ABSTRACT

BRIGGS, EMILEE ESTHER. The Impacts of Urbanization on Stream Fishes in the Southeastern United States. (Under the direction of Dr. Jesse R. Fischer).

Urbanization is a primary agent of stream ecosystem degradation. Watershed urbanization degrades stream ecosystems through a series of consistent physical and biogeochemical alterations such as increased high-flow events, stream channelization, and nutrient loading. The physical stream alterations reduce habitat complexity and suitability for aquatic biota, resulting in declines in biotic community richness and diversity. Fishes have been used to quantify the impacts of anthropogenic disturbances, such as landscape alterations, because they reactive sensitively to a wide array of environmental changes. However, there is limited understanding of the ecological implications of urbanization on stream fishes. In this thesis I describe how urbanization impacts stream fish ecology in the Southeastern United States, which is experiencing the greatest rate of urbanization in the nation. I describe two studies that address central questions in the study of urban fish ecology: 1) How does urbanization influence fish community structure? and 2) How do fish populations respond to increasing urbanization?

To determine how urbanization influences fish communities, I compared the physical habitat and fish assemblage structure in urban (n = 3) and non-urban (n = 6) headwater streams in the Upper Neuse River Basin in North Carolina. I conducted intensive fish sampling to calculate detailed density estimates for each species present in streams, and summarized assemblages taxonomically (species and family) and functionally (trophic guilds, tolerance to poor habitat). My results demonstrated assemblage and density shifts from diverse fish assemblages of ecological sensitive Cyprinids in non-urban streams towards tolerant and lentic adapted Centrarchid fishes in urban streams. The results of my research suggested that watershed-level characteristics were highly influential in structuring fish assemblages.

Additionally, I identified six species that accounted for almost 80% of the variation between urban and non-urban assemblages, and could be used as potential bioindicators to further understand degradation and monitor restoration efforts.

To understand how population characteristics respond to increasing urbanization, I conducted large-scale surveys in third-order streams (n = 22) in the Upper Neuse River Basin in North Carolina and documented changes to fish populations across an urban gradient. Specifically, I evaluated the population characteristics of three focal species: Green Sunfish (*Lepomis cyanellus*), Redbreast Sunfish (*L. auritus*), and Bluegill (*L. macrochirus*). Basic population characteristics (size distribution, condition, age, and growth) and elemental stoichiometric characteristics (%C, %N, %P, and C:N:P ratios) were quantified for each focal species and used to identify intercorrelations with environmental characteristics. My results revealed that Bluegill and Green Sunfish responded negatively (i.e., decreases in size and abundance) to watershed urbanization. Conversely, Redbreast Sunfish showed a strong, positive response to urbanization (i.e., increases in abundance). My study identifies Redbreast Sunfish as an excellent indicator of urbanization due to their tolerance to poor habitat and their life history characteristics.

Overall, my thesis research has shown that stream fish are impacted across a gradient of urbanization. As watersheds become urbanized, fish communities are increasingly dominated by tolerant centrarchid species. My results demonstrated that Redbreast Sunfish are uniquely impacted by urbanization and serve as excellent ecological indicators to monitor urban stream degradation and restoration. My findings provide valuable information to stream ecology and watershed management, including how urban watershed degradation affects fish ecology and potential bioindicators of urbanization in the Southeast United States.

The Impacts of Urbanization on Stream Fish in the Southeastern United States

by
Emilee Esther Briggs

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APPROVED BY:

Dr. Jesse R. Fischer
Committee Chair

Dr. James A. Rice

Dr. Stacy A. C. Nelson

DEDICATION

For my parents, Holly Elizabeth Briggs and Stephen Michael Briggs, to whom I owe everything.

BIOGRAPHY

I grew up in Raleigh, North Carolina, and have experienced firsthand the rapid urbanization in the Southeast U.S. My background and interests have centered around understanding the relationship between humans and their environments and have led me to pursue a career in freshwater ecology. My professional experiences have led me to research that takes an interdisciplinary and a collaborative approach to address applied aquatic conservation questions, and to promote the conservation and management of degraded stream ecosystems.

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To Chipper, you are my best friend.

"You can read every day were a dog saved the life of a drowning child, or lay down his life for his master. Some people call this loyalty. I don't. I may be wrong, but I call it love – the deepest kind of love." – Wilson Rawls, Where the Red Fern Grows

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CHAPTER 1: Effects of Urbanization on Stream Fish Assemblages in the Southeast U.S.

Abstract

Urbanization degrades stream ecosystems through a cascade of physical alterations (e.g., bank erosion and channelization) and biogeochemical changes (e.g., nutrient saturation and sediment loading). However, the impacts of urbanization on biotic assemblages in streams is poorly understood, particularly in the Southeast United States. In this study, I evaluated the effects of urbanization on fish assemblage structure in nine headwater streams in North Carolina, one of the most rapidly urbanizing states in the U.S. I quantified streams as urban (n = 3) or non-urban (n = 6) using GIS to delineate stream watersheds and quantify land cover area. Fishes were sampled using triple-pass electrofishing methods, and assemblages were analyzed taxonomically (species and family) and functionally (tolerance guilds and trophic guilds). Nonmetric multidimensional scaling (NMS) analysis was used to evaluate patterns in stream type and assemblage structure. The NMS identified shifts from high densities of diverse fish assemblages of ecologically sensitive taxa (e.g., cyprinids) in forested streams towards lower densities of assemblages dominated by tolerant and lentic adapted taxa (e.g., centrarchids) in urban streams. The similarity of percentages analysis (SIMPER) identified six species (Bluehead Chub *Nocomis leptocephalus,* White Shiner *Luxilus albeolus,* Swallowtail Shiner *Notropis procne*, Bluegill *Lepomis macrochirus*, Eastern Mosquitofish *Gambusia holbrooki,* and Redbreast Sunfish *L. auritus*) that explained 78% of the variation between stream types. The results from ordination of fish assemblages and evaluation of environmental correlations in urban and forested streams indicated that landscape characteristics (e.g., conductivity, instream velocity, and percent forested land cover) were important in structuring fish assemblages.

Introduction

Cities are growing at an unprecedented rate worldwide. In the United Sates, over 80% of the population is estimated to reside in urban areas by 2050 (Violin et al. 2007, US Census Bureau 2017, UN Population Review 2019). As a result of the rapid urban population growth, urban development is expected to increase by 150%, creating large metropolitan areas that span multiple states (White et al. 2009, Terando et al. 2014). Urbanization severely degrades stream ecosystems (Heaney & Huber 1984, Wenger et al. 2009) and is the sole cause of impairment for over 16% of streams and rivers in the United States (USEPA 2006). This makes urban development one of the primary causes of stream system degradation in the U.S. (Paul & Meyer 2001). Therefore, recognizing and understanding the consequences of urbanization on stream ecosystems is critical to the future conservation and restoration of urban streams.

The urbanization of stream watersheds is associated with a number of well-documented physical, biogeochemical, and biological responses that are collectively termed the urban stream syndrome (Heaney & Huber 1984**,** Paul & Meyer 2001, Walsh et al. 2005, Roy et al. 2016). For example, watershed urbanization is associated with increased frequency of short-duration flash flood events (i.e. flashy hydrography; Booth 1991, Walsh et al. 2005, DeVries et al. 2009). The flashy hydrography of urban streams results in increased natural runoff processes, such as channelization and increased delivery of sediments and nutrients (Klein 1979, Paul & Meyer 2001, Sterner & Elser 2002, Walsh et al. 2005), that can reduce habitat complexity and decrease suitability for sensitive aquatic biota (Paul & Meyer 2001, Walsh et al. 2005, Wenger et al. 2009). Although urban stream syndrome is well-documented (e.g., increased runoff and pollutants, decreased sensitive biota), the effects can vary widely among taxonomic groups (Tate et al. 2005, Meador 2019, Wenger et al. 2009) and regions (Paul & Meyer 2001, Tate et al.

2005). This suggests that there are still major gaps in our understanding of the relationship between urbanization and stream ecosystem degradation that may inhibit future research and management under the context of increasing urbanization and changing environmental conditions.

Fishes have long been used to quantify the impacts of anthropogenic disturbances, such as landscape alterations, because species may respond either positively or negatively to degradation depending on their species-specific life histories (Karr 1981, Chovanec et al. 2003, Meador et al. 2005, Hoeinghaus et al. 2007, Wenger et al. 2009). However, regional differences in species composition and habitats have limited our understanding of aquatic ecosystem degradation resulting from urbanization relative to non-urban streams (Paul & Meyer 2001, Hoeinghaus et al. 2007). Thus, the ecological implications of urbanization on fishes are poorly understood (Mulholland & Lenat 1992, Paul & Meyer 2001). Therefore, the goal of this study was to understand how fish assemblages and their habitats are influenced by urbanization in rapidly growing regions. Specifically, my objectives were to evaluate 1) relationships between stream environmental characteristics and urbanization, and 2) to identify potential effects of urbanization on taxonomic and functional fish assemblage structure.

Methods

Study System

This study was conducted in the headwater streams of the Neuse River, which flows 400 km east to the Pamlico Sound and covers a total watershed area of 16,000 km^2 (Phillips 1992, Lenat & Crawford 1994). All streams were located within the Upper Neuse River Basin (hereafter referred to as the Upper Neuse). The Upper Neuse watershed is 1,994 km^2 and spans

across the Piedmont ecoregion and Triassic Basin (Philips 19922, Griffith 2002, Kennen et al. 2005). Streams located within the Piedmont ecoregion are characterized by cobble, gravel, and sandy substrates (Phillips 1992, Lenat & Crawford 1994). Triassic Basin streams are characterized by erosive soil types and by sandy and clay substrates. Consequently, streams in this region are heavily influenced by stormwater runoff and sediment loading associated with watershed land cover types (Lenat & Crawford 1994, NCDENR 2009).

Land cover within the Upper Neuse is dominated by forested (61%) and agricultural (16%) land cover types (NCDENR 2009) but is becoming more urbanized due to the rapid growth of the Raleigh-Durham-Chapel Hill metropolitan area (RDU), which covers 14,270 km^2 and holds a population of 2.2 million (U.S. Census Bureau 2017). The RDU population grew by 40% from 2000 to 2012, making it the fastest growing metropolitan area in the United States (U.S. Census Bureau 2017). While the Upper Neuse subbasin includes only a portion of the RDU metropolitan area, growth in this area is expected to increase the human population by 50% and the developed land cover by 28% by 2025 (NCDENR 2009).

Site Selection

Nine third-order streams were selected to compare physical habitat and fish assemblages. Streams were sampled between May and August 2016. A single sample site was selected at each stream based on several criteria that minimized potential variation in specific environmental characteristics while maximizing contrast between urban and non-urban land use (Figure 1.1). For example, each sampling site was a 50-m, continuous, wadeable reach. Additionally, all stream reaches had similar natural characteristics such as catchment size and stream size, and were sampled during base flow conditions.

After appropriate study streams were selected, they were then classified as "urban" (n = 3) or "non-urban" (n = 6) based on the dominant catchment land use, as described below. The catchments for each sample reach were delineated using 1-arc second, 30-m resolution United States Geological Survey (USGS 2016) National Elevation Dataset digital elevation model (USGS 2014) and Arc Hydro tools methods in ArcGIS 10.5.1d (ESRI™, Redlands, California, USA). Catchment land cover was then extracted based on 30-m resolution 2011 National Land Cover Data (NLCD; Homer 2015) and summarized into eight major land cover types (Figure 1.1): water (open water), urban (barren, open space, low-, medium-, and high- intensity developed), forest (deciduous, evergreen, and mixed forest), shrubland, herbaceous, agriculture (hay/pasture and cultivated), and wetland (woody and emergent herbaceous). Each land cover category was quantified as the percent land cover of the total catchment area. Stream reach catchments with greater than 50% developed land were classified as urban, and stream catchments with greater than 50% forested land cover were classified as non-urban. The mean percent land cover was further summarized across each stream type (i.e., urban and non-urban). Effects of urban land cover on physical stream characteristics, mean physiochemical and hydromorphological variables were tested between urban and non-urban reaches using a t-test.

Physical habitat

Physiochemical characteristics, canopy cover, and substrate type were measured at a single point in the first run upstream of each study reach. Temperature, conductivity, dissolved oxygen, and pH were measured using a Yellow Spring Instruments (YSI) model Professional Pro Multiparameter handheld instrument (YSI Life Sciences, Yellow Springs, OH). Canopy cover was visually quantified by estimating the percent shading or cover of the stream and averaged for the entire habitat. Substrate particle size was quantified and classified based on a modified

Wentworth scale and included the following: boulder, cobble, gravel, sand, mud, and silt (Cummins 1962).

Additional instream characteristics were quantified to gain a coarse understanding of habitat structure. Stream wetted width, depth, and velocity were measured at a single point along three equidistant transects located at downstream, midstream, and upstream portions of each stream reach (0 m, 25 m, 50 m) using a transect-based method (McMahon et al. 1996). Stream wetted width and depth were measured with a meter stick, and stream depth was measured at the deepest point of the transect. Instream velocity was measured at the point of highest flow within each transect and determined by measuring the velocity at 60% of the water column height using a GEOPACK MFP51 Standard Stream Flowmeter (GEOPACKS, Hatherleigh, UK; McMahon et al. 1996).

Fish Sampling

Fish were sampled using a backpack-mounted pulsed-DC electrofisher (Smith-Root, Inc., Vancouver, Washington) using three successive passes. Prior to sampling, block nets (7 mm knotless mesh) were secured to the banks and substrate at the upstream and downstream ends of each reach to prevent fish from entering or exiting the study area. Electrofishing was conducted by crews of three to five people with a single backpack electrofisher and two to four netters, depending on stream width. Crews began sampling at the downstream reach and proceeded upstream, moving in a zigzag pattern to ensure coverage of the entire stream width and all available habitats (e.g., woody debris, boulders, undercut banks). Each pass was processed separately, and fish were immediately transferred to live wells (60L) on the stream bank after each pass. After the completion of each electrofishing pass, fish were identified to species, counted, measured to the nearest mm (total length [TL]), and weighed to the nearest 0.1 g. Fish

were released alive back into the stream after the completion of all three passes. Individuals that could not be identified were euthanized using rapid freezing methods outlined by the American Veterinary Medical Association guidelines (Leary et al. 2013) and transported back to the laboratory. Collected specimens were kept in a -20°C freezer until they could be processed and identified.

Statistical Analysis

Fish assemblage characteristics were quantified taxonomically and functionally to investigate the relationship between fish assemblage structure and land use characteristics. The taxonomic dataset included relative species abundance (number of species per stream), density of each species (individuals/100 m^2), and density of each family (individuals/100 m^2; *Aphredoderidae, Catostomidae, Centrachidae, Cyprinidae, Esocidae, Fundulidae, Ictaluridae, Percidae, Poecillidae,* and *Umbridae)*. Taxonomic group assemblages are often shaped by large-scale processes and can be used to understand regional-level impacts to stream systems, such as large-scale shrub and forest landscape cover (Hoeinghaus et al. 2007). Functional group assemblages were also analyzed because ecological responses are often more strongly influenced by and better predictors of environmental disturbances than taxonomic groups (Hoeinghaus et al. 2007, Fischer et al. 2009, Burgad et al. 2019). Functional groups were assigned based on ecological traits and included the following groups: trophic guild (piscivore, omnivore, and insectivore), tolerance to poor habitat and water quality (tolerant and intermediate), (Frimpong & Angermeir 2009, Kennen et al. 2005). The functional group dataset included the density of each trophic guild and the density of each tolerance-level (individuals/100 m^2). Fish density was estimated (individuals per m^2) according to species using a maximum-likelihood method for three-pass removals (Carle & Strub 1978, Seber 1982, Hayes et al. 2007) with the FSA package

(Ogle 2018) using the statistical software program R (version 3.5.1; R Core Team 2019). Density estimates were multiplied by an area correction factor ($100m^2$).

Nonmetric multidimensional scaling analysis (NMS) was used to investigate the similarities of fish assemblage structure among stream reaches. The NMS compares fish assemblages by constructing a multi-dimensional ordination to visualize relationships using a similarity index, with similar points reflecting more similar assemblage structure (Field et al. 1982). I constructed fish assemblage matrices based on fish density estimates using the Bray-Curtis similarity index (Bray and Curtis 1957). Rare species were not removed in order to look at the entire fish community and because the absence of rare species is often an important indicator of environmental stress (Chovanec et al. 2003, Poos & Jackson 2012, Burgad et al. 2019). Differences in fish assemblage structure between urban and non-urban reaches were tested using a PERMANOVA (Permutational Analysis of Variance). When PERMANOVA reveled significant differences, a SIMPER (Similarity of Percentages) analysis was used to identify species and families accounting for the most variation between assemblages. Environmental vectors were fit to the NMS ordinations to identify correlations between environmental variables and fish assemblage data. All analyses were performed using R 3.6.1 using Vegan and MASS packages (R Development Core Team, 2017; Oksanen et al. 2017). A type I error rate of 0.10 was used for all statistical tests.

Results

Watershed and Land Cover

The nine delineated stream catchments varied in area from 4 to 50 km^2 (17 ± 5 km^2 [overall mean ± *SE*]; Table 1.1) and did not significantly differ between urban and non-urban

streams ($P = 0.67$, $t = -0.49$). Urban catchments were dominated by developed land cover (91 ± 8%; Figure 1.2), followed by forested land cover (8 ± 6%). Non-urban stream catchments were characterized by forested (62 ± 8%; Figure 1.2) and agricultural land cover types (16 ± 11%).

