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Predictors of Fish Assemblage Structure and Dynamics in Atlantic Coastal Plain Streams

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PREDICTORS OF FISH ASSEMBLAGE STRUCTURE AND DYNAMICS IN ATLANTIC COASTAL PLAIN STREAMS

by

REBECCA SCOTT

(Under the direction of James H. Roberts)

ABSTRACT

Effective management of freshwater fishes requires a mechanistic understanding of the drivers of assemblage composition; in other words, what determines who is where and when. Stream fish assemblages are potentially influenced by environmental factors that act on multiple spatiotemporal scales, but the relative influence of these drivers may vary between geophysically distinct regions. This study sought to determine the patterns and drivers of fish taxonomic and functional assemblage composition in the coastal plain, a region possessing unique hydrologies, faunas, and physiochemical conditions. I addressed this goal using two complementary chapters, both of which utilized environmental and biotic data collected from twenty-six wadeable coastal plain streams in the Altamaha, Ogeechee, and Savannah River basins in Georgia during the summers of 2016 and 2017. In the first chapter, I compared the relative influence of both regional landscape-scale (e.g. land use, ecoregion memberships) and local habitat-scale (e.g. water chemistry, stream morphology) environmental factors on species richness and taxonomic assemblage composition. In the second chapter, I tested the abilities of six longstanding ecological models to predict observed longitudinal changes in habitat and fish assemblages in coastal plain streams. Results from this study indicate that both species richness and taxonomic composition of assemblages were influenced by environmental conditions acting at multiple scales, including drainage area, channel sinuosity, water chemistry, and substrate. In addition, coastal plain fish assemblages sorted spatially into two distinctive assemblage types (i.e.

“fluvial” and “nonfluvial”) that were characterized by differences in key environmental variables, most of them local in scale. Taxonomic assemblage composition remained stable over time, despite significant annual differences in hydrology. As frequently observed elsewhere, I detected increasing species richness in larger, downstream reaches. However, other longitudinal gradients in environmental conditions and species’ traits showed variable influence for stream size, providing substantial support for the River Continuum Concept, modest support for the Habitat Template Concept, and little support for four other models. I posit that this was because of the naturally harsh physiochemical regime and variable hydrology of coastal plain systems which limited the action of longitudinal filtering mechanisms observed in other regions. This study allows for a better understanding of how and why coastal plain stream fish assemblages are structured taxonomically and functionally, and lends insight into how communities may respond to environmental changes.

INDEX WORDS: Freshwater biology, Community ecology, Coastal plain

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by

REBECCA SCOTT

B.S. University of Rhode Island, 2013

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barriers, (4) low mesohabitat diversity, and (5) harsh physiochemical conditions, in ACP streams.

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Table 2.3: Relationship between Bray-Curtis and fluvial distance between pairs of sites for this study, as well as for 11 other streams where data were available. The linear slope and intercept of relationships as well as the statistical significant of associations are shown. Test statistics reflect the significance test used; linear regression models (for independent observations, F), Mantel tests, (for square pair-wise matrices, R), or permutation-based correlations (for non-square matrices, r). Only 4 and 8 sites were used from the Kiamichi River and Raystown Branch, respectively, in order to restrict streams to ≤50 km fluvial distance.

Table 2.4: Results of permutation-based correlations of each variable with drainage area, and comparison of observed results from this test and the longitudinal position test (DS-US effect size; Figure 2.1) with hypothesized relationships. Predicted or observed positive (+) or negative (-) relationships, or an observation of no effect (NE) are shown. Bolded variables were found to be significantly correlated with drainage area at $P < 0.05$.

Appendix 1: A list of all species sampled during summer sampling seasons (includes both 2016 and 2017). Bolded species were identified as “rare” (occurring at only one site at a level of less than 5 individuals) and were retained for analyses of richness but excluded from analyses of composition. Occurrence is the number of sampling events at which a species was captured, catch is the number of individuals captured across all sampling events.

Appendix 2: Descriptions of variables tested for longitudinal variation.

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respectively. Variables were considered to be consistently affected by position if the 95% CI did not include zero.

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Figure 2.3: Relationships between Bray-Curtis (BC) and fluvial distances between pairs of sites for this study (one point per stream; thick line shows linear trend) and other published longitudinal surveys of other streams (lettered trend lines). Solid lines represent a significant relationship between BC and fluvial distance; dashed lines were non-significant. Data points where fluvial distance was > 50 km were eliminated (C and G) so that relationships could be considered on a comparable scale. [A] Otter Creek, OK (Harrel et al. 1967), [B] Piasa Creek, IL (Smith et al. 1969), [C] Raystown Branch, PA (Barila et al. 1981), [D] Brier Creek, OK (Matthews 1986), [E] Buncombe Creek, OK (Dowell 1956 in Lienesch et al. 2000), [F] Big Sandy Creek, TX (Evans and Noble 1979), [G] Kiamichi River, OK (Matthew 1986), [H] Roanoke River, VA (Matthews 1986), [I] Little Stony Creek, VA (Hitt and Roberts 2012), [J] Spruce Run, VA (Hitt and Roberts 2012), [K] Sinking Creek, VA (Hitt and Roberts 2012).

GENERAL INTRODUCTION

Why Study Fish Assemblages?

An assemblage is a group of phylogenetically related organisms occupying a particular system at any given time (Fauth et al. 1996). Matthews (1998) defines a fish assemblage as "fish that occur together in a single place, such that they have at least a reasonable opportunity for daily contact with each other". Fish assemblages are composed of multiple species that are able to co-exist due to varying life history strategies and habitat requirements (i.e. varying ecological niches). A main goal of community ecology is understanding how fish assemblages are distributed (e.g. spatially, temporally), and what factors drive this distribution. For example, riverine ecosystems contain a variety of unique habitats including riffles, runs, pools, side channels, and floodplains, and the downstream flow and movement of aquatic organisms results in a dynamic system that varies across time and space (Fausch et al. 2002). Furthermore, although the United States is home to the greatest number of temperate freshwater fish species in the world (Warren and Burr 1994), increasing human population and therefore exploitation and degradation of freshwater habitat (e.g. impoundments, water pollution) has led to a decline in many freshwater fish taxa (Leidy and Moyle 1998, Fausch et al. 2002). Continued research into lotic systems and the way in which they impact fish populations would increase understanding of these complex processes and therefore benefit future conservation efforts.

Environmental Influences on Stream Fish Assemblages

Frissell et al. (1986) defined watersheds as "hierarchically organized systems incorporating, on successively lower levels, *stream segment, reach, pool/riffle* and *microhabitat* subsystems". Watersheds experience alteration through long-scale, high-impact changes such as glaciation or climatic shifts, causing variation among watershed- (or stream segment-) scale habitats to be influenced by changes in geology, topography, and climate. In contrast, reach systems experience alteration through more frequent, low-impact changes such as landslides and channelization that could lead to bank erosion and increased sedimentation. Reach-scale habitats may therefore vary in response to slope or bedrock relief, channel pattern, and bank composition, among others. On the smallest scale, microhabitat systems are influenced by seasonal variation in events such as precipitation and organic matter transport. Variation between microhabitats is therefore likely to be responsive to seasonal changes in these conditions that affect factors such as underlying substrate, water chemistry, and velocity (Frissell et al. 1986).

A main goal of my study is to understand the influence of environmental characteristics, acting at these multiple scales, on the organization of fish assemblages. I organized potential influences into groups, including (1) local instream conditions (e.g. water chemistry, physical habitat complexity) and (2) regional landscape-scale conditions (e.g. the biogeographic history of a basin precludes the occurrence of certain species) Regional-scale influences could be further sorted into “natural” (e.g. biogeographic history, geology) and “anthropogenic” (e.g. land use, hydrologic alteration) classes. For this study, the term “regional-scale” will refer to environmental attributes measured at the level of the watershed, whereas “local-scale” will refer to reach level or smaller (including microhabitat) attributes. This is because study areas (discussed in more detail in the methodology) were on the spatial scale defined as a stream reach, but microhabitat scale components (e.g. depth, velocity) were also considered. The way in which environmental variables influence fish assemblages at multiple scales (i.e. regional, local) is an important focus in stream fish ecology (Tonn et al. 1990, Angermeier and Winston 1998, Paller et al. 2016, Poff 1997), as understanding “what determines who will occur where” allows for more informed management.

Beginning at the local-scale, I first consider the abiotic conditions that fish experience as they interact directly with their habitat. Perhaps the most direct interaction that aquatic organisms have with their environment is with the water in which they occur; specifically, water chemistry components such as temperature, dissolved oxygen, and pH. Increased water temperatures lead to increased stress and physiological demands on fish, which combined with the resulting decrease in dissolved oxygen availability can limit the number of species that can occur or can result in mortality (Jackson et al. 2001). As some species are more tolerant to low levels of dissolved oxygen (e.g. fishes capable of air breathing), assemblage composition shifts to these species in low oxygen conditions (Jackson et al. 2001). Conversely, low water temperatures can also limit species occurrence and can result in slowed growth (Magnuson et al. 1979, Shuter et al. 1980). Decreased pH, or increased acidity, of the water has been shown to accompany a decrease in species richness (Somers and Harvey 1984), as only certain species are tolerant to acidic water and can therefore persist in acidic conditions. In addition to water quality, fish interact on the local scale with their physical habitat (e.g. channel dimensions, substrate, velocity). Depth, an important component of stream morphology, can impact assemblage structure as streams of greater depth are less likely to freeze in winter, deplete all dissolved oxygen, and see high water temperatures in the summer, and are therefore able to support a greater number of species (Schlosser 1987). Greater depth and habitat volume may also allow for the occurrence of larger-bodied species such as top predators (Schlosser 1987). Structural complexity, including substrate variability and presence of large rocks and woody debris, has been associated with an increase in fish diversity, as the structure provides increased refuge and foraging habitat (Gorman and Karr 1978, Angermeier and Karr 1984). These local-scale conditions may be affected by regional-scale conditions.

A watershed’s regional climate can directly impact the instream conditions experienced by aquatic organisms. Increased regional precipitation results in increased groundwater and surface flow, which may directly impact fish communities by shifting the species assemblage toward those species capable of persisting in higher velocities (Stazner et al. 1988, Jackson et al. 2001). Flood events may also connect communities to riparian floodplain habitats which may offer increased shelter and food resources (Bayley 1983). Increased freshwater inputs may result in changes local-scale conditions such as decreased water temperatures, increased dissolved oxygen, and increased turbidity, and high levels of flow may alter channel dimensions to provide wider, deeper habitat, and can increase the transport of fine sediment downstream which may prove problematic for species that require coarse substrate. Regional geology can also indirectly affect fish populations, as the bedrock of a watershed can influence the acidity of the water (Jackson et al. 2001).

Anthropogenic activities at the regional scale also impact the local-scale habitat with which fishes interact. Alteration of the floodplain and other riparian areas for anthropogenic land use can result in changes to both water chemistry and physical habitat. Increasingly acidic precipitation due to the burning of fossil fuels (Likens et al. 1972) and runoff of organic acids (Jackson et al. 2001) from urban and agricultural areas can increase stream acidity. Nutrient loadings of nitrogen and phosphorus from anthropogenic activities (e.g. livestock farms, agricultural fertilizer) in stream waters have been associated with loss of intolerant species, as the overabundance of nutrients results in a high abundance of primary producers which remove much of the dissolved oxygen in the water and produce harmful microbial products such as ammonia, creating inhabitable conditions for certain fish (Wang et al. 2003b, Wang et al. 2007). Increase urbanization may also lead to runoff of other contaminants such as salts and heavy metals (Sawyer et al. 2004). According to the EPA (1998), excessive nutrient loading is the second leading cause of impairment in lotic systems behind anthropogenic hydrologic alteration. Humans alter the natural flow of watersheds in many ways that may affect the amount of freshwater input to a stream habitat (e.g. impoundments, groundwater withdrawal). As discussed above, the amount of flow can influence both water chemistry and physical habitat at a local-scale. Other anthropogenic alterations such as channelization may influence local-scale factors characteristics such as channel dimensions, velocity, and amount of fine sediment due to erosion.

Although my study focuses primarily on interactions between abiotic factors and fish assemblages, studies have shown that biotic interactions may become more important in streams of larger size and/or stability (Strange and Moyle 1993, Schlosser 1987). The two biotic factors primarily influencing fish assemblages are predation and competition. Predation may directly impact an assemblage by causing fragmentation, as prey species tend to migrate to shallower microhabitats (e.g. riffles) and away from deeper pools typically occupied by predators (Fraser and Cerri 1982, Power 1987, Schlosser and Ebel 1989). The density of prey species is often therefore higher in tributaries versus the main, predator-dominated river channel (Angermeier and Karr 1983). Predation can also have indirect effects on assemblage structure through behavioral changes in prey including habitat choice and foraging behavior. These changes can have negative impacts on the growth and fitness of prey species and can result in smaller individuals with poor body condition (Jackson et al. 2001). Competitive interactions may influence assemblage structure through niche (specifically habitat) segregation (Grossman et al. 1998). It is argued that rather than resulting in competitive exclusion, competition can result in various forms of adaptation (e.g. behavioral, physiological) as a response to niche segregation, which has been demonstrated by an increase in species richness as environmental variability increases (Gilliam et al. 1993). The level of both predation and competition can be determined by historic, regional-scale conditions (e.g. past opportunities for a given species to colonize a particular area) as well as smaller, local-scale conditions (e.g. environmental conditions suitable for particular predator species) (Jackson et al. 2001). Although characterizing biotic interactions is not the focus of my study, in interpreting my findings, I consider ways in which these biotic interactions might influence fish assemblages.

Because stream systems vary geographically due to regional differences in geology and climate (Hughes and Larsen 1988), the local and regional environmental influences experienced by stream fish assemblages, as well as their relative impacts, can be expected to differ among study regions. The study of a particular stream fish assemblage must therefore take into consideration the suite of local and regional environmental factors most influential in that system when predicting how assemblage composition will react to changes.

Longitudinal Patterns in Stream Fish Assemblages

Stream fish assemblages are dynamic in nature and have been shown to vary spatially along several gradients including habitat complexity, movement permeability, temporal stability or disturbance, and habitat area or volume (Roberts and Hitt 2010). These gradients themselves tend to exhibit longitudinal gradients, increasing (area, volume, stability, complexity, permeability) or decreasing (disturbance) predictably between upstream and downstream reaches. The idea of a species-area relationship (i.e. increased species with increased area) is a pervasive concept in community ecology, with several possible explanations (Angermeier and Schlosser 1989). The first comes from MacArthur and Wilson's (1967) island biogeography theory, which suggests that the number of species present on an "island" (or in this case, a stream) is dependent on the rate at which new species immigrate to it and existing species go extinct. Larger islands are considered a larger target for immigrants, resulting in a higher number of species. This explanation relies on the ability of species to immigrate to other habitats, or habitat connectivity. A second possible explanation is that streams of larger area can house a wider variety of microhabitats and food resources, which creates more available ecological niches to be filled by a larger amount of species relative to smaller streams (Williams 1964). A third explanation suggests that the species-area relationship is an artifact of sampling, in that the increased volume of habitat is streams of larger area may simply provide room for more individuals, and this increase in abundance may allow for increased detection of multiple species (Connor and McCoy 1979). Angermeier and Schlosser (1989) found stream volume rather than stream area to be a better predictor of species richness, suggesting that stream depth may also be important in influencing species distributions.

Other potential longitudinal gradients within a stream might involve variation in the functional (i.e. species-trait) composition of fish assemblages. For example, the river continuum concept describes a shift in energy inputs and production in the downstream direction, and longitudinal organization of the fish community in response to this gradient (Vannote et al. 1980). In small headwater streams, autotrophic production is low as there is much shading from riparian vegetation (which contributes most of the production), and the fish community is characterized by surface-oriented feeders and benthic invertivores. As stream size increases, an increasingly open canopy allows for more autotrophic (i.e. phytoplankton) production, and large rivers see increased organic material and nutrients transported from upstream, resulting in a fish assemblage composed of invertivores, piscivores, detritivores, and planktivores (Vannote et al. 1980).

A study by Schlosser (1990) showed longitudinal variation in fish body size, with larger, downstream areas having species with a larger maximum body size on average, a pattern that was speculated to reflect the increased habitat volume in downstream areas that can support larger-bodied species. The suite of physical habitat conditions, or the “habitat template” of streams provides another longitudinal gradient along which species sort by traits, as traits are evolutionarily selected for to match habitat conditions (Southwood 1977, 1988, Townsend and Hildrew 1994). For example, species may sort by reproductive strategy, with broadcast spawners occurring more frequently in large downstream rivers (where there is more open water in which to broadcast eggs), and nest-builders preferring small headwater streams (where there is more substrate complexity on which to build nests and less flow to wipe out nests) (Goldstein and Meador 2004). The habitat heterogeneity of a site may also distribute species spatially based on their microhabitat specialization, specifically varying velocities, depths, and substrate types (Gorman and Karr 1978).

Although these predictions have shown general applicability to the stream systems where they were developed, some of the mechanisms that underlie the predictions (e.g. upstream sites having less stream area than downstream sites, variable connectivity of upstream and downstream sites for flow and movement of species) may be less applicable to environments where these gradients are less pronounced, such as the coastal plain region of the Southeastern United States, which possesses distinctive environmental and hydrologic regimes and fish faunas. The unique environmental characteristics of a stream system should be carefully considered when determining the applicability of broader spatial patterns.

Coastal Plain Ecoregions and Faunas

The coastal plain is a low-lying physiographic region adjacent to a coastline that undergoes high levels of seasonal variability. In contrast with more inland streams that typically consist of a single, flowing channel, coastal plain streams experience little change in elevation over their watershed area (i.e. are low gradient) and may therefore exhibit poorly defined channels, which may become braided in areas. Because of this, coastal plain rivers tend to experience less impoundment for hydroelectric use compared to other stream ecosystems, although smaller coastal plain streams may be impounded for recreational purposes (Benke 1990). Compared to other stream systems, coastal plain streams typically experience greater stressors such as (1) higher water temperatures, (2) lower levels of dissolved oxygen, and (3) greater acidity, which may be exacerbated seasonally as the streams dry and experience less flow (Felley 1992, Smock and Gilinsky 1992). High water temperatures and low dissolved oxygen are typical of coastal plain streams as their low gradient, braided nature results in generally shallower streams that may be stagnant for most of the year. Inputs of humic and fulvic acids (components of soil and organic matter) are high in coastal plain streams, which are typically swampy in nature and receive much input of organic matter from riparian areas or floodplains (Maxted et al. 2000, Junk et al. 1989)

Most unusual about coastal plain streams, however, is the relatively variable hydrology of ecosystems. Coastal plain streams experience extreme temporal hydrologic variation due to a seasonal flood pulse, which creates temporary connections between stream and their floodplains as stream channels fill and overflow their banks. This interconnection between a river and its floodplain, and in turn its interaction with biological communities, is referred to as the “flood pulse” (Junk et al. 1989). Whereas the river continuum concept discusses longitudinal connections and gradients between stream habitats, the flood pulse concept addresses lateral connections, and suggests that fish production is more dependent on lateral connections with the floodplain rather than the downstream flow of nutrients (Junk et al. 1989). Individual stream systems can experience periods of inundation and complete drying within the same year, and consecutive years can see significantly different flooding patterns (i.e. wet year vs dry year). The amount of flooding and resulting connection to its floodplain experienced by a stream in any given year is a function of the frequency, magnitude, duration, and timing of flood pulses (Figure 1.1). The magnitude of flood pulses, and the resulting amount of inundation, is strongly positively associated with river discharge, which is affected by precipitation, evapotranspiration, and dam release in regulated streams (Benke et al. 2000). In addition to downstream discharge, the filling of pools by local rainfall and groundwater inputs may inundate an additional 25% of a floodplain (Williams 1998). The low gradient and braided nature of the coastal plain renders it particularly susceptible to floodplain inundation in contrast with the more defined, steep channels of upland streams. Long term monitoring of the flood pulse and resulting floodplain inundation in an unregulated coastal plain stream showed predictable seasonal variability, with inundation being highest in the winter/spring months and beginning a drop in the early summer that continued until winter flooding returned (Benke et al. 2000). This suggests that evapotranspiration may be an important factor in influencing the timing of flood pulse events.

These floodplain connections provide valuable ecosystem services for aquatic organisms, such as increased habitat volume and the exchange of freshwater runoff, nutrients, and organic matter. In river floodplain systems, the biomass of fish is typically dominated by species that rely on access to the floodplain for feeding or some part of their life cycle (Welcomme 1979, Bayley 1983, 1991). Species richness has been shown to be higher in systems with strong floodplain connections versus in regulated streams where natural inundation patterns are interrupted (Sullivan and Watzin 2009). Many species spawn in the early stages of the flood pulse as the flood plain becomes colonizable in order for their young to take advantage of the increased availability of shelter and food resources (Bayley 1983, Welcomme 1985). Body condition has also been seen to improve along with connection to the floodplain as fish are able to exploit seeds, fruits, and terrestrial invertebrates from the riparian forest, whereas a loss of body fat and a general decrease in feeding is observed during times of receding inundation as fish return to the main river channel (Junk 1985, Goulding 1980, Welcomme 1979).

The timing of the start of the flood pulse in the winter can have significant effects on the recruitment and survival of young-of-the-year (YOY) fishes. Recruitment is highest in floodplain-oriented species if the rise of the flood pulse is concurrent with the seasonal rise in water temperature, as this allows YOY fish to have access to the food rich floodplain for much of the growing season (Junk et al. 1989). If the flood pulse occurs too late, or is not persistent through the spring growing season, YOY will not have access to the nutrients on the floodplain and recruitment will fall (Junk et al. 1989). In addition, over-wintering survival of YOY fish has been shown to increase if they are able to access flooded backwaters, which can have water temperatures higher than that of the deeper main channel and act as a thermal refuge (Sheehan et al. 1990).

Given these environmental distinctions of the coastal plain from other physiographic regions, the spatial patterns of environmental gradients and fish assemblages described in other lotic systems (discussed above) may not apply in coastal plain systems. For example, longitudinal patterns related to channel volume may be weakened in the coastal plain, as channel volume may not necessarily be greater downstream during periods of high flow and floodplain connectivity. Additionally, in reference to the River Continuum Concept (Vannote et al. 1980), the suggestion that downstream areas experience production primarily from autotrophic phytoplankton in the water column (in contrast with headwater streams) may not hold true in the coastal plain. The inundation of riparian floodplains allows for lateral exchange of production, and downstream areas are highly dependent on riparian organic input as there is relatively little autotrophic production due to its blackwater nature (Junk et al. 1989, Meyer and Edwards 1990). A more comprehensive investigation into how environmental regimes, and in turn fish assemblage patterns vary spatially in the coastal plain would therefore be beneficial to the understanding of these systems.

Thesis Goals

The goal of this thesis was to characterize the taxonomic and functional organization of coastal plain fish assemblages in the Atlantic coastal plain of Georgia, assess their variation over space and time, and determine the environmental factors most influential in driving these patterns. This was accomplished in two complimentary chapters. The objective of the first chapter was to characterize the richness and taxonomic composition of Atlantic coastal plain (ACP) fish assemblages among stream reaches, assess the stability of these patterns over time, and identify the environmental predictors (regional- and local-scale) most influential in driving assemblage variation. The second chapter focused on characterizing functional assemblage variation in terms of species traits (e.g. reproductive and trophic strategy) and explicitly testing hypothesized relationships between longitudinal position, stream size, assemblage composition, and habitat characteristics. Collectively, these two initiatives provide a deeper understanding of how Atlantic coastal plain fish assemblages vary in space and time, and the environmental drivers that may be responsible for these dynamics. These findings can be utilized in conservation biology applications to recommend strategies to managers regarding how best to manage populations, physical habitat, and water quality and quantity, and how best to measure habitat and community condition in Atlantic coastal plain streams.

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CHAPTER ONE: EVALUATING THE RELATIVE INFLUENCE OF LOCAL VERSUS REGIONAL CONTROLS ON FISH ASSEMBLAGE COMPOSITION IN ATLANTIC COASTAL PLAIN STREAMS

ABSTRACT

Atlantic coastal plain (ACP) streams are complex, dynamic ecosystems supporting diverse fish assemblages. Much of our knowledge about stream-fish assemblage ecology comes from upland streams, which may prove poor models for understanding ACP streams, which possess distinctive hydrologic regimes and faunas. A better ecological understanding of environmental controls on fish assemblages would therefore be beneficial and applicable to the conservation and management of ACP fishes. I examined various potential controls acting across regional (e.g. drainage area, elevation, anthropogenic land use) and local scales (e.g. water quality, physical habitat complexity) for their influence on species richness and assemblage structure in ACP streams of Georgia. Based on previous studies in other lowland systems, I hypothesized that (1) ACP fish assemblages would be more influenced by local than regional habitat conditions, and (2) at the regional scale, historical biogeographic factors such as basin, physiography, and stream size would be more influential than contemporary factors such as anthropogenic land use. Based on 38 assemblage samples collected across two summers, I found that both species richness and assemblage composition were influenced to varying extents by controls acting a both local (e.g. pH, conductivity, temperature, dissolved oxygen, sinuosity, and coarse substrate) and regional scales (e.g. stream size, ecoregion, and urban and agricultural land use). However, relations between assemblage composition and regional factors, particularly land use, were weak. Rather, assemblages primarily sorted into two groups, possessing species and local environmental characteristics typical of either “fluvial” of “nonfluvial” conditions. This

fluvial/nonfluvial distinction emerged in a variety of stream sizes across all three basins studied, and persisted across two hydrologically distinct summers, suggesting that this dichotomy might be a fundamental aspect of fish community structure in this region. Biologists should account for the importance of local control and the existence of alternative community states when testing ecological models, assessing biodiversity, and developing bioassessment tools in the Atlantic coastal plain.

INTRODUCTION

North American fluvial ecosystems such as streams, rivers, and their associated floodplain habitats harbor the greatest diversity of temperate freshwater fishes worldwide (Warren and Burr 1994, Fausch et al. 2002). Considerable research has been devoted to asking how environmental conditions "filter" regional species pools to produce local assemblages (Poff 1997, Southwood 1977, 1988, Townsend and Hildrew 1994). Answers to these questions have important implications for the maintenance of biodiversity, provisioning of ecosystem services (e.g. nutrient cycling, fisheries), and the ways in which rivers and adjacent landscapes are managed.

