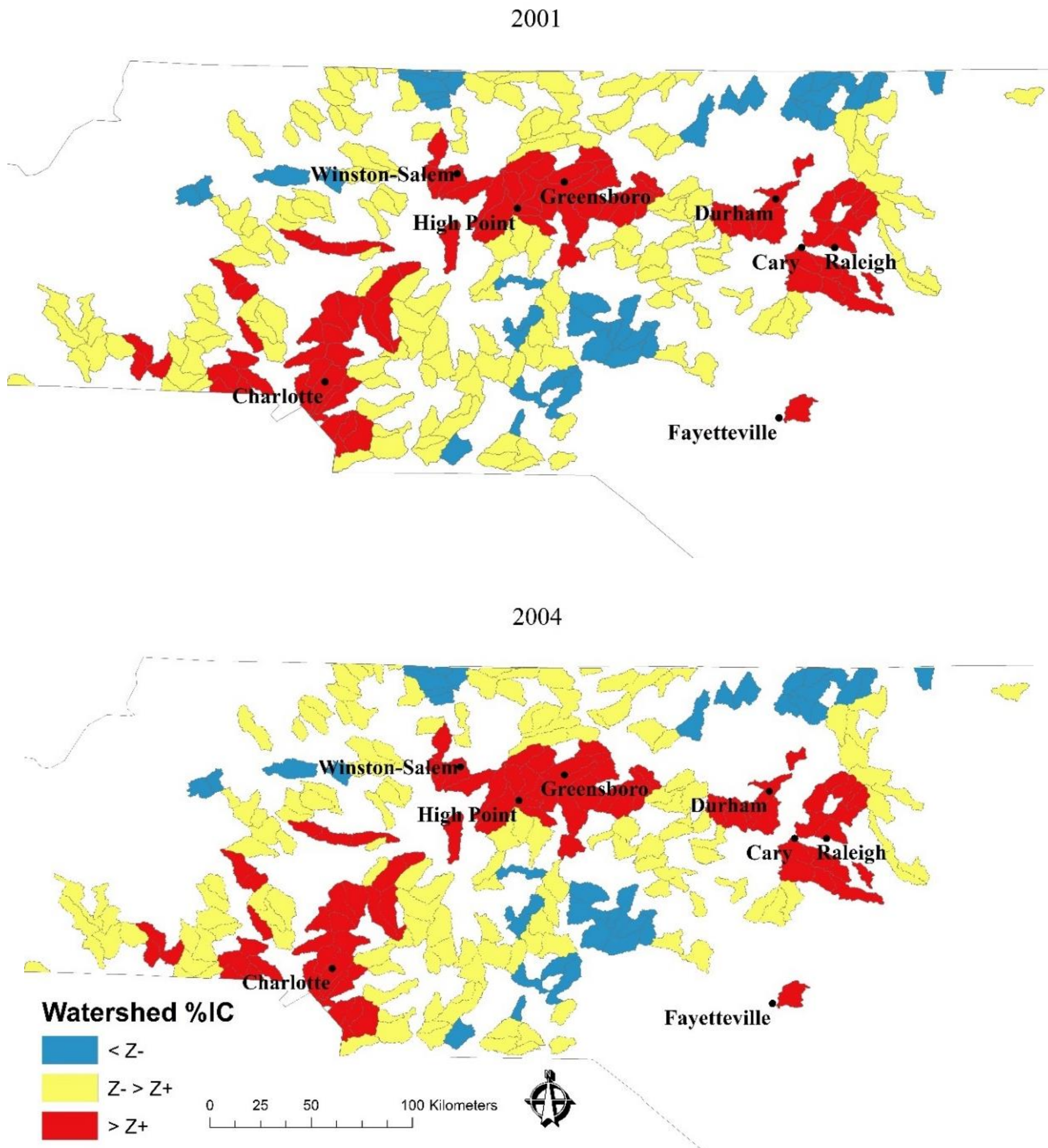
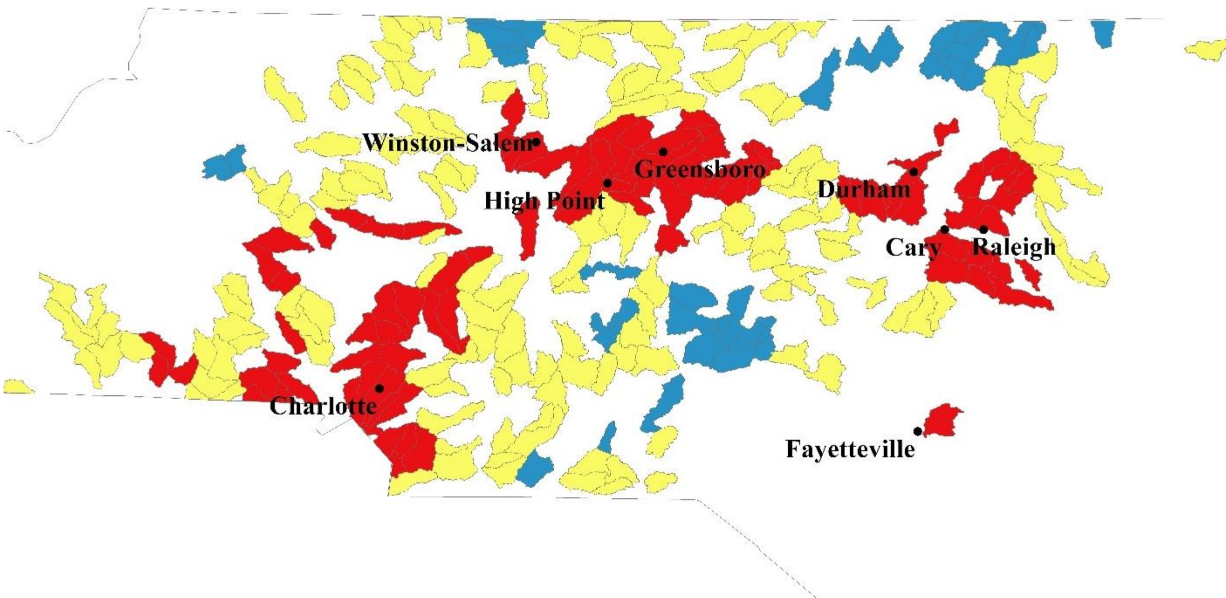


Figure 11. Changes in NCP watersheds classified by response group change points, 2001-2016. Color coded watershed denote the amount of %IC in relation to community response group change points. Z- taxa had a changepoint of 6.10%IC and Z+ taxa had a change point of 16.59 %IC.