Physical Habitat

In general, urban and non-urban streams differed in conductivity, substrate type, and instream velocity. Urban streams had significantly greater mean conductivity (306.0 ± 62.0) than non-urban stream reaches (110.8 ± 13.0; $P = 0.08$, $t = -3.08$, Table 1.1;). Stream substrate composition and instream velocity were highly variable between urban and non-urban streams (coefficient of variations ≥ 90%) but the differences were not statistically significant. Substrate composition across all stream reaches varied from 0-20% boulder, 0-67% gravel, 0-75% sand, and 0-80% silt (Table 1.1). Mean percent gravel substrate (CV = 303.0%) and mean percent silt substrate (CV= 100.8) were the most variable substrate types across all stream reaches. Urban streams were comprised of sand (41.0 ± 4.9%) and silt substrate cover (30.0 ± 15.3%) and had highly variable mean gravel substrate cover (Table 1.1). Conversely, non-urban streams had highly variable mean boulder, sand, and silt substrate cover and relatively consistent gravel substrate across sampled reaches (Table 1.1). Stream velocity varied from 0.0 to 0.48 m/s and was more variable among urban reaches (CV = 164.3%) than non-urban reaches (CV = 102.4%).

Fish Assemblage

A total of 1150 individuals from 24 species were sampled from all nine reaches. Species richness varied from 4 to 15 species (10.1 ± 1.2 species) across all stream reaches, and six species were commonly observed. Green Sunfish (*Lepomis cyanellus*) were present at all nine stream reaches. Redbreast Sunfish (*L. auritus*) were present at seven stream reaches, and Bluehead chub (*Nocomis leptocephalus*), Bluegill (*L. macrochirus*), White Shiner (*Luxilus*

albeolus), and Swallowtail Shiner (*Notropis procne*) were present at six of the sample reaches. Bluehead Chub also had the greatest population densities of all sampled species, but mean densities were highly variable among streams (0.0-98.1 fish/100 m^2; Table 1.2). Cyprinids (minnows) were the dominant family sampled and comprised over 60% of the total number of individuals sampled. Cyprinidae (minnows) had the greatest mean density of all families across all stream reaches (46.8 ± 18.6 fish/100 m^2; Figure 1.3), but Centrarchidae (sunfish) were the only family present at every site. Insectivores and intermediate-level tolerant individuals were the dominant functional groups across all sites, and had the greatest mean densities of all sampled functional groups (52.9 ± 11.5 fish/100 m^2,62.0 ± 19.9 fish/100 m^2, respectively; Figure 1.3)

Green Sunfish were ubiquitous across urban stream reaches, and all species present in urban stream reaches were also found in non-urban reaches. Eastern Mosquitofish (*Gabusia holbrooki*), Redbreast Sunfish, and Swallowtail Shiner were the most abundant species in urban reaches, with mean species densities of 8.7 ± 7.2 fish/100 m^2, 6.1 ± 5.5 fish/100 m^2, and 6.4 ± 6.3 fish/100 m^2, respectively (Table 1.2). Centrarchidae was ubiquitous and the most abundant sampled family in urban streams (Table 1.2), while Aphredoderidae (pirate perches), Catostomidae (sucker), Fundulidae (killifish), and Umbridae (mudminnow) were absent from urban stream reaches. Non-urban stream reaches had greater mean species richness (11.5 ± 0.9) and greater total fish density (109.0 ± 26.4 fish/100 m^2) than urban streams (7.3 ± 2.8; 31.4 ± 12.4 fish/100 m^2), but the differences were not significantly different ($P = 0.28$, $t = 1.40$; $P = 0.33$, $t = 1.05$; Figure 1.3). Bluehead chub were the most abundant species in non-urban stream reaches (38.4 ± 15.4 fish/100 m^2). Several species were exclusively present in non-urban reaches, including Pirate Perch (*Aphredoderus sayanus*), Creek Chubsucker (*Erimyzon*

oblongus), Northern Hogsucker (*Hypentelium nigricans*), Pumpkinseed (*L. Gibbosus*), Dusky Shiner (*N. cummingsae*), Creek Chub (*Semotiuls atromaculatus*), Speckled Killifish (*Fundulus rathbuni*), Johnny Darter (*Etheostoma nigrum*), Yellow Perch (*Perca flavescens*), and Eastern Mudminnow (*Umbra pygmaea*). Non-urban streams were characterized by high densities of Cyprinids and had significantly greater mean densities of Cyprinidae (66.5± 24.2 fish/100 m^2; $P = 0.07$, $t = 2.28$) than urban streams (Figure 1.3). Similarly, non-urban streams had significantly greater mean densities of intermediate-level tolerant individuals (86.9 ± 23.5 fish/100 m^2 $P = 0.06$, $t = 2.33$) than urban reaches (Figure 1.3).

NMS Analysis

A stable NMS ordination was obtained for the taxonomic fish assemblage structure for stream reaches and resulted in a final stress of 6.7% (Figure 1.4). Stress values less than or equal to 20% indicate that the resulting ordination provides an accurate representation of the actual assemblage configuration. A stable NMS ordination was also obtained for the family assemblage structure in for stream reaches and resulted in a final stress of 2.8% (Figure 1.5). The family NMS stress value indicates that the resulting ordination provides an excellent representation of the assemblage structure. Stable NMS ordinations could not be obtained for the tolerance and trophic functional groups. The NMS analyses indicated some overlap in fish assemblages among the two habitat types. However, the results from the PERMANOVA indicated that both species ($p = 0.08$, $F = 1.80$) and family ($p = 0.08$, $F = 2.30$) fish assemblage structures were dissimilar between urban and non-urban sites. Environmental vectors indicate the environmental variables that are highly correlated with the NMS configuration, and were fitted for both the species and family NMS (Figure 1.4 and 1.5). Species assemblage structure was related to conductivity, instream velocity, and land cover. Species assemblage structure at urban sites was correlated

with increasing conductivity ($r = 0.65$, $p = 0.04$), while non-urban sites were associated with increasing instream velocity ($r = 0.66$, $p = 0.04$), forested land cover ($r = 0.55$, $p = 0.08$), and herbaceous land cover ($r = 0.55$, $p = 0.08$; Figure 1.4). Similarly, family assemblage was related to land cover and several physical instream characteristics. Specifically, family structure in urban reaches was correlated with increasing developed land cover ($r = 0.59$, $p = 0.07$) and conductivity ($r = 0.75$, $p = 0.03$; Figure 1.5). Non-urban reaches were correlated with decreasing stream depth ($r = 0.78$, $p = 0.01$) and fine substrate type ($r = 0.58$, $p = 0.06$), and increasing herbaceous land cover (r = 0.69, $p = 0.03$), forested land cover ($r = 0.71$, $p = 0.02$), shrub land cover ($r = 0.53$, $p = 0.09$), and instream velocity ($r = 0.61$, $p = 0.05$; Figure 1.5). The species SIMPER analysis indicated that 78% of the dissimilarity between urban and non-urban streams was accounted for by six species (Table 1.3). Bluehead Chub accounted for 29% of the dissimilarity between urban and non-urban fish assemblage structure and were significant positive indicators of non-urban reaches. Similarly, the family SIMPER analysis indicated that the Cyprinidae family was a significant positive indicator of non-urban reaches and accounted for 63% of the dissimilarity between non-urban and urban stream reaches (Table 1.3).

Discussion

The results of this study demonstrate the impacts of urbanization on fish assemblage structure in headwater streams in North Carolina, one of the most rapidly growing metropolitan areas in the U.S. Few studies have attempted to evaluate the impacts of urbanization on fish assemblages using a multi-level approach and even fewer studies have evaluated stream ecosystems in the Southeast, which is expected to triple in total urban land area within the coming decades (Terando et al., 2014). Studies that have examined the impacts of urbanization

on stream fish communities have focused on species richness and have documented shifts from sensitive species to tolerant species with increasing urbanization (Karr 1981, Paul & Meyer 2001, Walsh et al. 2005, Kennen et al. 2005, Wenger et al. 2009, Meador et al. 2019). Similarly, the results from this study documented compositional shifts from sensitive cyprinid species in non-urban streams to less diverse assemblages of tolerant centrarchid species in steams with predominantly developed watersheds. However, the results of this study also documented shifts in species density and used density estimates to identify several species which were responsible for driving community shifts. Decreases in sensitive species density were attributed to reductions of Bluehead Chub, while the increase in tolerant species density in urban streams was attributed to increases in Green Sunfish and Redbreast Sunfish. The results of this study also indicated that fish assemblage structure was significantly influenced by watershed-level characteristics, which is consistent with knowledge that functional groups are sensitive to and structured by landscape-level changes (Wang et al. 2001, Hoeinghaus et al. 2007, Wenger et al. 2009, Kennen et al. 2015). Specifically, functional and taxonomic assemblages were negatively correlated with the percentage of developed land cover.

Fish communities have long been used as indicators of ecological degradation and to quantify anthropogenic effects on aquatic ecosystems (Karr et al. 1981, Chovanec et al. 2003, Hoeinghaus et al. 2007). Previous studies examining the impact of urbanization on fish communities have documented consistent shifts in fish assemblage composition in response to increasing urbanization (Walsh et al. 2005, Kennen et al. 2005, Wenger et al. 2009; Meador 2019). For example, fish assemblages warmwater streams have demonstrated consistent decreases in the richness of sensitive taxa (e.g., Bluehead Chub and Northern Hogsucker; Karr 1981, Kennen et al. 2005) and an increase in tolerant taxa (e.g., Green Sunfish and Redbreast

Sunfish; Paul & Meyer 2001) with increasing urbanization. Similarly, this study documented a shift from high densities of intermediate tolerance species (Cyprinids) in streams with forested watersheds to low densities of tolerant species (Centrarchids) in highly developed streams. The non-urban fish communities in this study were characterized by high densities of intermediate-tolerance level minnow species, such as the Bluehead Chub. While there were several species found exclusively in the non-urban streams (i.e. Northern Hogsucker, Pirate Perch, Creek Chubsucker, Pumpkinseed, Dusky Shiner, Johnny Darter, Yellow Perch, and Eastern Mudminnow) there were no sensitive or rare species found in forested watersheds. A plausible explanation for the absence of sensitive species is that current fish assemblages have been influenced by previous land use, such as agriculture, or the "ghost of disturbance past" (Harding et al. 1998). The urban stream fish communities in this study had lower overall densities and were comprised of tolerant species, such as Redbreast Sunfish and Green Sunfish. Similar studies in Piedmont streams throughout the Southeast have documented fish communities dominated by centrarchids (e.g., Redbreast Sunfish, Green Sunfish, and Bluegill) with increasing urbanization (Kennen et al. 2015, Meador et al. 2019). However, this study also indicated that mean centrarchid species densities (i.e. Redbreast Sunfish and Green Sunfish) increased in urban streams. Research has occasionally shown that the disappearance of sensitive species is accompanied by an increase in tolerant species, however these tolerant species are typically nonnative (i.e. Green Sunfish; Karr et al. 1981, Walsh et al. 2005, Wenger et al. 2009).

Additionally, the results from this study indicated that the shifts in fish assemblage with increasing urbanization were driven by decrease of the Bluehead Chub, and the establishment of tolerant species, Redbreast Sunfish and Green Sunfish. Bluehead Chub populations varied widely across urban and non-urban streams. Bluehead Chub had the greatest mean density of all

sampled species, experienced the greatest density declines with increasing urbanization, and were responsible for 30% of the variation between stream types. The species has long been used as an indicator of ecosystem health (Karr 1981, Lemly 1985) and is commonly the dominant species in pristine headwater streams (Lemly 1985, Weaver & Garman 1994, Montana 2018). Thus, similar studies have also documented dramatic declines in Bluehead Chub populations in response to urbanization (Weaver & Garman 1994, Kennen et al. 2005) and suggest that species decline is most likely due to the changes in substrate composition (e.g., siltation) and water quality (Peoples et al. 2011). The Bluehead Chub is an intermediate-level tolerant species that occurs in the pools and runs of headwaters, creeks, and small to medium sized streams with slow to medium current. Populations are found in streams with substrates composed of sand, rock, or gravel, which Male Bluehead Chub used to build nests (Frimpong & Angermeier 2010). As streams become urbanized, they experience more high-flow events and become heavily sedimented (Paul & Meyer 2001, Walsh et al. 2005). Thus, urbanization alters environmental characteristics (i.e., substrate type and stream flow) that are critical for stable Bluehead Chub populations. In contrast, urban streams were dominated by Redbreast Sunfish and Green Sunfish, which experienced increases population densities in urban streams. Both species are in the Centrarchidae family, which is a common indicator of aquatic degradation (Karr 1981). Redbreast Sunfish is a generalist and tolerant species that inhabits a variety of lotic and lentic habitats (Frimpong & Angermeier, 2010). Similarly, Green Sunfish is a tolerant, non-native species that inhabits pools and backwaters of streams and lakes (Frimpong & Angermeier, 2010). Similar studies on fish assemblage trends in urban streams have documented the proliferation of centrarchids (e.g., Redbreast Sunfish and Green Sunfish) with increasing urbanization (Kennen

et al. 2005, Roy et al. 2005, Wenger et al. 2009, Helms et al. 2019, Meador 2019), and attribute species dominance to their tolerance to habitat degradation (Lemly 1985, Wenger et al. 2009).

Fish assemblage structure in this study was strongly associated with watershed land cover and supports previous research focused on identifying mechanisms responsible for community shifts in urbanized streams (Kennen et al., 2005; King et al., 2005; Walsh et al., 2005; Wenger et al. 2009). A study by Wang (2001) identified percent urban land cover as an important indicator of stream fish assemblages. Specifically, the imperviousness of urban land cover affects stream hydrology (e.g., increased runoff and increased magnitude of high flow events) which causes stream degradation and resulting biotic responses (Wang et al. 2001). Alternatively, the relationship between forested land cover and non-urban fish assemblage suggests that it could also be related to forested connectedness. A study by Kennen et al. (2015) found a similar relationship between forested land cover fish assemblage structure, but specifically identified forest patch size as an indicator. The study documented changes associated with the conversion of forested land to urban land and suggested that forest fragmentation could impact aquatic ecosystems in a similar manner as terrestrial ecosystems. As fragmentation increases, forested, herbaceous, and shrubland land area (hereafter collectively referred to as vegetative land cover) becomes patchy and discontinuous. Decreased buffer area and riparian vegetation leads to altered water quality through soil erosion and runoff. Similarly, decreased vegetative land cover can result in decreased canopy cover and alterations to instream habitat and fish assemblage structure. Increases in impervious surface and developed land cover were indicators for urban assemblage structure with increases in runoff affecting sediment type, such as were seen for the functional group assemblage. While studies have highlighted the spatial factors and potential mechanisms influencing stream systems, the responses to urbanization vary and can be

disproportionate. For example, impacts to fish assemblages (e.g., decreased density, loss of sensitive species) can occur at as little as 5% developed land cover in the watershed (Paul & Meyer 2001, Miltner et al., 2004, King et al., 2005). Therefore, to elucidate urban impacts fish assemblages it is important to quantify watershed characteristics (King et al., 2005; Wenger et al., 2009).

Overall, this study is one of the few to examine the impacts of urbanization on stream fish communities and evaluate the impacts of rapid urbanization in the Southeastern United States. Currently, urbanization is expanding at an unprecedented rate and is expected to exponentially increase over the coming decades (Terando et al. 2014). Consequently, it is important to understand not only how fish communities respond, but to identify potential species driving community shifts. This study demonstrated the influence of land use changes within stream catchments on fish assemblage structure, and is one of the few to examine in-depth density shifts within a fish community in response to urbanization. Thus, my study provides a framework for evaluating the highly variable stream ecosystem responses to urbanization by evaluating assemblage structure to identify potential bioindicator species. This study indicates several excellent potential indicator species of urban stream degradation and urban stream restoration: Bluehead Chub, Green Sunfish, and Redbreast Sunfish. Future studies examining stream fish response to watershed urbanization should investigate species responses across a gradient of urbanization and identify population characteristic changes to link to potential mechanisms.

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TABLES

Table 1.1. Mean, standard error (SE), minimum (Min), maximum (Max), and coefficient of variation (CV) for catchment- and reach-scale environmental characteristics for urban and non-urban streams sampled in the Upper Neuse River Basin in North Carolina, 2016.

	Urban					Non-Urban					Overall				
Variables	Mean	SE	CV	Max	Min	Mean	SE	CV	Max	Min	Mean	SE	CV	Max	Min
Stream width (m)	5.7	0.9	26.5	7.4	4.5	4.6	0.5	24.6	5.9	2.6	5	0.4	26.3	7.4	2.6
Stream depth (m)	0.3	0.1	36.3	0.4	0.2	0.2	0	38.7	0.3	0.1	0.2	0	37.8	0.4	0.1
Stream velocity (m/s)	0.1	0.1	164.3	0.2	0	0.2	0.1	102.4	0.5	0	0.2	0.1	119.9	0.5	0
Dissolved oxygen (%L)	83.6	6.5	13.5	94.7	72.2	74.7	9.9	32.4	93.2	33.1	77.7	6.8	26.3	94.7	33.1
Temperature (°C)	22.9	0.6	4.8	24.1	22	22.5	0.7	7.2	24.7	22.1	22.6	0.5	6.2	24.7	22
Conductivity (µS/cm)	306	62	35.1	429.3	232.8	110.8	13	28.8	158.2	71.8	176	38.1	64.9	429.3	71.8
pH	7.5	0.1	1.9	7.6	7.4	7.6	0.1	2.6	7.8	7.4	7.6	0.1	2.4	7.8	7.4
Canopy cover (%)	55	22.7	77.7	95	10	65.5	7.5	28.2	93	40	62	8.8	42.6	95	10
Catchment area (km^2)	21.9	14.2	111.9	49.8	3.8	14.7	3.5	57.7	31.3	7.7	17.2	4.8	83.8	49.8	3.8
Boulder (%)	0	0	0	0	0	3.3	3.3	247.4	20	0	2.2	2.2	303	20	0
Gravel (%)	29	19	113.5	67	10	24.2	4.9	49.6	30	0	25.8	6.4	74.2	67	0
Sand (%)	41	4.9	20.8	50	33	35.8	16	109.8	75	0	37.6	10.5	83.7	75	0
Silt (%)	30	15.3	88.2	50	0	36.7	16.5	109.9	80	0	34.4	11.6	100.8	80	0

Table 1.2. Mean, standard error (SE), minimum (Min), and maximum (Max) densities (number per 100m^2) of fish sampled from streams in the Upper Neuse River Basin in North Carolina, 2016.