Past research indicates that local fish-assemblage richness and composition results from a complex interplay of environmental influences, acting across a range of spatial scales (Ricklefs 1987, Taylor 1996, Angermeier and Winston 1998, Marsh-Matthews and Matthews 2000, Hoeinghaus et al. 2007, Pease et al. 2011). At regional scales, assemblage richness and composition may be influenced by factors such as watershed area, zoogeographic breaks that occur at basin or ecoregion boundaries, and anthropogenic land use (Ricklefs 1987, Jackson and Harvey 1989, Angermeier and Winston 1998, Hoeinghaus et al. 2007). Ecoregions exhibit distinctive fish assemblages due to these distinguishing regional characteristics; for example, ecoregions of steeper gradient can support species that require consistent flow, and ecoregions with historical disturbance of riparian areas (e.g. conversion to agriculture) may contain species tolerant to turbidity and sedimentation (Legendre and Legendre 1992, Larsen et al. 1986, Pease et al. 2015). Assemblages may also separate across basins, due to dispersal barriers or basin endemism as a result of historic geographic isolation (Kaller et al. 2013, Paller et al. 2016). Because river basins may distribute throughout multiple ecoregions within their watershed, ecoregion membership may be more predictive of fish assemblage structure than basin membership (Pease et al. 2011). Fish assemblages also exhibit patterns within ecoregions. For example, a positive species-area relationship has been extensively described in lotic systems, as larger, downstream systems typically exhibit greater species richness and different assemblage composition than smaller, headwater systems. This species-area relationship has multiple potential explanations, including the theory of island biogeography, which suggests that larger habitat volume downstream is a bigger "target" for immigrants (MacArthur and Wilson 1967), a higher number of available ecological niches downstream due to greater microhabitat diversity (Gorman and Karr 1978, Townsend and Hildrew 1994), or the longitudinal shifts in energy and food resources described by the River Continuum Concept (Vannote et al. 1980).

In addition to natural variation introduced by stream size, ecoregions, and basin boundaries, regional anthropogenic activities can alter the environmental conditions of aquatic habitats, which may influence the structure and function of fish assemblages. For example, runoff from agricultural or urban areas can cause increased turbidity and fine sediment deposition, harmful nutrient loading leading to a decrease in dissolved oxygen, increased acidity due to organic acids, and inputs of urban pollutants such as salts and heavy metals (Jackson et al.

2001, Wang et al. 2003a, Sawyer et al. 2004). The removal of natural riparian vegetation for conversion to agriculture or urban areas may also lead to a decrease in bank stability and habitat heterogeneity, and an increase in sedimentation (Allan et al. 1997, Booth and Jackson 1997, Wang et al. 2001). Percent urban or impervious land cover has been shown to decrease richness, diversity, and density of fish communities (Wang et al. 2001). Agricultural land cover has been found to reduce species richness and functional diversity (Lammert and Allan 1999, Karr et al. 1986) and decrease stream habitat quality (Allan et al. 1997). For example, increased agriculture can lead to an increase in macrophyte growth and siltation, resulting in overall loss of functional diversity shown by an increase in macrophyte-related or herbivorous species and a loss of benthic-oriented species (Schlosser 1991, Dala-Corte et al. 2016). The relative influence of watershed versus local land use varies widely among studies. Roth et al. (1996) found that regional watershed-scale land use was significantly more influential than local riparian cover on habitat and biotic integrity, whereas other studies in the same watershed found local-scale land use within a 100-meter riparian buffer to be more predictive of the same integrity metrics (Lammert 1995, Lammert and Allan 1999). Anthropogenic groundwater withdrawal for agricultural purposes can alter the natural hydrologic regimes of lotic systems, lessening the availability of freshwater inputs that may otherwise provide beneficial flow, cooler temperatures, and dissolved oxygen. Another anthropogenic alteration of natural hydrology impacting stream systems is the channelization of streams channels for navigational and urban purposes, which can result in decreased structural complexity and habitat heterogeneity, leading to a decrease in species diversity (Booth and Jackson 1997, Schlosser 1991).

Local environmental conditions may also play a strong role in determining the richness and composition of fish assemblages. For example, harsher water quality conditions (e.g. high temperatures and suspended solids, low dissolved oxygen and pH) may shift assemblage composition to more tolerant species, with the loss of intolerant species leading to a decrease in species richness (Karr 1981, Meador and Carlisle 2007, Jackson et al. 2001). Other local-scale influences on fish communities include components of physical habitat complexity that contribute to microhabitat specialization (e.g. channel dimensions, substrate, structure, velocity). Variations in channel depth and velocity create microhabitats such as deep pools that provide refuge from strong currents or harsh seasonal water quality conditions, and shallow riffles that provide refuge from predators and increased flow for small-bodied species such as darters, madtoms, and some minnows that prefer current (Schlosser 1987, Fraser and Cerri 1982, Power 1987, Schlosser and Ebel 1989, Gorman and Karr 1978). Increased structural complexity (e.g. substrate variability, presence of large woody debris) can also increase community richness and diversity as structure provides increased refuge, foraging habitat, and substrate-specific reproductive strategies (Gorman And Karr 1978, Angermeier and Karr 1984). It is important to consider that local-scale factors can be influenced by regional-scale factors such as land use and ecoregion. For example, changes in riparian land use (e.g. conversion to agriculture) may result in increased siltation and sedimentation of local habitats (Larsen et al. 1986), and the natural geology of an ecoregion (e.g. bedrock) may influence water chemistry components such as acidity (Jackson et al. 2001). Furthermore, the relative importance of local- versus regional-scale factors may be region-dependent depending on regional levels of anthropogenic disturbance. Specifically, regional land cover may be more influential relative to local habitat conditions in highly disturbed systems (i.e. high levels of riparian agriculture and urbanization) (Wang et al. 2003b, Wang et al. 2006). In low-disturbance systems, there is a dynamic equilibrium between regional, riparian, and local instream factors that together create relatively stable instream habitat conditions along which species sort by life-history strategies and preferences (Allan 2004, Wang et al. 2006). As systems become more disturbed with increasing anthropogenic influences, this equilibrium is interrupted, and local instream factors reestablish according to changes in land cover (e.g. increased sedimentation). Fish assemblage composition is therefore a response to the altered instream habitat conditions that result from regional land cover disturbance (Wang et al. 2003b, Wang et al. 2006).

It is important to test these environment-assemblage relationships in a variety of settings to evaluate whether and when they can be generalized and produce accurate predictions. One ecoregion that has received relatively little study, relative to its drainage area, is the Atlantic Coastal Plain of the Southeastern United States. The Atlantic Coastal Plain (ACP) is characterized by its seasonal variability in freshwater inputs and hydrology (Benke et al. 2000). The ACP experiences a seasonal flood pulse in the winter and spring months that connects stream channels to floodplains, which provides ecosystem services for aquatic species such as nursery habitat and the exchange of nutrients (Bayley 1991). The extreme seasonal variation in hydrology can result in a single stream seeing occasions of both inundation and drying within a year, and flows may vary inter-annually depending on factors such as precipitation and evapotranspiration (Benke et al. 2000). ACP streams experience little change in elevation throughout their watershed area, which can result in poorly defined, braided, and slow-moving channels that are increasingly susceptible to inundation events. Because of this low-lying nature, ACP streams have few barriers or impoundments in comparison to upland systems. Organisms inhabiting ACP streams typically experience harsh water quality stressors such as high water temperatures and low dissolved oxygen during dry or stagnant periods, and high levels of acidity due to inputs of humic and fulvic acids from riparian organic matter (Maxted et al. 2000, Junk et al. 1989). ACP soils tend to be slow-draining, and land use in the region has been historically dominated by agricultural lands, livestock pastures, and silviculture (GA DNR 2007).

I hypothesize that the harsh physiochemical characteristics and dynamic hydrology of the ACP will affect the transferability of previous environmental-assemblage relationships to this ecoregion. In particular, I hypothesize that in the ACP, local environmental conditions will affect richness and composition more strongly than regional conditions, and that among regional factors, biogeographic variables such as stream size and ecoregion will be more influential than anthropogenic land use. Previous coastal plain literature shows little effect of anthropogenic land use on the taxonomic composition of coastal plain fish assemblages. Urbanization within a 30-meter riparian buffer was negatively correlated with the number of sensitive species in one study (Sawyer et al. 2004), and the amount of watershed development was positively correlated with the number and abundance of mosquitofish, chubsuckers, and some centrarchids (i.e. warmouth, bluegill, largemouth bass) in another (Paller et al. 2016); however, land-use at the watershed scale generally is not correlated with assemblage composition, as coastal plain species tend to be hardy and potentially insensitive due to naturally harsh physiochemical conditions (Paller 1994, Sawyer et al. 2004, Marion et al. 2015) (Table 1.1). Historically, the southern coastal plain was extensively deforested for agricultural use in the 18th and 19th centuries, but is more recently returning to open grassland and planted pine. It is possible that current coastal plain fish assemblages are an artifact of the historical landscape more so than a response to current land use (Marion et al. 2015). Additionally, the low-lying nature of the coastal plain naturally minimizes the magnitude of runoff and erosion, the most problematic land-use effects on instream habitat (Marion et al. 2015). Longitudinal species-area relationships described in other lotic systems (MacArthur and Wilson 1967, Schlosser 1990, Vannote et al. 1980) may be weakened or “reset” in the coastal plain during seasons of high flows when floodplain inundation leads to increased habitat area and volume and high movement connectivity system-wide, though a positive relationship between richness and stream may develop during periods of low flow (Paller 1994, Paller et al. 2016). Because endemism in the CP is low relative to upland systems, I expect basin boundaries and biogeographic history to contribute less to variation in the coastal plain than elsewhere, particularly when comparisons are restricted to the Atlantic slope (Marion et al 2015, Paller et al. 2016).

In contrast to regional factors, I predict that local-scale factors may be disproportionately influential in coastal plain streams relative to other regions due to 1) a naturally harsh environmental regime, and 2) the seasonal variability of habitat conditions. Coastal plain streams are characteristically acidic due to riparian input of organic acids, relatively low in structural complexity (i.e. are "habitat-starved"), and low-gradient, often resulting in stagnant waters with high temperature and low dissolved oxygen (Maxted et al. 2000, Dolloff and Warren 2003, Meffe and Sheldon 1988). Many coastal plain fish species are adapted to survive in these conditions, but reaches with more benign conditions (e.g. higher flows, less extreme water quality, more habitat structure) may attract more species and/or individuals. In addition, local instream factors influencing lotic fish communities (e.g. habitat heterogeneity, water quality) are inconsistent in the coastal plain due to its variable hydrology. For example, the number of microhabitats in a stream, and therefore the number of species it can support, will fluctuate as flow levels dictate channel volume and velocity. In other words, whereas regional-scale conditions remain relatively constant, local habitat conditions are not persistent in coastal plain streams. The importance of local habitat conditions in structuring ACP assemblages was evident in a study wherein defaunated reaches were rapidly recolonized by assemblages very similar in abundance, richness, and composition to pre-defaunation assemblages, suggesting that the suite of local habitat characteristics is highly predictive of the assemblage that will occur (Meffe and Sheldon 1990).

Regardless of transferability of local and regional spatial predictions to ACP streams, I predict that the temporal environmental variation of these streams will reduce the stability of CP fish assemblages over time. This should be particularly evident between wet and dry seasons, as I expect assemblage similarity among sites to increase during periods of high flow as environmental conditions homogenize system-wide (i.e. spring-early summer), and assemblages to separate during dry periods (i.e. late summer-fall) as some sites may dry and others may offer refuge. Coastal plain streams also experience inter-annual hydrologic variation among summer-fall dry seasons, due to differences in the annual rainfall and temperature and therefore timing, magnitude, frequency, and duration of the flood pulse (Junk et al. 1989). Variable conditions like these result in decreased stability of the fish community in other harsh systems such as prairie and desert streams, as species abundances respond to changes in abiotic environmental conditions (Ross et al. 1985, Constanz 1981, Grossman et al. 1982), whereas communities remain more stable in regions with milder environmental regimes. I therefore expect that coastal plain streams separate in assemblage structure across years of differing flow patterns (i.e. a “dry” versus a “wet” year); specifically, I expect individual coastal plain streams to maintain their overall assemblage characteristics across years of similar flow patterns, and to differentiate across years of dissimilar flows. The serendipitous timing of my study across two years with rather different flow conditions provided an opportunity to test this prediction.

I evaluated these hypotheses using data I collected on local fish assemblages and local- and regional-scale environmental conditions, over two summers in a series of streams in the

Atlantic coastal plain of Georgia. My specific objectives were to 1) characterize the richness and composition of fish assemblages in wadeable ACP streams, 2) evaluate relative influences of local- versus regional-scale environmental variation on assemblages, and 3) use inter-annual samples to assess the temporal stability of assemblage composition and environmental influences.

METHODS

Sample Site Selection

Thirteen study streams were selected within the Ogeechee, Altamaha, and Savannah River basins in the vicinity of Georgia Southern University. Eight study streams were located in the Ogeechee river basin, three within the Altamaha, and two within the Savannah. All streams were located within the Atlantic coastal plain, with most (11) located in the Southeastern Plains Level III ecoregion, and two located in the Southern Coastal Plain (distinguished as lower-gradient with slower-draining soils) (EPA 2013). All sample streams were located above tidal influence and therefore were not affected by tidal flow or salinity flux. Within each stream, a paired up- and downstream site were selected, for a total of 26 sample sites across the study area (Figure 1.2). The paired design within each watershed was selected in an attempt to capture longitudinal variation within each stream (a primary focus of chapter 2). Sites were selected to be wadeable (i.e. a depth and width suitable to be sampled via backpack electrofishing), non-braided streams with defined channels that were accessible with a short hike from a public road crossing. The beginning of each sample reach was always at least 50 meters from the bridge. Upstream sites were on average 17.0 km from downstream sites (range 2.4 - 36.9 km) and shared an average of 40.3% of downstream watershed area (range 12.6 - 76.8%). See Table 1.2 for a list of all sample sites.

All twenty-six sites were sampled during summer base flows of 2016. Although I intended to re-sample all sites during summer 2017 base flows, only 14 were wadeable during this time due to unusually high summer flows (see Table 1.2 and Figure 1.3). I subsequently removed data from one intermittent site (upstream Ogeechee Creek in 2016) and another sampling occasion (upstream Lotts Creek in 2017) in which few fish were captured. I thus retained 38 separate sampling events, with 25 occurring in 2016 and 13 occurring in 2017 (Table 1.2).

Fish Sampling

I sampled fishes using backpack electrofishers, which capture a broad range of sizes and species in comparison to other sampling techniques (Knight and Bain 1996, Reynolds 1996). All fish sampling took place during summer base flows (late June – early September) following the recession of the flood pulse, but before sites had disconnected or dried late summer/early fall months as sometimes occurs.

A 150-meter-long reach of the stream was isolated by setting up a block net at the upstream end of the reach. This reach length was in the range of reach lengths sampled in other similar studies, including those conducted in the southern coastal plain (Allan et al. 1997, Roberts and Hitt 2010, Sawyer et al. 2004, Marion et al. 2015). Field team members entered the reach at the downstream end and electrofishing occurred in the upstream direction, finishing at the upstream block net. At a typical site, two field team members were equipped with Halltech direct-current backpack electrofishers and dipnets while supplemental netters were also equipped with dip nets. If a stream was particularly narrow, only one electrofisher was used. Electrofishers were set at 60 Hz for all sampling, and voltage was adjusted as needed according to measured conductivity, ranging from 450-650 V. As the fish were stunned by the electrical current, they were netted and placed in holding buckets until they could be transferred to larger holding containers equipped with aerators.

Once all electrofishing was completed, sampled fish were individually counted, identified to species, and measured for mass (g), standard length (SL; mm) and total length (TL; mm). Only TL was recorded for fish lacking homocercal tails (American Eel, Bowfin, and Longnose Gar). Any abnormalities (e.g. lesions, parasites) or other observations (e.g. body condition) were also recorded. Fish were identified based on keys in Fishes of South Carolina (Rohde et al. 2009) and Fishes of the middle Savannah River Basin (Marcy et al. 2005). Crew members all were trained in ACP fish identification by Dr. Jamie Roberts, and whenever a fish could not be identified to species level in the field it was preserved in 10% formalin and transported back to the lab for subsequent identification by Dr. Roberts. Photo vouchers were collected for all but the most common species.

Local Environmental Variables

Water quality variables were measured immediately after arriving at the site. A YSI Pro2030 meter was used to measure water temperature (°C), dissolved oxygen (mgL^{-1}), ambient and specific conductivity (μScm^{-1}). A Eutech Instruments pHTestr 10 meter was used to measure pH, and a LaMotte 2020we turbidimeter to measure turbidity (NTU) (Table 1.3). Water quality measurements were collected at varying times of day, ranging from late morning to late afternoon. Due to diel fluctuations in water chemistry conditions, this may have injected random error into spatial and temporal comparisons of sites, particularly with regards to temperature. Although continuous water temperature data to determine diel variation was not available for most sites, USGS data for one site (downstream Williamson Swamp Creek, USGS gage 02201000) suggested that the average diel fluctuation in water temperature between late morning

(09:00) and late afternoon (15:00) during summer sampling months was approximately 0.9℃ and 0.7℃ in 2016 and 2017, respectively (U.S. Geological Survey 2016).

After electrofishing was completed, I conducted an assessment of instream physical habitat. I placed 15 transects perpendicular to the stream at 10-m intervals. At each transect, I measured bank height for each bank (i.e. the vertical distance from water level to the top of the bank) and stream wetted width. Stream depth and substrate type (mud, sand, gravel, detritus) were measured at 1-m intervals across transects. In the 10-m sections between transects, I counted the number of large woody debris (LWD) items (≥ 10 cm wide and ≥ 1.5 meters long; Kaeser and Litts 2008), and the presence/absence of submerged aquatic vegetation (SAV). Beginning in 2017, I used a Swoffer flowmeter to measure mean water-column velocity (at 0.6x depth) at the swiftest point along each transect.

Two reach-scale environmental variables were calculated post-hoc using remotely-sensed data in ArcGIS 10.4 (ESRI 2016). The sinuosity of each stream reach was calculated by dividing the actual reach length (150 m) by the straight-line distance between the start and end point of each sample reach. A perfectly straight channel would therefore have a sinuosity value of 1. The gradient of a sample reach was considered as the change in elevation over a 1-km-long segment, centered on the sampling site. This measurement segment was lengthened beyond the site boundaries in order to capture sufficient vertical variation given the vertical resolution of the DEM data. Gradient was calculated using differences in raster values from 1/3 arc-second (approximately 10 meter) Digital Elevation Model (DEM) data from the USGS National Map.

Regional Environmental Variables

Preliminary analyses showed little predictive power of land cover values in the upstream watershed area as a whole, so a more nuanced calculation of land cover was adapted. In order to effectively capture the relative influence of land cover at different distances upstream of a sample site, an inverse-distance-weighted (IDW) approach was used to calculate land cover. Upstream watershed boundaries were acquired through USGS StreamStats software. Using the methods of King et al. (2005) as a reference, upstream watershed buffers were created for 150, 250, 500, 1000, 5000, and 10000 meters upstream of the downstream start point at each sample site. The 150-meter buffer therefore represented the watershed area containing the 150 m sample reach. The amount of each land cover classification in each hollow buffer area (i.e. excluding the area within a buffer zone containing smaller buffers) was calculated using ArcGIS 10.4 (ESRI 2016) and land cover raster data from the Coastal Change Analysis Program Land Cover Atlas (C-CAP) (30-meter resolution; NOAA). C-CAP classes 2-4 were combined to create “percent developed” metric, classes 6 and 7 were combined to create “percent agriculture”, classes 9 and 11 were combined to create “percent deciduous/mixed forest”, and classes 13-15 were combined to create “percent wetland”. I then applied the following IDW equation to the land cover values for each buffer (King et al. 2005):

$$\text{IDW land cover \% in upstream watershed} \;=\; \frac{\sum_{i=1}^{C} n_X W_C}{\sum_{i=1}^{C} n_T W_C} \text{ x } 100$$

Where C refers to the number buffers or distance classes (in this case six), n_x is the number of raster cells in buffer i of the land cover classification of interest, W_c is the inverse-distance weight of buffer i, where the inverse-distance weight is equal to the maximum distance between a buffer’s edge and the start of the sample reach (i.e. 150, 250, 500, 1000, 5000, 10000) raised to the -1 power (d^{-1}), and n_T is the total number of raster cells within buffer i. The IDW equation therefore must be applied to each unique land cover classification of interest. By using an IDW approach to land cover, the relative importance of land cover closest to the sample reach is weighted the highest, with decreasing weight applied to each successively more distant buffer. This allows for the influences of upstream land cover to be considered over large distances (i.e. up to 10,000 meters), assigning the most influence to areas closest to the sample reach (i.e. 150 meters). Basin, Level-III ecoregion membership, and upstream drainage area (obtained from USGS StreamStats; streamstats.usgs.gov) were also included as predictor variables in analyses.

Data Analyses

Prior to further analyses, all environmental variables were tested for collinearity using a Pearson's correlation, and for pairs with a Pearson's correlation coefficient of $|r| > 0.7$ one of the two variables was excluded. Of correlated pairs, the variable thought to be most ecologically relevant was kept.

Mid-way through the 2016 sampling season I began keeping separate species lists for each of three contiguous 50-meter sections per site in order to estimate true species richness and the detection probability of my sampling method. Such data were collected for 28 out of 38 sampling events. The three 50-meter replicates were used to estimate true species richness using the "Chao2" (Chao 1987) bias-corrected nonparametric approach, as implemented in EstimateS version 9.1.0 (Colwell 2017). I divided observed (raw) by estimated richness to estimate my detection probability. However, because not all sampling events allowed the calculation of estimated richness, I used observed richness as the dependent variable in models investigating environmental influences (see below).

Environmental influences on assemblage structure were assessed using a series of three complementary analyses. All of these analyses included data from both summers (i.e. all 38 sampling events), as well as a binary "year" variable to account for temporal variation. First, I used random forest (RF) regression models to assess which environmental variables were most important in explaining variation in species richness among sites. Tree-based methods such as classification and regression trees (CART) and RF attempt to find the best predictive model, or "tree", that explains the variation in a continuous response variable given multiple predictor variables. In a regression tree analysis, a single best fitting tree is chosen based on the entirety of a data set (De'ath and Fabricius 2000). The model tree splits at "nodes" which represent a binary splitting rule of a given predictor variable, which are selected to minimize the within-group sum-of-squares for the response variable. Therefore, predictor variables identified by the model to be split at nodes can be considered as important to describing the variation in the response variable. Whereas a regression tree builds a single model based on the entire data set, an RF analysis builds multiple trees by partitioning random subsets of the data to identify recurring patterns between response and predictor variables (Cutler et al. 2007). Each tree will therefore vary slightly and can be considered a "vote" as to what predictor variables are important and should therefore be included in the final model. By incorporating multiple trees that have been built with different bootstrapped samples of the whole dataset, RF helps to avoid model over-fitting (Cutler et al. 2007).

To build the RF model of species richness I used the randomForest package (Liaw and Wiener 2002) in R 3.4.3 (R Core Team 2017). Five thousand trees were built for the model, and an mtry value, or number of variables randomly sampled at each split, of 3 was used as it minimized the out-of-bag error in comparison to other mtry values. Use of alternative mtry values had no substantive effect on model outcomes. I evaluated variable importance by the percent increase in model mean squared error (MSE) (i.e. decrease in accuracy) when values of a given predictor variable were randomly permuted among observations (i.e. sampling events). In this way, predictor variables with a higher increase in MSE can be considered as more important to the model, and therefore more predictive of the response variable. Variables with a % increase in MSE of > 10% were considered “important” and worthy of further interpretation (He et al. 2010). I then created partial dependence plots of important predictors to visualize the relationship between each predictor and the response of species richness while holding constant the effect of all other variables.

Second, I used the BIOENV procedure described by Clarke and Ainsworth (1993) to 1) visualize separation of assemblages in multivariate space and 2) determine which species, and which environmental variables, were most associated with this separation. The BIOENV procedure creates two non-metric multi-dimensional scaling (NMDS) ordinations based on pair-wise comparisons among sites: the first is created using a Euclidean distance matrix of environmental predictor variables and the second is created using a Bray-Curtis dissimilarity matrix of species abundances. The two distance matrices are then compared to each other using Spearman’s correlation coefficient with the statistical significance of these relationships assessed using a Mantel test. This is performed for multiple Euclidean distance matrices generated from random subsets of environmental variables to determine the subset of predictor variables that maximizes the correlation coefficient. If the subset of predictor variables identified includes variables most important for predicting assemblage structure, the two ordination plots would be expected to be very similar, whereas the exclusion of important predictor variables would cause the ordinations to deviate (Clarke and Warwick 2001). Variables included in this subset are then individually subjected to a Mantel test to determine the significance of their relation to community dissimilarity. In the second step of the BIOENV procedure, I conducted a type of indicator species analysis to identify which species are most influential in driving the loading of sites on the NMDS ordination (i.e. the variation in assemblage structure between sites) using a Mantel test for a significant association ($\alpha < 0.05$) between each species' abundance and community dissimilarity as described above for environmental variables. Significant species and environmental variables can be displayed on an NMDS ordination as vectors that are scaled by their correlation coefficients from permutation tests.

I conducted the BIOENV analysis using the bio.env function in the vegan package (Oksanen et al. 2018) of R 3.4.3 (R Core Team 2017). Environmental predictor variables were transformed using either an arcsine square root transformation (proportions, e.g. watershed land cover) (Ahrens et al. 1990), or $\log_{10}(x + 1)$ transformation (all other variables), and the abundance matrix was $\log_{10}(x + 1)$ transformed. Rare species, defined as any species occurring at only one site at a level of less than five individuals, were excluded from this analysis. Species identified as rare included Bluehead Chub (*Nocomis leptocephalus*), Christmas Darter (*Etheostoma hopkinsi*), Lined Topminnow (*Fundulus lineolatus*), Channel Catfish (*Ictalurus punctatus*), Creek Chub (*Semotilus atromaculatus*), and Eastern Mudminnow (*Umbra pygmaea*) (Appendix 1). In addition to rare species, *Gambusia holbrooki* (Eastern Mosquitofish) were excluded from this analysis because I was unable to sample them at a level representative of their true abundance at any given site.