2006



2008

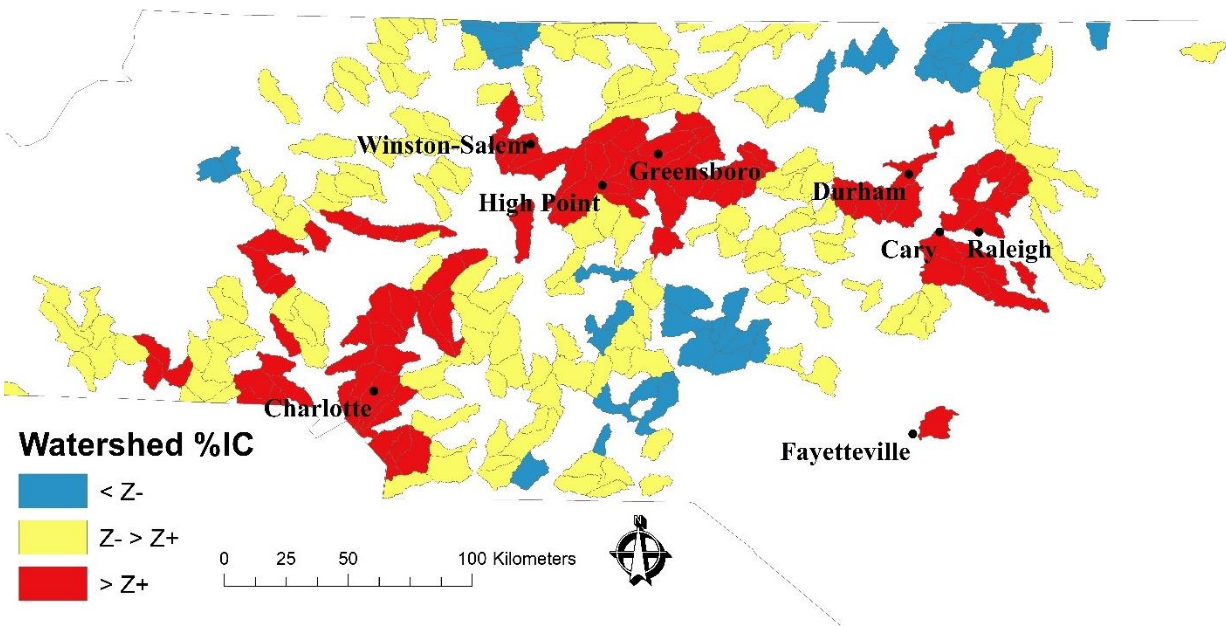
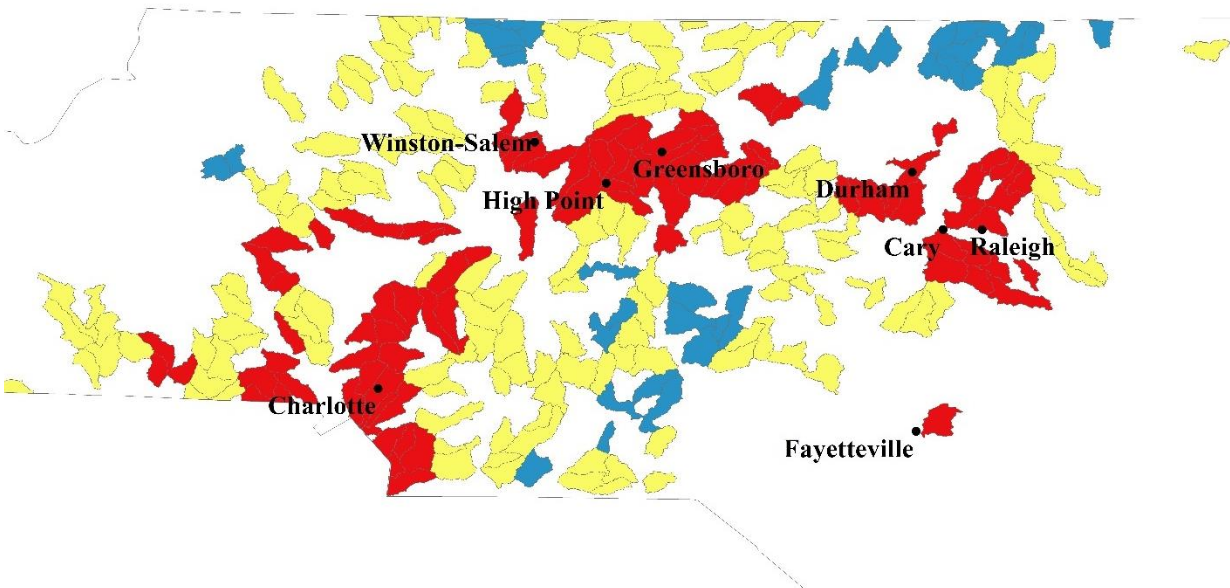
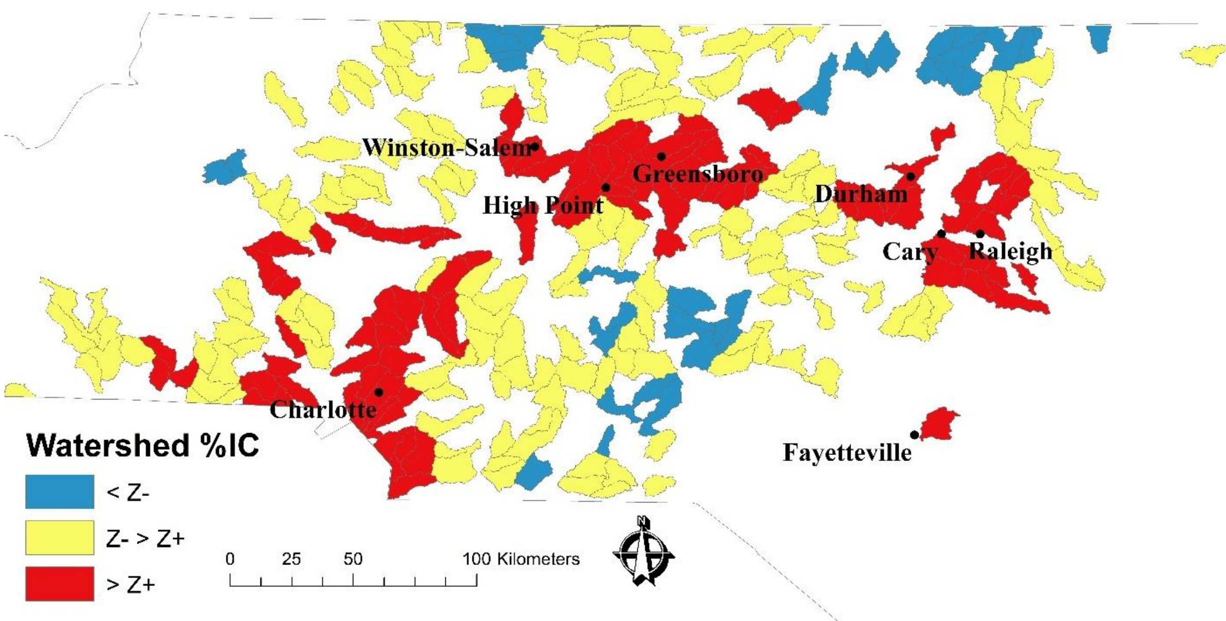