Family Common Name *Scientific Name*	Urban				Non-urban				Overall			
	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max
Aphredoderidae												
Pirate Perch (*Aphredoderus sayanus*)	0.0	0.0	0.0	0.0	0.6	0.4	0.0	2.2	0.4	0.3	0.0	2.2
Catostomidae												
Creek Chubsucker (*Erimyzon oblongus*)	0.0	0.0	0.0	0.0	0.4	0.4	0.0	2.3	0.3	0.3	0.0	2.3
Northern Hogsucker (*Hypentelium nigricans*)	0.0	0.0	0.0	0.0	0.6	0.3	0.0	2.0	0.4	0.2	0.0	2.0
Centrarchidae												
Redbreast Sunfish (*Lepomis auritus*)	6.1	5.5	0.0	17.0	6.8	4.7	1.0	30.0	6.6	3.4	0.0	30.0
Green Sunfish (*L. cyanellus*)	3.4	1.7	0.2	6.0	7.9	2.6	0.8	17.0	6.4	1.9	0.2	17.0
Pumpkinseed (*L. gibbosus*)	0.0	0.0	0.0	0.0	1.5	1.3	0.0	8.0	1.0	0.9	0.0	8.0
Bluegill (*L. marochirus*)	2.6	2.2	0.0	7.0	11.2	5.9	0.0	39.0	8.3	4.1	0.0	39.0
Largemouth Bass (*Micropterus salmoides*)	0.4	0.4	0.0	1.3	0.1	0.1	0.0	0.4	0.2	0.1	0.0	1.3
Cyprinidae												
White Shiner (*Luxilus albeolus*)	1.0	1.0	0.0	3.0	16.5	6.4	3.0	46.0	11.3	4.9	0.0	46.0
Bluehead Chub (*Nocomis leptocephalus*)	0.1	0.1	0.0	0.4	38.4	15.4	1.5	98.0	25.7	11.8	0.0	98.0
Dusky Shiner (*Notropis cummingsae*)	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.4	0.0	0.0	0.0	0.4
Swallowtail Shiner (*N. procne*)	6.4	6.3	0.0	19.0	9.0	2.9	0.0	21.0	8.1	2.6	0.0	21.0
Creek Chub (*Semotiuls atromaculatus*)	0.0	0.0	0.0	0.0	2.5	2.3	0.0	14.0	1.7	1.6	0.0	14.0
Esociade												
Redfin Pickerel (*Esox americanus*)	0.1	0.1	0.0	0.2	0.2	0.2	0.0	1.0	0.1	0.1	0.0	0.2

Table 1.2 (continued).

Fundulidae												
Speckled Killifish (*Fundulus rathbuni*)	0.0	0.0	0.0	0.0	1.6	1.5	0.0	9.0	1.0	1.0	0.0	9.0
Ictaluridae												
Yellow Bullhead (*Ameiurus natalis*)	0.9	0.5	0.0	1.7	4.8	2.3	0.0	13.0	3.5	1.6	0.0	13.0
Margined Madtom (*Noturus insignis*)	1.3	1.3	0.0	4.0	2.1	0.7	0.0	4.0	1.8	0.6	0.0	4.0
Percidae												
Johnny Darter (*Etheostoma nigrum*)	0.0	0.0	0.0	0.0	3.2	2.2	0.0	14.0	2.1	1.5	0.0	14.0
Tesseleated Darter (*E. olmstedi*)	0.3	0.3	0.0	0.8	1.4	1.3	0.0	8.0	1.0	0.9	0.0	8.0
Yellow Perch (*Perca flavescens*)	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.4	0.0	0.0	0.0	0.4
Poecillidae												
Eastern Mosquitofish (*Gambusia holbrooki*)	8.7	7.2	0.0	23.0	0.1	0.1	0.0	0.4	2.9	2.5	0.0	23.0
Umbriade												
Eastern Mudminnow (*Umbra pygmaea*)	0.0	0.0	0.0	0.0	0.3	0.2	0.0	1.0	0.2	0.1	0.0	1.0

Table 1.3. Analysis of similarity of percentage (SIMPER) of the nonmetric multidimensional scaling ordination (NMS) based on taxonomic (i.e., species and family) assemblage densities in the Upper Neuse River Basin in North Carolina, 2016. The individual contribution of each taxonomic group and the variation within each respective taxonomic assemblage is displayed.

NMS Type	Common Name	Individual contribution to variation (%)
Species	Bluehead Chub	28.5
	White Shiner	13.0
	Swallowtail Minnow	10.3
	Bluegill	9.7
	Eastern Mosquitofish	8.4
	Redbreast Sunfish	8.0
Total variation explained		77.9
Family	Minnow	63.2
	Sunfish	23.9
Total variation explained		87.1

FIGURES

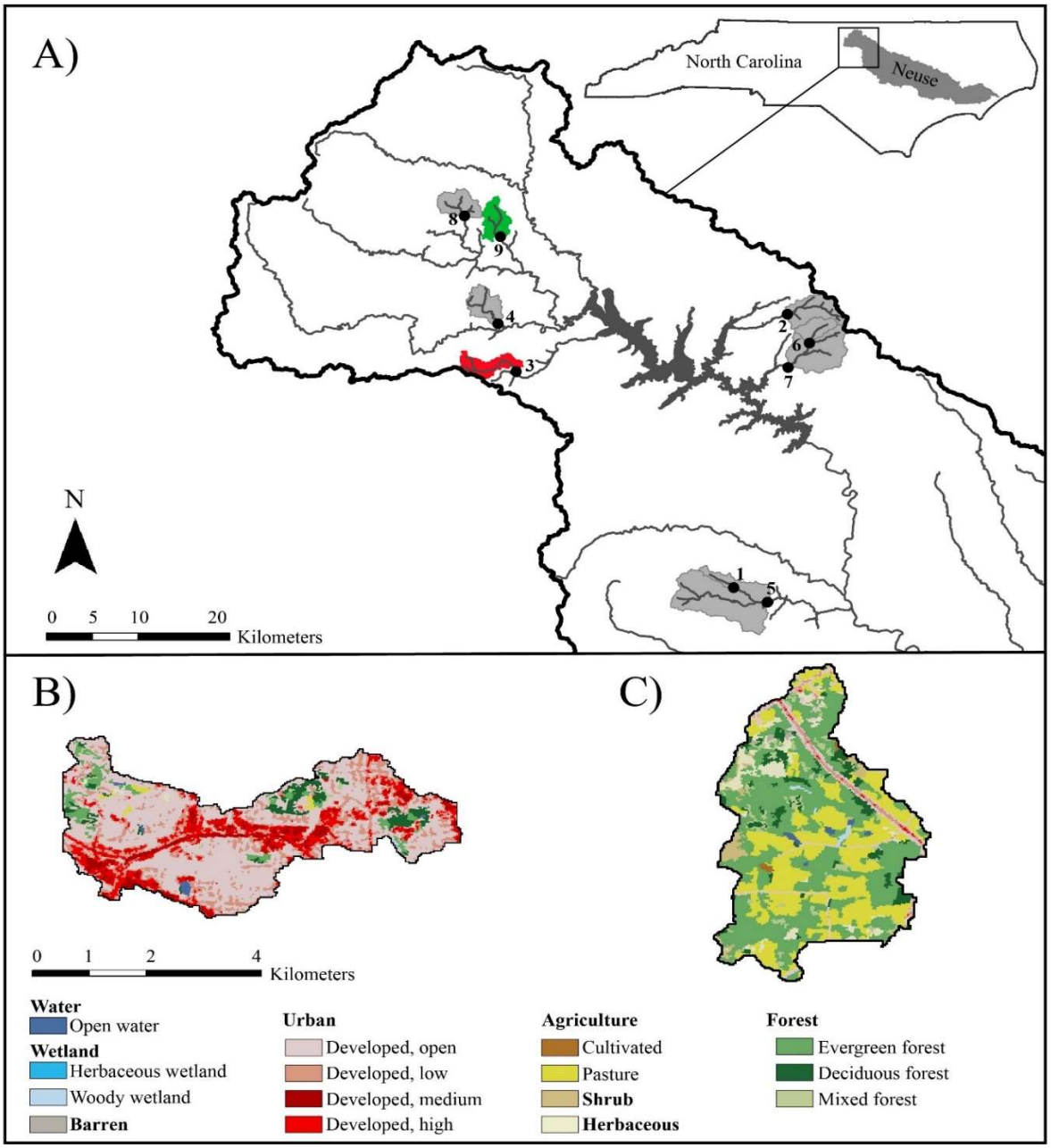

Figure 1.1. Upper Neuse River Basin, located in North Carolina, with locations of third order stream reaches sampled in 2016 and delineated catchments (A). Examples of urban (red) and non-urban (green) stream catchments are also highlighted. The same catchments are further enlarged to show examples urban (B) and non-urban (C) land cover classified using National Land Cover Data (NLCD 2011).

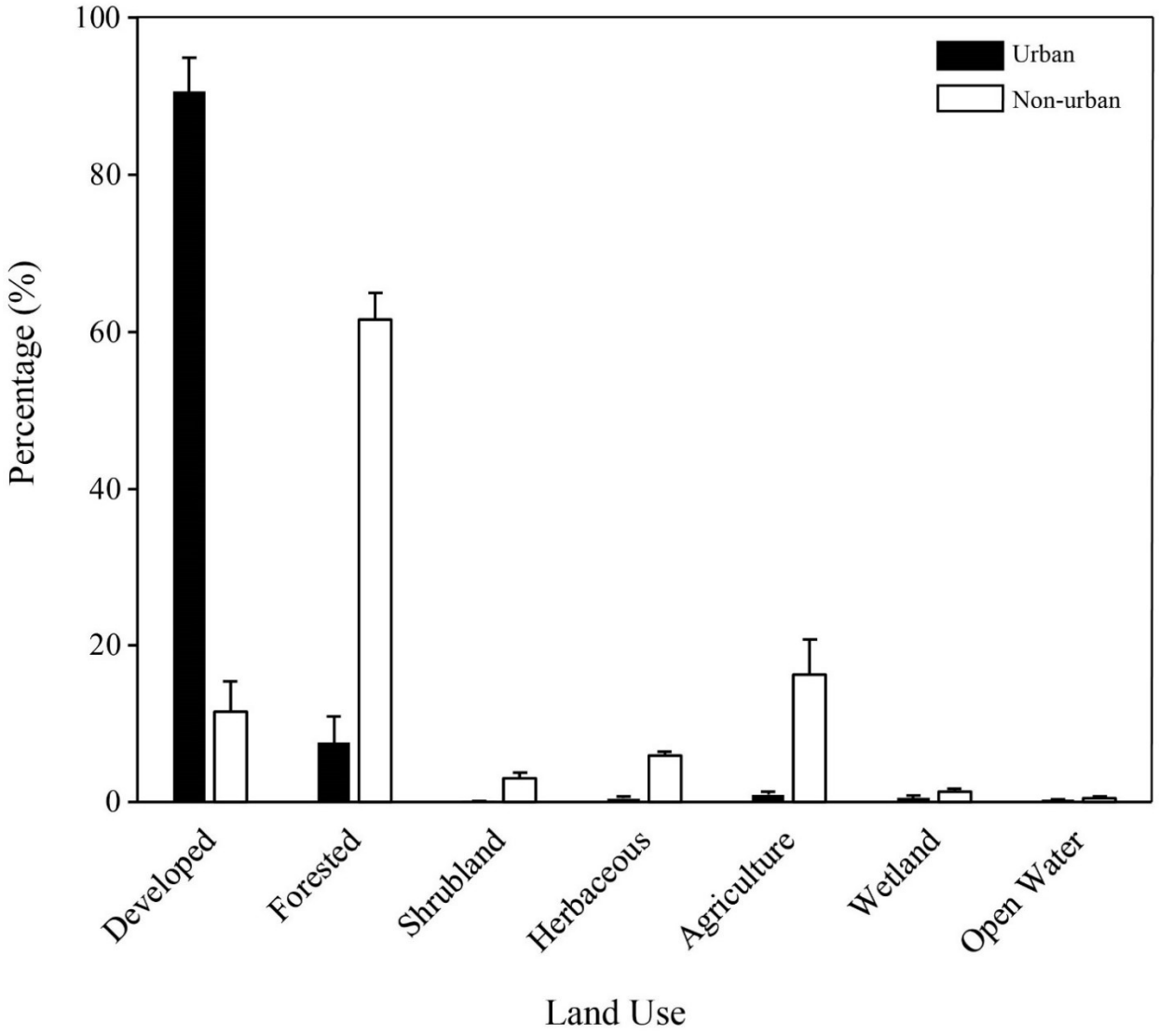

Figure 1.2. Mean percent land cover classifications using 2011 National Land Cover Database (NLCD 2011) for upstream delineated catchments of urban (*n*=3) and non-urban (*n*=6) stream reaches sampled in 2017. Bars represent one SE.

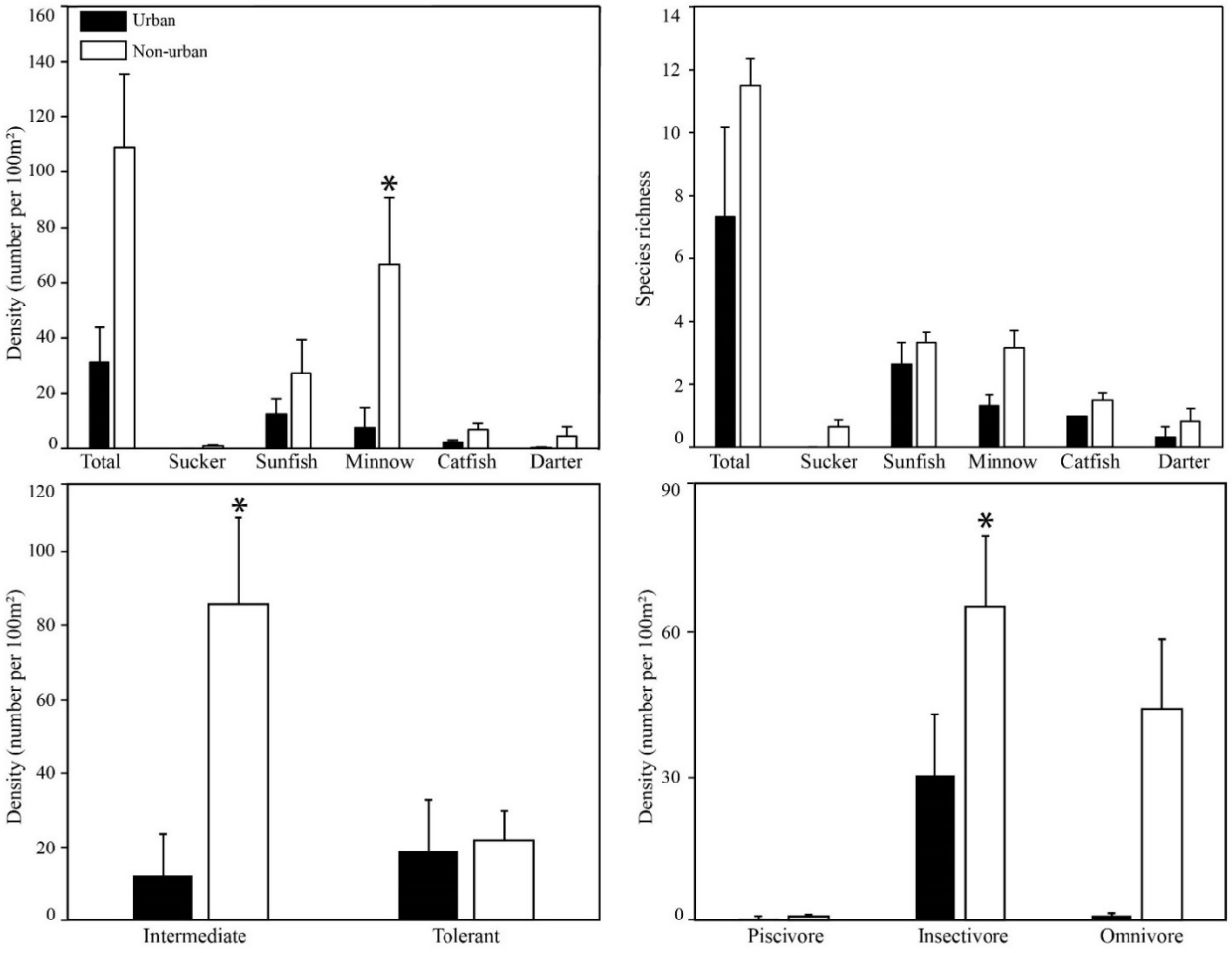

Figure 1.3. Mean density (number per 100 m^2) and species richness for fish sampled from streams within the Upper Neuse Watershed in North Carolina, 2016. Error bars represent one SE. Asterisks represent means with differences that are statistically significant.