Third, based on the clustering of sites in NMDS plots (see Results), I assigned sites to one of two groups and used RF to predict group membership of sites based on environmental variables. Classification random forests use the same statistical concepts as discussed above, but with predictor variables being evaluated in terms of their importance in correctly classifying an observation's membership to a particular group. By using a bootstrap of predictor variables and response data, the RF classification attempts to sort the bootstrapped observations (i.e. sites) into one of the two possible groups. From this, a confusion matrix is generated showing the classification error rate (i.e. how often a site was misclassified in the model). The best fitting model therefore has the lowest error rate. Five thousand trees and an mtry value of 4 were used for the model.

In order to statistically confirm that sites exhibited a true clustering pattern of two distinctive groups, I conducted an Analysis of Group Similarities (ANOSIM) using the anosim function in the vegan package (Oksanen et al. 2018) of R 3.4.3 (R Core Team 2017). ANOSIMs test for significant differences between defined groups in a way similar to other ANOVA-type tests, but look for between-group differences in distance matrices (e.g. Bray-Curtis) rather than raw data (Clarke 1993). I also conducted an ANOSIM analysis on sites sampled in both 2016 and 2017 to determine whether sites differed in assemblage composition between sampling years.

RESULTS

Environmental Influences on Species Richness

Over the course of all 38 sampling events, 5,788 individual fish representing 52 different species were captured (see Appendix 1). Observed species richness averaged 17.02 and ranged among events from 10 – 25 species. Mean richness estimated from the Chao2 approach was 20.9 (range 11.3 – 36.7). My detection probability (i.e. observed/estimated richness) averaged 0.86 with a range among sites of 0.59 to 0.98. None of the measured environmental variables were strongly correlated with sampling efficiency (all $|r| < 0.4$), indicating that my estimates of richness were relatively unbiased by environmental conditions. Furthermore, observed and estimated richness values were strongly correlated ($r = 0.87$), indicating that observed richness satisfactorily represented trends in the variation of richness among sites.

After testing for collinearity of environmental variables, percent evergreen forest was positively correlated with percent deciduous/mixed forest ($r = 0.79$), and percent mud substrate and percent detritus substrate were negatively correlated with percent sand/gravel substrate ($r = -0.71$ and $r = -0.78$, respectively). In each case, only the latter variable was retained for subsequent analyses. Channel volume was also excluded as it was highly correlated with both channel depth ($r = 0.88$) and channel width ($r = 0.76$).

The optimal RF regression model explained 18.1% of the variation in species richness among sites. Contrary to my hypothesis, both biogeographic regional and local factors were important predictors of species richness. The most important predictor variables were, in order of decreasing % increase MSE: sinuosity, drainage area, specific conductivity, temperature, % developed land cover, and pH (Figure 1.4). Sinuosity and developed land cover exhibited a negative relationship with species richness, whereas drainage area, conductivity, and pH exhibited positive relationships (Figure 1.5). Temperature exhibited a unimodal relationship, such that richness was highest in intermediate temperatures (~25-26°C) and decreased toward both extremes.

Environmental Influences on Taxonomic Composition

The two-dimensional NMDS ordination of all 38 assemblage samples in species space exhibited a stress of 0.15 (Figure 1.6a). NMDS stress values (a measure of goodness-of-fit) > 0.2 are commonly considered suspect or suggestive of a weak relationship whereas stress < 0.2 indicates acceptable fit (Boyra et al. 2004, Arthington et al. 2005). Based on the BIOENV analysis, the subset of environmental variables that were significantly associated with the species ordination included a mix of local-scale and biogeographic regional-scale factors, but local factors tended to be more important. These variables, in order of decreasing strength of correlation (rho), were: dissolved oxygen, % sand/gravel substrate, pH, sinuosity, specific conductivity, temperature, bank height, drainage area, and level III ecoregion (Table 1.4). Assemblage samples with higher scores along NMDS axis 1 tended to exhibit higher sinuosity, whereas samples with lower scores along axis 1 tended to exhibit greater dissolved oxygen, % sand/gravel substrate, pH, conductivity, temperature, bank height, and drainage area, and were more likely in the Southeastern Plains ecoregion than the Southern Coastal Plain (Figure 1.6a). Based on these environmental associations, it appeared that samples separated into two distinct groups possessing either more fluvial (i.e. associated with stream flow) characteristics (23 samples to the left) or more nonfluvial (i.e. associated with stagnant or low-flow conditions) characteristics (15 samples to the right). These two groups were confirmed to be statistically distinct from one another in assemblage composition by the ANOSIM test (R = 0.85, P = 0.001). I retained this post-hoc classification scheme for subsequent analyses.

In the indicator species analysis, I identified 15 species as significantly associated with fluvial assemblages (i.e. low NMDS axis 1 score) and 10 species as significantly associated with nonfluvial assemblages (i.e. high NMDS axis 1 scores) (Table 1.5). Exemplary fluvial species included Dusky Shiner, Coastal Shiner, Speckled Madtom, Redbreast, Tessellated Darter, and Blackbanded Darter, whereas exemplary nonfluvial species included Redfin Pickerel, Flier, and Warmouth (Figure 1.6a, Table 1.5).

Based on the classification of samples into fluvial (n = 23 samples) versus nonfluvial (n = 15 samples) groups, I used RF to predict the group membership based on the environmental variables described previously. The optimal model had an overall out-of-bag error rate of 10.5%;

all 23 fluvial samples were classified correctly, whereas 4 out of 15 (26.7%) nonfluvial samples were misclassified by the model as fluvial samples. Random Forest results agreed with those from the BIOENV analysis, indicating that assemblage type (fluvial versus nonfluvial) was influenced by a mix of local and regional factors, but with the most important factors being local. Unlike the BIOENV analysis, the RF indicated some importance of anthropogenic land use, but these variables were less important than biogeographic or local factors. Important predictor variables were, in order of decreasing % increase MSE: % sand/gravel substrate, dissolved oxygen, pH, specific conductivity, drainage area, level-III ecoregion, sinuosity, temperature, bank height, % developed land cover, and % agricultural land cover (Figure 1.7). Partial dependence plots indicated that membership to a fluvial assemblage was associated with higher water temperature, pH, dissolved oxygen, conductivity, substrate coarseness, and bank height (Figure 1.8). Furthermore, fluvial assemblages were more likely to occur in larger than smaller streams and more likely in the Southeastern Plains than the Southern Coastal Plain ecoregion.

Temporal Stability of Fish Assemblages

Seasonal stream flow patterns were notably different between sampling years, providing an opportunity to evaluate the effect of flow variation on assemblage variation. The first sampling year (2016) exhibited a pattern of high flows in winter and spring, tapering down to low base flows in the summer and early fall (during sampling). In the second year (2017), flows were lower than 2016 during the spring, but consistently higher than in 2016 throughout summer and early fall (Figure 1.3). These inter-annual differences were consistent throughout the study area.

A site's species richness in 2017 was strongly correlated with its richness in 2016 (r = 0.70), but species richness across sites did not significantly differ between years. The inter-annual stability of fish assemblage composition was assessed by visually examining a site's interannual change on the NMDS ordination. As sites are plotted on the NMDS axis in species space, movement along NMDS axes can be interpreted as a change in the abundance and/or presence of species (i.e. the assemblage composition). The particular direction in which a given species' abundance may shift a sampling event's placement on the axes can be visualized by indicator species' vectors (Figure 1.7a), which point in a direction of increasing abundance. For example, vertical movement of a site along an NMDS axis between years in a direction opposite to the way in which a species' abundance vector is aimed on the ordination suggests that the site experienced a lower abundance of that species over time. If 2016 and 2017 sampling events at a given site clustered together on the ordination, I considered the fish assemblage to be stable. Sites moved variable amounts on the ordination between years, but movement was primarily along NMDS axis 2, not axis 1, such that there was no change over time in the apparent fluvial or nonfluvial character of assemblages (Figure 1.7b). The vertical shifting along NMDS axis 2 suggests some differences in species' abundances at sample sites over time, but differences in assemblage composition between years was not enough to warrant an interruption in the apparent clustering pattern. In addition, an ANOSIM test found no distinctive grouping of sampling events by year for sites that were sampled in both 2016 and 2017 ($R = 0.04$, $P = 0.16$). Thus, summer assemblage composition appeared temporally stable despite significant interannual variation in environmental conditions preceding and during sampling.

DISCUSSION

Environmental Influences on Species Richness

Species richness was influenced by environmental factors acting at multiple scales. At the regional or watershed scale, species richness was positively related to stream size and negatively related to urban watershed land use, as expected (Figure 1.5). However, local-scale environmental factors such as sinuosity, water chemistry, and habitat structure were just as important in explaining spatial variation in species richness. In particular, richness was higher in sites that maintained a more fluvial character, typified by lower sinuosity and higher conductivity and pH (Figure 1.5).

Predictive relationships between richness and local-scale environmental factors can be reflective of idiosyncratic direct influences or artifacts of indirect influences of regional-scale factors that affect reach-level conditions. For example, the local water chemistry of a stream can directly influence the number of species than can occur, particularly at stressful extremes (i.e. only species tolerant to stressors can remain) and can be indirectly influenced by conditions at the watershed scale (e.g. downstream nutrient transport from agricultural runoff). In streams with low levels of anthropogenic disturbance, regional and local conditions are in dynamic equilibrium, and fishes are adapted to these equilibrium conditions (Wang et al. 2006). The fish assemblage is therefore a reflection of the direct effects of local conditions as well as the indirect effects of regional conditions impacting local factors (Wang et al. 2003b, Kauffman and Hughes 2006). I hypothesized that of environmental factors, local factors would be more influential on species richness in part due to the naturally harsh environmental regime of the coastal plain (e.g. high acidity, potentially stagnant waters) (Maxted et al. 2000, Dolloff and Warren 2003, Meffe and Sheldon 1988), which may drive a greater number of species to areas where these stressors were relatively more benign (e.g. higher flows). This hypothesis was not fully supported by my

RF analyses, as both local and regional factors were found to be important in predicting spatial variation in richness (Figure 1.4); however, the conceptual mechanisms behind this prediction may help to explain results. The local factors identified as important predictors of species richness by my RF analysis offer support for this idea, as richness increased as water chemistry became milder (e.g. higher pH, intermediate temperatures) (Figure 1.5).

I further hypothesized that of regional factors, biogeographic factors would be more influential than anthropogenic land cover. Despite high levels of historical land use for agricultural purposes, cover in the coastal plain has recently been returning to less disturbed cover of grassland and planted pine, and the low-gradient nature of the landscape minimizes impacts of erosion and runoff (Fry et al. 2009, Marion et al. 2015). Additionally, only two previous coastal plain studies of which I am aware have found relationships between land cover (i.e. development) and assemblage structure (Sawyer et al. 2004, Paller et al. 2016). Contrary to my hypothesis, both biogeographic (i.e. stream size) and anthropogenic (i.e. % developed cover) regional factors were important predictors of species richness. Similar to other coastal plain studies, my results suggest that development or urbanization is the only predictive land cover of species richness (Sawyer et al. 2004, Paller et al. 2016) (Figure 1.4). Specifically, species richness declines rapidly with even low levels of watershed development (<10%) (Figure 1.5). Of biogeographic regional factors, neither basin nor ecoregion membership were important predictors of species richness, as was predicted due to low endemism in the coastal plain (Marion et al. 2015, Paller et al. 2016); however, there was evidence of a species-area relationship as described in other regions (MacArthur and Wilson 1967, Schlosser 1990, Vannote et al. 1980). Although this relationship may weaken during periods of high flows when habitat volume increases system-wide, this study and other previous studies suggest that a positive relationship between stream size and richness may occur during dry periods, such as summer base flows (Paller 2004, Paller et al. 2016). The interaction of local and regional factors in predicting species richness suggests that my study area experiences relatively little anthropogenic disturbance (Wang et al. 2006).

It is important to consider, however, that the RF model predicting species richness left 82% of the variation in richness unaccounted for, which suggests that my study may have failed to include some potentially important environmental predictors. For example, I did not include velocity in my analyses as I did not acquire a flowmeter until the 2017 sampling season, yet given that richness increased in sites with fluvial characteristics it is intuitive that velocity may also be an important predictor. In addition to velocity, another potentially key component of hydrology that I was unable to measure (due to lack of available data at my sample site locations) is groundwater input, which may influence species richness and composition by impacting flow permanence and associated water quality conditions such as temperature and dissolved oxygen (Winter 1999). Land cover data of a finer resolution considered at a smaller scale may also be beneficial to understanding patterns of species richness but was not available to me for this study. Additionally, this study examined only abiotic influences on assemblage variation, but patterns in species richness also may be influenced by biotic interactions not captured by my data, such as predation and competition (Jackson et al. 2001, Power 1987, Grossman et al. 1998, Gilliam et al. 1993, Tonn and Magnuson 1982).

Environmental Influences on Taxonomic Composition

Based on taxonomic composition, fish assemblages clustered into two relatively discrete groups that were persistent over time, which I term “fluvial” and “nonfluvial” assemblages for reasons discussed below. Further analyses with BIOENV and RF indicated that the separation of these assemblages was driven more by local than regional environmental factors. In particular, fluvial assemblages tended to be associated with environmental conditions relating to the presence of flow, such as higher pH, dissolved oxygen, conductivity, substrate coarseness, and bank height, and lower sinuosity (Figure 1.7a). As discussed above, given this apparent separation in fluviality, another potentially key local factor influencing taxonomic composition is velocity. Although velocity was not measured during 2016 sampling, for the sampling events for which I collected velocity data, mean velocity was significantly greater in the fluvial (mean = $0.21\text{ft}^3\ \text{s}^{-1}$) than the nonfluvial group (mean = $0.07\ \text{ft}^3\ \text{s}^{-1}$) (Student's $t = 3.6$, $p < 0.05$, df =11.4). Fluvial sites did not necessarily have greater flow uniformly throughout the sample reach, but typically contained more patches of locally higher velocity than did nonfluvial sites (personal observation). Although less explanatory than local factors, stream size and ecoregion, two regional factors, were significantly related to assemblage grouping, with larger streams in the Southeastern Plains ecoregion more likely to contain a fluvial assemblage (Figure 1.9). These results agree with Meffe and Sheldon's (1990) findings that coastal plain assemblage composition is highly predicted by local habitat conditions, as a defaunated stream was rapidly recolonized by a taxonomically similar assemblage. Tonn and Magnuson (1982) identified two distinctive assemblages in Wisconsin lakes ("centrarchid-*Esox*" and "*Umbra*-cyprinid"), the dichotomy between which was also driven by local factors such as water chemistry and depth; however, the local environmental factors influencing species richness were different for each assemblage type. This provides additional postulation as to why my RF model explained only 18% of spatial variation in species richness; if different local factors are driving the richness of each assemblage type, this could explain why richness appears to be influenced by regional factors relatively more so than assemblage composition, as important local drivers may be being obscured by including all sample reaches in the model.

A species indicator analysis identified 15 species indicative of fluvial assemblages and 10 of nonfluvial assemblages (Table 1.5). These assignments matched expectations based on the morphology and ecology of the species and previous studies of ACP fishes. Species indicative of fluvial assemblages (e.g. shiners, darters, madtoms) prefer flow and coarse substrate, and typically are intolerant to stream drying and poor water quality (e.g. high temperatures and turbidity, low dissolved oxygen) (Rohde et al. 2009). Conversely, many nonfluvial indicator species (e.g. Golden Shiner, Bowfin), are tolerant to environmental stressors such as low flows, pH, and dissolved oxygen (Rohde et al. 2009, Meador and Carlisle 2007). Whereas all nonfluvial indicator species were also present at fluvial sites (although in lower abundances than at nonfluvial sites), ten out of fifteen fluvial indicator species were only present at fluvial sites (Snail Bullhead, Bannerfin Shiner, Savannah Darter, Tessellated Darter, Rosyface chub, Spotted Sucker, Dusky Shiner, Coastal Shiner, Speckled Madtom, Pugnose Minnow). Given that nonfluvial indicator species tended to occur across all sites, this suggests that nonfluvial assemblages are nested subsets of fluvial assemblages. If assemblages are completely distinct from each other, this suggests that biotic interactions may play a strong role in influencing assemblage composition (Tonn et al. 1990); however, if assemblages exhibit nestedness, this is evidence that assemblages are likely sorting based on environmental influences (Tonn et al. 1990). This was evident in a study comparing Wisconsin and Finnish lakes of historically similar geology and climate that identified equivalent assemblage types in both systems that were driven by the same local environmental conditions (e.g. one assemblage associated with larger lakes with higher pH, one with smaller, shallower lakes) (Tonn et al. 1990). Finnish lakes saw nestedness among assemblage types, whereas Wisconsin lakes saw almost completely distinct assemblages, with each lacking species indicative of the others (i.e. large predators) (Tonn et al. 1990, Tonn and Magnuson 1982). Given that both systems identified similar abiotic influences on assemblage composition, the distinctive assemblages seen in Wisconsin were likely a result of biotic interactions, whereas Finnish lakes were more influenced by environmental factors (Tonn et al. 1990). The apparent nestedness of assemblages found by this study suggests that coastal plain assemblages are more influenced by environmental factors that filter out fluvial species from some sites than by biotic interactions.

Species significantly associated with each assemblage type agreed with the results of an indicator species analysis of fishes in South Carolina coastal plain streams, which indicated that coastal plain assemblages could be classified as either “Fluvial”, “Nonfluvial”, “Centrarchid”, or “Eastern Mudminnow”, the latter including only the namesake species (Marion et al. 2015). Species classified to each assemblage type by Marion et al. were not exclusive and displayed some overlap between “Centrarchid” and both “Fluvial” and “Nonfluvial” (as “Centrarchid” species were habitat generalists), although “Fluvial” and “Nonfluvial” species did not overlap. Although I did capture Eastern Mudminnows at two sites, they were considered a rare species and were excluded from analyses. Of the 9 species identified by my study as significantly associated with a nonfluvial-type assemblage, 7 were identified as indicator species of “Nonfluvial” assemblage types in SC streams (i.e. Bowfin, Mud Sunfish, Pirate Perch, Flier, Redfin Pickerel, Chain Pickerel, Warmouth, Golden Shiner; Swampfish were not an indicator species for any group, and Bluegill were considered an indicator species of their “Centrarchid”-type assemblage). Of my 15 indicator species of fluvial-type assemblages, 13 were identified as indicator species by Marion et al. (2015); 7 were indicative of the “Fluvial” assemblage type (i.e.

Snail Bullhead, Savannah Darter, Tessellated Darter, Dusky Shiner, Speckled Madtom, Coastal Shiner, Pugnose Minnow, Blackbanded Darter), and 5 of the "Centrarchid" assemblage (i.e. American Eel, Redbreast, Spotted Sunfish, Spotted Sucker, Largemouth Bass). Interestingly, of the five fluvial indicator species from my study that were also found at nonfluvial sites, four were indicator species of Marion et al.'s (2015) "Centrarchid" assemblage (i.e. American Eel, Redbreast, Spotted Sunfish, Largemouth Bass). Neither Bannerfin Shiner nor Rosyface Chub were identified as an indicator species by Marion et al. (2015).

One of the most commonly utilized measures of stream health are bioassessment indices such as the Index of Biotic Integrity (IBI) (Karr 1981, Angermeier and Karr 1994). These protocols are customized to be region-specific (e.g. by state and ecoregion) and assess the biological health of streams based on the divergence of the ecological community from the expected or "reference" composition. Both my study and Marion et al.'s (2015) suggest that there are at least two potential reference habitats in the coastal plain that can be expected to have different assemblages. The "fluvial" and "centrarchid" assemblages in Marion et al.'s (2015) South Carolina streams were more likely to occur at sites with higher, permanent flows and environmental conditions associated with higher flows (e.g. higher dissolved oxygen), whereas "nonfluvial" and "Eastern Mudminnow" assemblages were typical of streams with little to no flows that may experience seasonal drying. The corroboration of these reference habitats and assemblages by my study suggests that IBI metrics may be applicable throughout the southeastern coastal plain (i.e. across state lines), but that separate protocols may be needed to detect future disturbances in fluvial versus nonfluvial streams.

Temporal Stability of Fish Assemblages

Atlantic coastal plain fish assemblages demonstrated high stability from one summer to the next despite significant inter-annual variation in the hydrology of sites. Multivariate assemblage composition exhibited little variation between summers, and assemblages maintained group membership (i.e. fluvial or nonfluvial) over time (Figure 1.7b). This result is reflective of Meffe and Sheldon's (1990) findings, where defaunated coastal plain streams rapidly recolonized with highly similar assemblages within a year, even despite drought conditions following defaunation. In Adams et al.'s (2004) study of coastal plain streams in Mississippi, assemblages were much more variable over time, and they became more similar during times of low flows and differentiated during high flows, which was not reflected by my results. My results suggest that coastal plain species may be resilient to periods of disturbance (e.g. periods of low flows or drying, extreme water quality), as assemblages return to approximately the same composition during summer months regardless of summer flow patterns or environmental conditions experienced during other seasons of varying flows (e.g. high winter flows). Tonn and Magnuson (1982) found that assemblage composition was highly predicted by disturbance level (e.g. seasonal depletion of dissolved oxygen and available refugia); given that fluvial assemblage types in my study are associated with more benign water chemistry, and potentially with higher velocity flow permanence, it's possible that my fluvial sites experience less disturbance (i.e. drying) than do nonfluvial sites. This idea is further supported in that larger streams were more likely to include a fluvial assemblage.

My study only captured a snapshot of temporal variation, as there was only one annual replicate and sites were sampled only during summer base flows. Results from this study suggest that spatial variation in ACP fish assemblages is driven predominantly by local-scale environmental factors, many of which may vary temporally given the extreme variation in seasonal flow levels (e.g. greater dissolved oxygen and depth, lower temperature during high flows). The stability of the fluvial/nonfluvial assemblage dichotomy may weaken with increased seasonal flows or rainfall amounts; given that fluvial assemblages are associated with higher dissolved oxygen, stream size, depth, pH, and mild temperatures, I would expect coastal plain assemblages to shift to mostly fluvial assemblages during seasons of high flows (e.g. winter and spring) and times of heavy rainfall. Additionally, the fluviality of assemblages may be sensitive to changes in land use, as sites of higher agricultural and developed cover were less likely to contain a fluvial assemblage, and increases in these cover types could lead to decreased pH, DO, and coarse substrate (Jackson et al. 2001, Wang et al. 2003a). Increases in these types of cover may cause coastal plain sites to shift towards the subset of species indicate of nonfluvial assemblages, as fluvial indicator species may be intolerant to resulting conditions. Similarly, environmental impacts of ongoing climate change (e.g. increasing water temperatures, decreasing dissolved oxygen and pH) may also result in a decrease or loss of fluvial assemblages. Sampling events spanning multiple years and seasonal flow conditions are therefore necessary to more effectively capture inter- and intra-annual variation and thereby test the broader transferability of relationships documented in my study. Of particular interest would be tests of whether the fluvial/nonfluvial assemblage distinction persists during both wet and dry seasons, and the degree to which each assemblage type might be affected by anthropogenic activities.

Conclusions

This study evaluated multi-scale environmental drivers of spatial variation of fish assemblages in the Atlantic coastal plain, a relatively understudied environment. Multiple factors at both the local- and regional-scale were important in explaining species richness and taxonomic composition. At the regional scale, natural biogeographic factors were more predictive than anthropogenic land-use. Atlantic coastal plain fish assemblages appear to sort into two distinct "types", suggested to be indicative of fluvial and nonfluvial environmental conditions, although the occurrence of each assemblage type may depend on seasonal flow patterns and the level of anthropogenic disturbance. While coastal plain assemblages may exhibit natural intra-annual variation with seasonal flow patterns, managers should consider that increasing development and agricultural use of watershed areas and anthropogenic climate change may result in a loss of certain species, such as darters and some minnows. Additionally, managers should consider the development of separate bioassessment protocols for fluvial and nonfluvial streams, as reference conditions and assemblages vary between the two. Future studies of coastal plain fish assemblage dynamics should focus on providing a thorough investigation into both intra- and interannual variation in environmental conditions and assemblage patterns.

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TABLES AND FIGURES

Table 1.1 A summary of coastal plain fish assemblage responses to various environmental features that have been described in previous literature.

Environmental Feature	Fish response	References
Local factors		
Dissolved oxygen (DO) (+)	+ abundance	Sawyer et al. (2004)
	+ darters, madtoms, shiners	Marion et al. (2015)
Dissolved oxygen (DO) (-)	+ tolerant species	Marion et al. (2015)
pH (+)	+ darters, madtoms, shiners	Marion et al. (2015)
pH (-)	+ tolerant species	Marion et al. (2015)
Total suspended solids (TSS) (+)	- richness	Sawyer et al. (2004)
	- # intolerant fish	Sawyer et al. (2004)
Stream depth (+)	+ centrarchids, *Ictalurus* sp., *Erimyzon sp.*	Meffe and Sheldon (1988)
Stream width (+)	Shannon diversity	Sawyer et al. (2004)
	+ darters, madtoms	Sawyer et al. (2004)
	+ centrarchids, *Ictalurus* sp., *Erimyzon sp.*	Meffe and Sheldon (1988)
Velocity (+)	+ darters, madtoms, shiners	Marion et al. (2015), Meffe and Sheldon (1988)
Velocity (-)	+ tolerant species	Marion et al. (2015)
Biogeographic factors (regional)		
Stream size/watershed area (+)	+ richness	Paller (1994), Paller et al. (2016)
	+ piscivores, benthic insectivores	Paller (1994)
Stream size/watershed area (-)	+ large-bodied fish	Paller (1994)
	+ generalized insectivores	Paller (1994)
	+ small-bodied fish	Paller (1994)
Canopy cover (+)	+ evenness	Sawyer et al. (2004)
Elevation (+)	+ darters, madtoms, shiners	Marion et al. (2015)
	+ richness	Paller (1994)
	+ generalized insectivores	Paller (1994)
	+ small-bodied fish	Paller (1994)
Anthropogenic factors (regional)		
% urbanization within 30 m (+)	- darters, madtoms	Sawyer et al. (2004)
% developed cover in watershed	+ mosquitofish, some centrarchids, *Erimyzon* sp.	Paller et al. (2016)

Table 1.2 List of twenty-six sample sites. Sites with the same alphanumeric prefix (e.g. A1) were located upstream (U) or downstream (D) in the same stream, with the exception of Jack's Branch (S2U), which served as the upstream comparison to Ebenezer Creek (S2D). Coordinates represent the downstream end of sample reaches. Assemblage type refers to the environmental conditions and resulting taxonomic assemblage composition occurring at each site, with "fluvial" referring to environmental conditions and species typically associated with the presence of flow.