Figure 11. Changes in NCP watersheds classified by response group change points, 2001-2016. Color coded watershed denote the amount of %IC in relation to community response group change points. Z- taxa had a changepoint of 6.10%IC and Z+ taxa had a change point of 16.59 %IC (Continued).

2011



2013



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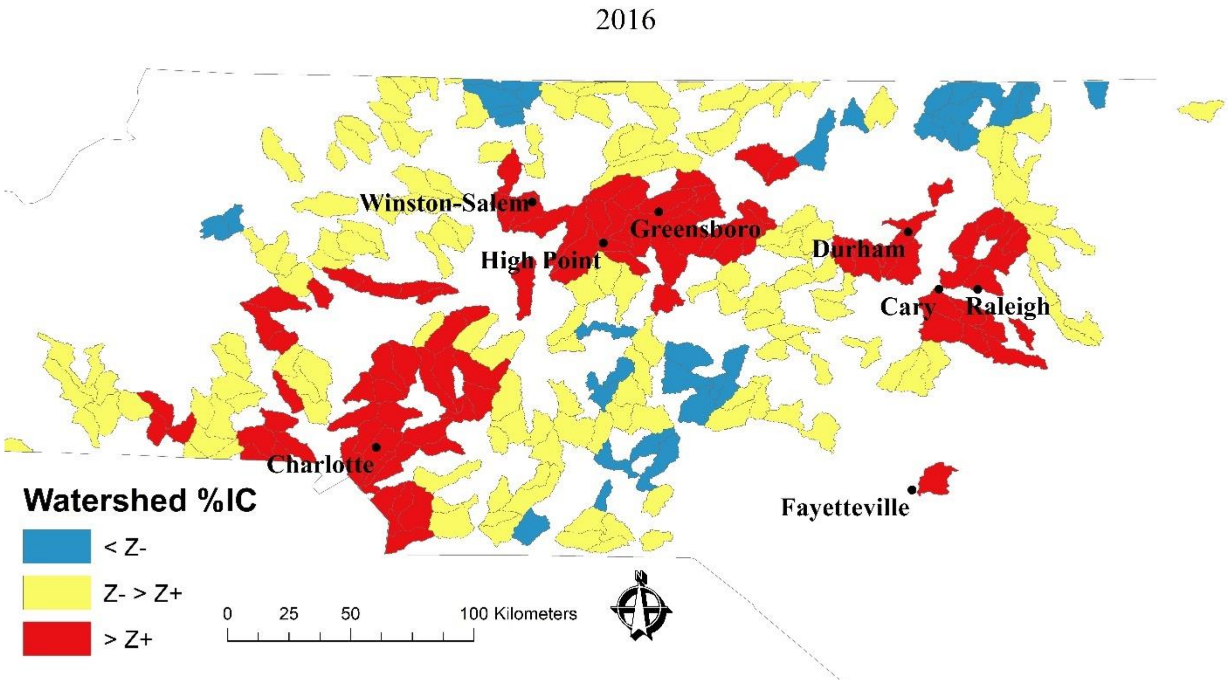

Figure 11. Changes in NCP watersheds classified by response group change points, 2001-2016. Color coded watershed denote the amount of %IC in relation to community response group change points. Z- taxa had a changepoint of 6.10%IC and Z+ taxa had a change point of 16.59 %IC (Continued).

CHAPTER 5: CONCLUSION

Although this research has gained insight into the NCP fish assemblage, more data needed for more accurate representation of the NCP urban thresholds. More sampling efforts are needed to fill the data gaps for representing sampling sites with higher percent IC and for rare species occurrences. As I previously mentioned, sampling sites with percent IC > 50% IC accounted for less than 8% of total sample sites, which could misrepresent the upper limits of IC thresholds for the community and individual Z+ taxa and perhaps even Z- taxa metrics. Likewise, excluding rare species from TITAN due to lack of required occurrences in the dataset, potentially misrepresents the lower limits of the Z- community thresholds.

The use of TITAN to identify and quantify community and individual thresholds of the NCP fish assemblage have allowed for better understanding of how important watershed development regulations are to preserve stream health. North Carolina's aquatic biodiversity crisis have been exacerbated by watershed development regulations that do not reflect our current understanding of the relationship between aquatic biodiversity and urbanization. Although more comprehensive regulations can and are implemented at the river basin and/or municipality level, regulations at the state level still allow for development of ~88% of the state's watersheds to exceed IC thresholds of ~75% of taxa within the NCP fish assemblage (NCDEQ 2011).

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APPENDIX A: WATERSHED LAND COVER DELINEATION MODEL DETAILS

Step	Geoprocessing tool	Geoprocessing tool Summary[1]	Dataset		Notes
			Input(s)	Output(s)	
1	XY Table to Point	Creates a new point feature classed based on x-, y-, and z- coordinated from a table.	Sampling Site Coordinate	Point feature class for sampling site locations	The input dataset was from a query results using SQLite to return unique sample site coordinates in the Piedmont ecoregion.
2	Select Layer by Location	Selects features in a layer based on a spatial relationship to features in another layer.	Point features for sampling site locations and NC watershed boundaries (10 HUC)	Selected watershed boundaries within the study area	
3	Extract by Mask	Extracts the cells of a raster that correspond to the areas defined by a mask.	Land Cover Data (NLCD) and Selected watersheds	Land Cover for study area	
4	Reclassify	Reclassifies (or changes) the values in a raster	Land Cover for study area	Binary land cover	Land cover were reclassified into two categories: "developed" (Developed open space, low, medium, and high intensity) and "other" (open water, barren land, deciduous forest, etc.). Developed open spaced areas are categorized as a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Low intensity developed area IC accounts for 20% to 49% mostly single-family housing, Medium IC accounts for 50-79% single family housing, High Intensity developed areas 80 to 100% and exampled include apartment complexes, row homes and commercial/industrial
5	Raster to Polygon	Converts a raster dataset to polygon features.	Binary land cover (Raster)	Binary land cover (Shapefile)	
6	Intersect	Computes a geometric intersection of the input features. Features or portions of features which overlap in all layers and/or feature classes will be written to the output feature class.	Binary land cover (Shapefile)	Binary land cover (shapefile) for indvidual watersheds	This function assigns each "developed" polygon to its respected watershed.
7	Select Layer by Attribute	Adds, updates, or removes a selection based on an attribute query.	Binary landcover (Shapefile) for individualwatersheds	"Developed" selected gridcode datalayer	By selecting only "Developed polygons" it allows us to decrease the amount of data
8	Table to Excel	Converts a table to a Microsoft Excel file (.xls or .xlsx).	"Developed" selected gridcode datalayer	Microsoft Excel Spreadsheet	