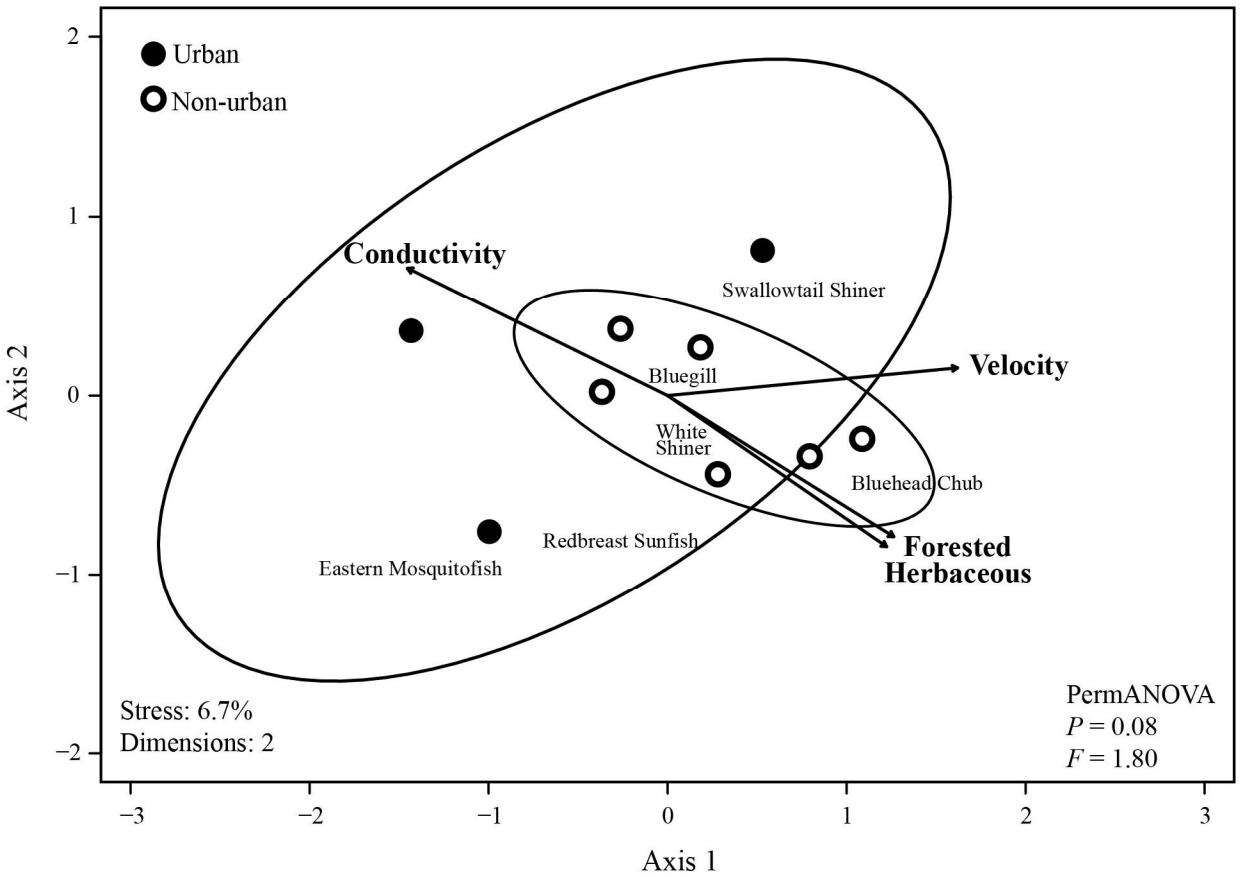

Figure 1.4. Nonmetric multidimensional scaling ordination of urban (closed circles) and non-urban (open circles) stream reaches using fish densities and environmental vector fitting from streams in North Carolina, 2016, with *P*-values and *F*-values from permutational analysis of variance (PERMANOVA). Ellipses denote grouping (urban or non-urban) and show the 90% CI around the centroid of each group. Species shown account for the most variation between urban and non-urban assemblages (78%).

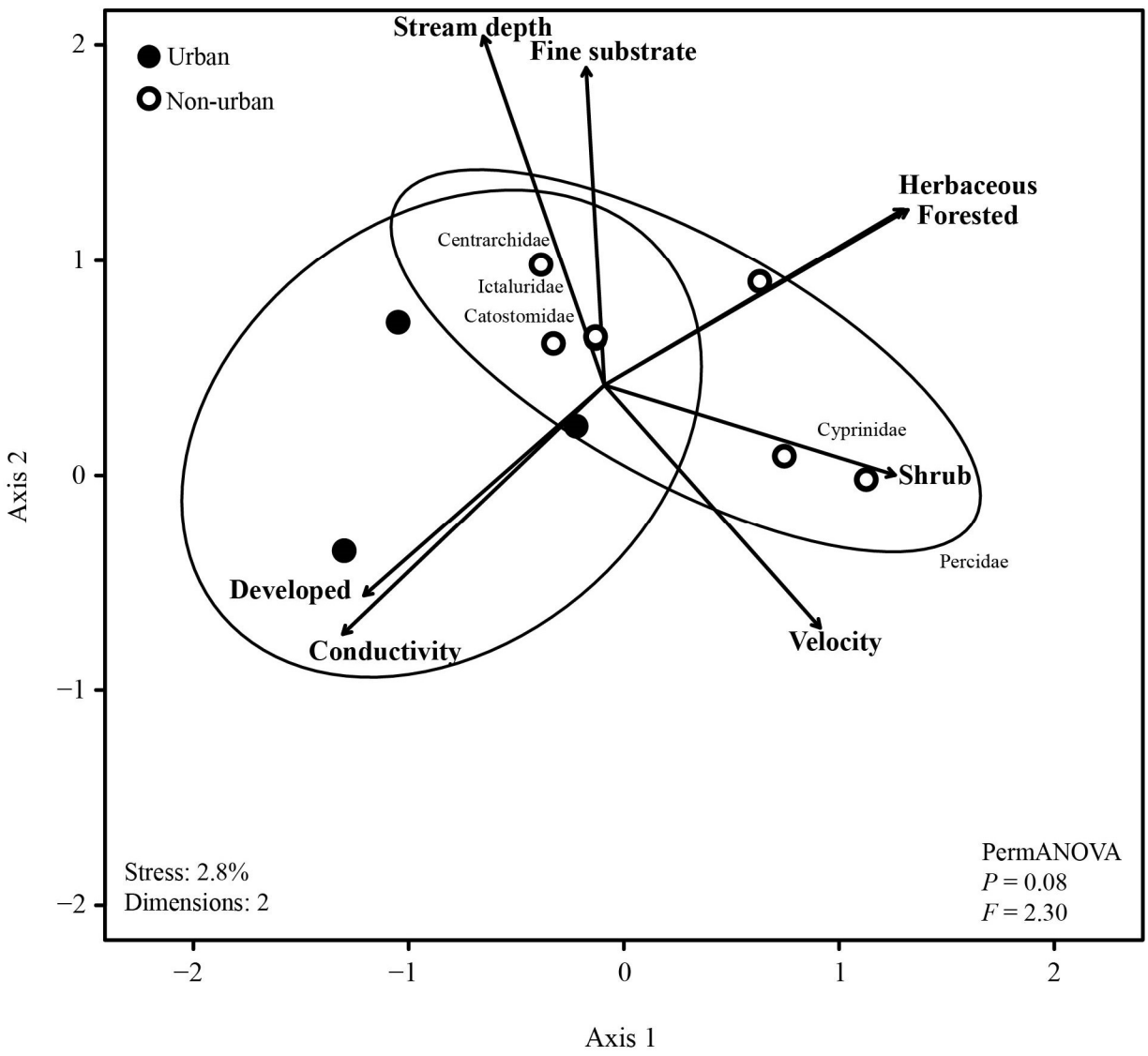

Figure 1.5. Nonmetric multidimensional scaling ordination of urban (closed circles) and non-urban (open circles) stream reaches using family densities and environmental vector fitting from streams in North Carolina, 2016, with *P*-values and *F*-values from permutational analysis of variance (PERMANOVA). Ellipses denote grouping (urban or non-urban) and show the 90% CI around the centroid of each group. Families shown account for the most variation between urban and non-urban assemblages (63%).

CHAPTER 2: Variation in Population Characteristics and Ecological Stoichiometry of Three Sunfishes Across an Urbanization Gradient

Abstract

Urbanization degrades stream ecosystems and reduces habitat complexity. Urban stream degradation consistently results in decreased richness and density of sensitive taxa with increased abundance of tolerant species. However, relationships between urbanization and aquatic biota are complex and include multiple stressors. Thus, the mechanisms by which urbanization affects aquatic biota are poorly understood, particularly for stream fishes. In this study I evaluated fish populations across an urban environmental gradient to identify potential mechanisms of urbanization that drive fish responses. I conducted large-scale surveys in third-order streams (n=22) in the Upper Neuse River Basin in North Carolina to evaluate the population characteristics of three Centrarchid species: Green Sunfish (*Lepomis cyanellus*), Redbreast Sunfish (*L. auritus*), and Bluegill (*L. machrchirus*). Basic population characteristics (size distribution, condition, age, and growth) and elemental stoichiometric characteristics (%C, %N, %P, and C:N:P ratios) were quantified for each focal species. Principal component analysis (PCA) was used to reduce the dimensionality of catchment and reach environmental characteristics and provide an interpretable gradient. Spearman Rank Correlation analysis was then used to identify intercorrelations between environmental characteristics and focal species characteristics. Bluegill and Green Sunfish had a weak negative association with increasing watershed urbanization. Redbreast Sunfish were strongly and positively associated with urbanization. These results revealed disparities in age and size structure between species found in both habitats suggesting that altered resource quality and differences in competition dynamics may be simultaneously responsible for observed population characteristics.

Introduction

Stream ecosystems have been severely degraded by numerous anthropogenic disturbances (Lenat & Crawford 1994, Paul & Meyer 2001, Kennen et al. 2005, Walsh et al. 2005, USEPA 2006, Wilson et al. 2011). Urbanization has been identified as a primary degrading anthropogenic influence on stream ecosystems and is the sole cause of impairment for almost 20% of streams in the United States (Paul & Meyer 2001, Walsh et al. 2005, USEPA 2006, Wenger et al. 2009). As watersheds become urbanized, numerous negative physical, biogeochemical, and biological responses collectively termed the urban stream syndrome have been well-documented (Meyer et al. 2005, Walsh et al. 2005, Wenger et al. 2009). Specifically, as watersheds become urbanized there are increases in the frequency of short-duration flash flood events, which affect the runoff processes and leads to an increase in the amount of sediments and nutrients delivered to streams (Paul & Meyer 2001, Walsh et al. 2005). As a result of the increased flow, stream banks become eroded and streams become deeper and more channelized (Paul & Meyer 2001, Walsh et al. 2005). Additionally, high concentrations of nutrients and increased intense flow events lead to nutrient loading of streams (Paul & Meyer 2001) and changes to the spatial and temporal availability of nutrients to aquatic biota (Sterner & Elser 2002). The physical and chemical changes to urban streams decrease the complexity and stability of streams, and result in increasing habitat suitability for tolerant species and decreasing habitat suitability for all other aquatic biota (Paul & Meyer 2001, Walsh et al. 2005, Helms et al. 2005, Kennen et al. 2005, Wenger et al. 2009).

Degradation of urban stream habitat has been repeatedly associated with declines in species richness, density, and overall shifts in community structure in streams (Karr 1981, Wang et al. 2000, Walsh et al. 2005, Roy et al. 2005, Tsoi et al. 2011, Weaver et al 2011). Fish

communities are highly sensitive to urban stream degradation (Karr 1981, Paul & Meyer 2001, Chovanec et al. 2003), and have demonstrated responses (e.g., declines of sensitive species and functional groups) at low levels of watershed urbanization (5-10% total urban area; Paul & Meyer 2001, Wenger et al. 2009). Often, the decline of fish sensitive species in urban streams is accompanied by an increase in tolerant species (Karr et al. 1981, Wenger et al. 2009, Helms et al. 2018). For example, studies evaluating fish assemblages in the Southeast U.S. have documented a proliferation in tolerant Green Sunfish populations in urbanized streams (Karr 1981, Roy et al. 2005, Helms et al. 2005, Walsh et al. 2005, Chapter 1). While fish community responses to urbanization are well-documented (Paul & Meyer 2001, Walsh et al. 2005, Tsoi et al. 2011, Meador et al. 2019), few studies have evaluated the impacts of urbanization on populations of specific species. Fish populations are highly structured by environmental conditions and are commonly used to quantify ecosystem stability (MacArthur 1955, Karr 1981, Lemly 1985). Specifically, fish species represent specific ecological traits or functions (e.g., trophic guild, functional feeding guild) that can change as a result of environmental variation (Hoeinghaus et al. 2007, Sammons et al. 2009, Helms et al. 2018). For example, studies have documented shifts in diet, growth, and trophic position between centrarchid populations in urban and degraded streams Lenat & Crawford 1994, Helms et al. 2018). Fish populations could, therefore, be used as bioindicators of degradation and could be used to quantify anthropogenic decline by quantifying fish fitness and provide a more accurate relationship to ecosystem health (Chovanec et al. 2003, Hoeinghaus et al. 2007).

Ecological stoichiometry provides an additional framework to quantify anthropogenic impacts to fish populations across disturbance gradients (Glibert 2012). Ecological stoichiometry examines the transfer, flow, and movement of nutrients in ecological systems (Sterner & Elser

2002, Tsoi et al. 2011, Milanovich et al. 2014) and can quantify the nutrient loading and spatial variability in nutrients in urban streams (Paul & Meyer 2001, Tsoi et al. 2011). Ecological stoichiometric theory is based on the assumption that all living organisms are hardwired to maintain a strict homeostasis of elemental composition and that the ratio of elements remains (C:N:P) relatively balanced between consumers and food resources (Sterner & Elser 2002, McIntyre & Flecker 2010, Tsoi et al. 2011, Glibert 2012). A key understanding of ecological stoichiometry is that the species are constrained by the relative availability of elements (McIntyre & Flicker 2010). Therefore, while species strive to maintain a strict homeostasis dramatic shifts in available nutrients can cause species to deviate from their specific elemental composition (Cross et al. 2003, Winemiller et al. 2011, Milanovich et al. 2014). Producers have a relatively plastic elemental homeostasis and are likely to be impacted by shifts in nutrient availability (Tsoi et al. 2011, Glibert 2012), however vertebrates maintain a strict homeostasis of elemental composition of C:N:P (Stener & Elser 2002). Therefore, small shifts in vertebrate stoichiometry suggest large or dramatic shifts in the transfer, availability and flow of elements to a system (Sterner & Elser 2002, Hendrixson et al. 2007, Tsoi et al. 2011). For example, as urbanization causes nutrient loading in streams (Walsh et al. 2005, Grimm et al. 2005, Tsoi et al. 2011), increased nutrient availability to aquatic biota can alter the elemental composition of producers and causes primary consumers become nutrient-rich in both nitrogen and phosphorous (Cross et al. 2003, Milanovich et al. 2014). Recent studies have shown that the consumer-resource elemental imbalances propagate up the food web and ultimately change the stoichiometry of vertebrates (Sterner & Elser 2002, Feijoo et al. 2014). The deviations in vertebrate stoichiometry can consequently impacts their physiology and ecological processes, such as growth or reproduction (Sterner & Elser 2002, Montana et al. 2018). Therefore,

ecological stoichiometry can be used to provide an integrated assessment of ecosystem changes when incorporated with fish population characteristics (McIntyre et al. 2010).

The relationship between urbanization and stream ecosystems is complex, and often includes multiple stressors (Wang et al. 2000, Wang et al. 2001, Wenger et al. 2009). While urbanization is often categorized as a discrete group (i.e., urban or non-urban; Kennen et al. 2005, Tsoi et al. 2011, King et al. 2015, Montana et al. 2018), there is no universal threshold of percent impervious or urban land cover at which stream ecosystems respond (Wenger et al. 2009). It is important to consider urbanization across a gradient and understand impacts across a gradient of scales, rather than focus on catchment-level factors (Wenger et al. 2009, Engman et al. 2012, King et al. 2015). Therefore, the goal of this study was to use a multi-concept approach to understand the how the populations of three focal species change across an urban environmental gradient. The objectives of this study were to characterize watershed traits across an environmental gradient, to identify influential abiotic factors across multiple scales, and to link important environmental variables to the population characteristics and elemental stoichiometry of three centrarchid species in North Carolina.

Methods

Study Area

This study was conducted in the Neuse River watershed in North Carolina, U.S (Figure 2.1). The watershed includes the Neuse River, which flows 400 km to the Pamlico Sound and has a watershed area greater than 16,000 km^2 (NCDENR 2009). Sampling occurred within the Upper Neuse River Subbasin (hereafter referred to as the "Upper Neuse") from May to August 2017. The Upper Neuse has a total watershed area of 1,994 km^2 and is predominantly forested

land area (61%), but also includes urban/developed (17%) and agricultural (16%) land types. Land cover is rapidly changing within the Upper Neuse, primarily driven by the Raleigh-Durham metropolitan area. The Raleigh-Durham area holds 2.2 million people over 14,270 km^2 and has been one of the fastest-growing cities in the United States (4% population increase every year since 2010; Ewing et al. 2002; Kennen et al. 2005; U.S. Census Bureau 2017, UN Population Division 2019). Predictions suggest that developed area within the Upper Neuse is expected to grow 28% by 2025 in response to a nearly 50% increase in population size (NCDENR 2009).

Site Selection

Twenty-two third-order streams were selected throughout the Upper Neuse across an urban-forest land cover gradient (Figure 2.1). Streams were sampled between May and August 2017. A single sample site was selected at each stream based on several criteria that minimized potential variation in specific environmental characteristics. For example, all stream reaches had similar natural characteristics such as catchment size and stream size, and were sampled at base flow. Each sampling site was a continuous, wadable reach that length varied from 50-300 m. Stream reach length varied due to the highly variable fish densities between urban and non-urban streams (Chapter 1), and were sampled in equal 50 m intervals until a minimum of 10 individuals (per focal species) were collected or a maximum of 300 m was sampled.

Watershed and Land Cover

To determine the land cover for each stream site, I delineated the stream reach catchment and quantified land use as percent land cover of the total stream reach catchment area. Stream catchments were characterized as the entire area draining to a sampling point. The coordinates for the farthest upstream sampling point, or batch point, were used to delineate each catchment. Catchments were delineated using 1-arc second, 30-m resolution United States Geological

Survey (USGS) National Elevation Dataset digital elevation model (USGS 2016) and Arc Hydro tools methods in ArcGIS 10.5.1 (ESRI™, Redlands, California, USA). Stream catchment boundaries were overlaid on 30-m resolution 2016 National Land Cover Data (NLCD; Yang 2018), and land cover class percentages were calculated for each catchment. The NLCD land cover data included 18 minor land cover types (Yang 2018), which were summarized into eight major land cover types (Figure 2.1; Wang et al. 2000, King et al. 2005, Gabriel et al. 2018): water (open water), urban (open space, low-, medium-, and high- intensity developed), barren (barren land, rock, sand, and clay), forest (deciduous, evergreen, and mixed forest), shrubland, herbaceous, agriculture (hay/pasture and cultivated crops), and wetland (woody wetlands and emergent herbaceous wetlands).