Site Name	Site ID	Latitude	Longitude	Observed richness (2016)	Observed richness (2017)	Assemblage type
Altamaha Basin						
Ohoopee River	A1D	32.44051	-82.38322	25		Fluvial
Ohoopee River	A1U	32.47139	-82.4474	22		Fluvial
Little Ohoopee River	A2D	32.50668	-82.42987	25		Fluvial
Little Ohoopee River	A2U	32.75397	-82.53706	13	13	Nonfluvial
Pendleton Creek	A3D	32.19112	-82.26239	13		Fluvial
Pendleton Creek	A3U	32.24646	-82.28275	21		Fluvial
Ogeechee Basin						
Buckhead Creek	O1D	32.945503	-82.06768	23	23	Fluvial
Buckhead Creek	O1U	32.983401	-82.133453	20	17	Fluvial
Williamson Swamp Creek	O2D	32.950796	-82.556066	24	22	Fluvial
Williamson Swamp Creek	O2U	33.02116	-82.66362	22	15	Fluvial
Ogeechee Creek	O3D	32.52561	-81.53922	23		Fluvial
Ogeechee Creek	O3U	32.75283	-81.68938	6		Not classified
Mill Creek	O4D	32.42678	-81.61569	14	16	Fluvial
Mill Creek	O4U	32.47358	-81.75408	11	12	Nonfluvial
Black Creek	O5D	32.21947	-81.52109	17		Nonfluvial
Black Creek	O5U	32.27639	-81.62849	18	18	Nonfluvial
Canoochee River	O6D	32.20053	-81.95438	22	21	Fluvial
Canoochee River	O6U	32.35626	-82.09016	10	13	Nonfluvial
Fifteenmile Creek	O7D	32.44865	-82.06181	12	13	Nonfluvial
Fifteenmile Creek	O7U	32.59036	-82.09482	10		Nonfluvial
Lotts Creek	O8D	32.26538	-81.80882	15		Fluvial
Lotts Creek	O8U	32.44814	-81.95569	12	5	Nonfluvial
Savannah Basin						
Beaverdam Creek	S1D	32.821549	-81.623882	20	15	Fluvial
Beaverdam Creek	S1U	32.87331	-81.67543	20	11	Fluvial
Ebenezer Creek	S2D	32.3922	-81.30475	14		Nonfluvial
Jack's Branch	S2U	32.37612	-81.31721	12		Nonfluvial

Table 1.3 Environmental variables measured and method of measurement. Variables in bold were used in BIOENV and random forest (RF) analyses; others were excluded due to collinearity with one or more other variables (see text).

Variable	Code	Mean (Range)
Water temperature (°C)	Temperature	25.7 (23.6-27.1)
Dissolved oxygen (mg L^{-1})	DO	3.4 (0.19-6.8)
Specific conductivity (µS cm^{-1})	S.Conductivity	100.7 (38.6-263.7)
pH	pH	6.9 (5.8-8.3)
Mean bank height (m)	Mean.bankheight	0.9 (0.2-1.8)
Mean stream width (m)	Mean.width	7.3 (3.6-12.2)
Mean stream depth (m)	Mean.depth	0.5 (0.3-1.3)
Channel volume (m^2)	-	4.1 (1.3-9.9)
Velocity (m sec^{-1})	-	0.15 (0-0.33)
% mud substrate	-	7.6 (0-38.3)
% sand/gravel substrate	Sand.Gravel	64.5 (11.3-100)
% detritus substrate	-	26.3 (0-74.5)
Mean large woody debris (LWD) (count)	Mean.LWD	4.0 (1.6-8.7)
Reach gradient (m)	Gradient	0.7 (0-3.0)
Sinuosity	Sinuosity	1.2 (1.0-1.9)
Basin	Basin	-
EPA Level III ecoregion	L3Ecoregion	-
Drainage area (km^2)	Drainage.area	370.8 (51.8-1432.3)
% developed land cover (%)	Developed.cover	2.4 (0.3-10.0)
% deciduous/mixed forest land cover (%)	DeciduousMixed.cover	5.4 (1.9-14.6)
% evergreen forest land cover (%)	-	17.5 (8.3-35.8)
% agricultural land cover (%)	Agriculture.cover	22.4 (7.0-37.2)
% wetland land cover (%)	Wetland.cover	30.1 (16.7-46.3)

Table 1.4 Loadings of environmental variables on the NMDS ordination for the BIOENV analysis (Clarke and Ainsworth 1993). Bolded variables were found to be significantly correlated with assemblage variation between sites based on Mantel tests (*P* <0.05).

	Variable scale	NMDS1	NMDS2	R^2	*P*
DO	**Local**	**-0.783**	**0.622**	**0.617**	**0.001**
Sand.Gravel	**Local**	**-0.870**	**0.493**	**0.472**	**0.001**
Ph	**Local**	**-0.999**	**-0.044**	**0.381**	**0.001**
Sinuosity	**Local**	**0.821**	**0.572**	**0.235**	**0.009**
S.Conductivity	**Local**	**-1.000**	**0.003**	**0.233**	**0.012**
Temperature	**Local**	**-0.998**	**-0.061**	**0.208**	**0.014**
Mean.bankheight	**Local**	**-0.885**	**0.466**	**0.204**	**0.017**
Drainage.area	**Regional**	**-0.476**	**-0.879**	**0.169**	**0.033**
L3Ecoregion	**Regional**	**-0.851**	**0.525**	**0.163**	**0.038**
Year		-0.177	0.984	0.152	0.054
Developed.cover	Regional	0.348	0.938	0.128	0.102
Mean.LWD	Local	-0.961	0.275	0.091	0.182
Wetland.cover	Regional	0.402	-0.916	0.074	0.263
Mean.width	Local	-0.508	-0.861	0.073	0.247
is.Ogeechee	Regional	-0.244	-0.970	0.022	0.684
Gradient	Local	0.972	-0.236	0.019	0.734
DeciduousMixed.cover	Regional	-0.617	-0.787	0.011	0.842
Mean.depth	Local	-0.243	0.970	0.008	0.876
Agriculture.cover	Regional	0.175	0.985	0.006	0.9
is.Savannah	Regional	-0.116	0.993	0.002	0.969

Table 1.5 Loadings of species on the NMDS ordination for the BIOENV analysis (Clarke and Ainsworth 1993). Bolded species were found to be significantly correlated with assemblage variation between sites based on Mantel tests (*P* <0.05). Species with a significant positive or negative association with NMDS axis 1 were considered indicators of nonfluvial (NF) or fluvial (F) assemblage types, respectively.

	Species code	NMDS1	NMDS2	R^2	*P*	Indicator Type
Lepomis gulosus	Lgulo	**0.601**	**-0.800**	**0.609**	**0.001**	**NF**
Centrarchus macropterus	Cmacr	**0.718**	**-0.696**	**0.501**	**0.001**	**NF**
Esox americanus	Eamer	**0.893**	**-0.450**	**0.466**	**0.001**	**NF**
Lepomis macrochirus	Lmacr	**0.489**	**-0.872**	**0.394**	**0.001**	**NF**
Notemigonus crysoleucas	Ncrys	**0.953**	**-0.302**	**0.348**	**0.001**	**NF**
Aphredoderus sayanus	Asaya	**0.312**	**-0.950**	**0.313**	**0.001**	**NF**
Esox niger	Enige	**0.942**	**-0.334**	**0.235**	**0.006**	**NF**
Amia calva	Acalv	**0.374**	**-0.928**	**0.202**	**0.017**	**NF**
Acantharchus pomotis	Apomo	**0.668**	**-0.744**	**0.190**	**0.028**	**NF**
Chologaster cornuta	Ccorn	**0.273**	**-0.962**	**0.190**	**0.016**	**NF**
Notropis cummingsae	Ncumm	**-0.684**	**-0.729**	**0.744**	**0.001**	**F**
Percina nigrofasciata	Perni	**-0.724**	**-0.690**	**0.723**	**0.001**	**F**
Etheostoma olmstedi	Eolms	**-0.855**	**-0.519**	**0.582**	**0.001**	**F**
Notropis petersoni	Npete	**-0.783**	**-0.622**	**0.551**	**0.001**	**F**
Lepomis auritis	Lauri	**-0.945**	**-0.329**	**0.468**	**0.001**	**F**
Noturus leptacanthus	Nlept	**-0.716**	**-0.699**	**0.445**	**0.001**	**F**
Micropterus salmoides	Msalm	**-0.489**	**-0.873**	**0.360**	**0.001**	**F**
Lepomis punctatus	Lpunc	**-0.600**	**-0.800**	**0.339**	**0.003**	**F**
Minytrema melanops	Mmela	**-0.903**	**-0.430**	**0.330**	**0.002**	**F**
Anguilla rostrate	Arost	**-0.685**	**-0.729**	**0.325**	**0.005**	**F**
Ameiurus brunneus	Abrun	**-0.881**	**-0.474**	**0.305**	**0.001**	**F**
Opsopoeodus emiliae	Oemil	**-0.997**	**0.081**	**0.257**	**0.004**	**F**
Hybopsis rubrifrons	Hrubr	**-0.936**	**-0.352**	**0.198**	**0.018**	**F**
Cyprinella leedsi	Cleed	**-0.621**	**-0.784**	**0.183**	**0.025**	**F**
Etheostoma fricksium	Efric	**-0.547**	**-0.837**	**0.172**	**0.032**	**F**
Notropis lutipinnis	Nlutr	-0.594	-0.805	0.119	0.091	
Noturus gyrinus	Ngyri	-0.055	-0.999	0.109	0.122	
Erimyzon oblongus	Eoblo	0.005	-1.000	0.106	0.138	
Etheostoma inscriptum	Einsc	-0.461	-0.888	0.101	0.140	
Labidesthes vanhyningi	Lvanh	-0.477	-0.879	0.091	0.185	
Notropis maculatus	Nmacu	-0.232	-0.973	0.091	0.187	
Enneacanthus gloriosus	Eglor	0.592	0.806	0.080	0.242	
Pteranotropis stonei	Pston	-0.873	-0.489	0.066	0.314	
Ameiurus natalis	Anata	0.796	0.605	0.062	0.317	

Etheostoma serrifer	Eserr	-0.077	-0.997	0.058	0.343
Lepisosteus osseus	Losse	-0.767	-0.641	0.054	0.360
Pomoxis nigromaculatus	Pomni	-0.383	0.924	0.053	0.392
Erimyzon sucetta	Esucc	0.681	-0.732	0.052	0.401
Lepomis microlophus	Lmicr	0.080	-0.997	0.050	0.418
Notropis chalybaeus	Nchal	-0.999	-0.039	0.046	0.442
Lepomis marginatus	Lmarg	0.972	0.235	0.044	0.467
Ameiurus nebulosus	Anebu	0.905	-0.425	0.036	0.531
Elassoma zonatum	Ezona	0.631	0.776	0.036	0.564
Etheostoma fusiforme	Efusi	0.741	0.672	0.036	0.547
Notropis hudsonius	Nhuds	-0.839	-0.544	0.022	0.712

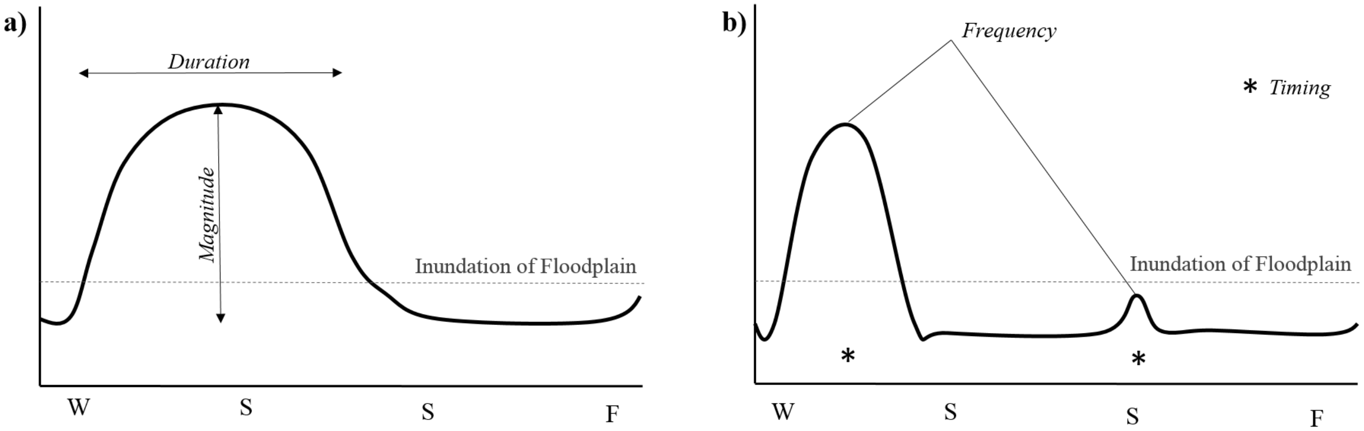

Figure 1.1 A conceptual model demonstrating two contrasting hydrographs of freshwater flow and floodplain inundation. Hydrograph **a)** represents a wet year when the floodplain is inundated for the entirety of the spring growing season, allowing for young-of-the-year (YOY) fishes to have access to floodplain habitats and food resources throughout the entire spring. Recruitment is high. Hydrograph **b)** represents a dry year where the floodplain does *not* remain inundated for the entirety of the spring growing season. Recruitment is low.

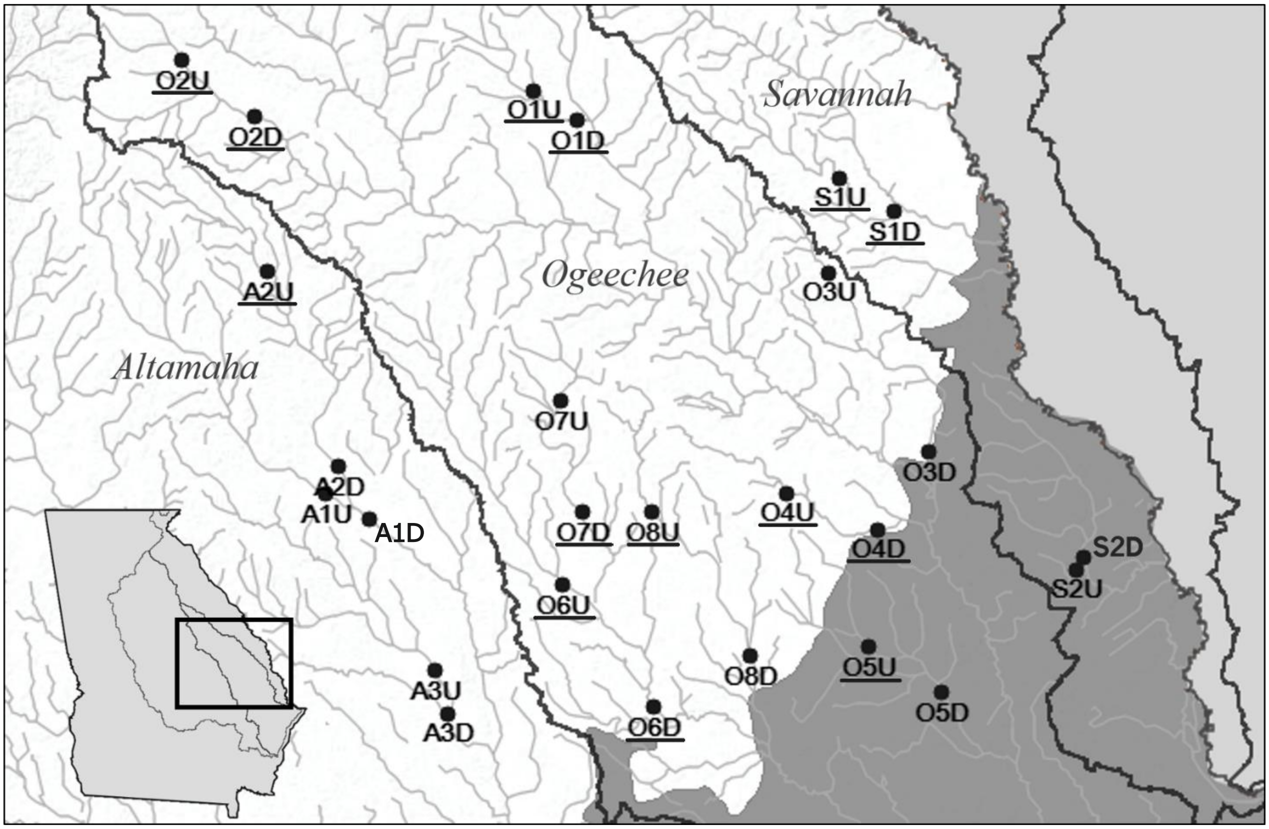

Figure 1.2 Locations of the twenty-six sites. Site ID codes are shown and can be used to reference stream names in Appendix 3. The first letter in the code references the river basin in which it is located (A = Altamaha, O = Ogeechee, S = Savannah). The last letter refers to the treatment of the sample site (D = downstream, U = upstream). Underlined sites were sampled in both years. Major river basins are labeled. Within Georgia, white and shaded areas indicate the Southeastern Plains and Southern Coastal Plain Level III ecoregions, respectively.

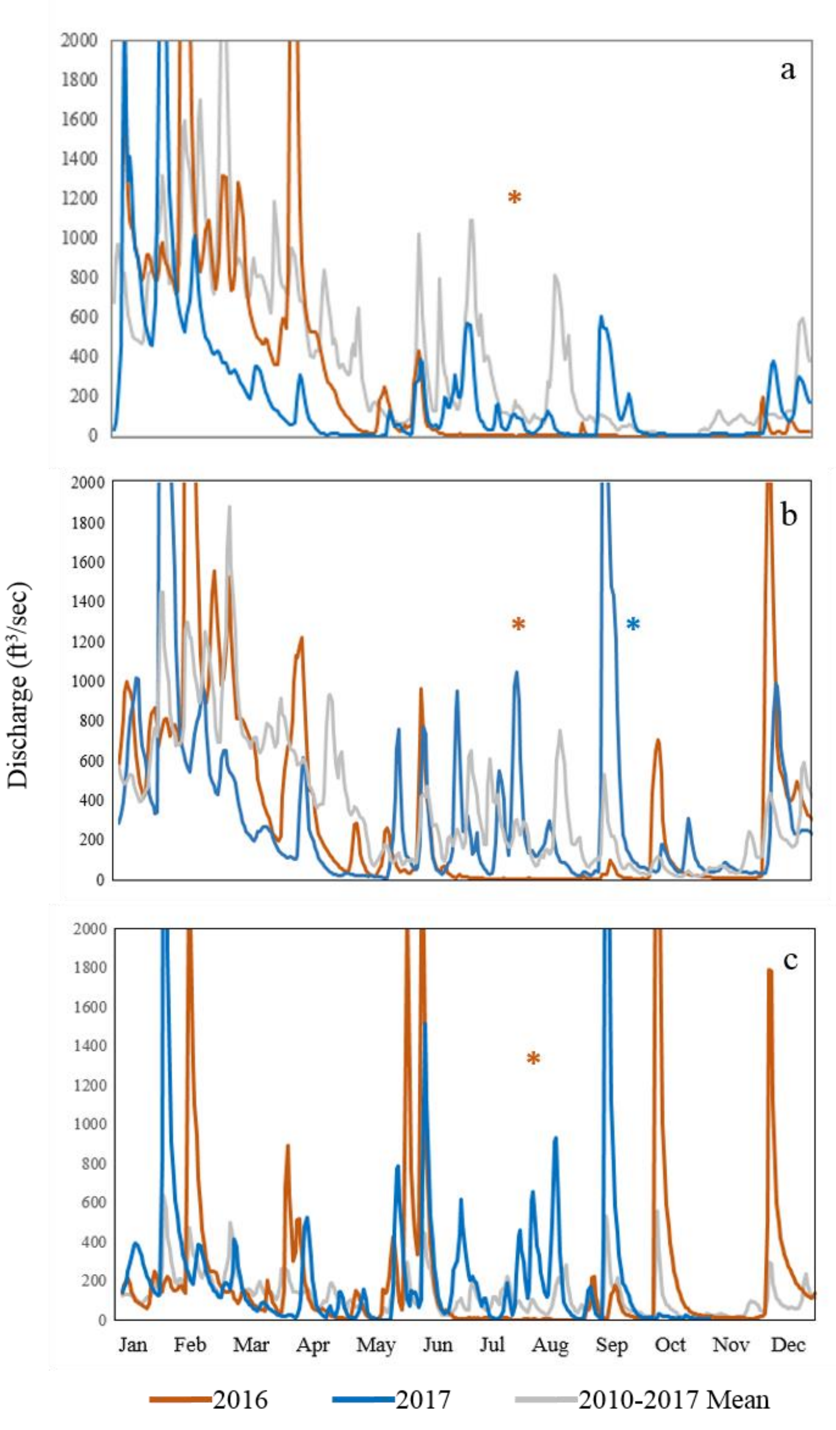

Figure 1.3 Hydrographs from USGS gages at three sample sites (a) Ohoopee River, Altamaha River basin, USGS 02225270 b) Canoochee River, Ogeechee River basin, USGS 02203518, c) Ebenezer Creek, Savannah River basin, USGS 02198690) that demonstrate the contrast in discharge between the two sampling years. Asterisks indicate timing of sampling events. Due to high flows, the Ohoopee River and Ebenezer Creek were not sampled in 2017.

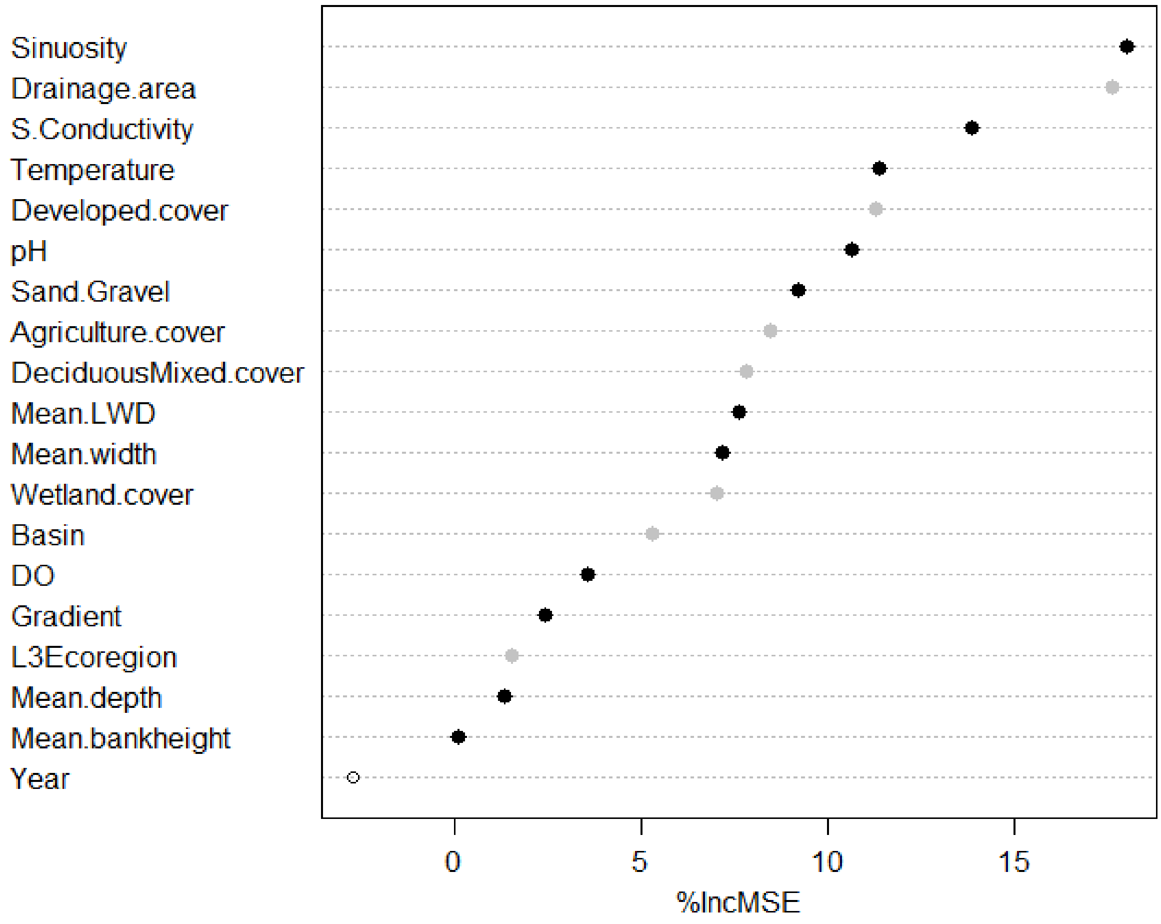

Figure 1.4 Variable importance plot of all tested environmental predictor variables of species richness from random forests regression. The x-axis demonstrates the increase in mean squared error (%IncMSE) of the model if a particular variable were to be excluded; predictor variables with higher %IncMSE were therefore more influential to species richness. Black circles represent environmental variables measured on the local scale; gray circles represent regional-scale variables. R^2 = 18.1.