[1] Source (ESRI 2016)

APPENDIX B: POLLUTION AND GUILD CLASSIFICATIONS OF THE NCP FISH ASSEMBLAGE

Species	Family	Trophic Guild	Pollution Tolerance	Spawning Guild
Anguilla rostrata	Anguillidae	Piscivore	Intermediate	NA
Aphredoderus sayanus	Aphredoderidae	Insectivore	Intermediate	Speleophil[1]
Catostomus commersonii	Catostomidae	Omnivore	Tolerant	Lithophil[7]
Erimyzon oblongus		Omnivore	Intermediate	Lithophil[2]
Hypentelium nigricans		Insectivore	Intermediate	Lithophil[5 9]
Hypentelium roanokense		Insectivore	Intermediate	Lithophil[7]
Moxostoma cervinus		Insectivore	Intermediate	Lithophil[7]
Moxostoma collapsum		Insectivore	Intermediate	Lithophil[7]
Moxostoma erythrurum		Insectivore	Intermediate	Lithophil[7]
Moxostoma pappillosum		Insectivore	Intermediate	Lithophil[7]
Moxostoma rupiscartes		Insectivore	Intermediate	Lithophil[7]
Minytrema melanops		Insectivore	Intermediate	NA
Lepomis auritus	Centrarchidae	Insectivore	Tolerant	Lithophil[9]
Acantharchus pomotis		Insectivore	Intermediate	Phytophil[5]
Pomoxis annularis		Piscivore	Intermediate	Phytophil[7]
Ambloplites cavifrons		Piscivore	Intermediate	Polyphil[3 11 15]
Ambloplites rupestris		Piscivore	Intolerant	Polyphil[5]
Centrarchus macropterus		Insectivore	Intermediate	Polyphil[12]
Enneacanthus gloriosus		Insectivore	Intermediate	Polyphil[12]
Lepomis cyanellus		Insectivore	Tolerant	Polyphil[9]
Lepomis gibbosus		Insectivore	Intermediate	Polyphil[9]
Lepomis gulosus		Insectivore	Intermediate	Polyphil[5]
Lepomis macrochirus		Insectivore	Intermediate	Polyphil[12]
Lepomis marginatus		Insectivore	Intermediate	Polyphil[7]
Lepomis microlophus		Insectivore	Intermediate	Polyphil[7]
Micropterus dolomieu		Piscivore	Intolerant	Polyphil[4]
Micropterus punctulatus		Piscivore	Intermediate	Polyphil[1]
Micropterus salmoides		Piscivore	Intermediate	Polyphil[7]
Pomoxis nigromaculatus		Piscivore	Intermediate	Polyphil[5]
Dorosoma cepedianum	Clupeidae	Omnivore	Intermediate	Lithopelagophil[7]
Dorosoma petenense		Omnivore	Intermediate	Phytophil[7]
Notropis hudsonius	Cyprinidae	Omnivore	Intermediate	Lithopelagophil[1]
Campostoma anomalum		Herbivore	Intermediate	Lithophil[9 16]
Clinostomus funduloides		Insectivore	Intermediate	Lithophil[1]
Exoglossum maxillingua		Insectivore	Intolerant	Lithophil[18]
Luxilus albeolus		Insectivore	Intermediate	Lithophil[7]
Luxilus cerasinus		Insectivore	Intermediate	Lithophil[7]
Luxilus coccogenis		Insectivore	Intermediate	Lithophil[7]
Lythrurus ardens		Insectivore	Intermediate	Lithophil[20]
Nocomis leptocephalus		Omnivore	Intermediate	Lithophil[7]
Nocomis raneyi		Omnivore	Intermediate	Lithophil[7]

Notropis altipinnis		Insectivore	Intermediate	Lithophil[7]
Notropis amoenus		Insectivore	Intermediate	Lithophil[9]
Notropis chiliticus		Insectivore	Intermediate	Lithophil[9]
Notropis cummingsae		Insectivore	Intermediate	Lithophil[6]
Notropis petersoni		Insectivore	Intermediate	Lithophil[7]
Notropis procne		Insectivore	Intermediate	Lithophil[7]
Semotilus atromaculatus		Insectivore	Tolerant	Lithophil[7]
Cyprinella lutrensis		Insectivore	Tolerant	Phytolithophil[5]
Cyprinus carpio		Omnivore	Tolerant	Phytolithophil[5]
Hybognathus regius		Herbivore	Intermediate	Phytolithophil[1]
Notemigonus crysoleucas		Omnivore	Tolerant	Phytophil[7]
Chrosomus oreas		Herbivore	Intermediate	Polyphil[17]
Cyprinella analostana		Insectivore	Tolerant	Speleophil[8,9]
Cyprinella chloristia		Insectivore	Intermediate	Speleophil[9]
Cyprinella nivea		Insectivore	Intermediate	Speleophil[9]
Cyprinella pyrrhomelas		Insectivore	Intolerant	Speleophil[7]
Cyprinella zanema		Insectivore	Intolerant	Speleophil[7]
Pimephales promelas		Omnivore	Tolerant	Speleophil[9]
Cyprinella labrosa		Insectivore	Intolerant	NA
Hybopsis hypsinotus		Insectivore	Intolerant	NA
Lythrurus matutinus		Insectivore	Intolerant	NA
Notropis alborus		Insectivore	Intermediate	NA
Notropis chlorocephalus		Insectivore	Intermediate	NA
Notropis scepticus		Insectivore	Intermediate	NA
Rhinichthys obtusus		Insectivore	Intermediate	NA
Esox americanus	Esocidae	Piscivore	Intermediate	Phytophil[1]
Esox niger		Piscivore	Intermediate	Phytophil[1]
Fundulus rathbuni	Fundulidae	Insectivore	Intermediate	Lithophil[13]
Ameiurus melas	Ictaluridae	Insectivore	Tolerant	Lithophil[5,19]
Ameiurus brunneus		Insectivore	Intermediate	Speleophil[7]
Ameiurus catus		Omnivore	Tolerant	Speleophil[7]
Ameiurus natalis		Omnivore	Tolerant	Speleophil[7]
Ameiurus nebulosus		Omnivore	Tolerant	Speleophil[7]
Ameiurus platycephalus		Insectivore	Tolerant	Speleophil[7]
Ictalurus punctatus		Omnivore	Intermediate	Speleophil[7]
Noturus insignis		Insectivore	Intermediate	Speleophil[9]
Morone americana	Moronidae	Piscivore	Intermediate	Phytolithophil[7]
Percina crassa	Percidae	Insectivore	Intolerant	Lithophil[10]
Percina nevisense		Insectivore	Intolerant	Lithophil[10]
Percina roanoka		Insectivore	Intolerant	Lithophil[10]
Perca flavescens		Piscivore	Intermediate	Phytolithophil[7]
Etheostoma collis		Insectivore	Intermediate	Speleophil[1,7]
Etheostoma flabellare		Insectivore	Intermediate	Speleophil[9]
Etheostoma nigrum		Insectivore	Intermediate	Speleophil[7]
Etheostoma olmstedi		Insectivore	Intermediate	Speleophil[10]
Etheostoma brevispinum		Insectivore	Intermediate	NA
Etheostoma fusiforme		Insectivore	Intermediate	NA
Etheostoma podostemone		Insectivore	Intolerant	NA