Physical habitat

Stream reaches varied from 50-300 m in total length, and total length was determined using the criteria described above. Study stream reaches were sampled in 50 m transects, and habitat data was taken in the middle of the farthest upstream run of every 50 m transect. Temperature, conductivity, dissolved oxygen, and pH were measured using a Yellow Spring Instruments (YSI) model Professional Pro Multiparameter handheld instrument (YSI Life Sciences, Yellow Springs, OH). Stream wetted width and depth were measured, and instream velocity was determined by measuring the velocity at 60% of the water column height using a GEOPACK MFP51 Standard Stream Flowmeter (GEOPACKS, Hatherleigh, UK, McMahon et al. 1996). Canopy cover and the dominant substrate types were visually determined at each transect and averaged for the entire stream reach. Canopy cover was estimated as the percent shading or cover of the stream. Substrate cover types were based on a modified Wentworth scale and included the following: boulder, cobble, gravel, sand, mud, and silt (Cummins 1962). All

physical stream characteristics were measured the same day as fish sampling (May-August 2017). Mean physical habitat characteristics were summarized for all stream reaches.

Focal species

The population characteristics of three centrarchid species – Bluegill (*Lepomis machrchirus*), Redbreast Sunfish (*L. auritus*), and Green Sunfish (*L. cyanellus*) – were evaluated to understand the relative influence of environmental characteristics across an urban landscape. The three focal species were common throughout the study area (Menhinick 1991, Kennen et al. 2005, Chapter 1) and are established bioindicators of habitat degradation (Karr et al. 1981, Chovanec et al. 2003, Roy et al. 2005, Helms et al. 2018) but still abundant in undisturbed habitats (Kennen et al. 2005, Chapter 1). Focal species were selected because they represented different functional feeding groups, tolerance levels, and habitat requirements. Bluegill are insectivores that inhabit streams with macrophyte cover and slow currents and have an intermediate level tolerance to poor stream quality (Frimpong et al. 2010). Redbreast Sunfish are insectivores that inhabit slow streams with fine, sand, gravel, and cobble substrate (Cooner and Bayne 1982, Rohde et al. 1994, Frimpong & Angermeir 2009). Green Sunfish are insectivores that inhabit slow to medium current streams with fine, clay, sand, and gravel substrates and macrophyte cover (Werner et al. 1977, Frimpong & Angermeir 2009). Both Redbreast Sunfish and Green Sunfish are tolerant to habitat degradation (Karr et al. 1981) and are associated with increases in urbanization (Lemly et al. 1985, Weaver et al. 1994, Kennen et al. 2005, Roy et al. 2005, Helms et al. 2018). The functional traits of each species are dependent upon environmental variables, and would be expected to change with increasing catchment urbanization (Figure 2.2; Hoeinghaus et al. 2007, Helms et al. 2019). Therefore, changes to functional traits can be used to infer the influence of environmental variation on population responses (Hoeinghaus et al. 2007).

The focal species also exhibit common stoichiometric characteristics that make them ideal for comparison (Figure 2.2; Montana et al. 2018). Elemental composition varies among species, however, fishes in the centrarchid family have similar elemental ratios (Sterner & George 2000). Centrarchids are heavily ossified, and therefore have higher P content compared to fishes in other families (Sterner & George 2000). Body size is also an important factor and driver of overall individual elemental composition, and studies show that that C:P ratios decrease with increasing body size (Sterner & George 2000; Hendrixson et al. 2007, McIntyre & Flecker 2010). Comparisons of centrarchid species in degraded habitats have used the functional variation between centrarchids to identify specific environmental mechanisms driving degradation (Osenberg et al. 1988, Lenat & Crawford 1994, Kennen et al. 2005, Devries et al. 2009, Werner et al. 2009, Montana et al. 2018, Burgad et al. 2019).

Fish sampling and collection

Fish were sampled using a backpack-mounted pulsed-DC electrofisher (Smith-Root, Inc., Vancouver, Washington) with two to four netters in a single upstream pass. Stream reaches were sampled following the continuous 50 m transects as described above, moving upstream in a zigzag pattern to ensure all available habitats were sampled. At the end of each 50 m interval all fish from the transect were immediately processed. All sampled individuals were identified to species, and counted. After fish were processed, they were held in aerated live wells outside the stream. Fish were released into the stream after all transects were sampled. Individuals that could not be identified or were a focal species were euthanized using rapid freezing methods outlined by the American Veterinary Medical Association guidelines (Leary et al. 2013) and immediately transported back to the laboratory.

In the laboratory, specimens were measured to the nearest mm (total length [TL]) and weighed to the nearest 0.1 g. After all individuals of a focal population were processed, six to eight specimens were selected for stoichiometric analysis and kept in a -20°C freezer until further analyses. The six to eight individuals of each focal species collected from each stream reach for elemental analysis were pooled to analyze whole body C, N, and P (Sterner & Elser 2002, Tsoi 2011, Mehler 2013). Individuals were selected out of the larger population to include a wide distribution of the lengths of each focal species (Sterner & Elser 2002). All other individuals were used for age and growth analysis and otoliths were extracted before fish were preserved in 70% ethanol.

The relative abundance of centrarchids in each stream reach was quantified as the number of fish per 50 m^2. Population size structure was described using proportional size distribution (PSD) of quality length fish (PSD-Q) (Anderson 1976). PSD was calculated using standard lengths defined by Gabelhouse (1984) following the methods outlined in Ogle (2016). Condition was estimated for Green Sunfish and Bluegill using relative weight (no estimates exist for Redbreast Sunfish; Wege & Anderson 1978, Neumann et al. 2012) based on methods detailed by Ogle (2016). Mean population characteristics were estimated by species for all stream reaches.

Length at age

Sagittal otoliths were removed from all sampled fish at each site. Whole otoliths were read under a compound microscope and assigned ages using the methods described in Buckmeier & Howells (2003). Otolith diameter at each annulus was measured from the focus to the outer edge of the annulus to the nearest µm using image pro analysis (Image-Pro Plus, Media Cybernetics Silver Springs, Maryland). Back-calculated lengths at age were determined using the direct proportional method (Devries & Frie 1996). Mean back-calculated length at age, mean

age, and age-specific abundance (number of fish at age interval per 50 m^2) were summarized by species for each stream reach.

Elemental analysis

Whole fish elemental analysis was conducted following methods outlined by Montana et al. (2018) and Sterner et al. (2002). At 6 of 22 stream sites one or more of the focal species were not abundant enough to allow for elemental analysis. Redbreast Sunfish were analyzed at 8 of 22 sites, Green Sunfish were analyzed at 7 of 22 sites, and Bluegill were analyzed at 9 of 22 sites. In the laboratory, individuals collected for elemental analysis were measured to the nearest mm (total length), weighed to the nearest 0.01 g and kept in a -20°C freezer until they were analyzed. Individuals were then pooled together by focal species and stream location, and the wet mass of each sample was measured to the nearest 0.01g. All samples were dried in an oven at 60°C for up seven days, or until sample weights stabilized. Samples were homogenized into a fine powder using a mortar and pestle and stored in a desiccator until analysis. For P analysis, 17 to 21mg of dried content was weighed to the nearest µg. Samples were combusted in a muffle furnace at 500°C for 4 hours and digested in 5ml of 0.2 HCL at 60°C for 60 minutes (Fourqurean & Zieman 1992). The digested samples were analyzed for SRP (soluble reactive phosphorous) using the acid-molybdate method (Parson et al. 1984). Samples were run on Evolution 201 UV-Visible Spectrophotometer (Thermo Fisher Scientific, Bermen, Germany) and converted to % P. Pine needle (NIST #1575) was used as the standard reference and the efficiency of P extraction was >95%. For analysis of C and N content all dried and ground samples were analyzed at the University of Georgia Stable Isotope Laboratory, University Georgia, U.S. and results presented at percent values (%). All C, N, and P samples were run in triplicate and contents were

represented by % mass, and were converted to C:N, C:P, N:P molar ratios. The mean elemental composition data were summarized by species for each stream reach.

Statistical Analysis

A principal component analysis (PCA) was used to reduce the dimensionality of catchment and reach environmental characteristics and create new variables that best characterize the underlying gradient of fish assemblage and population structure. The PCA was performed using 21 abiotic variables (Table 2.1) for each stream reach. All variables were tested for normality using an Anderson-Darling test. A multiple analysis of variance (MANOVA) was used to determine whether population or elemental characteristics differed among focal species (Johnson 1998). If the MANOVA results were statistically significant, then one-way ANOVA was used to determine how focal species characteristics differed. A Spearman rank correlation test was used to examine the intercorrelations between population characteristics (Table 2.2) and environmental characteristics (Table 2.1) for each focal species. All analyses were performed using R 3.6.1 (R Development Core Team, 2017). A type I error rate of 0.10 was used for all statistical tests.

Results

Abiotic Characteristics

Sampled stream watersheds spanned the land-use gradient and encompassed a variety of land use types, but were dominated by developed (38 ± 7%), or forested (43 ± 5%) and agricultural (13 ± 3%) land use types (Table 2.1). Six stream catchments were majority urban land cover (64.5-93.3%; Table 2.3), eight study stream watersheds were majority forested (52.5-

95.6%; Table 2.3), and the remaining eight watersheds were neither dominated by forested nor developed land cover (Table 2.3).

The environmental PCA resulted in a three principal components solution which explained a cumulative 47.7% of the variance among stream reaches (Table 2.1, Figure 2.3-2.5). The PCA reduced the abiotic dataset and identified three orthogonal environmental gradients. The first principal component (PC1) identified watershed-level variables that explained 21.9% of the variance. Percent forested land cover, percent developed land cover, and percent herbaceous land cover had the highest loadings in PC1. Overall, PC1 identified a land-use gradient, moving from heavily forested to heavily urbanized stream watersheds (Figure 2.3). Principal component 2 (PC2) accounted for 14.5% of the variance between stream sites. The highest loadings for PC2 were stream catchment area, stream width, stream velocity, and instream temperature, indicating stream reach characteristics that were associated with watershed land cover. Streams with urbanized watersheds were related to small catchment sizes and low stream velocity, while forested streams were related to faster stream velocities with large watershed sizes (Figure 2.3-2.5, Table 2.3). Principal component 3 identified instream variables that accounted for 11.4% of the variance between stream reaches. The highest loadings for PC 3 were gravel substrate, and fine substrate. Principal component 3 indicated that instream factors such as substrate composition were important in further separating streams, creating a gradient from streams composed of predominantly gravel in urban watersheds to streams with fine substrate in non-urban watersheds.

Population Characteristics

A total of 2,464 individuals from 30 species were sampled across all stream reaches. Fish population characteristics were summarized across all stream sites (Table 2.2, Table 2.4).

Bluegill (557 total individuals) and Green Sunfish (604 total individuals) were present at 12 of the 22 stream reaches. Redbreast Sunfish (498 total individuals) were collected from 9 stream reaches. Mean population characteristics differed significantly among centrarchids, however separate ANOVAs revealed that only PSD differed among populations (MANOVA $p < 0.05$). The mean PSD varied across focal species (ANOVA $p < 0.05$, $F = 50.25$, $df = 2$) and was greatest for Redbreast Sunfish populations (Table 2.2). However, mean PSD values were relatively low for all species, suggesting most sampled locations had few to no stock length fish. Spearman rank correlations were used to determine if environmental characteristics were associated with population characteristics of the centrarchid species. There were few significant correlations between Bluegill populations and environmental characteristics, but I did find a negative association between Bluegill relative weight and canopy cover ($\rho = -0.76$, $P < 0.05$). Bluegill had the greatest mean relative weight (79.8 ± 2.1) of the three focal species. Bluegill relative weight was relatively low and indicated that on average bluegill weighed 80% of the standard Bluegill weight (Wege & Anderson 1978). Similarly, I found that Green Sunfish mean relative weight (72.3 ± 1.6) had a weak, negative association with temperature ($\rho = -0.44$, $P = 0.15$). The strongest correlation for Redbreast Sunfish population characteristics was between mean PSD and percent shrub land cover ($\rho = 0.73$, $P = 0.03$), followed by mean Redbreast Sunfish abundance and percent forested land cover ($\rho = -0.66$, $P = 0.05$).

Age Characteristics

Totals of 485 Bluegill, 532 Green Sunfish, and 444 Redbreast Sunfish were aged across stream reaches. I found that the age characteristics varied among focal species (MANOVA $p < 0.05$; Table 2.2, Figure 2.6). Mean age of focal species age was significantly different (ANOVA $p < 0.05$, $F = 17.90$, $df = 2$) and was the highest for Redbreast Sunfish populations (2.5 ± 0.3

years). Additionally, the mean back-calculated-length at age-1 (ANOVA P < 0.01, F = 8.52, df = 2) and age-2 (P = 0.02, F = 4.76, df = 2) was substantially greater for Bluegill than for the other two focal species (Table 2.6). Spearman rank correlation analyses were used to compare centrarchid population and environmental characteristics. There were limited significant correlations between Bluegill age characteristics and environmental variation. The strongest relationship was a weak, negative association between Bluegill mean back-calculated-length at age-2 and percent agricultural land cover (ρ = -0.53, P = 0.08). I found several significant relationships between Green Sunfish age characteristics and PC3. Green sunfish mean age (ρ = 0.69, P = 0.01) and age-5 abundance (ρ = 0.61, P = 0.03) were positively correlated with PC3. Conversely, PC3 was negatively associated with the mean Green Sunfish age-1 abundance (ρ = -0.56, P = 0.06). Similarly, Redbreast Sunfish age characteristics were related to PC1. I found that Redbreast Sunfish mean age was negatively correlated with PC 1 (ρ = -0.62, P = 0.08).

Elemental Stoichiometry Characteristics

Whole fish stoichiometry for Bluegill, Green Sunfish, and Redbreast Sunfish was summarized across a subset of stream reaches (9, 7, and 8 stream reaches, respectively; Table 2.2, Figures 2.6-2.7). Fish stoichiometry varied across reaches but was not significantly different. Bluegill populations were characterized by high %N and low %C values that remained relatively consistent across stream sites. Mean %N was negatively correlated with PC 3 (ρ = -0.70, P = 0.04), indicating that populations in forested watersheds had lower %N than populations in highly urbanized watersheds. Green Sunfish populations had highly variable mean %N across stream reaches, and correspondingly low N/P ratios, with the lowest %N values in streams with highly urbanized catchments. Similarly, highly urbanized stream watersheds were positively correlated with low mean %P in Green Sunfish populations (ρ = -0.86, P = 0.13). Redbreast

Sunfish populations had variable mean %N across streams. The highest mean %N and the lowest %N were observed in the same stream reaches as for Green Sunfish. Population %N was positively correlated with age 2 and age 3 abundance ($\rho = 0.62$, $P = 0.09$; $\rho = 0.72$, $P = 0.04$), indicating mean %N was associated with high abundances of older individuals. There was a positive correlation between %P and body size and age across all centrarchid species, which was consistent across stream reaches.

Discussion

This study examined the population and elemental characteristics of three centrarchid species across a diverse landscape in North Carolina undergoing rapid urbanization. Few studies have evaluated fish population responses to urbanization, but previous research on stream fish responses to stream degradation has demonstrated that centrarchids are tolerant to poor habitat conditions (Karr 1981, Paul & Meyer 2001, Walsh et al. 2005). However, this study demonstrated that centrarchid species have varying responses to urban stream degradation. Redbreast Sunfish were positively associated with urbanization and their juvenile abundance increased as watersheds became more urban. Conversely, Green Sunfish responded negatively to urbanization and demonstrated decreases in mean age and abundance with increasing urbanization. Similarly, Bluegill responded negatively to urbanization, but there were few significant relationships between Bluegill populations and urban characteristics. Additionally, few studies examined the ecological stoichiometry of fishes in urban streams or attempted to link the traits to population characteristics. The results in this study identified associations in whole body stoichiometry and watershed urbanization. Redbreast Sunfish juvenile populations had P-enriched diets in urban streams, whereas Green Sunfish %P was negatively associated with

increasing urbanization. My results agree with other studies showing decreased abundances and elemental shifts with increasing urbanization (Montana et al 2018, Wenger et al 2009, Tsoi et al 2011, Glibert et al 2012, Kennen et al 2015).

Redbreast sunfish are a ubiquitous species that is tolerant to degradation (Karr et al. 1981, Roy et al. 2005, Helms et al. 2005), but few studies have attempted to understand population responses to watershed urbanization (Helms et al. 2005). This study found a positive association between Redbreast Sunfish population characteristics and watershed urbanization. Specifically, I observed a positive association between age-1 abundance and increasing developed land cover. Similarly, a study by Kennen et al. (2005) found an increased abundance of Redbreast Sunfish in watersheds with heavily fragmented forested areas. There was also a negative association in the mean and minimum age of Redbreast Sunfish populations with increasing urbanization. The relationship between juvenile abundance with increasing urbanization suggests that Redbreast Sunfish populations were the only focal species to experience recruitment in urban streams. While this study was unable to capture recruitment trends across multiple years, previous studies examining Redbreast Sunfish have documented recruitment in unstable environments (such us urban streams) that allow populations to persist in degraded environments (Lemly 1985, Sammons & Maceina 2009). The relationship between urbanization and Redbreast Sunfish recruitment could have several explanations. First, Redbreast Sunfish in urban streams could experience an increase in reproduction. Urban streams are characterized by frequent high flow events (Paul & Meyer 2001, Walsh et al. 2005, Wenger et al. 2009), which has been shown to have negative impacts on the nesting success of several centrarchid species (Devries et al. 2009). However, studies suggest that Redbreast Sunfish are uniquely adapted to tolerate the flow variability in urban streams (Sammons et al. 2009). For example, Redbreast sunfish spawn in late

May, the latest of all focal species evaluated, in pool habitats and construct nests of small gravel and sand (Lukas et al. 2011). The late spawning period allows Redbreast Sunfish to avoid the highest flow periods and significantly increases Redbreast Sunfish nest survival relative to other Centrarchid species (Sammons et al. 2009). A second potential explanation for increased Redbreast Sunfish recruitment is decreased juvenile mortality due to unique habitat preferences. Juveniles inhabit shallow, open areas as refuge, which decrease their predation risk in areas without vegetative cover (i.e., urban streams; Devries et al. 2009) relative to Bluegill and Green Sunfish. Redbreast Sunfish juveniles are similarly tolerant to high flow events and move into new or underutilized stream areas during intense periods of high flow to seek refuge, whereas other centrarchids (i.e., Green Sunfish) were less likely to move from their preferred habitat (Roy et al. 2005, Sammons et al. 2009).