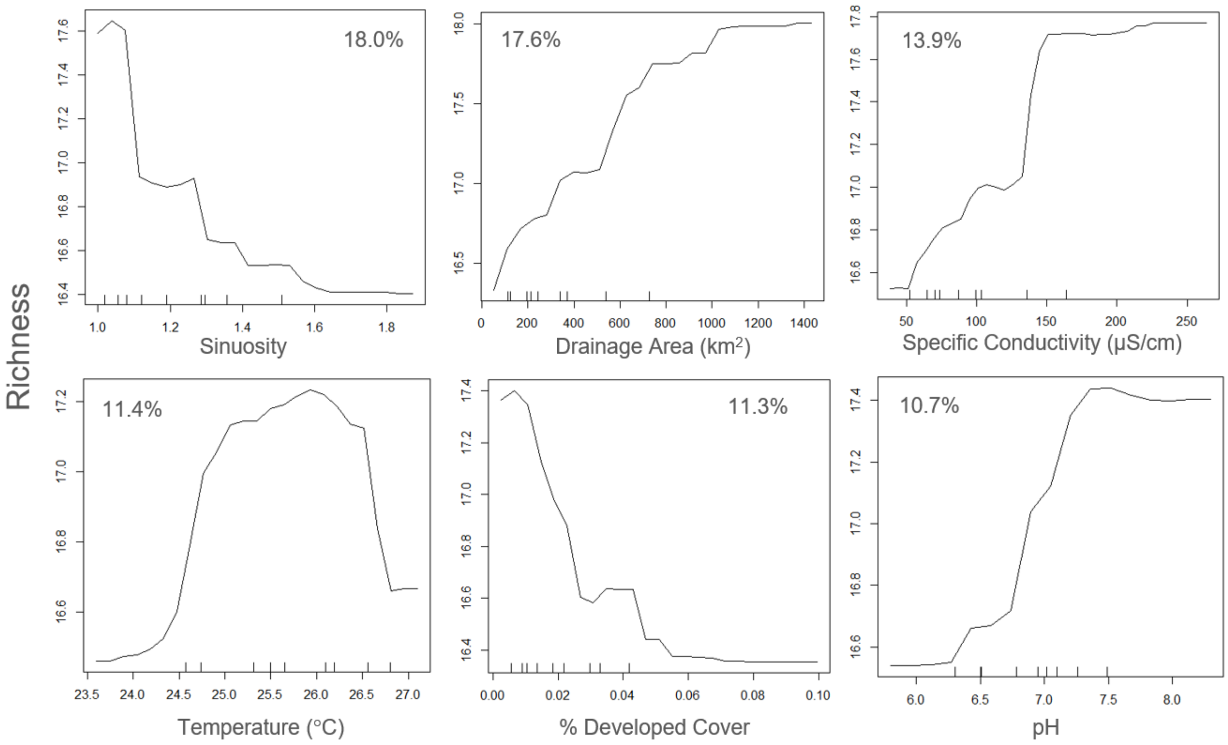

Figure 1.5 Partial dependence plots of top-ranking environmental variables as predictors of species richness from random forests classification. Predictor variables were considering top-ranking if they showed an increase in mean squared error (%IncMSE; overlaid on plots) of ≥10%. Axis scaling varies among plots.

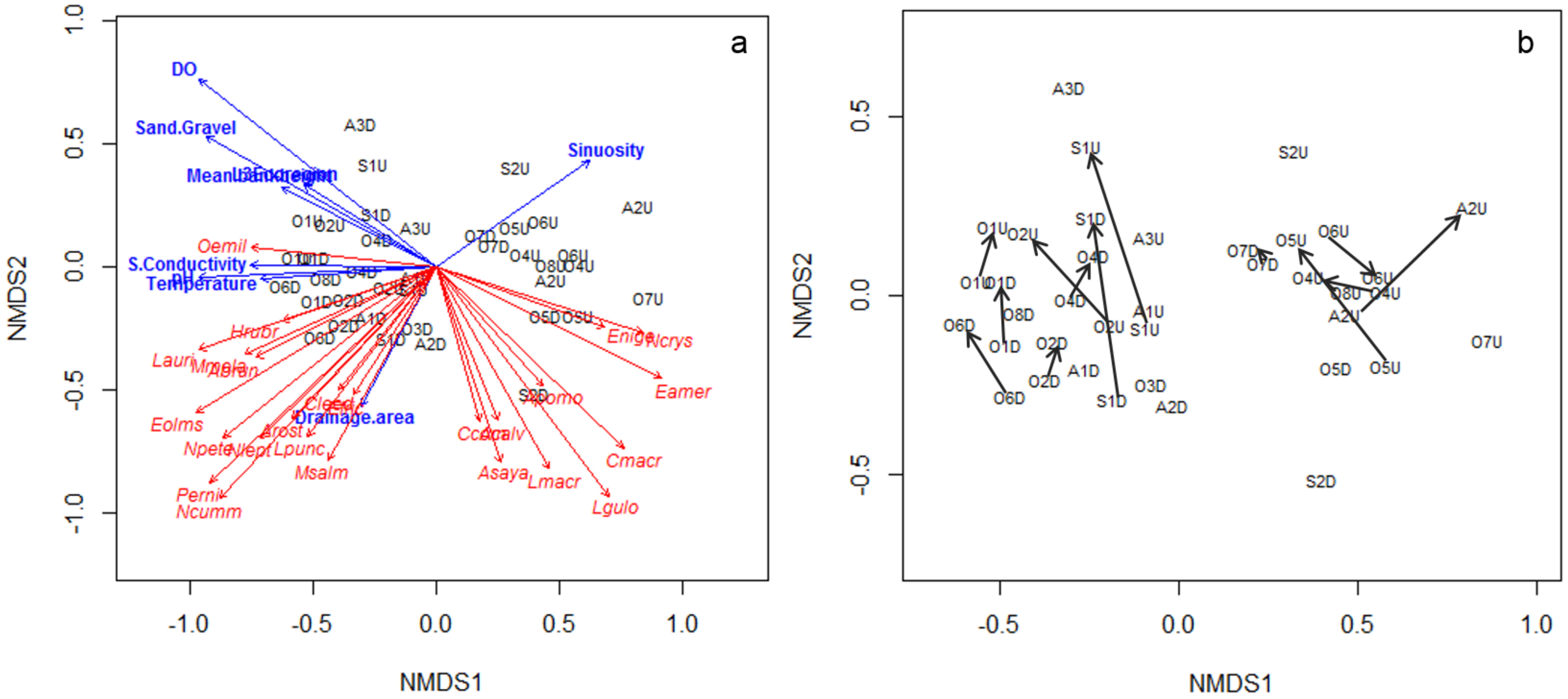

Figure 1.6 a) Tri-plot output from a BIOENV analysis (Clarke and Ainsworth 1993). Sampling events are shown as site codes plotted in species space on an NMDS ordination. Species codes of species that were determined to be significantly correlated to assemblage variation between sites ($P < 0.05$) are shown in red. Significant environmental covariates are shown in blue. Vectors indicate direction and strength of correlation, **b)** sampling events shown on the same ordination with arrows connecting temporal replicates; arrows show change in loadings from 2016 to 2017. Stress = 0.15.

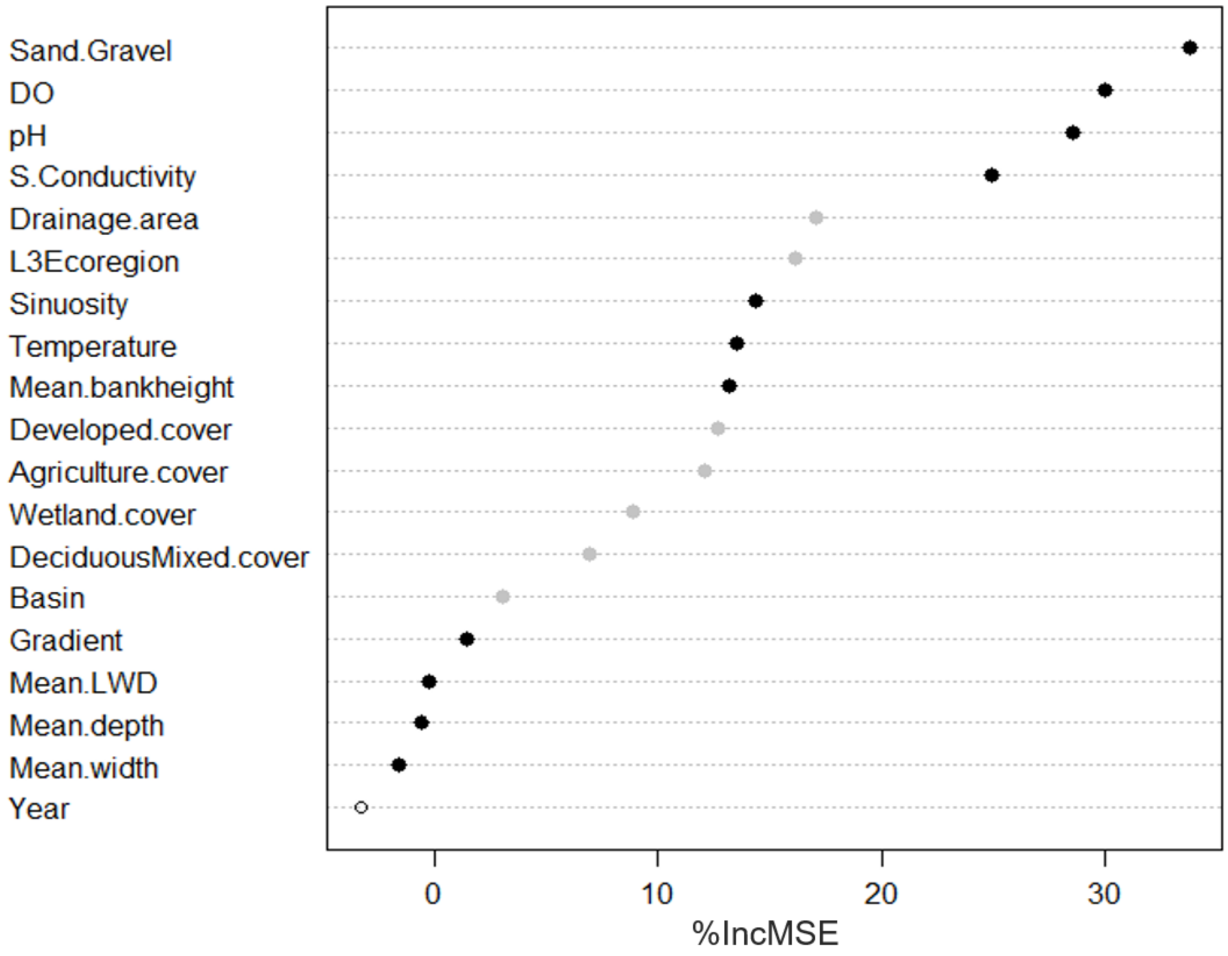

Figure 1.7 Variable importance plot of all tested environmental variables for predicting group classification (“fluvial” vs “nonfluvial”) from random forests classification. The x-axis demonstrates the increase in mean squared error (%IncMSE) of classification if a particular variable were to be excluded; predictor variables with higher %IncMSE were therefore more important to classification. Black circles represent environmental variables measured on the local scale; gray circles represent regional-scale variables. Out-of-bag error rate is 10.53%.

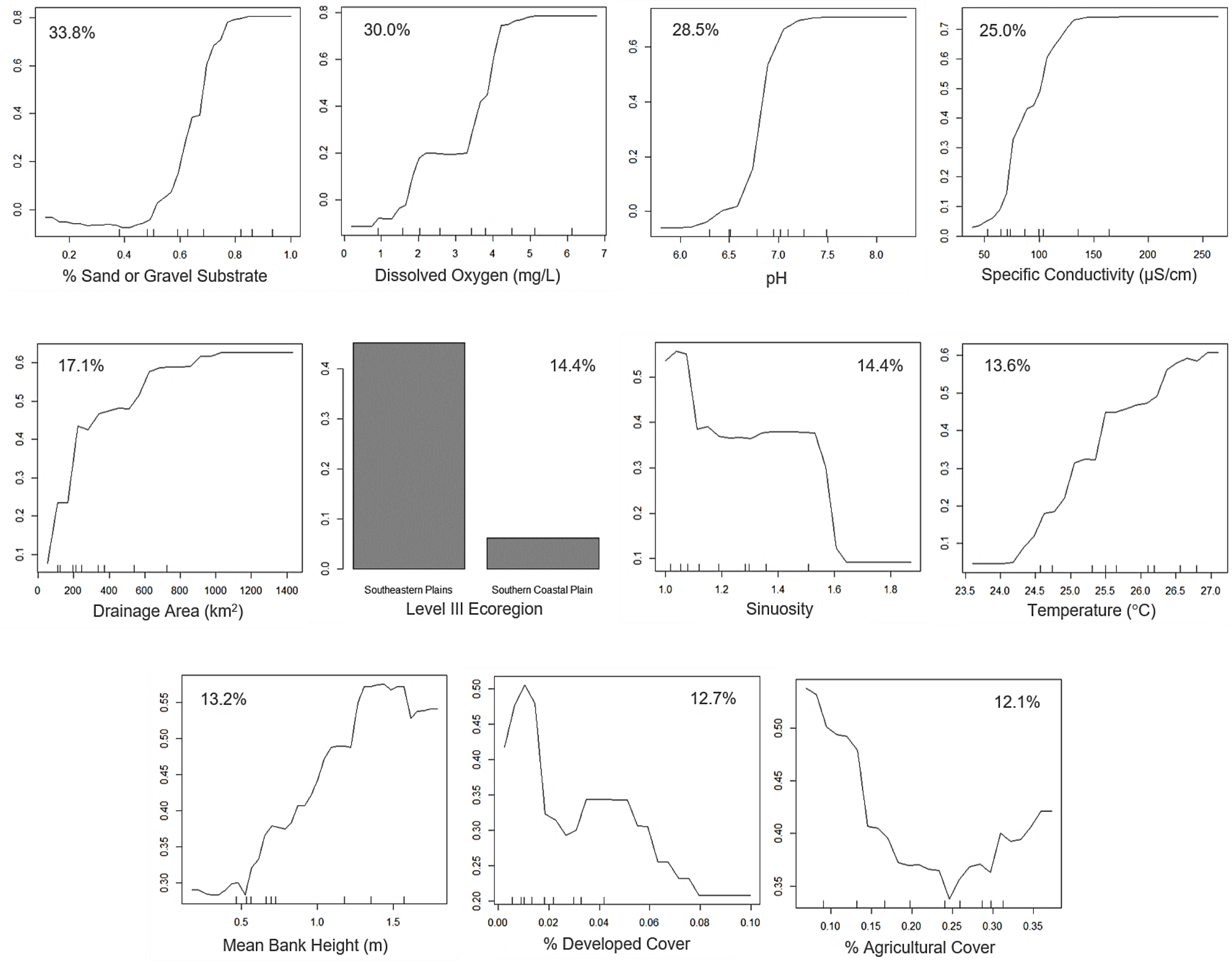

Figure 1.8 Partial dependence plots of top-ranking environmental variables as predictors of group membership. Y-axes can be interpreted as the likelihood of a site being assigned to class “fluvial”. Predictor variables were considering top-ranking if they showed an increase in mean squared error (%IncMSE; overlaid on plots) of ≥10%. Axis scaling varies among plots.

CHAPTER TWO: PATTERNS AND MECHANISMS OF LONGITUDINAL FISH COMMUNITY STRUCTURE IN COASTAL PLAIN STREAMS

ABSTRACT

Fish species richness increases downstream across a wide range of riverine environments, yet the ecological mechanisms that produce this ubiquitous pattern are unexplored in coastal plain streams. I synthesized six models predicting longitudinal gradients in environmental conditions and fish assemblage composition and tested their applicability to longitudinal data I collected in coastal plain streams of Georgia. Due to the unusual environmental characteristics of the coastal plain region, it was hypothesized that the applicability of some longitudinal models would be weakened in these systems. Environmental and assemblage data were collected from paired upstream (US) and downstream (DS) sites during summer base flows. Species richness increased with stream size, and magnitudes and rates of longitudinal species turnover was comparable to that observed in other regions. Of the six predictive models examined, the River Continuum Concept had the strongest applicability in coastal plain streams, as trophic diversity increased with stream size with the addition of specialized feeders. The Habitat Template Concept was somewhat supported: environmental stability and intolerant species increased downstream, but life history strategies did not respond predictably to this gradient. Four other models involving longitudinal gradients in habitat volume, niche diversity, and migrant accessibility generally were not supported by data, lending some support to my hypotheses about the uniqueness of coastal plain environments for fish ecology. Further research into the temporal stability of these findings would benefit understanding of coastal plain fish communities and better inform management decisions.

INTRODUCTION

A key goal of community ecology is understanding how the taxonomic and functional organization of communities is shaped by the environment. Stream ecologists have long considered how fish assemblages are filtered by biotic and abiotic environmental conditions across a variety of spatiotemporal scales, thereby giving rise to the local assemblage (Poff 1997). A better understanding of how assemblage composition may vary in response to changing environmental conditions is essential for effective management and conservation of stream fishes.

One of the most ubiquitous taxonomic patterns in stream-fish ecology is an increase in fish species richness with increasing stream size. This is because streams and rivers exhibit predictable longitudinal gradients (i.e. changes with stream size) in a number of physical and chemical characteristics that differentially influence the distribution and abundance of fish species. These gradients involve longitudinal variation in energy sources, habitat area and volume, habitat diversity, environmental stability, permeability to movement, and proximity to external migrant pools, and have been used in the past to develop alternative explanations to explain longitudinal assemblage change in stream fishes (Vannote et al. 1980, Roberts and Hitt 2010). These explanations can be formalized as six alternative, though not mutually exclusive, conceptual models. First, the Species-Area Relationship (SAR) (Angermeier and Schlosser 1989) predicts that downstream increases in habitat area or volume will provide room for more individuals, thus intercepting a larger sample of the regional species pool and resulting in higher species richness and abundance in downstream areas (Connor and McCoy 1979, Angermeier and Schlosser 1989). The SAR acts as a kind of “null” model, in that it makes no assumptions about longitudinal variation in habitat conditions or diversity, or functional differences among species. In contrast, The Niche Diversity Model (NDM) (Roberts and Hitt 2010) suggests that the variety of habitat configurations (i.e. potential ecological niches) increases downstream, allowing larger streams to support a greater diversity of autoecological types, and thus species (Lowe-McConnell 1975, Schlosser 1982). Further, as microhabitats tend to be added rather than replaced downstream, upstream areas are expected to exhibit nested subsets of downstream niches and species (Schlosser 1987). This notion of niche diversification is extended by the River Continuum Concept (RCC) (Vannote et al. 1980) which predicts longitudinal changes in canopy cover, riparian inputs, autotrophy, and organic matter cycling, which correspondingly allows for diversification of trophic strategies and species downstream. In contrast to the previous three models, which assume static habitat conditions, the Habitat Template Concept (HTC) (Southwood 1977, Townsend and Hildrew 1994) [similar to Roberts and Hitt's (2010) "Environmental Stability Model"], predicts higher species richness downstream due to greater environmental stability provided by greater habitat volume and permanence and lower hydrologic variation (Schlosser 1990, Taylor and Warren 2001). Upland streams are more likely to experience seasonal drying, creating temporally unstable environmental conditions that may result in a smaller species pool due to extinction of less resilient species (Schlosser 1990, MacArthur and Wilson 1967).

The last two conceptual models emphasize the role of regional fish dispersal and migration patterns in influencing the distribution of species. The Immigrant Accessibility Model (IAM) (Roberts and Hitt 2010) hypothesizes that downstream reaches are more permeable to fish movement as they are deeper and typically experience fewer barriers to movement due to their lower gradient nature (MacArthur and Wilson 1967, Taylor and Warren 2001, Robinson and Rand 2005, Grossman et al. 2010). Similar to the IAM, the Adventitious Stream Effect (ASE) (Gorman 1986) expects DS reaches that are close to main channels to see increased richness due to immigration by riverine migrants (Osborne and Wiley 1992, Schaefer and Kerfoot 2004, Matthews 1986, Hitt and Angermeier 2008, Roberts and Hitt 2010).

Although changes of richness and nestedness with stream size are relatively well-studied (reviewed by Roberts and Hitt 2010), longitudinal variation in functional organization (i.e. species traits) has received less study. Poff (1997) suggested that the composition of a fish assemblage is strongly influenced by abiotic environmental “filters” that must be matched by species’ traits in order to be passed through. In this way, sites with similar environmental regimes can be expected to have assemblages with similar trait characteristics. These filters can act on multiple spatial scales and can filter species by many trait-types (e.g. tolerance, life-history, trophic, reproductive). By considering each of the previously described mechanistic models as longitudinal “filters”, we can predict how species traits might respond to environmental gradients. For example, the amount of available habitat volume, as considered by the SAR, can filter species based on body size (i.e. larger streams can support larger-bodied species) (Schlosser 1982, Schlosser 1990, Gilliam et al. 1993, Poff 1997). Streams with smaller habitat volume may therefore filter out large-bodied species, resulting in lower species richness in upstream reaches (Table 2.1). Based on the IAM, upstream reaches also are more likely to filter out species that are poorer dispersers, and based on the ASE, are less likely to contain species with larger-stream affinities or temporary riverine migrants (Hitt and Angermeier 2008) such as large catostomids and ictalurids (Table 2.1). The longitudinal gradient of energy inputs as described by the RCC may further filter species by trophic strategy. For example, as stream size increases, wider streams with an increasingly open canopy allow for autotrophic (i.e. phytoplankton) production, which combined with increased stream size and food-web complexity would allow for increasing numbers of piscivores, detritivores, planktivores, and other specialized feeding groups (e.g. benthic insectivores), and a corresponding reduction in the number of generalist strategies (Vannote et al. 1980, Paller 1994, Goldstein and Meador 2004) (Table 2.1). Finally, the HTC predicts that species whose tolerances of life history strategies are not tuned to upstream disturbance regimes will be filtered from those assemblages. The HTC predicts that upstream species will be the subset of all species that are most tolerant to hydrologic instability, harsh water quality conditions, and stream desiccation (Matthews and Styron 1981, Townsend and Hildrew 1994). This tolerance to desiccation may result in a loss of species with high levels of parental care, as energy spent on nest construction may be futile in unstable habitats (Townsend and Hildrew 1994). Temporally unstable environments also may filter out species that are late-maturing with long generation times, as intermittency may restrict the reproductive success of these life-history strategies (Gray 1981). In other words, downstream sites may be more favorable to species of an “equilibrium” life-history strategy (i.e. high parental care, longer lived) whereas upstream sites may favor species towards an “opportunistic” life-history (i.e. low generation time, low parental care) (Winemiller 2005). However, in contrast to the HTC, Goldstein and Meador (2004) suggested that nest-building species may prefer small headwater streams with more substrate complexity, and be filtered out of larger streams where high flows could destroy nests. Results of some large-scale analyses (e.g. Lamouroux et al. 2002, Mims and Olden 2012, McManamay et al. 2015) have supported some of these predictions, but by focusing on a large number of streams may have had reduced the ability to parse the effects of stream size from other factors that may differ among those streams, such as hydrology and degree of human disturbance. Intensive surveys of functional organization within one stream or watershed are less common but can control for these factors and may shed additional insight.

It is crucial to test these longitudinal predictions in a variety of environmental settings, to assess the generality of different stream-size gradients as influences on taxonomic and functional assemblage organization. The majority of North American research on this topic has occurred in either montane or Great Plains streams, whereas coastal plain streams have received relatively little study in proportion to their geographic area (Matthews 1998, Marsh-Matthews and Matthews 2000). This disparity is significant, because some longitudinal predictions, particularly those developed in montane systems, may be less applicable to coastal plain streams. Streams in the coastal plain exhibit particularly harsh physiochemical conditions (i.e. highly variable water temperatures, dissolved oxygen and pH) as their low gradient nature results in streams that may remain stagnant for much of the year (Felley 1992, Smock and Gilinsky 1992). Inputs of humic and fulvic acids from riparian organic matter are high due to lateral floodplain connections, resulting in high levels of acidity (Maxted et al. 2000, Junk et al. 1989). Streams are low in mesohabitat diversity (i.e. riffle-pool development) and structural complexity relative to montane streams, with wood being the primary component of physical habitat (Maxted et al. 2000, Meffe and Sheldon 1988). Most distinctively, coastal plain streams experience extreme temporal hydrologic variation due to a winter-spring flood pulse, which creates temporary connections between stream organisms and floodplains as stream channels fill and overflow their banks followed by severed connections as rivers retreat to their channels during the summer-fall dry season. This temporal variation can cause a single stream system to experience periods of inundation and complete drying within the same year. Thus, depending on the time of year, I predict that the unusual habitat and disturbance patterns of the coastal plain might weaken or strengthen the six longitudinal models' abilities to predict assemblage composition.