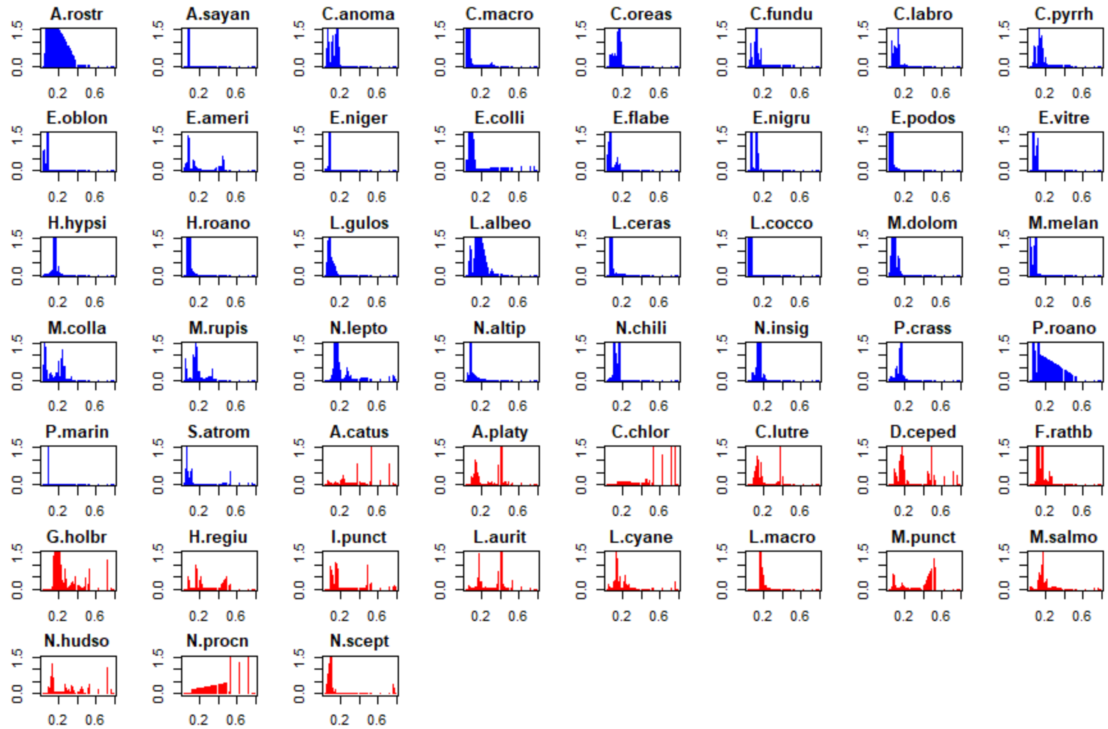
Etheostoma thalassinum		Insectivore	Intolerant	NA
Etheostoma vitreum		Insectivore	Intermediate	NA
Petromyzon marinus	Petromyzontidae	Parasitic	Intermediate	Lithophil[7]
Gambusia holbrooki	Poecilidae	Insectivore	Tolerant	NA
Salmo trutta	Salmonidae	Piscivore	Intermediate	Lithophil[7]
Umbra pygmaea	Umbridae	Insectivore	Intermediate	Polyphil[1]

[1] Arndt and Foltz (2009)
[2] Boschung and Mayden (2004)
[3] Breder and Rosen (1966)
[4] Carlander (1997)
[5] Etnier and Starnes (1993)
[6] Fletcher (1993)
[7] Frimpong and Angermeier (2009)
[8] Gale and Buynak (1978)
[9] Jenkins and Burkhead (1993)
[10] Kuehne and Barbour (2015)
[11] Lee et al. (1980)
[12] Marcy (2005)
[13] McCormick et al. (2001)
[14] Raney (1947)
[15] Smith (1972)
[16] Sublette et al. (1990)
[17] Thompson et al. (2017)
[18] van Duzer (1939)
[19] Wallace (1967)
[20] Yokely Jr (1974)

APPENDIX C: COMPLETE LIST OF TITAN DATA OUTPUTS

Titan Data Outputs		
Data Indices		**Notes**
Community	Change Point	defined by the sum(z) maximum
	5%, 10%, 50%, 90%, 95%	selected quantiles of CP determined by resampling the observed data
Individual	ienv.cp	envrionemtal change point for each taxon based on IndVal maximum (used id imax = TRUE)
	zenv.cp	envrionmental change point for each taxon based on z maximum (default, imax = FALSE
	freq	number of non-zero abundance values per taxon
	maxgrp	1 if z- (negative response); 2 if z+ (positive response)
	IndVal	Dufrene and Legendre 1997 IndVal statistic. Scaled 0-100%
	obsiv.prob	the probability of obtaining an equal or larger IndVal score frm random daa; (number of random IndVals > = observed IndVal/ numPerm
	zscore	IndVal Z score
	5%, 10%, 50%, 90%, 95%	change point quantiles among bootstrap replicates
	purity	proportion of replicates matching observed maxgrp assignment
	reliability	proportion of replicate obsiv.prob values < = 0.05
	z.median	median score magnitude across all bootstrap replicates
	filter	logical (if>0) indication whether each taxa met purity and reliability criteria, value indicates maxgrp assigment.