Green Sunfish are an ecologically tolerant species whose presence and abundance are often an indicator of aquatic degradation, such as urbanization (Lemly 1985, Karr 1981, Chovanec et al.2003, Kennen et a. 2005, Walsh et al. 2005, Wenger et al. 2009). However, my study observed a limited number of associations between Green Sunfish and urbanization. Specifically, Green Sunfish mean age was positively associated with PC3, which represented shifts in dominant substrate (gravel to fine). Finer substrates are often associated with urban streams (Paul & Meyer 2001, Walsh et al. 2005, Wenger et al. 2009) and although PC3 was not a direct measure of urbanization, it provides guidance on environmental factors that are relevant to Green Sunfish. These results suggest that Green Sunfish were influenced by instream characteristics associated with urbanization, rather than direct watershed-level variation. The association between Green Sunfish population age and PC3 reflects a truncated age structure and suggests a decrease in recruitment in urban streams. There are several possible explanations for

the decrease in recruitment. First, decreased recruitment could be the result of increased juvenile predation. Juvenile recruitment is heavily influenced by habitat structure, such as substrate type (i.e., cobble versus fine sediment). Studies have shown that substrate is directly linked to predation risk and juvenile survival (Hunt et al. 2002; DeVries et al. 2009), and that increases in fine sediment lead to decreased juvenile mortality (Devries et al. 2009). For example, shifts in substrate composition (e.g., from gravel to fine sediment) can result in decreased juvenile survivorship by altering macrophyte density, which juveniles use as refuge from predators (Hunt et al. 2002; DeVries et al. 2009). Even in streams without vegetation, substrate type has been shown to provide refuge for juveniles and influence predation rates (Devries & Frie 1996, DeVries et al. 2009). Second, lack of Green Sunfish recruitment could be the result of interspecific competition with Redbreast Sunfish populations. My study observed that decreased age-1 and age-2 Green Sunfish abundance in urban streams was accompanied by an increase in age-1 Redbreast Sunfish abundance. The shift in centrarchid abundance suggests competition for critical juvenile habitat, which is supported by previous studies that have documented interspecific competition between congener Centrarchid species in overlapping environments (Lemly 1985; Werner & Hall 1997; Sammons et al. 2009). Green Sunfish compete well with congener species, and have adverse effects resulting in habitat shifts or their population decline (Lemly 1985). However, in third order streams (such as this study) increased congener density further constrains Green Sunfish distribution and habitat, impacting growth and reproduction (Werner 1985). A third explanation for the observed Green Sunfish pattern is intraspecific competition caused by increased stream temperature. This study found that temperature was negatively associated with Green Sunfish mean relative weight. Green Sunfish populations in the study had a low mean relative weight, with sampled individuals weighing on average 72% of the

standard weight of Green Sunfish of the same size (Wege & Anderson 1978). A relative weight between 80-100% is not ideal and individuals are considered severely thin, but relative weights are still within the range of a healthy population (Wege & Anderson 1978). However, the mean relative weight of Green Sunfish in this study is outside the range of a healthy population. The documented trends suggest that as urbanization increases, the altered instream temperature will drive Green Sunfish population decline. Urban streams are characterized by an increase in mean instream temperature (Walsh et al. 2005, Wenger et al. 2009), which is an important determining factor in centrarchid recruitment, affecting spawning, hatch time, and the growth of fish (DeVries et al. 2009, Osenburg et al. 1987). Increased stream temperature has been shown to decrease hatch time, increase egg development and larval growth, and cause earlier spawn times in centrarchids (Devries et al. 2009). Therefore, in species such as Green Sunfish increased temperatures could cause an early ontogenetic niche shift in juveniles (Elliot 1975, Osenberg et al. 1987, Pilati 2007). The resulting shift results in diet overlap between juveniles and adults, creating intraspecific competition and limiting resources during a period that is critical for juvenile growth and survivorship. Ultimately, the resulting changes to habitat (e.g., substrate composition, temperature dynamics) with increasing urbanization impact abiotic factors that are important for the survival and recruitment of juvenile centrarchids.

This study did not identify any meaningful associations between Bluegill population characteristics and urbanization. I expected Bluegill populations be negatively impacted by increasing urbanization due to their sensitivity to degraded stream systems and increased competition from more tolerant congener species, such as Redbreast Sunfish and Green Sunfish (Lemly 1985, Osenberg et al. 1988, Sammons et al. 2009, Wenger et al. 2009). While fish responses to urbanization are often inconsistent, the decrease of sensitive species with increasing

urbanization is a well-documented, consistent symptom of urban stream syndrome (Paul & Meyer 2001; Walsh et al. 2005; Roy et al. 2005; Wenger et al. 2009). Therefore, the absence of a correlation between Bluegill and urbanization was unexpected. One potential explanation may be previous land use legacies (Harding et al. 1998) where loss of sensitive species may be determined by long-term disturbance and modifications from past land use, such as agriculture. The Upper Neuse watershed has long been an important agricultural region in North Carolina (NCDENR 2009). As a result, the Upper Neuse watershed has experienced degradation from agricultural runoff and decreased water quality (NCDENR2009) that precedes the rapid urbanization and resulting urban stream degradation. While this study found a limited number of significant associations, I found a negative relationship percent agricultural land cover and Bluegill mean back-calculated-length at age-2. Therefore, Bluegill population characteristics may have been highly structured and affected by previous agricultural degradation and reflect previous land use alterations (Harding et al. 1998; Fischer et al. 2010; Surasinghe & Baldwin 2014).

This study also examined looked at the elemental stoichiometry of each focal species. Fish strive to maintain a strict homeostasis of their elemental stoichiometry (i.e. percent composition and C:N:P ratios are constant) (Sterner & Elser 2002). However, extreme nutrient loading or changes in spatial availability of nutrients can cause small, quantifiable shifts in the ecological stoichiometry of fishes. Studies have shown that in highly urbanized systems fishes have increased elemental P values (Sterner & Elser 2000, Montana et al. 2018) but urbanization causes nutrient enrichment that can cause considerable variation in elemental composition of food web components that propagates through the food web (Cross et al. 2003). Similarly, the results from this study found that focal species elemental composition deviated from a strict

homeostasis and found that focal species stoichiometry was associated with catchment land cover alterations driven by urbanization. Redbreast Sunfish %N and N:P ratios decreased as substrate composition shifted from predominantly fine substrate to predominantly gravel substrate. Urbanization has been shown to alter stream substrate composition and sediment texture (e.g., decreased fine sediment; Paul & Meyer 2001). Therefore, while PC3 was not a direct measure of urbanization, the relationship between PC3 and Redbreast Sunfish suggests that urban environmental characteristics, such as increases in fine sediment, influence their populations. The relationship between PC3 and elemental N and P could be explained by trophic relations. Studies have shown that Redbreast Sunfish in urban streams have altered diets than populations in forested streams and altered diets from congener species in urban streams (Helms et al 2018). This relationship could also be explained by the age structure of Redbreast Sunfish. Juveniles are higher in elemental N than elemental P, and do not readily uptake P until they begin the ontogenetic shift as adults (Sterner & George 2000). Additionally, Redbreast %N was positively correlated with high mean age and increased abundance of older individuals, while %P was negatively associated with the same characteristics. Centrarchids are heavily ossified fish and therefore %P would be expected to increase with increasing age and abundance (Sterner & Elser 2002, Hendrixson et al. 2007, Pilati & Vanni 2007), thus the results of this study are contrary to what was expected. However, my findings are supported by recent studies which found that fish populations in urban streams consumed P-enriched basal resources that resulted in urban populations with higher whole body %P (Montana et al. 2018). Conversely, I did not find evidence to support an enriched-P diet in urban Green Sunfish populations. My results documented a negative association between Green Sunfish %P and PC2, which represented an environmental shift from wide, fast streams to slower, smaller streams. While PC2 is not a direct

measure of urbanization, it indicates that Green Sunfish %P is negatively associated with reach-scale characteristics in urban streams. In contrast to the results for Redbreast Sunfish, these findings are consistent with previous studies on centrarchid stoichiometry, which document increases in %P with increasing age and abundance (Sterner & Elser 2002, Hendrixson et al. 2007, Pilati & Vanni 2007). I observed a similar pattern in Bluegill populations. Bluegill %N and C:N were negatively correlated with PC3, which represents increasing gravel substrate in urban streams. This pattern reflects the decreased Bluegill abundance in urban streams and is consistent with the previous literature and the knowledge that centrarchid %N and %P are related to population abundance and density (Sterner & George 2000, McIntyre & Flecker 2010, Montana et al. 2018) since fishes are pools of nitrogen and phosphorous (Sterner & Elser 200)

This study observed contradictory associations with urbanization for three ecologically important centrarchid species. The results of this study provide an understanding of fish response to urbanization, outline potential mechanisms driving changes for population, and identify important indicator species. For example, while Green Sunfish have long been considered an important indicator of urbanization (Kar 1981, Chovanec et al. 2003), Redbreast Sunfish were the only species to be positively associated with urbanization in this study. Therefore, Redbreast Sunfish appear to be an excellent indicator of urbanization due to their tolerance and unique life history characteristics. This study provides a framework for incorporating population characteristics and ecological stoichiometry into analysis of urbanization impacts. Few studies have evaluated population-level structure and elemental stoichiometry in response to catchment-, reach-, and instream-level environmental changes across a gradient or developed catchments (Paul & Meyer 2001, McIntyre & Flecker 2010, Gilbert 2012). While the results of this study identify population and ecological responses, additional future studies are needed to capture the

variation in urban effects and identify potential pathways influencing stream response. Therefore, further studies examining stream fish responses to catchment urbanization that incorporate multiple ecological concepts across a spatial scale are needed to identify mechanisms driving fish structure.

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TABLES

Table 2.1. Mean, standard error (SE), minimum (Min), maximum (Max), and eigenvectors for catchment- and reach-scale environmental variable for streams sampled in the Upper Neuse River Basin in North Carolina, 2017. The eigenvalues of each principle component, percent of variance explained by each principle component, and cumulative percent variance explained by each principle component are displayed.

Variable	Mean	SE	Min	Max	Eigenvectors		
% Developed	38.10	7.00	0.70	96.10	**0.43**	0.03	-0.09
% Forested	42.70	5.20	3.10	95.60	**-0.36**	0.13	0.08
% Shrubland	0.90	0.30	0.00	5.60	-0.26	-0.20	0.12
% Herbaceous	2.20	0.30	0.20	5.40	**-0.37**	-0.17	-0.12
% Agricultural	13.20	3.00	0.00	55.60	-0.27	-0.20	0.11
% Wetland	1.90	0.40	0.00	6.50	-0.25	-0.23	-0.27
Catchment Area (km^2)	11.70	2.20	2.30	52.50	0.00	**-0.31**	0.19
Width (m)	4.70	0.30	2.70	8.10	0.02	**-0.38**	-0.10
Depth (m)	0.30	0.00	0.10	0.50	-0.01	0.13	0.23
Velocity (m/s)	314.90	53.30	0.00	813.00	-0.04	**-0.34**	0.28
Dissolved Oxygen (%L)	72.60	5.20	18.30	92.80	0.17	-0.13	0.14
Temperature (°C)	23.47	0.48	19.30	28.77	0.08	**-0.35**	-0.30
Conductivity (µS/cm)	116.90	10.40	65.00	247.70	0.24	-0.08	-0.19
pH	7.10	0.10	6.40	7.60	0.34	-0.22	-0.02
% Fine substrate	22.70	5.90	0.00	80.00	-0.07	0.33	**-0.35**
% Sand substrate	33.90	4.80	0.00	80.00	0.02	-0.18	0.26
% Gravel substrate	18.20	3.30	0.00	50.00	0.21	0.09	**0.46**
% Boulder substrate	12.00	3.90	0.00	60.00	0.01	-0.18	0.17
% Bedrock substrate	8.00	5.50	0.00	95.00	-0.03	-0.18	-0.28
% Woody substrate	4.30	1.70	0.00	30.00	-0.22	0.19	0.16
% Canopy cover	62.50	4.00	20.00	90.00	-0.20	0.04	0.07
	Eigenvalues				**4.59**	**3.04**	**2.39**
	Percent variance explained				**21.88**	**14.48**	**11.37**
	Cumulative percent variance explained				**21.88**	**36.36**	**47.73**

Table 2.2. Mean, standard error (SE), minimum (Min), and maximum (Max) for population characteristics of Bluegill (*Lepomis macrochirus*), Redbreast Sunfish (*L. auritus*), and Green Sunfish (*L. cyanellus*) sampled from streams in the Upper Neuse in North Carolina, 2017.

	Bluegill				Redbreast Sunfish				Green Sunfish			
Variable	Mean	Se	Min	Max	Mean	Se	Min	Max	Mean	Se	Min	Max
Abundance (per 50m^2)	15.8	6.1	3.8	81.0	15.0	3.6	5.3	40.0	12.1	2.0	4.0	30.7
Mean age	1.1	0.1	0.0	4.0	2.5	0.3	0.0	7.0	1.6	0.1	0.0	5.0
Mean abundance at age 1 (per 50m^2)	7.4	2.9	2.0	39.0	2.5	0.9	0.0	7.0	5.1	1.1	1.8	15.7
Mean abundance at age 2 (per 50m^2)	4.9	2.2	0.3	26.0	6.8	2.7	0.5	27.7	4.3	0.8	0.8	10.7
Mean total length	90.7	3.8	44.0	188.0	86.5	3.3	36.0	175.0	80.7	2.8	38.0	161.0
Mean back calculated length at age 1	74.9	3.8	48.7	92.4	55.3	3.5	33.8	71.5	62.1	2.7	47.2	81.9
Mean back calculated length at age 2	88.5	4.8	53.5	116.6	74.7	4.7	47.5	97.0	72.1	2.8	57.9	88.7
Mean relative weight *	79.8	2.1	48.1	125.3	-	-	-	-	72.3	1.6	29.5	229.8
Mean proportional size distribution	2.7	1.8	0.0	21.0	34.8	4.9	12.0	59.0	0.5	0.4	0.0	4.0
%C	39.4	0.8	34.6	43.2	42.5	0.6	40.4	44.8	40.4	0.9	37.5	44.5
%P	1.7	0.1	1.4	1.9	1.7	0.1	1.3	2.0	1.7	0.0	1.5	1.9
%N	10.5	0.2	9.5	11.2	10.3	0.4	8.9	11.4	10.1	0.3	9.0	11.2
C/P	23.5	1.1	20.4	29.5	25.2	1.7	20.0	34.6	23.6	0.9	19.4	26.3
C/N	3.8	0.1	3.6	4.5	4.2	0.2	3.6	5.1	4.0	0.1	3.8	4.2
N/P	6.3	0.2	5.7	7.7	6.0	0.3	4.7	6.9	5.9	0.2	4.7	6.6

Table 2.3. Summarized environmental variables, including mean and standard error (SE), for each sampled stream in the Upper Neuse River Basin in North Carolina, 2017. The principle component (PC) loadings of each reach are displayed.

Variable	Stream Reach ID										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
PC1	-2.6	-2.2	3.0	1.0	-1.5	-2.1	-3.4	3.5	-0.3	-1.7	-0.7
PC2	-1.4	4.0	-0.4	0.9	1.4	-3.6	2.6	0.0	0.1	0.9	0.0
PC3	1.0	-0.8	1.1	0.7	1.3	-2.2	-1.6	1.4	2.6	2.0	2.2
% Developed	4.3	0.7	79.7	31.1	8.6	9.4	3.4	93.3	31.7	10.2	28.5
% Forested	65.8	95.6	17.2	61.1	30.6	44.9	73.4	6.0	52.5	69.6	44.6
% Shrub	5.6	0.0	0.0	0.1	0.2	0.6	0.6	0.0	0.3	1.5	1.3
% Herbaceous	2.8	2.3	0.5	0.5	1.3	4.5	3.9	0.2	2.6	3.5	3.5
% Agricultural	17.6	1.3	0.4	4.2	55.6	31.3	14.3	0.0	11.7	12.3	19.4
% Wetland	3.2	0.0	0.6	2.3	2.5	5.0	3.2	0.0	0.4	0.1	1.9
Catchment area (km^2)	7.8	3.5	16.1	21.2	2.3	11.2	7.9	10.0	15.8	9.0	10.4
Dissolved oxygen (%)											
Mean	76.1	53.1	90.7	81.3	82.0	92.8	33.5	91.5	90.6	79.0	89.5
SE	2.1	5.1	1.4	0.4	1.8	0.7	1.6	1.6	0.3	1.0	0.6
Temperature (C°)											
Mean	22.0	20.2	23.7	22.6	19.3	28.8	21.6	25.1	19.9	24.1	20.6
SE	0.0	0.1	0.8	0.0	0.1	0.3	0.1	0.9	0.0	0.1	0.1
Conductivity (µS/cm)											
Mean	71.2	70.0	136.4	179.8	76.7	67.7	65.0	138.7	82.8	74.0	87.4
SE	0.2	1.5	2.0	0.4	0.1	0.4	0.2	0.1	0.1	0.1	0.2
pH											
Mean	7.1	6.4	7.2	7.2	6.8	6.9	6.5	7.3	7.2	6.9	7.0
SE	0.3	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0
Width (m)											
Mean	5.2	2.9	5.6	4.0	3.8	6.6	5.4	4.4	4.2	2.9	3.2
SE	0.4	0.6	1.2	1.4	1.3	2.0	0.8	0.5	0.8	0.4	0.4
Depth (m)											
Mean	0.2	0.2	0.2	0.4	0.4	0.2	0.3	0.5	0.3	0.3	0.3
SE	0.0	0.0	0.1	0.1	0.3	0.1	0.1	0.1	0.0	0.0	0.1
Velocity (m/s)											
Mean	0.28	0.06	0.35	0.39	0.13	0.74	0.0	0.41	0.69	0.54	0.56
SE	0.13	0.05	0.15	0.08	0.12	0.14	0.0	0.13	0.25	0.15	0.13
% Fine substrate	0.0	80.0	0.0	40.0	20.0	0.0	80.0	0.0	0.0	0.0	0.0
% Sand substrate	30.0	0.0	50.0	20.0	50.0	5.0	0.0	40.0	60.0	40.0	50.0
% Gravel substrate	10.0	10.0	50.0	40.0	20.0	0.0	0.0	40.0	30.0	30.0	40.0
% Boulder substrate	60.0	10.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	10.0
% Bedrock substrate	0.0	0.0	0.0	0.0	0.0	95.0	0.0	0.0	0.0	0.0	0.0
% Woody debris	0.0	0.0	0.0	0.0	10.0	0.0	20.0	0.0	10.0	30.0	0.0
% Canopy cover	90.0	80.0	50.0	70.0	60.0	65.0	50.0	50.0	80.0	50.0	85.0

Table 2.3 (continued).