Although habitat volume may homogenize throughout a system during extreme seasonal flooding, the longitudinal increases in habitat volume described by the SAR likely still hold true in coastal plain systems during periods of low flows. As such, I expect an overall downstream increase in species richness. Because I expect downstream areas to maintain a larger habitat area, and because coastal plain streams contain few obvious barriers (e.g. waterfalls, dams), I expect longitudinal patterns of species richness predicted by the IAM and ASE to be less applicable in the coastal plain; if there are fewer species upstream, it is not because there are barriers to the upstream movement of riverine migrants. I therefore also expect weaker longitudinal patterns in the stream-size preferences of species. Due to the naturally low habitat diversity and complexity of the coastal plain, I expect even large downstream areas to be low in microhabitat diversity and available ecological niches, thereby weakening the longitudinal gradient of richness and habitat specialization (e.g. substrate or depth requirements) predicted by the NDM. The longitudinal variation of energy inputs described by the RCC may not be as distinct in coastal plain streams as their blackwater nature may inhibit sunlight penetration and therefore autotrophic phytoplankton. In addition, streams system-wide experience high levels of energy inputs from riparian sources due to frequent lateral connection to their floodplains. As the longitudinal shift in energy inputs described by the RCC may be weakened in the coastal plain, I also expect the longitudinal sorting of traits and species and in response to these energy sources to weaken. For example, the downstream addition of certain trophic strategies (e.g. planktivores, detritivores) in response to available energy resources may not play out on the coastal plain, as blackwater prevents phytoplanktonic production in larger streams, and all sites are high in riparian energy inputs through temporary floodplain connections. Functional trophic composition of communities may therefore be similar system-wide. The HTC predicts that the temporal instability of small upstream reaches results in the extirpation of species intolerant to desiccation. Due to their variable hydrology, small upland coastal plain streams are likely to be even more environmentally harsh and unstable than upstream sites in other regions. This instability is especially extreme during summer base flows, when upstream sites are likely to experience desiccation and associated harsh environmental conditions (e.g. high temperature, low dissolved oxygen). I therefore expect longitudinal patterns of environmental stability predicted by the HTC such as the upstream loss of species to be even more pronounced in the coastal plain, with only the most tolerant species remaining. However, it is worth considering the alternate prediction that coastal plain communities may be composed of hardy, tolerant species system-wide due to naturally harsh physiochemical conditions year-round. Additionally, Winemiller's (2005) triangular model of life-history strategies predicts that increasing environmental disturbance filters for species with lowered generation time, juvenile survivorship, and fecundity. Given that I predict extreme environmental disturbance upstream in the coastal plain, I also predict that these life-history traits will sort accordingly with downstream sites containing longer lived, highly fecund species that exhibit relatively higher levels of parental care than upstream areas. Finally, because some environmental gradients may be weaker in the coastal plain, I expect that longitudinal assemblage variation (i.e. the rate of species turnover with increasing fluvial distance) might be weaker in coastal plain streams than in streams in other regions, where longitudinal variation has exerted strong filters on local assemblages (e.g. Sheldon 1968, Taylor and Warren 2001, Roberts and Hitt 2010).

I tested these longitudinal hypotheses using original fish and habitat data I collected from paired upstream (US) and downstream (DS) sites in each of 12 replicate coastal plain streams in summer 2016. Two complementary approaches assessed whether environmental and fish assemblage characteristics varied with stream size or longitudinal position in the directions hypothesized by the six aforementioned longitudinal models. First, I asked whether longitudinal position (US versus DS) consistently affected environmental and assemblage variables in hypothesized directions. Second, I looked across all sample sites, and asked whether variables were correlated with stream size (upstream drainage area) in hypothesized directions. To contextualize my results with previous studies, I also calculated the magnitude and longitudinal rate-of-change of species turnover in my study stream and compared these to estimates calculated from published longitudinal studies in other regions.

METHODS

Site Selection

Initially, thirteen coastal plain stream systems were selected in the Altamaha, Ogeechee, and Savannah River basins of southeast Georgia. Sample streams spanned two Level-III ecoregions (i.e. Southeastern Plains, Southern Coastal Plain), with most (11) falling in the Southeastern Plains (EPA 2013). In order to capture longitudinal variation within sample streams, each stream had a paired up- (US) and downstream (DS) sample reach (Table 1.2, Figure 1.2). Paired sites were on average 17.0 km from each other (range 2.4 - 36.9 km) and shared an average of 40.3% of their upstream watershed area (range 12.6 - 76.8%). Sites were selected to be wadeable (i.e. able to be sampled via backpack electrofishing) with a defined channel, and able to be accessed via a short hike from a public road crossing. All sample reaches began at least 50 meters from a road or bridge crossing. Subsequent to sampling, I excluded one site (upstream Ogeechee Creek) from analysis as it was intermittent and few fish were captured there, resulting in 24 paired sites plus an additional unpaired site.

Fish Sampling

The fish assemblage was sampled within the stream channel of each site during 2016 summer base flows (late June-early September) following recession of water from the floodplain. A 150-meter-long reach was isolated using a block net placed at the upstream end of the reach, and fish were collected via single-pass backpack electrofishing in an upstream direction. Stunned fish were collected with dip nets and placed in aerated holding buckets until they could be identified, weighed for mass (g), and measured to total length (TL; mm) and standard length (SL; mm, if applicable). Fish were identified in the field using keys in regionally appropriate guidebooks (Rohde et al. 2009, Marcy et al. 2005), or were preserved in 10% formalin and taken back to the lab for subsequent identification.

Environmental Variables

Water quality parameters were measured upon arrival to a sample site using a YSI Pro2030 meter (water temperature, dissolved oxygen, ambient and specific conductivity) and a Eutech Instruments pHTestr 10 (pH). Following fish sampling, I measured instream habitat characteristics. At 10-m intervals, I measured wetted width (cm), stream depth (cm), and substrate type (mud, sand, gravel, detritus) at 1-m intervals across transects. In the 10-m sections between transects, I counted the number of large woody debris (LWD) items, defined as ≥ 10 cm wide and ≥ 1.5 meters long (Kaeser and Litts 2008). In addition to the water quality data measured at the time of fish sampling, the coefficient of variation (CV) was also calculated for temperature, pH, and DO using measurements taken at three different temporal snapshots (late spring, summer base flows, late fall). Stream gradient, or the change in elevation over the course of a sample reach, was calculated post-hoc for each stream using remotely-sensed data in ArcGIS 10.4 (ESRI 2016). Elevation values were retrieved from 1/3 arc-second (approximately

10-meter resolution) Digital Elevation Model (DEM) data from the USGS National Map. In order to account for vertical resolution error in the DEM, gradient was measured over a 1-km long segment, which was centered on the sample reach. Microhabitat diversity was calculated as the Shannon diversity of unique combinations of depth, substrate type, and LWD density (Gorman and Karr 1978). For this index, each transect was binned for LWD density (≤5, >5), mean depth (≤50, 50-100, >100 cm), and the presence of coarse substrate (sand or gravel present versus absent).

Data Analyses

I compiled species traits descriptive of life history, spawning mode, trophic strategy, tolerance, and stream-size preference from existing literature (Table 2.2). Life history, spawning mode, and stream size preference traits for most fish were compiled from the FishTraits database (Frimpong and Angermeier 2009), with the exception of traits for American Eel (*Anguilla rostrata),* which were taken from Jenkins and Burkhead (1994). Trophic strategy classifications were taken from Paller (1994), which I considered most appropriate for coastal plain populations of these species, and tolerance rankings were synthesized from multiple sources (Meador and Carlisle 2007, NC DENR 1995, GA DNR 2005, Barbour et al. 1999). Tolerance, as considered by these sources, refers to a species' tolerance to environmental disturbance and associated changes in physical habitat and water quality. Species are commonly sorted into three tolerance categories: tolerant, moderate, and intolerant (Halliwell et al. 1999). Once species' traits were compiled, they were used to derive additional metrics descriptive of site assemblage composition. To prevent abundant species from overwhelming results, I used species occurrence data, not individual counts, when calculating the proportional composition of different trait-state categories in the assemblage. Metrics included site averages for continuous variables (e.g.

fecundity, longevity, maximum body size), proportions of species for categorical variables (e.g. trophic strategies, spawning modes), and the Shannon diversity of trophic strategies and spawning modes (Appendix 2). Parental care was numerically coded based on spawning-mode (1 = open-spawner, 2 = brood-hider, 3 = substrate-chooser, 4 = nest-builder, 5 = bearer), and mean degree of parental care was calculated for sites using these values. Stream size preferences were also coded numerically (1 = creek, 2 = small river, 3 = large river) and mean preference values were calculated for each species, as many had multiple preferences. Mean stream-size preference was determined for each site by averaging across size preferences of all present species. Species were considered to be "late-maturing" if they reached sexual maturity at 1.5 years or older. American Eels were excluded from any metric relating to reproduction (e.g. age at maturity, fecundity, spawning mode metrics) as they are catadromous and do not spawn in coastal plain streams (Jenkins and Burkhead 1994). A total of 32 possible response variables were tested for a longitudinal relationship (Table 2.1).

I tested for longitudinal position effects on environmental and assemblage variables by estimating the standardized mean differences of each variable between paired DS and US sites. I standardized variables to a mean of 0 and a standard deviation of 1, and subtracted the US from the DS value. I then used a custom R script (R Core Team 2017) to estimate a bootstrapped (10^4 resamples) mean and 95% confidence interval (CI) from these 12 differences for each variable. Variables were considered to differ consistently between longitudinal positions if the 95% CI of differences did not overlap with zero (i.e. < 5% chance that the mean difference between US and DS sites was zero). Data from lower Ogeechee Creek (O3D) were excluded from this paired analysis as its upstream pair had been previously excluded. Because some DS sites in this analysis were relatively "upstream" in the river continuum, I complemented this analysis by pooling all sites (ignoring stream membership) and testing for a significant monotonic relationship between each variable and stream size (i.e. upstream drainage area). I calculated Pearson's correlation coefficient, and tested whether $|r| > 0$ based on a two-tailed permutation-based test in using a custom script in R. Before correlation analyses, all variables as proportions (e.g. proportion generalized insectivores) were transformed using an arcsine-square-root transformation, whereas all other variables were transformed using a $\log_{10}(x + 1)$ transformation. All test results were evaluated at $\alpha = 0.05$.

To test whether longitudinal assemblage turnover in my study streams was similar to other systems I conducted an "isolation-by-distance" analysis analogous to Hitt and Roberts (2012). I compared fluvial and Bray-Curtis (BC) distances between US and DS site pairs. The Bray-Curtis distance ranges from 0 to 1, with higher values indicating greater assemblage dissimilarity. Fluvial distance was calculated as the distance in kilometers. All else being equal, we expect assemblage dissimilarity to increase with fluvial distance, though the slope and intercept of this relationship might vary among streams and faunas. The intercept of this relationship is thus indicative of the magnitude of species turnover, and the steepness of the slope is indicative of the rate of longitudinal turnover (Hitt and Roberts 2012). For comparison, I compiled fish assemblage data from studies spanning a range of systems, including montane (Barila et al. 1981, Matthews 1986, Hitt and Roberts 2012), Great Plains (Harrel et al. 1967, Matthews 1986, Dowell 1956 in Lienesch et al. 2000), Mississippi Valley (Smith et al. 1969), and other coastal plain streams (Evans and Noble 1979) (Table 2.3). To be included, the study needed to contain both (a) counts of species by site, and (b) either a text description of distances among sites or a map from which these distances could be derived. I restricted the analysis to site comparisons $\leq$ 50 km apart, in order to interpret all studies on a comparable scale. In addition, I removed data from one system in my study (Ebenezer Creek/Jack's Branch), as the US site was a tributary of the DS site (i.e. not part of the same river continuum), which caused fluvial distance between the two to be an outlier. Depending on whether the dataset consisted of independent observations (my study), a square pair-wise matrix (most other streams), or a non-square matrix (Raystown Branch and Kiamichi River), I tested the significance of relationships using linear regression models, Mantel tests, or permutation-based correlations, respectively. I also fit a linear trend line to all datasets in order to compare slopes and intercepts.

RESULTS

Based on 25 sampling events, 4,217 individual fish from 47 different species were captured (Appendix 1). Richness and Shannon diversity among sites ranged from 10 to 25 and 1.25 to 2.59, respectively. Of captured species, 14 were benthic insectivores, 13 were generalized insectivores, 11 were surface-water insectivores, and 7 were invertivore-piscivores (Table 2.2). Eighteen species were open-spawners, 6 were brood-hiders, 2 were substrate-choosers, 19 were nest-spawners, and 2 were bearers (Table 2.2). Of life-history metrics, mean maximum body size ranged among sites from 23.8 to 46.0 cm TL, mean longevity from 5.5 to 9.2 years, mean fecundity from 11253.4 to 31781.2, proportion late-maturing species from 0.56 to 0.91, and mean stream size preference from 1.8 to 2.0 (Appendix 3). Proportion of species as open-spawners, brood-hiders, substrate-choosers, and nest-spawners ranged from 0.09 to 0.40, 0.06 to 0.26, 0 to 0.11, and 0.39 to 0.73, respectively (Appendix 3). Proportion of species as bearers was not considered as a variable as only two species were included in this category (i.e. Swampfish, *Chologaster cornuta,* and Eastern Mosquitofish, *Gambusia holbrooki*). The mean degree of parental care at sites ranged from 2.6 to 3.6, and spawning-mode Shannon diversity ranged from

0.90 to 1.38 (Appendix 3). Proportion of species as surface-water insectivores, generalized insectivores, benthic insectivores, and invertivore-piscivores ranged from 0 to 0.29, 0.21 to 0.59, 0.06 to 0.38, and 0.13 to 0.42, respectively (Appendix 3). Trophic Shannon diversity ranged from 0.90 to 1.38. Proportion of tolerant species ranged from 0 to 0.20, and proportion of intolerant species from 0 to 0.30. A complete list of species' traits can be found in Table 2.2. As expected, species richness was greater at downstream than upstream sites, and was significantly positively correlated with stream size (Table 2.4, Figures 2.1 and 2.2). However, there was no effect of longitudinal position or stream size on species diversity.

The predictions of the SAR, NDM, IAM, and ASE were not strongly supported by my test results. Stream width, a measure of habitat area, was positively correlated with stream size (Table 2.4, Figure 2.2); however, width did not consistently vary with longitudinal position (Figure 2.1). In addition, stream volume, stream depth, and the mean body size of species (predictions of the SAR), the microhabitat diversity index (prediction of the NDM), stream gradient (IAM), and the stream-size preference (ASE) of species all failed to vary consistently with either longitudinal position or stream size.

Some environmental and biotic predictions of the HTC were supported by test results, though few variables were correlated with stream size and all life-history relationships ran counter to expectations (Table 2.4). The only variable significantly correlated with stream size was the CV of temperature, which was lower in larger streams, as expected. In longitudinal position tests, as hypothesized, pH and water temperature were higher at DS than US sites, whereas the temporal CV of dissolved oxygen was higher at upstream than downstream sites (Figure 2.1). Of fish-assemblages metrics, the only observation that was consistent with expectations was that the proportion of intolerant species was higher at DS than US sites. In contrast, all life-history metrics either were unrelated to stream size or ran counter to the predictions of the HTC. For example, contrary to expectations, the proportion of brood-hiding species was higher at DS than US sites, whereas the proportion of substrate-choosing species and mean fecundity were higher at US than DS sites.

Of the six conceptual models considered, the predictions of the RCC were most consistently supported by test results. As hypothesized, the diversity of trophic strategies and the proportion of benthic insectivores were significantly positively correlated with stream size and were greater at DS than US sites, whereas the proportion of generalized insectivores decreased significantly with stream size and was greater at US than DS sites (Table 2.4, Figures 2.1 and 2.2). In addition, the proportion of surface-water insectivores was greater at DS than US sites as predicted but was not significantly correlated with stream size. Contrary to predictions of the RCC, the abundance of large woody debris was higher at DS than US sites.

The magnitude and longitudinal rate-of-change of species turnover in my coastal plain streams were within the range observed in other studies in other physiographic areas of the United States. For the 11 upstream-downstream site-pairs used in the species-turnover analysis, the fluvial distance separating sites ranged from 8.5 to 36.9 kilometers. There was a significant positive relationship between Bray-Curtis distance and fluvial distance ($F = 8.18_{(1,9)}$, $P = 0.02$)(Table 2.3, Figure 2.3). Among the other 11 published datasets considered, Bray-Curtis and fluvial distance were significantly positively associated in all but 3 (Raystown Branch, Spruce Run, Kiamichi River). The intercept and slope of the relationship for my site-pairs were 0.27 and 0.01, respectively, similar to the relationships observed in studies in other regions (mean intercept = 0.38, range 0.10 - 0.71; mean slope = 0.02, range 0.002 – 0.03).

DISCUSSION

In this study, I tested the ability of six longstanding conceptual ecological models to predict longitudinal changes in fish assemblages and environmental characteristics in coastal plain streams. I hypothesized that some features particular to coastal plain environments such as intrinsically low habitat diversity and harsh physiochemical conditions would weaken the applicability of some of the models, as evidenced by weaker spatial gradients in environmental and fish-assemblage variables. These hypotheses were partially supported by my findings. Much like previous longitudinal studies in other physiographic regions, I found that species richness increased with stream size (Table 2.4). I also found that the magnitude and rate of longitudinal species turnover in my study streams was on the low end of, but similar to, that observed in other systems (Table 2.3, Figure 2.3). The trophic composition of fish assemblages varied in ways consistent with the RCC, suggesting that this model can provide sound ecological predictions in blackwater coastal plain streams. On the other hand, measured physical and chemical habitat gradients generally were weak, which may explain why predictions based on the often-invoked HTC and SAR, along with other models considered, generally were inconsistent with assemblage variation observed in my study. In the following text, I consider each model and its predictions in detail, and explain mechanisms that may have contributed to its applicability (or lack thereof) in my study system.

The SAR predicts a downstream increase in species richness in response to increasing habitat area or volume. Indeed, I found find higher species richness in larger, downstream sites, and a significant positive relationship between stream width and stream size (Table 2.4). However, width did not consistently increase with longitudinal position, and other longitudinal environmental patterns predicted by the SAR (i.e. depth, habitat volume) did not exhibit any consistent longitudinal relationships. This suggests that habitat area increases in larger coastal plain streams as streams become wider downstream but not necessarily deeper, and therefore that the “null” model of the SAR cannot be fully rejected. However, given that habitat volume has been found to be more predictive of species richness than habitat area (Angermeier and Schlosser 1989), my results suggest that there are likely other mechanisms contributing to the longitudinal sorting of species. The SAR further predicts a longitudinal biotic response of increasing body size in response to greater habitat area, which also was not found by this study. All species with a maximum TL of > 50 cm (Bowfin, American Eel, Brown Bullhead, Chain Pickerel, Longnose Gar, Largemouth Bass, Spotted Sucker) (Table 2.2) occurred at both up- and downstream sites, as did most species with a maximum TL < 10 cm (e.g. darters, many minnows). This may be because habitat volume (specifically depth), which did not increase downstream in my study, is more important for supporting larger-bodied species than is habitat area (Schlosser 1982). These results suggest that the applicability of the SAR is weakened in the coastal plain, even during periods of base flows when sampling occurred, which is contrary to initial hypotheses. As this study was conducted during periods of lowest flows, when a longitudinal gradient in habitat area would be most distinctive, the weakening of the SAR in the coastal plain may remain true throughout the year, as habitat area will further homogenize longitudinally during wetter seasons when upstream sites experience greater flow permanence.

In contrast with the NDM, the diversity of microhabitats (i.e. unique combinations of depth, substrate, and large woody debris) showed no consistent relationship with either longitudinal position or stream size (Table 2.4). This aligned with my hypothesis that the NDM would be weakened in the coastal plain, as the coastal plain is naturally low in habitat diversity, so an increase in available microhabitat niches in the downstream direction is potentially unlikely. Discrete habitat units (e.g. riffles) are rare in the coastal plain; rather, components of habitat characterization (e.g. velocity, depth) are continuous environmental gradients along which species sort gradually (Meffe and Sheldon 1988). It is important to note, however, that velocity is an important consideration in microhabitat characterization and specialization (Gorman and Karr 1978) that was not examined in this study as I did not have access to a flowmeter during sampling. Another coastal plain study found that species sorted along habitat gradients of stream size, velocity, substrate, and cover, with depth and velocity being the most predictive of assemblage composition (Meffe and Sheldon 1988). It is possible that if variations in current were considered in the calculation of microhabitat diversity I might have detected some longitudinal differences; although flows were low during times of sampling I observed that downstream sites tended to have more patches of noticeable flow than did upstream sites. Additionally, this study tested only one environmental variable (i.e. microhabitat diversity) and no biotic factors related to the NDM. Other potentially useful factors that could be examined in future studies would be the longitudinal sorting of species by habitat preferences (e.g. current, substrate). An investigation into the influence of floodplain habitat characteristics (e.g. complexity) would also be interesting, given the importance of lateral connections to some coastal plain species for foraging, nursery, or refuge habitat (Junk et al. 1989).

Many previous studies have shown that temporal environmental stability increases with stream size, and as a result, the HTC predicts that species traits will sort accordingly based on their adaptability to unstable conditions (Townsend and Hildrew 1994). Results from this study suggest that the HTC has moderate applicability in the coastal plain. As predicted by the HTC, water quality was somewhat harsher and more variable in smaller, upstream sites, which could explain why intolerant species (e.g. Sawcheek Darter, Turquoise Darter, Tessellated Darter, Speckled Madtom, Taillight Shiner, Spottail Shiner) were more prevalent in downstream sites where environmental stability was higher. On the other hand, the lack of a longitudinal difference in the proportion of tolerant species may be a result of hardy species being present system-wide in the coastal plain due to naturally harsh conditions; upstream assemblages may be nested subsets of downstream assemblages, as tolerant species seem to occur everywhere whereas intolerant species are more prevalent in downstream areas. Analyses found three other biotic gradients described by the HTC to exhibit longitudinal variation, although in opposite directions of HTC predictions. Brood-hiding species were more prevalent in downstream sites, whereas the proportion of substrate-choosing species and mean fecundity decreased downstream (Table 2.4). This unexpected pattern in fecundity could be an artifact of the gradient (or lack thereof) in body size found by this study, as most highly fecund species were also some of the largest-bodied species (e.g. Bowfin, Largemouth Bass, Longnose Gar, Spotted Sucker) and the least fecund were small-bodied species (e.g. minnows, darters, madtoms); due to a lack of longitudinal depth variation, downstream sites were not necessarily able to support larger, more fecund species relative to upstream sites. Of brood-hiders occurring in my study streams, four were lithophils that hide their eggs in coarse, rocky substrate (i.e. Dusky Shiner, Yellowfin Shiner, Savannah Darter, Blackbanded Darter), and two were cavity spawners (i.e. Bannerfin Shiner, Pirate Perch) (Frimpong and Angermeier 2009). Although I did not test for this, this gradient in brood-hiders could imply that substrate coarseness increases downstream in the coastal plain. The coastal plain naturally lacks large rocky substrate system-wide (Meffe and Sheldon 1988), so longitudinal patterns in substrate coarseness may be weakened as even upstream sites have relatively fine substrate compared to other systems. Only two species were considered substrate choosers (i.e. Banded Pygmy Sunfish and Swamp Darter), both of which are phytophiles, meaning they prefer to spawn on aquatic macrophytes (Frimpong and Angermeier

2009). As there was no true submerged aquatic vegetation at any of my sites, the presence of absence of these substrate-choosing species may not reflect true ecological patterns. Although my findings of trophic patterns were not consistent with many original predictions of the HTC as defined by Townsend and Hildrew (1994), results were somewhat consistent other empirical studies that also found a decrease in substrate-choosers downstream (Goldstein and Meador 2004, McGarvey and Hughes 2008). Goldstein and Meador's (2004) study across multiple systems and ecoregions also found patterns in open-water-spawners and nest-spawners opposite to Townsend and Hildrew (1994).

Although I hypothesized that the RCC would be less applicable to coastal plain systems due to their blackwater nature and high lateral connectivity, the RCC was the most consistently supported of tested models (Table 2.4). My assemblages lacked herbivores, planktivores, or detritivores, so I could not assess longitudinal patterns in these trophic groups. However, patterns in other groups were predictable from the RCC. Specifically, trophic diversity and the proportion of benthic insectivores and surface-water insectivores increased downstream, and generalized insectivores increased upstream, all of which is predicted by the RCC due to variation in energy sources and the downstream transport of organic matter. Surface-water insectivores were found to be more prevalent in downstream areas, which is intuitive given that I found a positive correlation between stream size and width, suggesting an increasingly open canopy downstream that is generally associated with surface-oriented feeders (Vannote et al. 1980). Many studies have also found a downstream increase in benthic insectivores (Schlosser 1982, Paller 1994, McGarvey and Hughes 2008), although this has been attributed to a downstream increase in deep pool habitat and body size that was not found by this study. The benthic insectivores in my streams were darters, ictalurids, and catostomids (Table 2.2), many of which were intolerant species that may be more prevalent in downstream areas due to increased environmental stability (discussed with the HTC). My results suggest that there were more specialist feeders (i.e. surface-water insectivores, benthic insectivores) downstream; generalist feeders were present system-wide, but the absence of surface-water and benthic insectivores upstream resulted in a significantly higher proportion of species as generalized insectivores in these areas. This result further suggests that upstream assemblages are nested subsets of downstream assemblages. I found no longitudinal patterns in the prevalence of top predators (i.e. invertivore-piscivores), potentially because top predators are typically larger-bodied (e.g. bowfin, longnose gar, largemouth bass) and occupy deep pools (Power 1987, Schlosser 1982, Schlosser 1987), and I found no significant longitudinal variation in stream depth. Contrary to RCC predictions, but consistent with my coastal plain hypotheses, was a downstream increase in the abundance of large woody debris. Vannote et al. (1980) suggested a downstream decrease in coarse particulate organic matter (CPOM) as CPOM such as large wood is typically more prevalent in upstream areas with high riparian inputs, and is broken down by flows and deposited downstream as fine particulate organic matter (FPOM). While I did not test for longitudinal patterns in substrate coarseness or accumulation of FPOM, large woody debris (LWD) is the primary structural component in coastal plain streams and riparian debris inputs are high system-wide due to frequent system-wide lateral connections to forested floodplains (Meffe and Sheldon 1988), which may explain high LWD density downstream.

No longitudinal environmental or biotic predictions of the IAM or ASE were found by this study to be applicable to the coastal plain (Table 2.4). A potential explanation for the weakening of these models may be the low number of movement barriers system-wide as the coastal plain experiences few anthropogenic (e.g. impoundments for hydroelectric use) or natural barriers (e.g. waterfalls) due to its low-gradient nature (Benke 1990). Fishes may therefore be able to move more freely throughout the continuum in the coastal plain than in other regions. However, I did not directly measure regional connectivity, and had relatively few satisfactory surrogates for connectivity, so this test of the IAM should be considered tentative. I attempted to classify mainstream- versus headwater-oriented species using stream size preferences from Frimpong and Angermeier (2009), which classified stream sizes as “large river”, “small river”, and “creek”. However, most species were associated with more than one size category; only one of my species (Bannerfin Shiner) was listed as occurring only in large rivers, and only two species (Banded Pygmy Sunfish, Ironcolor Shiner) were listed as occurring only in creeks. This suggests that most species were not truly mainstem- or headwater-oriented, as all but these three species occur in intermediate “small rivers”, which may make it difficult to quantify mainstem migrants (and therefore the applicability of the ASE) in coastal plain streams. Additionally, upstream movement of main channel individuals may have been low during times of sampling as tributaries were experiencing base flows; upstream movement may be greater during wet periods as habitat volume in tributaries will increase and potentially better support larger-bodied species (Angermeier and Schlosser 1989, Schlosser 1982). In order to improve understanding of the applicability of the IAM and ESM in the coastal plain, future studies should test for additional gradients in environmental characteristics (e.g. proximity to a main channel, number of movement barriers) and species’ traits (e.g. mobility, dispersal).