APPENDIX D: NCP FISH ASSEMBLAGE IC TOLERANCE MATRIX



APPENDIX E: TITAN RESULTS

Species Code	Species	ienv.cp	zenv.cp	freq	maxgrp	IndVal	obsiv.prob	zscore	Change Point Quantiles 5%	10%	50%	90%	95%	purity	reliability	z.median	filter
A.pomot	Acantharchus pomotis	5.54%	8.12%	4	1	1.78	0.044	2.51	5.54%	5.59%	7.98%	40.83%	41.11%	0.78	0.642	3.80342	0
A.cavif	Ambloplites cavifrons	3.93%	5.98%	3	1	4.34	0.008	5.7	3.93%	3.94%	6.04%	8.14%	11.22%	0.93	0.764	6.20258	0
A.rupes	Ambloplites rupestris	11.64%	12.78%	3	1	1.23	0.204	1.7	7.29%	7.31%	11.07%	12.86%	13.12%	0.88	0.332	2.07969	0
A.brunn	Ameiurus brunneus	77.42%	76.57%	42	2	26.72	0.016	4.26	7.89%	11.79%	76.57%	77.42%	77.42%	0.90	0.92	5.44679	0
A.catus	Ameiurus catus	57.37%	52.85%	18	2	24.09	0.004	11.41	20.64%	22.71%	52.85%	62.72%	72.60%	0.98	0.996	11.6398	2
A.melas	Ameiurus melas	3.37%	5.71%	6	1	8.05	0.004	7.6	3.43%	5.66%	7.07%	76.99%	77.42%	0.55	0.984	10.0284	0
A.natal	Ameiurus natalis	3.37%	3.37%	69	1	51.43	0.004	5.1	3.37%	3.37%	6.91%	77.42%	77.42%	0.86	0.992	6.37965	0
A.nebul	Ameiurus nebulosus	3.37%	8.67%	22	1	5.44	0.024	2.85	3.30%	3.31%	8.05%	16.59%	23.54%	0.85	0.876	4.17811	0
A.platy	Ameiurus platycephalus	77.42%	40.97%	280	2	52.1	0.004	8.58	10.30%	11.57%	40.83%	41.11%	41.11%	1.00	1	8.91184	2
A.rostr	Anguilla rostrata	8.11%	8.11%	35	1	14.18	0.004	9.67	7.88%	7.97%	8.12%	8.64%	8.67%	0.99	1	10.4417	1
A.sayan	Aphredoderus sayanus	3.37%	7.90%	135	1	48.35	0.004	21.06	7.70%	7.73%	7.97%	8.42%	8.59%	1.00	1	22.2501	1
C.anoma	Campostoma anomalum	5.71%	16.07%	42	1	14.19	0.004	8.94	5.71%	5.71%	11.32%	16.58%	16.60%	1.00	1	9.44143	1
C.comme	Catostomus commersonii	3.94%	8.57%	228	2	32.58	0.004	3.96	4.02%	5.67%	8.47%	25.30%	45.74%	0.72	1	4.54149	0
C.macro	Centrarchus macropterus	3.37%	6.25%	15	1	10.09	0.004	6.82	3.37%	5.66%	6.18%	7.70%	8.65%	0.99	0.98	7.47312	1
C.oreas	Chrosomus oreas	16.17%	16.58%	32	1	10.53	0.004	6.92	6.27%	6.91%	16.28%	16.59%	16.61%	1.00	1	7.3369	1
C.fundu	Clinostomus funduloides	77.42%	11.57%	217	1	36.03	0.004	7.56	6.18%	6.26%	11.75%	16.58%	16.60%	1.00	1	7.91954	1
C.analo	Cyprinella analostana	67.25%	7.73%	192	2	32.86	0.004	5.44	7.39%	7.70%	7.76%	67.25%	72.60%	0.77	1	6.03037	0
C.chlor	Cyprinella chloristia	76.57%	57.37%	38	2	62.66	0.004	23.2	51.87%	52.70%	62.72%	76.56%	76.56%	1.00	1	22.6611	2
C.labro	Cyprinella labrosa	6.26%	12.69%	15	1	5.44	0.004	4.92	6.25%	6.26%	10.51%	12.86%	13.12%	1.00	0.996	6.05524	1
C.lutre	Cyprinella lutrensis	39.44%	38.05%	30	2	13.97	0.004	8.11	11.06%	11.28%	18.03%	39.44%	39.44%	1.00	1	8.66312	2
C.nivea	Cyprinella nivea	77.42%	77.42%	28	2	21.64	0.024	3.14	7.00%	7.97%	72.60%	77.42%	77.42%	0.93	0.884	4.72333	0
C.pyrrh	Cyprinella pyrrhomelas	12.69%	12.79%	35	1	9.12	0.004	5.12	7.97%	7.97%	12.78%	19.87%	20.28%	0.99	0.996	5.92314	1
C.zanem	Cyprinella zanema	11.28%	11.25%	5	2	2.15	0.048	2.71	10.96%	11.07%	11.25%	12.80%	12.87%	0.64	0.614	2.77848	0
C.carpi	Cyprinus carpio	14.45%	14.18%	3	2	1.63	0.064	2.61	8.91%	13.48%	14.25%	15.80%	21.57%	0.95	0.59	2.84824	0
D.ceped	Dorosoma cepedianum	76.57%	49.00%	31	2	20.35	0.004	8.56	8.12%	14.57%	23.63%	52.55%	72.60%	1.00	1	10.4533	2
D.peten	Dorosoma petenense	45.14%	44.85%	3	2	1.35	0.032	0.89	8.12%	8.41%	8.73%	44.85%	45.14%	0.47	0.384	2.1157	0
E.glori	Enneacanthus gloriosus	3.37%	3.43%	7	1	16.13	0.008	4.43	3.30%	3.30%	3.47%	26.84%	27.55%	0.71	0.85	4.39983	0
E.oblon	Erimyzon oblongus	3.43%	7.76%	189	1	38.74	0.004	9.95	3.49%	3.93%	7.75%	8.42%	8.57%	1.00	1	10.4709	1
E.ameri	Esox americanus	44.85%	8.11%	58	1	11.21	0.012	3.29	6.16%	6.74%	8.53%	45.14%	45.48%	0.98	1	4.33523	1
E.niger	Esox niger	3.47%	8.12%	21	1	12.96	0.004	13.3	7.70%	7.75%	8.04%	8.12%	8.23%	1.00	1	14.0281	1