	12	13	14	15	16	17	18	19	20	21	22
PC1	0.2	-3.0	-1.8	2.7	-0.5	1.4	0.6	3.7	1.6	0.6	1.6
PC2	-1.8	-2.5	0.8	0.5	-3.4	0.5	0.0	0.0	0.8	0.4	-0.1
PC3	-0.8	-1.1	0.0	0.2	1.8	-1.9	-2.5	-1.6	-0.8	0.3	-1.2
% Developed	21.9	19.6	18.9	76.6	5.6	86.3	33.1	96.1	64.5	39.4	74.9
% Forested	44.7	42.4	50.5	17.9	59.3	9.2	50.5	3.1	28.2	54.3	17.2
% Shrub	0.2	2.8	3.0	0.0	1.8	0.0	0.8	0.0	0.1	0.4	0.2
% Herbaceous	4.2	5.4	2.9	0.3	1.6	1.0	3.2	0.3	0.9	1.0	1.4
% Agricultural	25.1	22.1	20.9	4.2	30.3	1.7	7.6	0.2	5.5	2.2	1.8
% Wetland	2.5	6.5	3.3	0.0	0.8	0.0	4.6	0.1	0.6	1.7	3.3
Catchment area (km^2)	5.6	17.4	6.4	3.1	52.5	9.8	11.8	8.0	5.1	13.2	10.4
Dissolved oxygen (%)											
Mean	89.3	18.3	31.0	20.2	80.3	66.6	88.7	89.3	86.1	88.0	79.7
SE	0.6	2.0	1.3	1.5	2.2	1.9	1.9	1.7	2.6	1.1	4.1
Temperature (C°)											
Mean	25.5	26.1	24.2	25.7	23.7	24.0	25.2	22.8	23.7	23.3	24.3
SE	0.1	0.1	0.1	0.3	0.0	0.0	0.3	0.1	0.2	0.1	0.6
Conductivity (µS/cm)											
Mean	204.0	136.1	110.1	153.5	89.5	84.5	247.7	156.2	108.9	114.1	118.4
SE	6.9	0.5	4.2	0.7	0.1	16.5	12.2	6.7	1.4	0.0	1.3
pH											
Mean	7.5	6.8	7.2	7.3	7.3	7.1	7.6	7.5	7.4	7.4	7.4
SE	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0
Width (m)											
Mean	4.7	6.7	2.7	4.0	8.1	4.9	2.8	6.6	4.6	4.7	4.5
SE	0.4	0.2	0.7	0.9	1.7	0.5	0.8	0.9	0.4	1.3	0.7
Depth (m)											
Mean	0.1	0.2	0.5	0.3	0.4	0.3	0.3	0.1	0.1	0.4	0.2
SE	0.0	0.1	0.4	0.1	0.0	0.0	0.2	0.0	0.0	0.1	0.1
Velocity (m/s)											
Mean	0.3	0.4	0.0	0.3	0.6	0.1	0.4	0.1	0.3	0.4	0.0
SE	0.1	0.1	0.0	0.1	0.3	0.1	0.2	0.1	0.1	0.1	0.0
% Fine substrate	10.0	10.0	20.0	0.0	10.0	10.0	80.0	40.0	50.0	30.0	20.0
% Sand substrate	80.0	80.0	40.0	20.0	20.0	10.0	10.0	30.0	30.0	40.0	40.0
% Gravel substrate	10.0	5.0	15.0	40.0	10.0	0.0	0.0	10.0	20.0	10.0	10.0
% Boulder substrate	0.0	0.0	15.0	40.0	60.0	0.0	10.0	10.0	0.0	10.0	20.0
% Bedrock substrate	0.0	0.0	0.0	0.0	0.0	80.0	0.0	0.0	0.0	0.0	0.0
% Woody debris	0.0	5.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0
% Canopy cover	60.0	80.0	70.0	40.0	40.0	90.0	40.0	20.0	80.0	60.0	65.0

Table 2.4. Summarized population characteristics for focal species at each sampled stream in the Upper Neuse River Basin in North Carolina, 2017.

Species	Site	Mean abundance (per 50 m2)	Mean age	Mean total length (mm)	Mean relative weight	PSD	Mean age 1 abundance (per 50 m2)	Mean age 2 abundance (per 50 m2)	%C	%P	%N	C/P	C/N	N/P
Green Sunfish *L. cyanellus*	1	17.3	1.7	74.3	69.5	0.0	5.5	6.3	-	-	-	-	-	-
	2	15.7	1.5	71.5	66.2	0.0	4.0	6.7	-	-	-	-	-	-
	3	6.8	2.1	80.1	79.6	0.0	2.0	3.3	-	-	-	-	-	-
	4	12.3	1.6	101.5	79.6	4.0	6.0	5.0	-	-	-	-	-	-
	8	4.0	1.8	71.3	64.2	0.0	1.8	0.8	41.3	1.7	10.0	25.1	4.1	6.1
	9	9.5	1.5	83.5	76.9	0.0	3.8	3.8	39.1	1.7	10.0	22.4	3.9	5.7
	12	10.5	1.2	80.2	76.2	0.0	6.5	3.0	42.0	1.8	11.2	23.1	3.8	6.1
	14	6.3	2.0	85.8	72.7	0.0	1.8	3.0	-	-	-	-	-	-
	15	8.0	2.0	68.1	71.7	0.0	2.3	3.5	40.4	1.5	10.2	26.3	4.0	6.6
	17	12.2	1.0	87.2	76.2	2.0	8.0	1.6	37.9	1.6	9.5	23.1	4.0	5.8
	18	11.5	1.3	90.4	66.0	0.0	4.5	4.5	44.5	1.7	10.8	25.7	4.1	6.2
	22	30.7	1.5	74.0	68.5	0.0	15.7	10.7	37.5	1.9	9.0	19.4	4.2	4.7
Redbreast Sunfish *L. auritus*	3	8.0	1.8	81.7	-	19.0	2.3	3.0	43.5	1.7	9.7	25.9	4.5	5.8
	6	7.0	2.4	86.8	-	40.0	1.0	3.7	44.8	1.6	10.8	28.6	4.2	6.9
	8	16.8	1.1	84.5	-	37.0	7.0	4.3	44.8	1.3	8.9	34.6	5.1	6.8
	9	5.3	4.1	68.9	-	12.0	0.3	0.5	41.0	1.9	8.9	21.7	4.6	4.7
	13	19.7	3.0	89.3	-	42.0	1.0	5.3	40.6	1.9	10.6	21.8	3.8	5.7
	14	8.0	2.3	94.8	-	59.0	0.8	3.8	-	-	-	-	-	-
	16	11.3	2.9	103.4	-	47.0	0.0	5.3	40.4	2.0	11.4	20.0	3.6	5.6
	19	40.0	1.9	90.5	-	34.0	7.0	27.7	42.6	1.6	10.7	26.4	4.0	6.6
	22	19.3	2.6	78.7	-	23.0	3.0	8.0	42.1	1.9	11.3	22.6	3.7	6.1
Bluegill *L. macrochirus*	5	6.0	0.9	77.2	88.1	0.0	2.0	1.0	-	-	-	-	-	-
	7	9.3	0.6	100.0	79.7	5.0	2.7	1.3	-	-	-	-	-	-
	8	6.5	1.3	95.2	88.9	0.0	4.5	1.3	43.2	1.5	9.7	29.5	4.5	6.6
	9	3.8	0.7	105.4	73.6	0.0	2.0	0.3	34.6	1.7	9.5	20.8	3.6	5.7

Table 2.4. (continued).

Bluegill *L. macrochirus*	10	21.8	1.6	94.9	76.2	0.0	5.8	13.5	40.9	1.9	10.8	21.6	3.8	5.7
	11	81.0	1.3	87.1	73.8	0.0	39.0	26.0	40.4	1.7	10.4	24.2	3.9	6.2
	12	8.0	0.9	57.8	90.9	0.0	5.0	1.3	36.9	1.7	10.3	21.9	3.6	6.1
	13	10.3	1.1	95.6	74.5	0.0	5.7	3.0	40.6	1.7	11.2	23.8	3.6	6.5
	14	14.0	1.1	96.0	74.3	6.0	6.5	4.3	-	-	-	-	-	-
	20	7.6	0.6	95.1	72.4	0.0	4.0	0.4	40.6	1.4	11.0	28.4	3.7	7.7
	21	9.0	1.2	103.0	90.1	21.0	6.0	1.5	38.2	1.8	10.7	21.2	3.6	5.9
	22	12.3	1.4	81.2	75.3	0.0	5.3	5.0	39.4	1.9	11.0	20.4	3.6	5.7

FIGURES

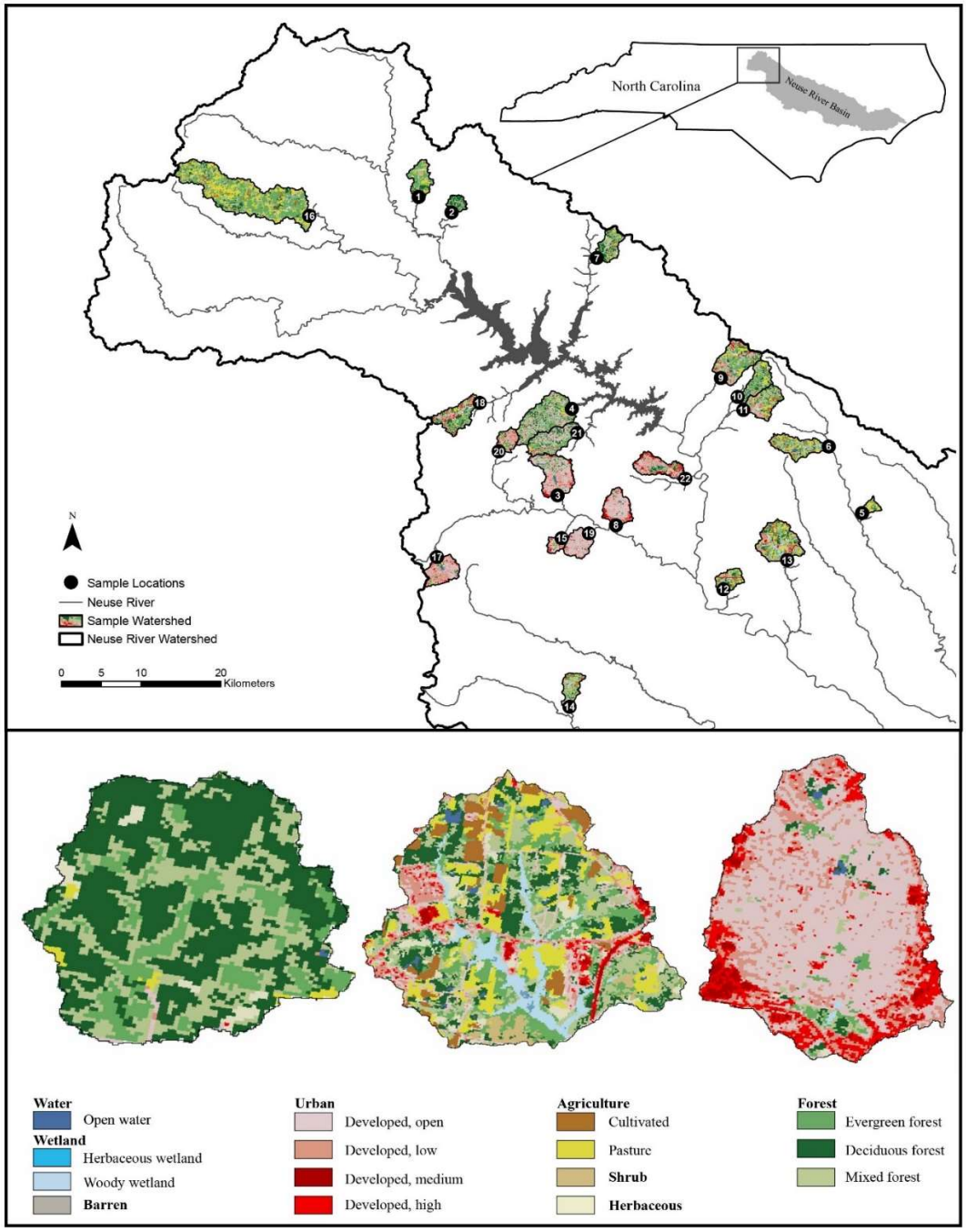

Figure 2.1. Upper Neuse River Basin, located in North Carolina, with locations of third order stream reaches sampled in 2017 and delineated catchments (A). Examples of the environmental gradient across delineated catchments classified using National Land Cover Data (B) (NLCD 2011).

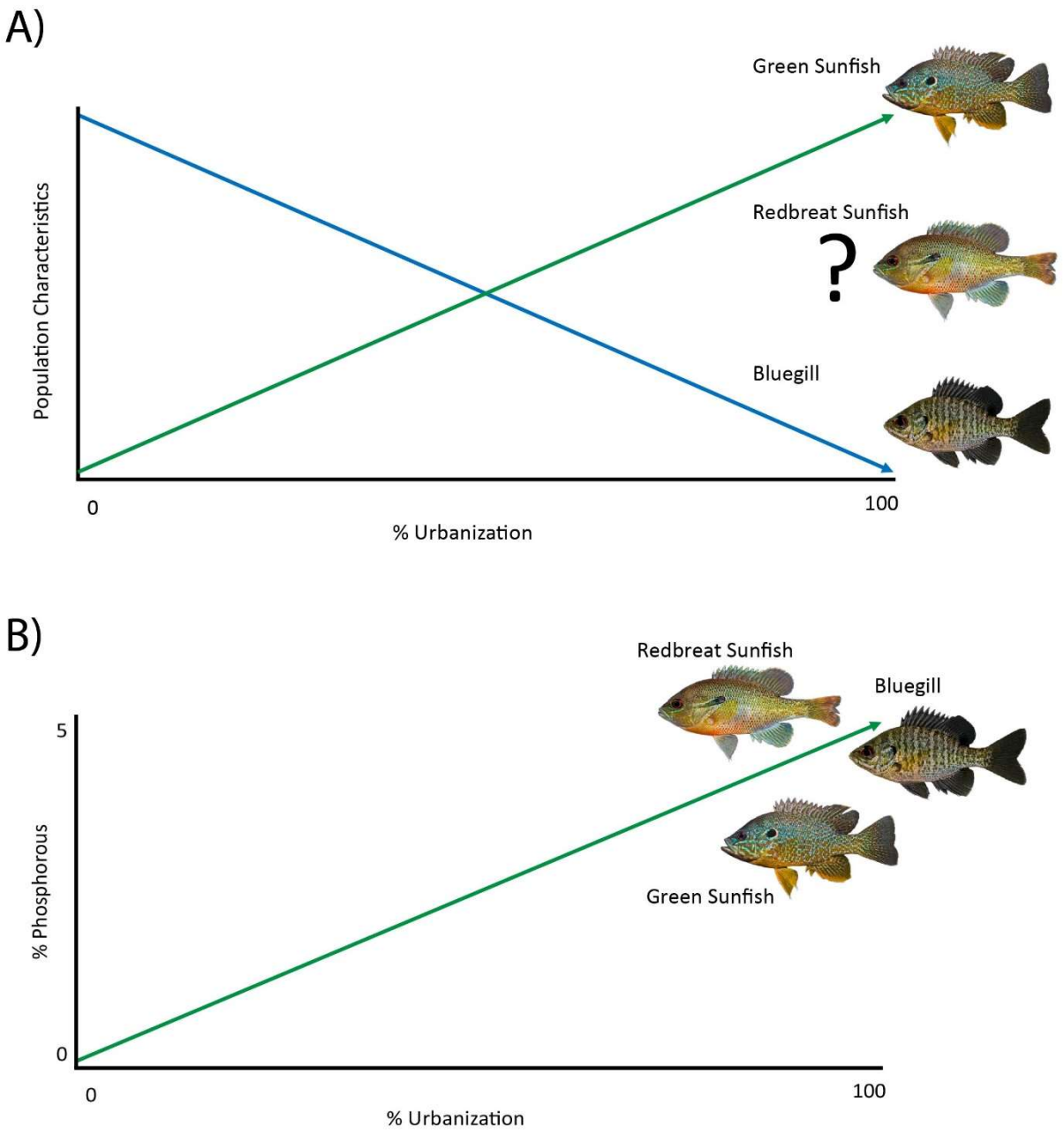

Figure 2.2. A conceptual diagram of the expected population (A) and stoichiometric (B) responses to increasing urbanization.