Conclusions

By understanding how coastal plain fish assemblages sort along environmental gradients, we can better predict how the community may respond to environmental changes or disturbance (e.g. channelization, land-use changes, impoundments). The results of this study reinforce the need for ecoregion-specific investigations, as environment-trait relationships developed in other systems may not readily transfer to the coastal plain. Regional nuances in species-environment relationships should be considered when making management decisions that may impact environmental or habitat conditions. Importantly, although this study has furthered our understanding of coastal plain assemblage regulation during the dry season, it is important to recognize that these results only capture a snapshot of the dynamic conditions that coastal plain streams exhibit over the course of a year. This is common in existing research as base flows facilitate accessibility and therefore ease of sampling; however, many of the underlying mechanisms regulating fish assemblage structure, both longitudinally and laterally, may differ during the wet season. Although logistically challenging, future studies in coastal plain streams and elsewhere should therefore attempt to capture and classify environmental and assemblage variation across all flow periods to fill in critical gaps in our knowledge of fish ecology.

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TABLES AND FIGURES

Table 2.1 Longitudinal gradients tested in this study, organized by six conceptual models. I indicate the direction of the relationship as described in previous literature, as well as the hypothesized applicability of each gradient in the coastal plain. Potential explanations for altered applicability include (1) seasonal flooding and drying, (2) low-gradient, (3) lack of barriers, (4) low mesohabitat diversity, and (5) harsh physiochemical conditions, in ACP streams.

	Described relation with stream size	Literature reference	Hypothesized applicability in ACP	Hypothesized relation with stream size in ACP
Species-Area Relationship				
Channel volume	+	Schlosser 1982; Vannote et al. 1980; Angermeier and Schlosser 1989; Sheldon 1968; Hitt and Angermeier 2008	Same	+
Stream depth	+	Schlosser 1982; Schlosser 1990; Angermeier and Schlosser 1989; Sheldon 1968	Same	+
Stream width	+	Schlosser 1982; Angermeier and Schlosser 1987; Sheldon 1968	Same	+
Maximum body size	+	Schlosser 1982; Schlosser 1990; Gilliam et al. 1993; Poff 1997	Same	+
Niche Diversity Model				
Microhabitat diversity	+	Schlosser 1982; Lowe-McConnell 1979; McGarvey and Hughes 2008; Poff 1997; Angermeier and Karr 1984	Weaker (1, 2, 4)	+
Habitat Template Concept				
pH	+	Burton and Odum 1945	Same	+
pH (CV)	-	Schlosser 1990	Stronger (1, 5)	-
Dissolved oxygen	+	Matthews and Styron 1981	Same	+
Dissolved oxygen (CV)	-	Schlosser 1990	Stronger (1, 5)	-
Temperature	+	Troia and Gido 2014	Same	+
Temperature (CV)	-	Schlosser 1990	Stronger (1, 5)	-
Fecundity	+	Townsend and Hildrew 1994; Goldstein and Meador 2004	Stronger (1,5)	+
Longevity	+	Schlosser 1990; Townsend and Hildrew 1994	Stronger (1,5)	+
Proportion later-maturing species	+	Schlosser 1990; Townsend and Hildrew 1994; Gray 1981	Stronger (1,5)	+
Degree of parental care	+	Schlosser 1990; Townsend and Hildrew 1994	Stronger (1,5)	+
Degree of parental care	-	Goldstein and Meador 2004		
Spawning mode diversity	-	Townsend and Hildrew 1994 ; Goldstein and Meador 2004	Stronger (1,5)	-
Proportion open-water-spawners	-	Townsend and Hildrew 1994	Stronger (1,5)	-
Proportion open-water-spawners	+	Goldstein and Meador 2004		

Proportion brood-hiders	-	Townsend and Hildrew 1994; Goldstein and Meador 2004; McGarvey and Hughes 2008	Stronger (1,5)	-
Proportion substrate-choosers	+	Townsend and Hildrew 1994	Stronger (1,5)	+
Proportion substrate-choosers	-	Goldstein and Meador 2004; McGarvey and Hughes 2008		
Proportion nest-spawners	+	Townsend and Hildrew 1994	Stronger (1,5)	+
Proportion nest-spawners	-	Goldstein and Meador 2004; McGarvey and Hughes 2008		
Proportion intolerant species	+	Matthews and Styron 1981; Townsend and Hildrew 2004	Stronger (1,5)	+
Proportion tolerant species	-	Matthews and Styron 1981; Townsend and Hildrew 2004	Stronger (1,5)	+
River Continuum Concept				
Large woody debris	-	Vannote et al. 1980	Weaker (1)	-
Trophic diversity	+	Vannote et al. 1980; Paller 1994; Goldstein and Meador 2004	Weaker (1)	+
Proportion benthic insectivores	+	Vannote et al. 1980; Paller 1994; McGarvey and Hughes 2008; Schlosser 1982; Hitt and Angermeier 2008	Weaker (1)	+
Proportion generalized insectivores	-	Vannote et al. 1980; Paller 1994; Goldstein and Meador 2004; McGarvey and Hughes 2008	Weaker (1)	-
Proportion invertivore-piscivores	+	Vannote et al. 1980; Paller 1994; Goldstein and Meador 2004; McGarvey and Hughes 2008; Schlosser 1982	Weaker (1)	+
Proportion surface-water insectivores	+	Vannote et al. 1980; Paller 1994; Schlosser 1982; Hitt and Angermeier 2008	Weaker (1)	+
Immigrant Accessibility Model				
Gradient	-	Robinson and Rand 2005; Burton and Odum 1945; Grossman et al. 2010; Sheldon 1968	Weaker (2, 3)	-
Adventitious Stream Effect				
Stream-size preference	+	Schaefer and Kerfoot 2004; Gorman 1986; Osborne and Wiley 1992; Hitt and Angermeier 2008	Weaker (2, 3)	+

Table 2.2 Species trait-states compiled and analyzed in this study, including spawning mode, degree of parental care, longevity, fecundity, maximum body size, age at maturity, trophic strategy, tolerance, and size preference. See Appendix 2 for a description of each trait.

Scientific Name	Spawning mode	Degree of parental care	Longevity (years)	Fecundity (ova)	Maximum total length (cm)	Age at maturity (years)	Trophic Strategy	Tolerance Ranking	Size preference
Acantharchus pomotis	NS	4	4.0	11838	21.0	1.0	GNI	MOD	CRK, SMR
Ameiurus brunneus	NS	4	6.0	1742	29.0	3.0	BNI	MOD	SMR, LGR
Ameiurus natalis	NS	4	7.0	7000	47.0	2.5	BNI	TOL	CRK, SMR, LGR
Ameiurus nebulosus	NS	4	11.0	13000	55.0	2.5	BNI	MOD	CRK, SMR, LGR
Amia calva	NS	4	25.0	64000	109.0	4.0	IVP	MOD	SMR, LGR
Anguilla rostrate	OS	1	20.0	2500000	100.0	8.6	IVP	MOD	CRK, SMR, LGR
Aphredoderus sayanus	BH	2	4.0	160	14.0	2.0	GNI	MOD	CRK, SMR, LGR
Centrarchus macropterus	NS	4	7.0	37500	29.2	1.0	GNI	MOD	CRK, SMR
Chologaster cornuta	BR	5	3.0	426	6.8	1.0	GNI	MOD	CRK, SMR
Cyprinella leedsi	BH	2	4.0	1000	10.0	1.5	SWI	MOD	LGR
Elassoma zonatum	SC	3	2.0	970	4.7	1.0	GNI	MOD	CRK
Enneacanthus gloriosus	NS	4	6.0	635	9.5	2.0	GNI	MOD	CRK, SMR
Erimyzon oblongus	OS	1	5.5	83013	36.0	2.0	BNI	MOD	CRK, SMR
Erimyzon sucetta	OS	1	12.0	18478	41.0	3.0	BNI	INT	CRK, SMR
Esox americanus	OS	1	7.0	4584	37.6	2.5	IVP	MOD	CRK, SMR
Esox niger	OS	1	9.0	8000	99.0	2.0	IVP	MOD	CRK, SMR, LGR
Etheostoma fricksium	BH	2	3.5	300	7.4	1.0	BNI	INT	CRK, SMR
Etheostoma fusiforme	SC	3	1.5	50	5.9	0.5	BNI	MOD	CRK, SMR
Etheostoma inscriptum	OS	1	3.0	120	8.0	1.0	BNI	INT	CRK, SMR
Etheostoma olmstedi	NS	4	3.0	1435	11.0	1.0	BNI	INT	CRK, SMR
Etheostoma serrifer	OS	1	2.0	100	6.8	1.0	BNI	INT	CRK, SMR
Fundulus lineolatus	OS	1	2.5	200	8.5	1.0	SWI	MOD	CRK, SMR
Gambusia holbrooki	BR	5	1.0	315	4.0	0.3	SWI	MOD	CRK, SMR
Hybopsis rubrifrons	OS	1	3.0	1000	8.4	1.0	SWI	INT	CRK, SMR

Labidesthes vanhyningi	OS	1	2.0	785	13.0	1.0	GNI	MOD	CRK, SMR, LGR
Lepisosteus osseus	OS	1	26.0	77156	200.0	5.0	IVP	TOL	SMR, LGR
Lepomis auratus	NS	4	6.0	10000	30.5	2.0	GNI	MOD	CRK, SMR, LGR
Lepomis gulosus	NS	4	8.0	63000	31.0	1.5	IVP	MOD	CRK, SMR, LGR
Lepomis macrochirus	NS	4	10.0	50000	41.0	2.0	GNI	MOD	CRK, SMR, LGR
Lepomis marginatus	NS	4	6.0	600	12.0	2.0	GNI	MOD	CRK, SMR, LGR
Lepomis microlophus	NS	4	5.0	80000	43.2	2.0	GNI	MOD	CRK, SMR, LGR
Lepomis punctatus	NS	4	5.0	15000	20.0	2.0	GNI	MOD	CRK, SMR, LGR
Micropterus salmoides	NS	4	16.0	109314	97.0	2.5	IVP	TOL	CRK, SMR, LGR
Minytrema melanops	OS	1	6.0	40000	50.0	3.0	BNI	MOD	CRK, SMR
Notemigonus crysoleucas	OS	1	8.0	4700	30.0	1.0	GNI	TOL	SMR, LGR
Notropis chalybaeus	OS	1	3.0	300	6.5	1.0	SWI	INT	CRK
Notropis cummingsae	BH	2	3.0	300	7.2	1.0	SWI	MOD	CRK, SMR
Notropis hudsonius	OS	1	4.5	3709	15.0	1.5	SWI	INT	SMR, LGR
Notropis lutipinnis	BH	2	3.0	800	7.5	1.0	SWI	MOD	CRK, SMR
Notropis maculatus	OS	1	2.0	431	7.6	0.5	SWI	INT	SMR, LGR
Notropis petersoni	OS	1	4.0	854	8.2	2.0	SWI	MOD	CRK, SMR, LGR
Noturus gyrinus	NS	4	4.0	400	13.0	1.5	BNI	MOD	CRK, SMR, LGR
Noturus leptacanthus	NS	4	2.5	45	9.4	1.0	BNI	INT	CRK, SMR
Opsopoeodus emiliae	NS	4	3.0	600	6.4	1.5	SWI	INT	CRK, SMR, LGR
Percina nigrofasciata	BH	2	2.5	250	11.0	1.0	BNI	MOD	CRK, SMR
Pomoxis nigromaculatus	NS	4	8.0	188000	49.0	2.5	IVP	TOL	SMR, LGR
Pteronotropis stonei	OS	1	2.0	800	6.6	1.0	SWI	MOD	CRK, SMR

Spawning-mode: OS = Open-spawner, BH = Brood-hider, SC = Substrate-chooser, NS = Nest-spawner, BR = Bearer
Trophic strategy: BNI = Benthic insectivore, GNI = Generalized insectivore, SWI = Surface-water insectivore, IVP = Invertivore-piscivore
Tolerance ranking: INT = Intolerant, MOD = Moderate, TOL = Tolerant
Size preference: CRK = Creek, SMR = Small river, LGR = Large river

Table 2.3 Relationship between Bray-Curtis and fluvial distance between pairs of sites for this study, as well as for 11 other streams where data were available. The linear slope and intercept of relationships as well as the statistical significant of associations are shown. Test statistics reflect the significance test used; linear regression models (for independent observations, F), Mantel tests, (for square pair-wise matrices, R), or permutation-based correlations (for non-square matrices, r). Only 4 and 8 sites were used from the Kiamichi River and Raystown Branch, respectively, in order to restrict streams to $\leq$50 km fluvial distance.

Stream	State	Region	Reach length (km)	Gradient (m/km)	Sites	Species	Intercept	Slope	Test statistic	P	Reference
Various Coastal Plain streams	GA	Coastal Plain	8.5 - 36.9	0-3	2 per stream	47	0.27	0.01	F= 8.18 (1,9)	0.019	This study
Big Sandy Creek	TX	Coastal Plain	34.6	1.9	7	46	0.35	0.01	R= 0.84	0.003	1
Otter Creek	OK	Great Plains	27.0	1.8	12	18	0.51	0.02	R= 0.62	0.001	2
Brier Creek	OK	Great Plains	18.0	2.1	6	22	0.47	0.02	R= 0.74	0.025	3
Buncombe Creek	OK	Great Plains	9.9	3.2	4	40	0.42	0.03	R= 0.66	0.042	4
Piasa Creek	IL	Mississippi Valley	28.0	2.1	9	40	0.51	0.02	R= 0.45	0.036	5
Raystown Branch	PA	Appalachian Highlands	97.0	1.9	6	32	0.71	0.00	r = 0.17	0.349	6
Roanoke River	VA	Appalachian Highlands	27.0	3.5	6	30	0.36	0.01	R= 0.45	0.049	3
Sinking Creek	VA	Appalachian Highlands	40.4	4.1	13	27	0.14	0.01	R= 0.67	0.001	7
Spruce Run	VA	Appalachian Highlands	6.0	21.5	10	8	0.10	0.02	R= 0.29	0.080	7
Little Stony Creek	VA	Appalachian Highlands	18.4	33.0	12	15	0.19	0.03	R= 0.67	0.001	7
Kiamichi River	OK	Ouachita Highlands	164.0	1.2	6	31	0.46	0.00	r= 0.24	0.331	3
Average (other studies)			42.8	6.9	8.3	28.1	0.38	0.02			

[1] Evans and Noble (1979), [2] Harrel et al. (1967), [3] Matthews (1986), [4] Dowell (1956) in Lienesch et al. (2000), [5] Smith et al. (1969), [6] Barila et al. (1981), [7] Hitt and Roberts (2012)

Table 2.4 Results of permutation-based correlations of each variable with drainage area, and comparison of observed results from this test and the longitudinal position test (DS-US effect size; Figure 2.1) with hypothesized relationships. Predicted or observed positive (+) or negative (-) relationships, or an observation of no effect (NE) are shown. Bolded variables were found to be significantly correlated with drainage area at *P* <0.05.

	Correlation with drainage area		Relationship with stream size		
Environmental or assemblage attribute	*r*	*P*	Correlation	Position test	Hypothesized
Species richness	0.47	**0.010**	+	+	+
Species diversity	0.17	0.093	NE	NE	+
Species-Area Relationship					
Stream volume	0.21	0.187	NE	NE	+
Stream depth	-0.02	0.253	NE	NE	+
Stream width	0.48	**0.010**	+	NE	+
Mean body size	0.07	0.268	NE	NE	+
Niche Diversity Model					
Microhabitat diversity	0.06	0.202	NE	NE	+
Habitat Template Concept					
pH	0.19	0.278	NE	+	+
CV of pH	-0.04	0.225	NE	NE	-
Dissolved oxygen	0.05	0.289	NE	NE	+
CV of dissolved oxygen	-0.05	0.398	NE	-	-
Temperature	0.05	0.379	NE	+	+
CV of temperature	-0.34	**0.027**	-	NE	-
Mean fecundity	-0.28	0.076	NE	-	+
Mean longevity	0.03	0.464	NE	NE	+
Proportion later-maturing species	-0.04	0.497	NE	NE	+
Parental care index	0.03	0.485	NE	NE	+
Spawning mode diversity	0.23	0.120	NE	NE	-
Proportion open-water spawners	-0.07	0.276	NE	NE	-
Proportion brood hiders	0.16	0.162	NE	+	-
Proportion substrate choosers	0.30	0.086	NE	-	+
Proportion nest spawners	-0.05	0.370	NE	NE	+
Proportion intolerant species	0.04	0.280	NE	+	+
Proportion tolerant species	-0.07	0.361	NE	NE	-
River Continuum Concept					
Large woody debris abundance	0.30	0.065	NE	+	-
Trophic diversity	0.40	**0.047**	+	+	+
Proportion benthic insectivores	0.45	**0.011**	+	+	+
Proportion generalized insectivores	-0.42	**0.007**	-	-	-
Proportion invertivore-piscivores	-0.07	0.383	NE	NE	+
Proportion surface-water insectivores	0.09	0.198	NE	+	+

Immigrant Accessibility Model					
Stream gradient	-0.01	0.474	NE	NE	-
Adventitious Stream Effect					
Stream-size preference	0.25	0.091	NE	NE	+

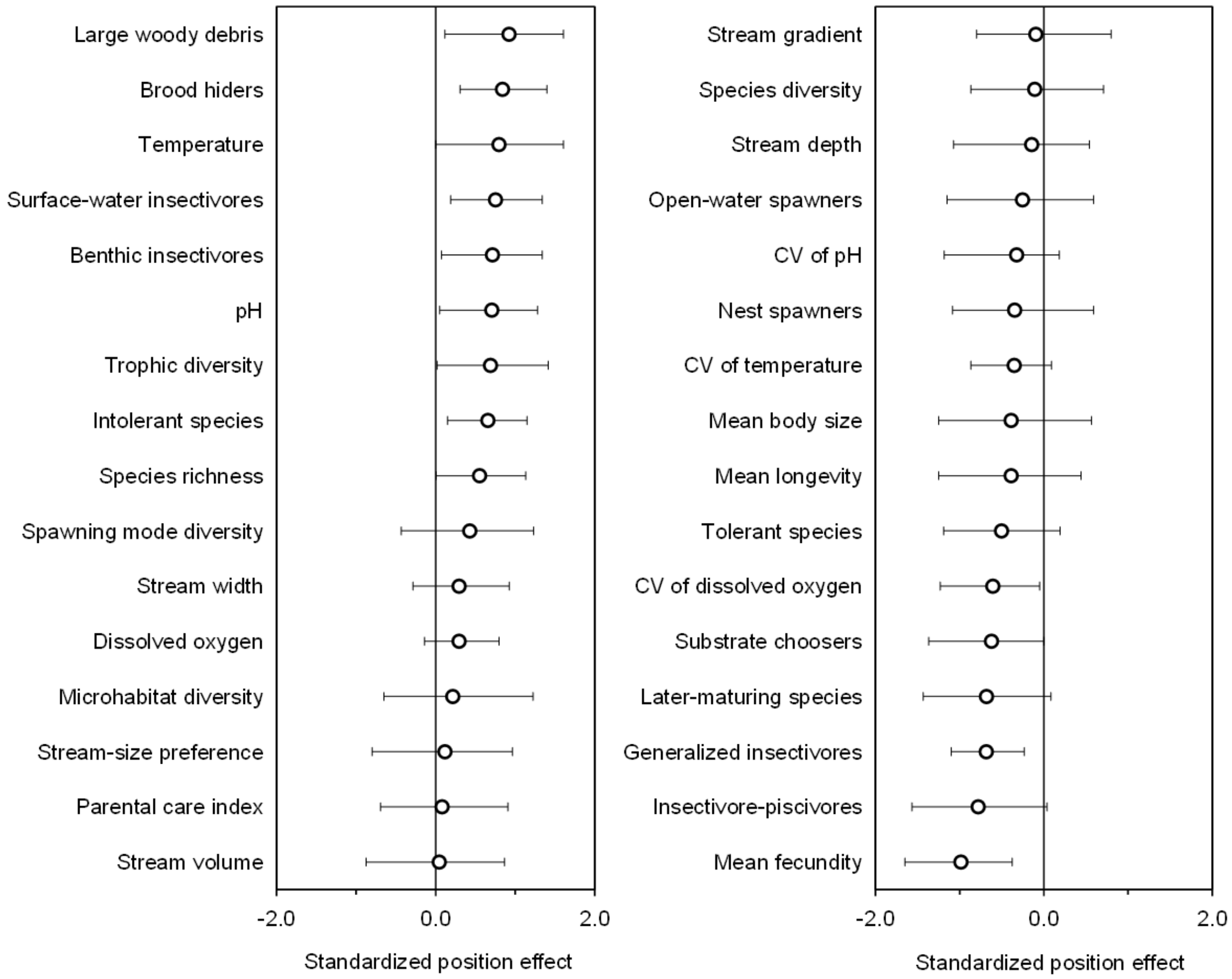

Figure 2.1 The effect of longitudinal position on each variable, as measured by the mean and 95% confidence interval (CI) of the standardized difference between DS (n=12) and US (n=12) sites. Means greater or less than zero were greater downstream or upstream, respectively. Variables were considered to be consistently affected by position if the 95% CI did not include zero.

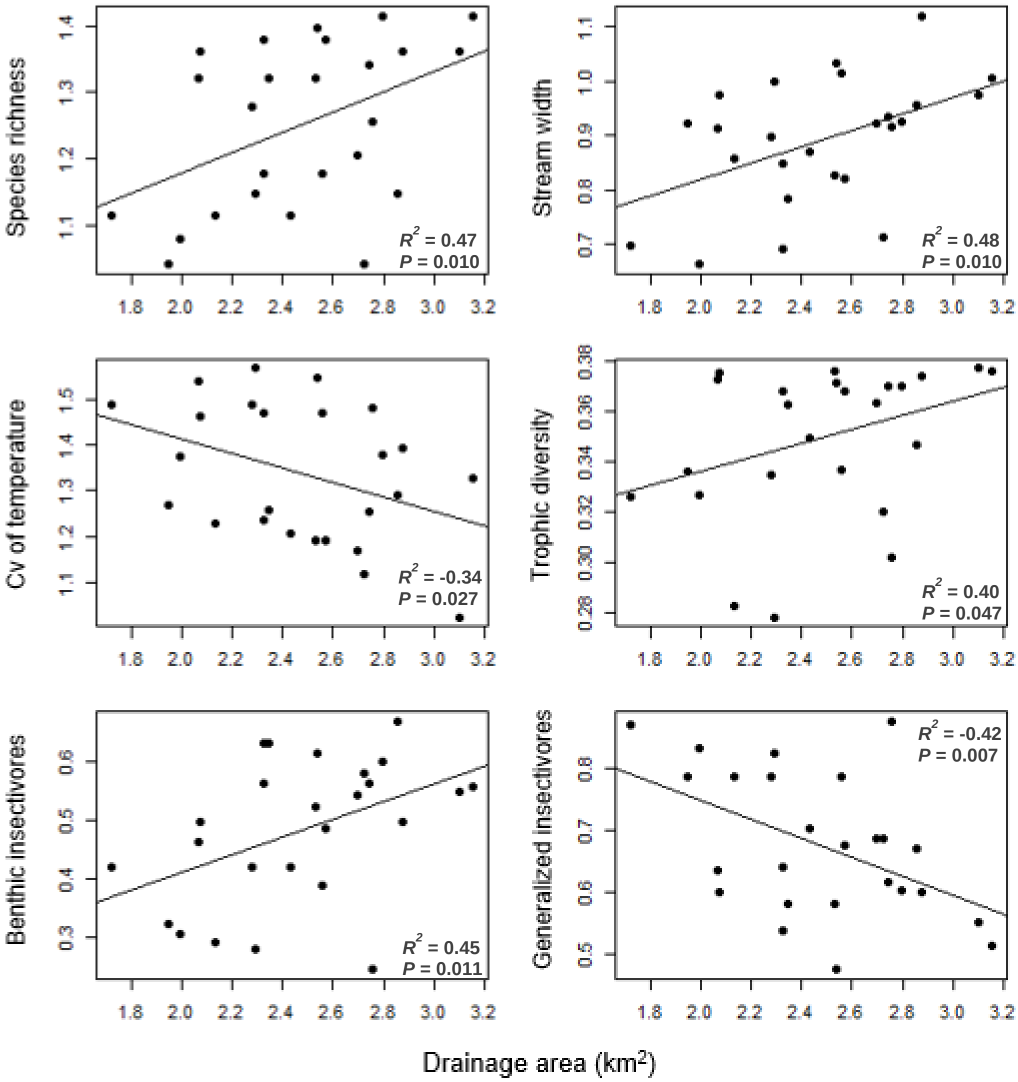

Figure 2.2 Bivariate relationships between drainage area (a measure of stream size) and each of six variables found to exhibit a significant ($P < 0.05$) correlation with drainage area. Linear trend lines are for visualization purposes only. Proportion-type variables were transformed using an arcsine-square-root transformation and all others were transformed using $\log_{10}(X+1)$.