E.brevi	Etheostoma brevispinum	52.85%	29.83%	107	1	22.3	0.004	6.17	8.57%	10.61%	25.97%	29.63%	29.87%	0.91	1	6.36611	0
E.colli	Etheostoma collis	3.37%	7.16%	39	1	16.74	0.004	9.58	3.49%	5.79%	7.28%	7.70%	7.76%	0.97	1	10.0474	1
E.flabe	Etheostoma flabellare	4.26%	7.16%	110	1	30.7	0.004	10.85	4.26%	4.76%	6.99%	14.18%	14.91%	1.00	1	11.0085	1
E.fusif	Etheostoma fusiforme	3.37%	3.37%	3	1	19.59	0.012	7.93	3.30%	3.31%	5.64%	31.93%	37.88%	0.83	0.818	7.64195	0
E.nigru	Etheostoma nigrum	5.71%	5.71%	34	1	21.88	0.004	10.68	5.71%	5.71%	5.95%	12.78%	12.86%	1.00	1	10.977	1
E.olmst	Etheostoma olmstedi	77.42%	3.98%	359	1	53.75	0.02	2.73	3.94%	3.98%	7.70%	28.63%	76.57%	0.74	0.982	3.86694	0
E.podos	Etheostoma podostemone	5.71%	5.90%	6	1	11.23	0.004	14.51	5.71%	5.71%	5.91%	6.25%	6.32%	0.99	0.98	13.01	1
E.thala	Etheostoma thalassinum	8.57%	8.57%	7	2	2.38	0.096	2.26	8.27%	8.36%	9.30%	15.43%	15.97%	0.80	0.686	2.73884	0
E.vitre	Etheostoma vitreum	5.71%	5.87%	16	1	12.04	0.004	7.47	5.71%	5.71%	6.27%	11.22%	11.28%	1.00	0.998	8.04302	1
E.maxil	Exoglossum maxillingua	5.71%	6.86%	3	1	4.48	0.004	9.43	5.71%	5.71%	5.98%	6.32%	6.91%	0.97	0.856	10.3646	0
F.rathb	Fundulus rathbuni	16.54%	17.15%	104	2	31.55	0.004	15.48	11.08%	11.28%	13.39%	22.11%	24.23%	1.00	1	16.342	2
G.holbr	Gambusia holbrooki	72.60%	15.99%	165	2	37.22	0.004	12.42	16.04%	16.17%	26.01%	72.60%	72.60%	1.00	1	14.0699	2
H.regiu	Hybognathus regius	51.19%	16.07%	27	2	7.04	0.004	3.78	7.31%	7.63%	16.78%	50.09%	51.19%	0.99	0.978	5.17414	2
H.hypsi	Hybopsis hypsinotus	3.60%	16.58%	148	1	36.67	0.004	12.23	16.05%	16.07%	16.58%	17.15%	19.48%	1.00	1	12.7008	1
H.nigri	Hypentelium nigricans	8.47%	8.47%	27	2	6.83	0.004	3.6	7.70%	8.12%	8.63%	16.61%	19.89%	0.61	0.988	4.43045	0
H.roano	Hypentelium roanokense	5.71%	5.78%	18	1	14.79	0.004	8.86	5.71%	5.71%	6.27%	10.30%	10.44%	1.00	1	9.21021	1
I.punct	Ictalurus punctatus	77.42%	14.91%	20	2	7.22	0.004	6.12	7.98%	8.12%	15.80%	51.19%	53.07%	1.00	0.998	7.27408	2
L.aurit	Lepomis auritus	67.25%	18.74%	448	2	53.46	0.004	6.66	16.27%	16.60%	37.88%	52.55%	57.37%	1.00	1	6.99248	2
L.cyane	Lepomis cyanellus	77.42%	22.37%	319	2	44.9	0.004	5.58	10.47%	10.82%	13.11%	25.97%	76.56%	0.97	1	6.45296	2
L.gibbo	Lepomis gibbosus	3.94%	21.27%	129	2	22.75	0.004	4.35	7.69%	13.12%	21.55%	40.83%	40.98%	0.92	0.988	5.04046	0
L.gulos	Lepomis gulosus	5.59%	7.70%	137	1	31.23	0.004	8.8	5.54%	5.59%	7.70%	8.47%	8.57%	1.00	1	9.34624	1
L.macro	Lepomis macrochirus	17.15%	16.61%	387	2	56.92	0.004	11.94	16.28%	16.58%	16.72%	19.31%	20.28%	1.00	1	11.6266	2
L.margi	Lepomis marginatus	7.97%	8.12%	3	1	1.12	0.128	1.15	7.97%	7.98%	8.12%	20.28%	21.55%	0.67	0.286	1.68368	0
L.micro	Lepomis microlophus	72.60%	52.85%	93	2	29.59	0.008	4.16	7.07%	10.74%	52.70%	76.56%	76.56%	0.89	0.998	5.48683	0
L.albeo	Luxilus albeolus	7.00%	12.99%	60	1	13.63	0.004	5.74	6.99%	7.02%	12.78%	28.27%	30.68%	1.00	1	6.79171	1
L.ceras	Luxilus cerasinus	6.04%	7.00%	37	1	19.88	0.004	11.98	6.04%	6.16%	7.00%	7.07%	7.29%	1.00	1	12.2498	1
L.cocco	Luxilus coccogenis	5.09%	5.71%	5	1	12.5	0.004	16.14	5.02%	5.09%	5.54%	5.99%	6.24%	1.00	0.996	16.5158	1
L.arden	Lythrurus ardens	5.71%	7.00%	45	1	17.81	0.004	8.58	5.71%	5.88%	6.99%	48.81%	48.81%	0.80	1	9.79017	0
L.matut	Lythrurus matutinus	4.55%	4.91%	5	1	3.88	0.104	1.89	4.55%	4.61%	10.51%	11.28%	12.60%	0.71	0.626	2.73439	0
M.dolom	Micropterus dolomieu	3.49%	6.25%	27	1	18.31	0.004	10.86	6.24%	6.25%	7.65%	12.10%	12.78%	1.00	1	11.3805	1
M.punct	Micropterus punctulatus	52.55%	51.87%	26	2	15.33	0.004	5.49	8.23%	8.35%	51.19%	51.87%	52.55%	0.97	0.97	5.74448	2
M.salmo	Micropterus salmoides	3.43%	16.78%	227	2	35.6	0.004	5.59	11.50%	12.46%	16.60%	20.64%	23.12%	0.95	0.998	6.29465	2
M.melan	Minytrema melanops	3.37%	8.68%	25	1	11.24	0.004	10.31	3.37%	3.37%	8.42%	8.73%	8.73%	1.00	1	10.8582	1