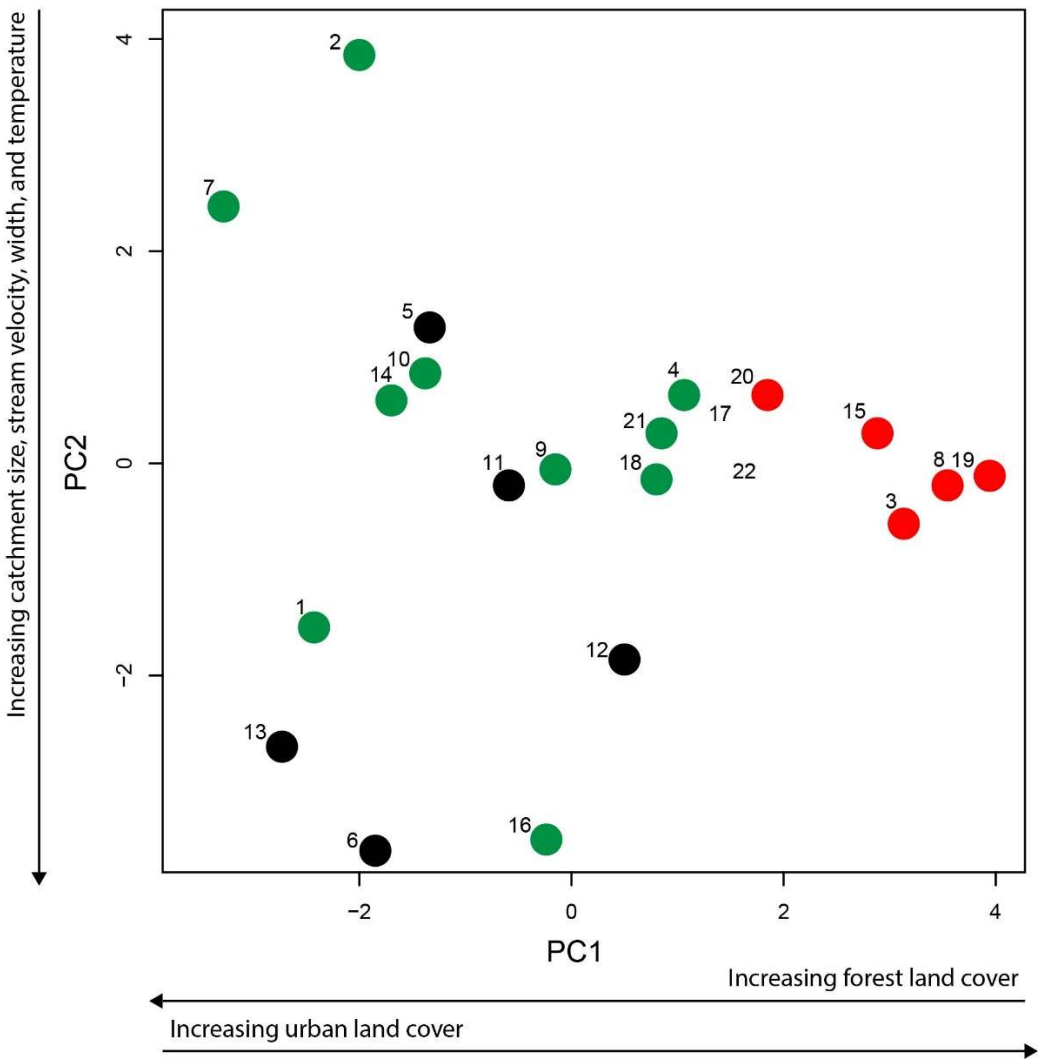

Figure 2.3. PCA of the catchment- and reach-scale environmental characteristics (PC 1 and PC 2). The location of each sample reach is identified with a number (1-22) that corresponds to the sample reach ID displayed in Figure 2.1 and a point that corresponds to majority land cover type. Sites with majority urban land cover are shown in red, majority forest and cover are shown in green, and with mixed land use are shown in black. Arrows along axis one and two are labeled with the environmental variables that had eigenvectors with an absolute value greater than or equal to 0.30. Eigenvalues of each principle component, percent of variance explained by each principle component, and cumulative percent variance explained by each principle component are displayed in Table 2.1.

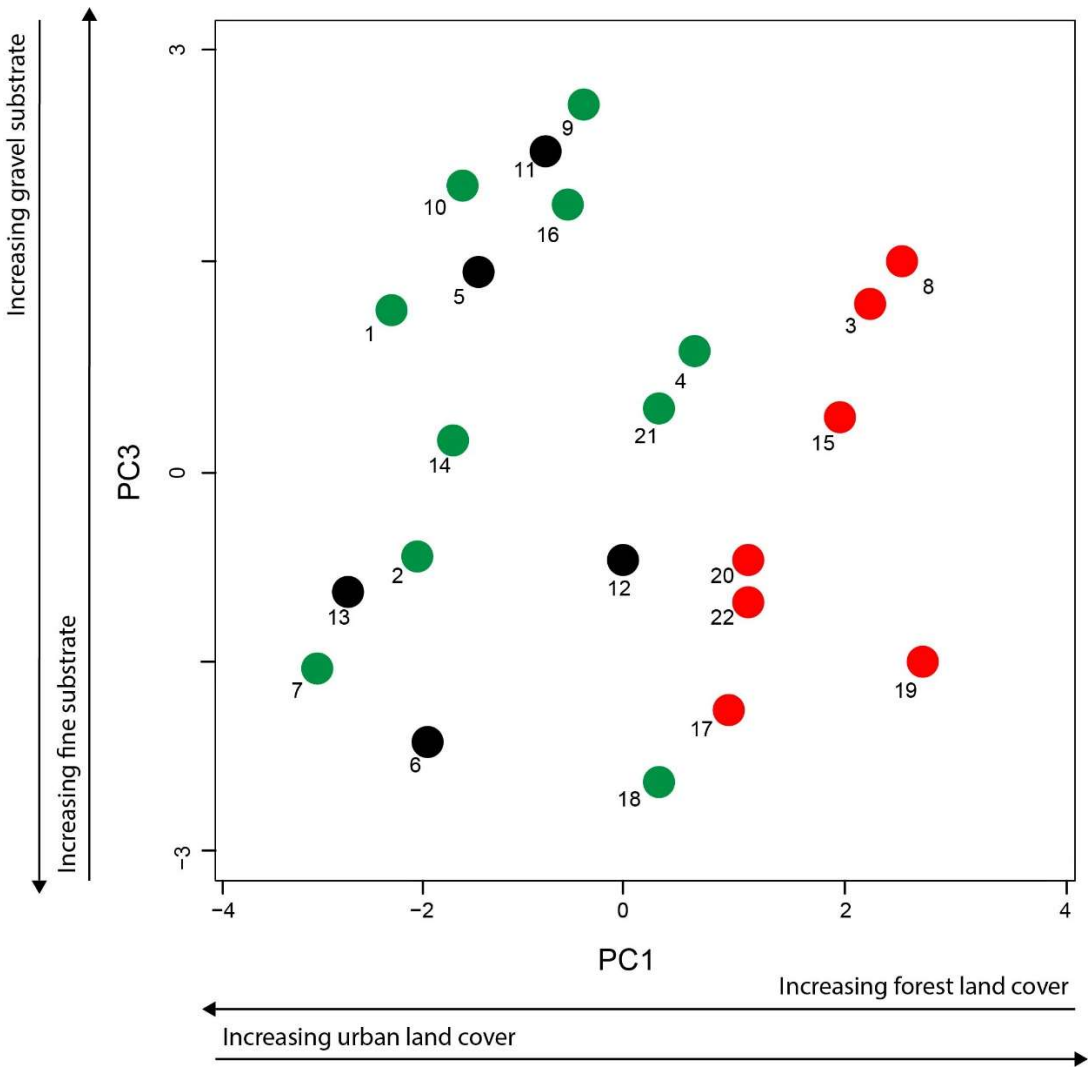

Figure 2.4. PCA of the catchment- and reach-scale environmental characteristics (PC 1 and PC 3). The location of each sample reach is identified with a number (1-22) that corresponds to the sample reach ID displayed in Figure 2.1 and a point that corresponds to majority land cover type. Sites with majority urban land cover are shown in red, majority forest and cover are shown in green, and with mixed land use are shown in black. Arrows along axis one and two are labeled with the environmental variables that had eigenvectors with an absolute value greater than or equal to 0.30. Eigenvalues of each principle component, percent of variance explained by each principle component, and cumulative percent variance explained by each principle component are displayed in Table 2.1.

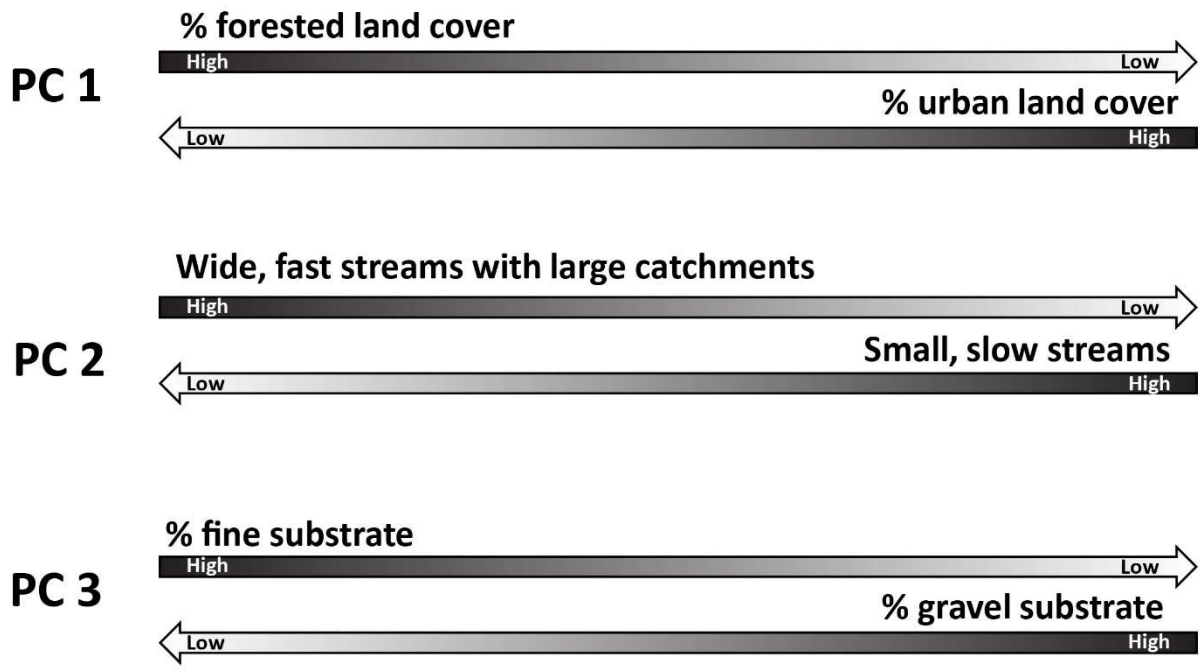

Figure 2.5. The three main environmental gradients indicated by the PCA. Each principle component (PC) and corresponding variables are shown.

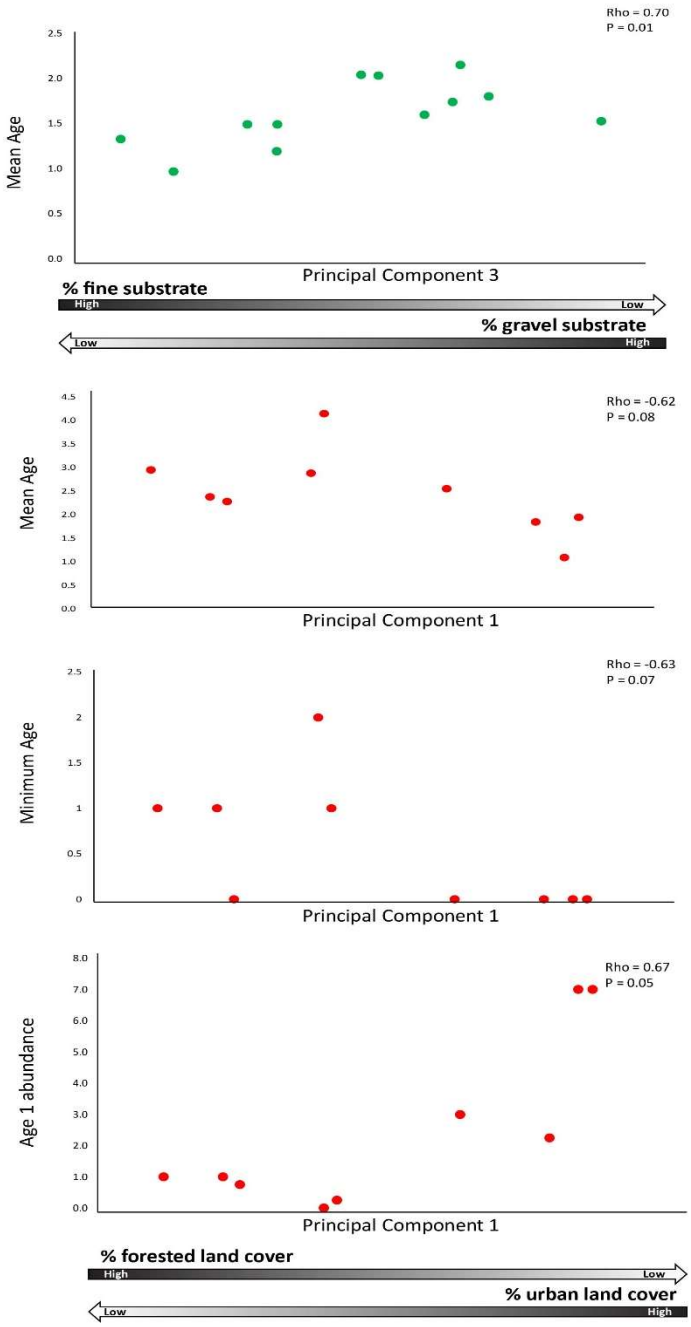

Figure 2.6. Population characteristics for Bluegill (shown in blue), Redbreast Sunfish (shown in red), and Green Sunfish (shown in green) plotted against the component loadings of PC1, PC2, and PC3, with *P*-values and rho values for spearman rank correlation tests.

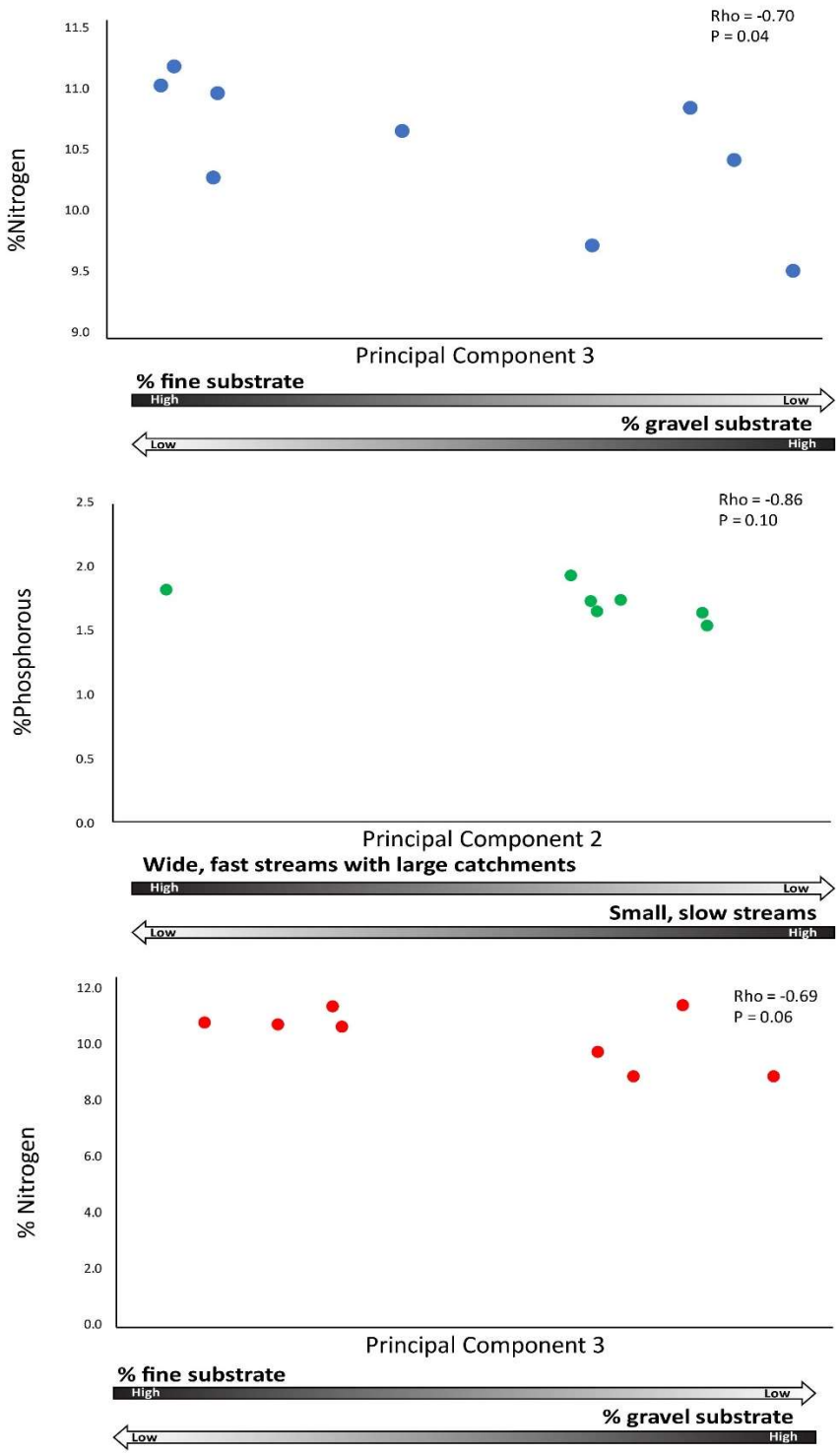

Figure 2.7. Stoichiometric characteristics for Bluegill (shown in blue), Redbreast Sunfish (shown in red), and Green Sunfish (shown in green) plotted against the component loadings of PC1, PC2, and PC3, with *P*-values and rho values for spearman rank correlation tests.