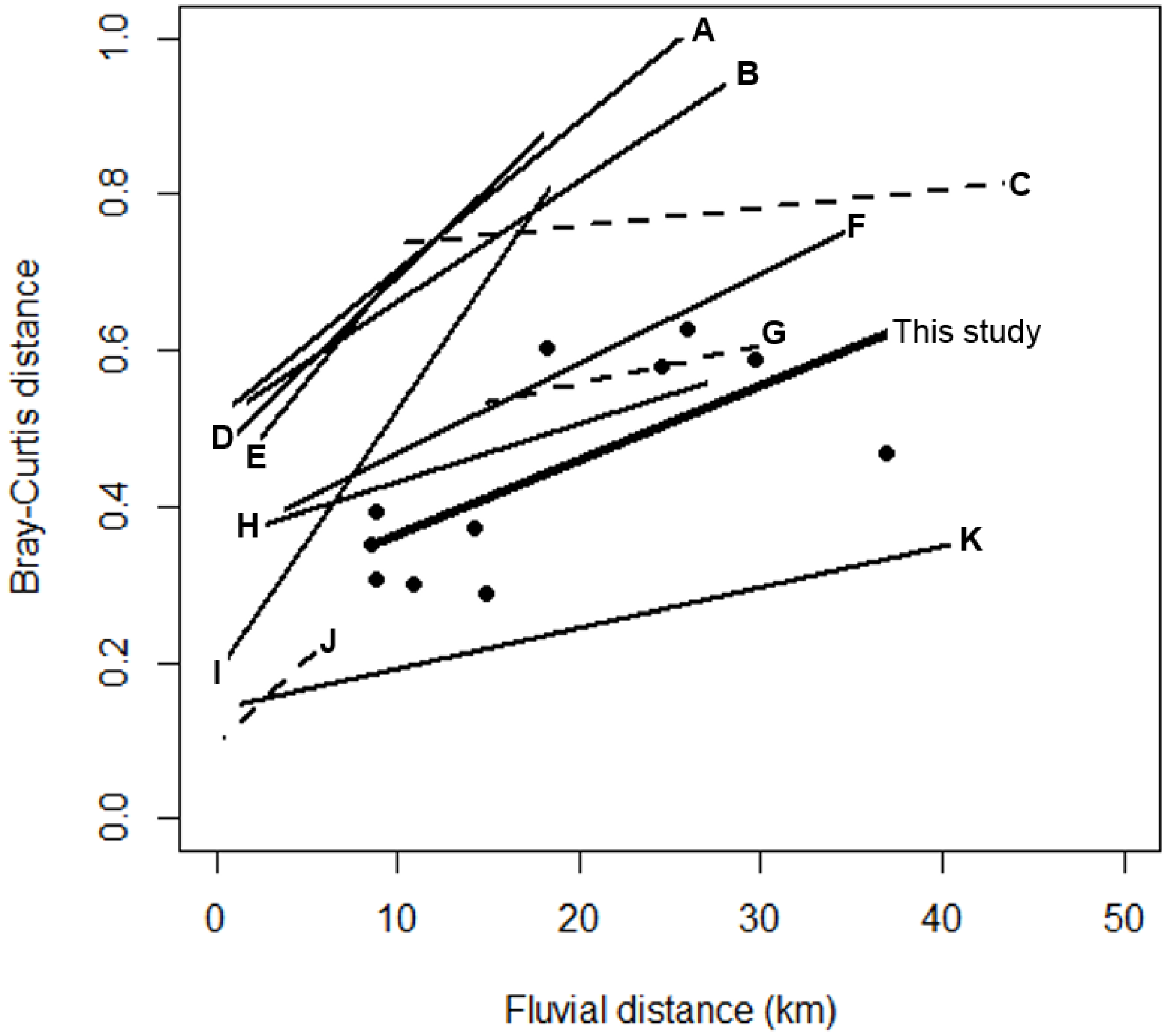

Figure 2.3 Relationships between Bray-Curtis (BC) and fluvial distances between pairs of sites for this study (one point per stream; thick line shows linear trend) and other published longitudinal surveys of other streams (lettered trend lines). Solid lines represent a significant relationship between BC and fluvial distance; dashed lines were non-significant. Data points where fluvial distance was > 50 km were eliminated (C and G) so that relationships could be considered on a comparable scale. [A] Otter Creek, OK (Harrel et al. 1967), [B] Piasa Creek, IL (Smith et al. 1969), [C] Raystown Branch, PA (Barila et al. 1981), [D] Brier Creek, OK (Matthews 1986), [E] Buncombe Creek, OK (Dowell 1956 in Lienesch et al. 2000), [F] Big Sandy Creek, TX (Evans and Noble 1979), [G] Kiamichi River, OK (Matthew 1986), [H] Roanoke River, VA (Matthews 1986), [I] Little Stony Creek, VA (Hitt and Roberts 2012), [J] Spruce Run, VA (Hitt and Roberts 2012), [K] Sinking Creek, VA (Hitt and Roberts 2012).

GENERAL CONCLUSIONS

The main goals of this study were to (1) characterize the taxonomic and functional organization of coastal plain fish assemblages in the Atlantic coastal plain of Georgia, (2) assess their variation over space and time, and (3) determine the environmental factors most influential in driving these patterns. The interaction of communities with their environment is an extensive area of research in stream fish ecology, yet most studies that seek to characterize these interactions have been conducted in upland systems. Due to its distinctive hydrologic patterns and harsh physiochemical regime, it is intuitive that community-environment relationships in the coastal plain may differ from those in other systems, yet limited research has been conducted in this region. This study sought to help fill these gaps through an extensive survey of spatial environmental and assemblage variation in coastal plain systems.

I characterized spatial patterns in both the taxonomic and functional composition of coastal plan stream fish assemblages. This study found that spatial variation in the taxonomic composition of assemblages is driven by environmental factors on multiple scales, including biogeographical regional factors such as drainage area and ecoregion, and local instream factors such as water chemistry and substrate. Anthropogenic land use was found to have limited influence on the species make-up of coastal plain fish assemblages, but there was a negative association between species richness and the amount of upstream development. Assemblages appear to sort into “fluvial” and “nonfluvial” type by species composition, with fluvial sites being characterized as having higher temperature, dissolved oxygen, and coarse substrate, among others. Nonfluvial assemblages were nested subsets of fluvial assemblages, potentially composed of species tolerant to harsh environmental conditions (e.g. lower dissolved oxygen); anthropogenic disturbance such as increased development of watershed areas or global climate change may therefore result in unfavorable conditions and a subsequent loss of fluvial species.

However, membership to these assemblage types was consistent across two summers of noticeably different flow patterns, suggesting assemblages may be resilient to disturbance. Given the environmental predictors and composition of these assemblage types, the development of separate bioassessment protocols for fluvial and nonfluvial streams may be warranted, as reference conditions and assemblages vary between the two.

Coastal plain streams appear to exhibit several longitudinal gradients in both environmental and biotic factors, including higher species richness in large, downstream reaches and greater temporal instability of environmental conditions upstream. Species' traits also somewhat sorted longitudinally by both spawning-mode and trophic strategy, although many longitudinal gradients described in other lotic systems had weakened applicability in the coastal plain due to its distinctive hydrology and physiochemical regime. These results further imply the need for research and management practices that consider region-specific nuances in environmental-trait relationships, as models developed in other systems may have limited applicability in the coastal plain.

This study used data from two sampling seasons, both of which occurred during summer base flows. Given the extreme seasonal hydrologic variation in the coastal plain, environmental conditions are likely to exhibit great temporal variation as systems see increased flows, flooding, and lateral connection to floodplains. While this study provided insight as to drivers of spatial assemblage variation during dry months, a great deal could be learned from investigating the same research question during seasons or years of differing flow patterns. This knowledge gap is likely also applicable to systems other than the coastal plain, as many sampling-based studies take place during base flows that facilitate accessibility. Stream fish community ecology as a whole would likely benefit from increased sampling effort during periods of high flow, if an effective sampling protocol could be established. In addition, as this study only captured two years of variation, there would be much benefit to continued replication of the sampling effort in order to assess long-term variation in spatial patterns, as well as temporal stability of communities.

APPENDICES

Appendix 1 A list of all species sampled during summer sampling seasons (includes both 2016 and 2017). Bolded species were identified as "rare" (occurring at only one site at a level of less than 5 individuals) and were retained for analyses of richness but excluded from analyses of composition. Occurrence is the number of sampling events at which a species was captured, catch is the number of individuals captured across all sampling events.

Species	Species code	Common name	Occurrence	Catch
Acantharchus pomotis	Apomo	Mud Sunfish	6	26
Ameiurus brunneus	Abrun	Snail Bullhead	12	28
Ameiurus natalis	Anata	Yellow Bullhead	13	31
Ameiurus nebulosus	Anebu	Brown Bullhead	4	4
Amia calva	Acalv	Bowfin	12	29
Anguilla rostrata	Arost	American Eel	22	74
Aphredoderus sayanus	Asaya	Pirate Perch	37	695
Centrarchus macropterus	Cmacr	Flier	14	78
Chologaster cornuta	Ccorn	Swampfish	3	5
Cyprinella leedsi	Cleed	Bannerfin Shiner	5	205
Elassoma zonatum	Ezona	Banded Pygmy Sunfish	3	9
Enneacanthus gloriosus	Eglor	Bluespotted Sunfish	3	5
Erimyzon oblongus	Eoblo	Creek Chubsucker	12	55
Erimyzon sucetta	Esucc	Lake Chubsucker	3	3
Esox americanus	Eamer	Redfin Pickerel	34	661
Esox niger	Enige	Chain Pickerel	18	33
Etheostoma fricksium	Efric	Savannah Darter	6	18
Etheostoma fusiforme	Efusi	Swamp Darter	5	8
Etheostoma hopkinsi	**Ehopk**	**Christmas Darter**	**1**	**1**
Etheostoma inscriptum	Einsc	Turquoise Darter	4	13
Etheostoma olmstedi	Eolms	Tessellated Darter	21	96
Etheostoma serrifer	Eserr	Sawcheek Darter	3	8
Fundulus lineolatus	**Fline**	**Lined Topminnow**	**1**	**1**
Hybopsis rubrifrons	Hrubr	Rosyface Chub	6	31
Ictalurus punctatus	**Ipunc**	**Channel Catfish**	**1**	**2**
Labidesthes vanhyningi	Lvanh	Golden Silverside	17	37
Lepisosteus osseus	Losse	Longnose Gar	6	9
Lepomis gulosus	Lgulo	Warmouth	27	135
Lepomis marginatus	Lmarg	Dollar	31	210
Lepomis auritus	Lauri	Redbreast	35	905
Lepomis macrochirus	Lmacr	Bluegill	37	489
Lepomis microlophus	Lmicr	Redear Sunfish	6	8
Lepomis punctatus	Lpunc	Spotted Sunfish	35	331
Micropterus salmoides	Msalm	Largemouth Bass	28	84
Minytrema melanops	Mmela	Spotted Sucker	13	24
Nocomis leptocephalus	**Bhchb**	**Bluehead Chub**	**1**	**3**

Notropis chalybaeus	Nchal	Ironcolor Shiner	5	10
Notemigonus crysoleucas	Ncrys	Golden Shiner	10	25
Notropis cummingsae	Ncumm	Dusky Shiner	22	362
Notropis hudsonius	Nhuds	Spottail Shiner	2	4
Notropis lutipinnis	Nlutr	Yellowfin Shiner	8	72
Notropis maculatus	Nmacu	Taillight Shiner	2	3
Notropis petersoni	Npete	Coastal Shiner	23	130
Noturus gyrinus	Ngyri	Tadpole Madtom	9	18
Noturus leptacanthus	Nlept	Speckled Madtom	13	29
Opsopoeodus emiliae	Oemil	Pugnose Minnow	8	15
Percina nigrofasciata	Perni	Blackbanded Darter	25	231
Pomoxis nigromaculatus	Pomni	Black Crappie	2	2
Pteronotropis stonei	Pston	Lowland Shiner	3	9
Semotilus atromaculatus	**Satro**	**Creek Chub**	**1**	**4**
Umbra pygmaea	**Upygm**	**Eastern Mudminnow**	**1**	**1**

Appendix 2 Descriptions of variables tested for longitudinal variation.

Category		Description
Habitat area/volume	Species richness	The count of species captured
	Species diversity	Shannon diversity index
	Channel volume	Mean channel width multiplied by mean depth (m^2)
	Stream depth	The mean of all depths measurements at a given site (m)
	Stream width	The mean of all width measurements at a given site (m)
	Maximum body size	Maximum total length (TL; mm). Values from FishTraits database (Frimpong and Angermeier 2009). Values for *A. rostrata* from Jenkins and Burkhead (1994). Values for all species present at a site were averaged together to determine average maximum body size of the assemblage.
Habitat diversity	Microhabitat diversity	Three-digit codes were created for each transect at a sample site, determined by a transect's values of depth, coarse substrate, and large woody debris. The Shannon diversity index was calculated for each site using three-digit codes in place of species (Gorman and Karr 1978).
Environmental variation/stability	pH	The level of acidity of the water; measured at time of fish sampling
	pH (CV)	The coefficient of variation of pH measured at three distinct temporal snapshots (Spring, Summer, Fall)
	Dissolved oxygen	The amount of dissolved oxygen in the water; measured at time of fish sampling (mg/L)
	Dissolved oxygen (CV)	The coefficient of variation of dissolved oxygen measured at three distinct temporal snapshots (Spring, Summer, Fall)
	Temperature	The temperature of the water; measured at time of fish sampling (°C)
	Temperature (CV)	The coefficient of variation of water temperature measured at three distinct temporal snapshots (Spring, Summer, Fall)
	Fecundity	Values from FishTraits database (Frimpong and Angermeier 2009). Values for *A. rostrata* from Jenkins and Burkhead (1994). Fecundity values for all species present at a site were averaged together to determine average fecundity of the assemblage.
	Longevity	Values from FishTraits database (Frimpong and Angermeier 2009). Values for *A. rostrata* from Jenkins and Burkhead (1994). Longevity values for all species present at a site were averaged together to determine average longevity of the assemblage.
	Proportion later-maturing species	The proportion of species at a site reaching sexual maturity at or older than 1.5 years. Age-at-maturity values from FishTraits database (Frimpong and Angermeier 2009). Values for *A. rostrata* from Jenkins and Burkhead (1994).
	Degree of parental care	Values from FishTraits database (Frimpong and Angermeier 2009). Degree of parental care was coded as follows: Open-water-spawners = 1, Brood-hiders = 2, Substrate-choosers = 3, Nest-spawners = 4, Bearers = 5. Parental care values for all species present at a site were averaged together to determine the average degree of parental care of the assemblage.
	Spawning mode diversity	Shannon diversity index calculated using the five spawning-modes listed above in place of species.

	Proportion open-water-spawners	The proportion of species at a site classified as open-water-spawners. Species classifications from FishTraits database (Frimpong and Angermeier 2009).
	Proportion brood-hiders	The proportion of species at a site classified as brood-hiders. Species classifications from FishTraits database (Frimpong and Angermeier 2009).
	Proportion substrate-choosers	The proportion of species at a site classified as substrate-choosers. Species classifications from FishTraits database (Frimpong and Angermeier 2009).
	Proportion nest-spawners	The proportion of species at a site classified as nest-spawners. Species classifications from FishTraits database (Frimpong and Angermeier 2009).
	Proportion intolerant species	The proportion of species at a site classified as intolerant. Tolerance classifications were compiled from Meador and Carlisle (2007), North Carolina DENR (1995), Georgia DNR (2005), and Barbour et al. (1999).
	Proportion tolerant species	The proportion of species at a site classified as tolerant. Tolerance classifications were compiled from Meador and Carlisle (2007), North Carolina DENR (1995), Georgia DNR (2005), and Barbour et al. (1999).
River Continuum Concept	Large woody debris	The average density of LWD per 10-meter reach
	Trophic diversity	Shannon diversity index calculated using the four trophic strategies below in place of species.
	Proportion benthic insectivores	The proportion of species at a site classified as benthic insectivores. Species classifications determined using Paller (1994) as a guide.
	Proportion generalized insectivores	The proportion of species at a site classified as generalized insectivores. Species classifications determined using Paller (1994) as a guide.
	Proportion invertivore-piscivores	The proportion of species at a site classified as invertivore-piscivores. Species classifications determined using Paller (1994) as a guide.
	Proportion surface-water insectivores	The proportion of species at a site classified as surface-water insectivores. Species classifications determined using Paller (1994) as a guide.
Movement permeability	Gradient	The change in elevation over a 1 km reach, centered around the 150 m sample reach
Adventitious stream effect	Stream-size preference	Values from FishTraits database (Frimpong and Angermeier 2009). Preferences were coded as follows: Creek = 1, Small River = 2, Large River = 3. If a species was listed as having more than one preference, preference values were averaged together for that species. Stream-size preferences for all species present at a site were then averaged together to determine the average stream-size preference of the assemblage.

Appendix 3 Raw data for assemblage and habitat metrics assessed for longitudinal variation at 25 coastal plain sites. LWD = large woody debris, CV = coefficient of variation, DO = dissolved oxygen.

Site ID	Upstream drainage area (km^2)	Mean channel volume (m^2)	Mean depth (m)	Mean width (m)	Microhabitat diversity	Mean LWD count	Temp (°C)	CV Temp	pH	CV pH	DO (mg/L)	CV DO
A1D	1432.3	3.7	0.4	9.1	1.71	4.3	25.7	20.3	6.9	3.7	1.9	46.9
A1U	753.7	6.7	0.6	12.2	1.20	3.1	25.5	23.8	7.1	2.4	2.0	74.6
A2D	621.6	2.4	0.3	7.4	1.31	5.3	25.5	22.8	7.0	1.4	0.9	105.2
A2U	194.0	4.5	0.5	9.0	1.58	2.2	24.0	36.0	6.8	1.4	1.5	82.3
A3D	714.8	6.3	0.8	8.0	1.26	3.1	26.7	18.6	7.7	3.9	4.8	9.4
A3U	549.1	4.9	0.6	7.6	1.86	4.3	24.6	16.9	6.5	6.5	4.5	12.3
O1D	210.0	3.8	0.6	6.1	1.67	5.3	25.3	16.2	7.4	2.3	4.5	23.5
O1U	116.5	5.0	0.7	7.2	1.24	2.7	26.5	33.8	8.3	5.9	6.8	5.2
O2D	341.9	5.6	0.6	9.7	1.32	6.1	26.1	34.2	7.4	2.0	5.4	34.3
O2U	116.8	2.4	0.3	8.4	1.62	3.5	26.6	28.1	7.0	131.6	5.8	16.9
O3D	373.0	2.2	0.4	5.6	1.42	2.4	26.3	14.5	7.3	10.6	2.2	34.9
O4D	212.1	1.6	0.4	3.9	1.25	3.8	27.1	28.7	7.0	4.5	4.4	33.4
O4U	98.4	1.4	0.4	3.6	1.24	1.6	24.5	22.7	6.3	1.6	0.2	82.1
O5D	567.2	2.8	0.4	7.2	1.49	2.9	25.8	29.2	6.6	13.9	1.8	48.0
O5U	188.6	3.0	0.4	6.9	1.66	2.2	26.1	29.7	6.4	0.9	0.7	143.6
O6D	1261.3	5.3	0.6	8.4	1.77	5.5	26.0	9.6	6.8	5.0	2.3	35.3
O6U	525.8	1.2	0.3	4.2	1.51	3.7	23.6	12.2	6.3	0.9	2.0	97.2
O7D	269.4	2.0	0.3	6.4	1.93	4.7	25.4	15.0	7.1	3.9	3.2	39.1
O7U	88.3	9.9	1.3	7.4	0.72	2.6	24.7	17.6	6.5	6.9	1.0	127.6
O8D	494.7	5.3	0.7	7.4	1.43	3.7	26.4	13.8	7.1	4.5	4.1	7.4
O8U	134.4	3.7	0.6	6.2	1.78	4.0	24.2	15.9	6.6	2.3	3.3	13.8
S1D	336.7	2.3	0.4	5.7	1.53	2.3	25.5	14.6	7.2	4.7	3.5	25.4
S1U	219.9	1.8	0.4	5.1	1.64	4.8	26.6	17.2	7.1	2.1	2.3	35.5
S2D	362.6	6.9	0.7	9.3	1.81	5.8	26.2	28.5	7.1	20.3	0.8	68.2
S2U	51.8	2.5	0.6	4.0	1.75	4.3	26.1	29.8	6.5	9.2	0.9	61.7

Appendix 3 Continued.

Site ID	Gradient (m km^{-1})	Species richness	Species diversity	Mean max TL (mm)	Mean longevity	Mean fecundity	Prop. late-maturing	Prop. OpnSpwnr	Prop. Brdhdr	Prop. SubChooser	Prop. NstSpwnr	Mean degree of parental care
A1D	1	25	2.58	38.9	7.5	18858.5	0.7	0.3	0.2	0.0	0.5	2.7
A1U	1	22	2.41	38.6	7.5	27625.7	0.8	0.3	0.1	0.0	0.5	2.9
A2D	0	25	2.37	29.2	6.0	16734.3	0.6	0.3	0.2	0.0	0.5	3.0
A2U	0	13	1.91	43.9	8.6	31781.2	0.8	0.4	0.1	0.0	0.5	2.7
A3D	1	13	1.78	36.4	6.8	11253.4	0.8	0.3	0.2	0.0	0.6	2.9
A3U	2	21	2.30	29.0	5.6	23871.5	0.7	0.2	0.1	0.1	0.5	2.9
O1D	0	23	2.59	27.9	6.3	16953.5	0.6	0.3	0.2	0.0	0.5	2.7
O1U	0	20	2.48	34.1	6.6	18563.1	0.6	0.3	0.2	0.0	0.5	2.8
O2D	3	24	2.58	26.2	5.5	20175.3	0.6	0.3	0.3	0.0	0.4	2.6
O2U	0	22	2.51	29.3	6.8	17265.2	0.6	0.2	0.2	0.0	0.6	2.9
O3D	0	23	2.41	31.8	7.0	21158.1	0.6	0.3	0.1	0.0	0.5	3.0
O4D	0	14	1.49	26.0	5.5	14457.2	0.7	0.3	0.2	0.0	0.5	2.7
O4U	1	11	2.07	32.3	6.2	17999.7	0.7	0.2	0.1	0.0	0.6	3.4
O5D	0	17	1.96	35.1	7.9	23293.3	0.6	0.1	0.1	0.1	0.6	3.4
O5U	1	18	2.20	39.3	7.5	30504.1	0.7	0.2	0.1	0.1	0.6	3.2
O6D	1	22	2.36	31.3	6.1	14991.2	0.7	0.3	0.2	0.0	0.5	2.8
O6U	0	10	1.86	37.4	8.1	16488.2	0.9	0.1	0.1	0.1	0.7	3.3
O7D	2	12	1.55	37.3	7.8	23670.3	0.9	0.1	0.1	0.0	0.7	3.6
O7U	2	10	1.81	37.0	7.1	26673.7	0.7	0.4	0.1	0.0	0.4	2.7
O8D	0	15	1.50	27.2	6.1	13628.6	0.6	0.1	0.2	0.0	0.6	3.2
O8U	1	12	2.15	46.0	9.2	28785.1	0.9	0.3	0.1	0.0	0.6	3.0
S1D	0	20	1.71	36.5	7.2	19928.1	0.6	0.3	0.2	0.0	0.4	2.7
S1U	1	20	2.58	34.3	6.6	20789.2	0.7	0.3	0.2	0.0	0.5	2.6
S2D	1	14	2.47	44.4	8.0	23356.6	0.6	0.2	0.1	0.0	0.5	3.2
S2U	1	12	1.25	23.8	5.9	21229.8	0.7	0.3	0.2	0.0	0.5	3.0

Appendix 3 Continued

Site ID	Spawning mode diversity	Trophic diversity	Prop. SWInsct	Prop. GenInsct	Prop. BenInsct	Prop. InvPisc	Prop. tolerant	Prop. intolerant	Mean stream size pref.
A1D	1.15	1.38	0.2	0.2	0.3	0.3	0.1	0.2	1.9
A1U	1.28	1.37	0.2	0.3	0.2	0.3	0.1	0.0	1.8
A2D	1.20	1.34	0.2	0.3	0.3	0.2	0.1	0.1	1.8
A2U	0.90	0.90	0.0	0.5	0.1	0.4	0.2	0.0	2.0
A3D	0.96	1.22	0.1	0.4	0.4	0.2	0.1	0.1	1.9
A3U	1.18	1.34	0.1	0.3	0.3	0.2	0.1	0.1	1.8
O1D	1.17	1.33	0.3	0.3	0.3	0.1	0.0	0.3	1.8
O1U	1.19	1.36	0.3	0.4	0.2	0.2	0.1	0.3	1.8
O2D	1.22	1.35	0.3	0.2	0.3	0.2	0.0	0.2	1.8
O2U	0.98	1.37	0.2	0.3	0.2	0.2	0.0	0.1	1.8
O3D	1.10	1.33	0.2	0.4	0.2	0.2	0.1	0.1	1.9
O4D	1.03	1.33	0.2	0.4	0.3	0.1	0.1	0.1	1.9
O4U	1.03	1.12	0.1	0.5	0.1	0.3	0.1	0.0	1.8
O5D	1.28	1.01	0.1	0.6	0.1	0.3	0.1	0.0	1.8
O5U	1.12	1.16	0.1	0.5	0.2	0.3	0.2	0.0	1.9
O6D	1.17	1.38	0.2	0.3	0.3	0.2	0.1	0.1	1.9
O6U	1.00	1.09	0.0	0.4	0.3	0.3	0.2	0.0	1.9
O7D	0.89	1.24	0.1	0.4	0.2	0.3	0.2	0.0	1.9
O7U	1.19	1.17	0.1	0.5	0.1	0.3	0.1	0.1	1.9
O8D	1.12	1.31	0.2	0.4	0.3	0.1	0.1	0.1	1.9
O8U	0.86	0.92	0.0	0.5	0.1	0.4	0.1	0.1	1.9
S1D	1.21	1.38	0.2	0.3	0.3	0.3	0.2	0.2	1.8
S1U	1.05	1.31	0.1	0.3	0.4	0.3	0.1	0.1	1.8
S2D	1.23	1.17	0.1	0.5	0.1	0.3	0.1	0.0	1.9
S2U	1.20	1.12	0.1	0.6	0.2	0.2	0.1	0.0	1.8