M.ameri	Morone americana	16.59%	16.58%	4	2	1.76	0.04	2.2	8.31%	8.39%	16.58%	21.27%	22.09%	0.86	0.542	2.41092	0
M.cervi	Moxostoma cervinus	5.71%	5.98%	6	1	6.29	0.008	7.16	5.71%	5.71%	5.88%	11.22%	11.28%	1.00	0.906	6.33297	0
M.colla	Moxostoma collapsum	3.37%	23.63%	106	1	25.17	0.004	7.86	5.15%	5.34%	19.87%	25.24%	26.01%	1.00	1	9.16528	1
M.eryth	Moxostoma erythrurum	6.04%	6.04%	7	1	5.74	0.012	4.46	5.71%	5.85%	6.22%	12.46%	12.78%	1.00	0.912	5.37466	0
M.pappi	Moxostoma pappillosum	6.04%	6.60%	15	1	7.69	0.008	5.16	5.87%	5.90%	6.45%	16.68%	32.19%	0.97	0.934	5.65066	0
M.rupis	Moxostoma rupiscartes	5.03%	16.58%	46	1	11.87	0.004	6.05	5.03%	5.15%	16.02%	32.19%	32.77%	1.00	1	6.68193	1
N.lepto	Nocomis leptocephalus	77.42%	13.23%	406	1	54.5	0.004	9.56	12.12%	12.78%	16.07%	27.55%	57.64%	1.00	1	10.5342	1
N.raney	Nocomis raneyi	57.37%	17.39%	5	2	2.51	0.02	3.14	10.62%	10.96%	18.35%	52.85%	57.37%	0.98	0.796	4.12708	0
N.cryso	Notemigonus crysoleucas	49.00%	50.09%	78	1	15.22	0.112	1.46	3.30%	5.01%	13.25%	48.81%	50.09%	0.73	0.796	2.77511	0
N.albor	Notropis alborus	3.37%	3.37%	79	1	48.15	0.02	4.43	3.37%	3.37%	7.70%	48.06%	48.81%	0.94	1	4.79009	0
N.altip	Notropis altipinnis	3.82%	7.97%	122	1	34.53	0.004	14.06	7.70%	7.73%	7.90%	8.39%	8.42%	1.00	1	14.3758	1
N.amoen	Notropis amoenus	3.37%	3.43%	8	1	13.82	0.088	2.73	3.30%	3.30%	7.07%	16.75%	20.28%	0.96	0.74	3.97176	0
N.chili	Notropis chiliticus	3.93%	11.01%	219	1	42.77	0.004	12.41	10.50%	10.62%	11.28%	16.54%	16.58%	1.00	1	12.9336	1
N.chlor	Notropis chlorocephalus	9.39%	9.24%	4	2	1.5	0.096	1.77	8.76%	8.91%	14.25%	20.28%	21.13%	0.92	0.494	2.31957	0
N.cummi	Notropis cummingsae	3.98%	4.76%	5	1	13.61	0.004	10.81	3.98%	3.98%	4.91%	76.56%	76.57%	0.78	0.946	10.4573	0
N.hudso	Notropis hudsonius	77.42%	67.25%	152	2	57.97	0.004	9.9	11.57%	11.64%	44.11%	72.60%	72.60%	1.00	1	10.0377	2
N.peter	Notropis petersoni	3.98%	3.98%	15	1	19.64	0.004	9.15	3.93%	3.98%	4.61%	72.60%	72.60%	0.80	0.982	8.93823	0
N.procn	Notropis procne	57.37%	72.60%	82	2	75.5	0.004	19.44	52.55%	52.70%	57.37%	72.60%	72.60%	1.00	1	19.873	2
N.scept	Notropis scepticus	77.42%	8.39%	97	2	19.88	0.004	6.88	8.33%	8.41%	8.65%	13.66%	76.99%	1.00	1	6.74128	2
N.insig	Noturus insignis	76.57%	16.58%	265	1	51.27	0.004	13.38	11.07%	12.55%	14.59%	16.67%	17.27%	1.00	1	13.9029	1
P.flave	Perca flavescens	16.38%	16.07%	31	2	8.57	0.004	4.84	4.61%	5.87%	16.07%	16.58%	16.59%	0.71	0.992	5.63224	0
P.crass	Percina crassa	3.47%	16.58%	129	1	29.5	0.004	9.32	10.51%	13.22%	16.58%	16.62%	17.39%	1.00	1	9.77788	1
P.nevis	Percina nevisense	5.71%	5.71%	7	1	3.66	0.072	2.57	5.71%	5.71%	8.73%	19.87%	20.28%	0.92	0.674	3.29232	0
P.roano	Percina roanoka	5.71%	5.90%	25	1	13.39	0.008	6.14	5.71%	5.71%	11.07%	12.78%	12.82%	1.00	1	7.35687	1
P.marin	Petromyzon marinus	7.97%	8.12%	8	1	5.37	0.004	7.41	7.75%	7.76%	7.98%	8.12%	8.23%	1.00	0.992	7.92169	1
P.prome	Pimephales promelas	46.31%	11.25%	12	1	2.5	0.204	0.88	6.97%	8.12%	11.35%	46.31%	48.90%	0.52	0.694	2.73878	0
P.annul	Pomoxis annularis	57.37%	52.85%	3	2	3.29	0.12	2.76	8.42%	8.65%	50.43%	57.37%	57.37%	0.67	0.388	2.35725	0
P.nigro	Pomoxis nigromaculatus	52.85%	16.58%	36	2	8.29	0.012	3.55	5.79%	6.19%	16.59%	52.85%	52.85%	0.83	0.942	4.53817	0
R.obtus	Rhinichthys obtusus	15.87%	13.76%	3	2	1.6	0.036	2.82	3.47%	13.85%	15.32%	15.88%	15.99%	0.92	0.626	2.88728	0
S.trutt	Salmo trutta	5.02%	6.24%	3	1	5.56	0.004	11.57	3.91%	5.02%	5.71%	6.60%	6.97%	0.95	0.878	10.1922	0
S.atrom	Semotilus atromaculatus	52.85%	6.26%	263	1	41.08	0.008	3.53	5.09%	5.84%	9.11%	53.30%	61.90%	0.99	0.99	4.75611	1
U.pygma	Umbra pygmaea	6.45%	6.60%	3	1	2.98	0.008	4.67	5.87%	5.89%	6.67%	17.27%	21.55%	0.84	0.682	4.75763	0