An Examination of Stream Fishes at 3 Levels: Species-Area and Environmental Interactive Influences on Fish Species Richness, Stream Type Influences on Assemblage Membership, and Variation in Weight-Length Parameters by Stream Type & Fish Trait

by

Susan A.R. Colvin

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Approved by

Jack W. Feminella, Co-chair, Professor of Biological Sciences
Dennis R. DeVries, Co-chair, Professor of Fisheries, Aquaculture, and Aquatic Sciences
Brian S. Helms, Associate Research Professor of Biological Sciences
Russell A. Wright, Associate Professor of Fisheries, Aquaculture, and Aquatic Sciences

Abstract

Obtaining an understanding of the multi-scale influences on species richness is of concern not only to theoretical development but to building an understanding of potential mechanisms of rapid species loss. To address modern conservation and management concerns we must further our understanding of fundamental ecological patterns. One of the most basic ecological phenomenon is the increase in the number of species with an increase in area. Though an oft-studied phenomenon the mechanisms that underlie this basic ecological pattern are not well understood. Though contributions to our understanding of this phenomenon have been gleaned from research conducted in many systems the most well-known of the species-area theories, island biogeography, credits lotic systems with being an especially useful ecotype to study the underlying mechanisms of these phenomena.

Within streams many environmental variables influence fish assemblage membership across regions and within basins at the stream level. Current stream theoretical approaches explaining the hierarchical influences of fish assemblage membership within a basin lack the precision to predict differing assemblages at a relatively close spatial scale, although this is known to occur. To better develop our understanding of what dictates fish assemblage further research must address what the fundamental environmental differences are between streams in close proximity, even within a basin, with different assemblages. Indeed, because closely spaced streams within the same or nearby basins presumably share a similar regional species pool identifying differences in the fish assemblage membership and correlated environmental parameters would provide greater insight into the determinants of assemblage composition.

The 1st chapter of my dissertation discusses the levels of investigation I conducted in subsequent chapters to examine influences on stream fish richness and assemblage membership at multiple scales. In the 2nd chapter I used multiple linear regression and 3 model selection techniques (stepwise, all-subsets, and AIC_c) and 1 model shrinkage technique (Lasso) to determine which environmental parameters were most important predictors of native fish species richness as singular and interactive terms with stream flow. I found that most of the environmental predictors investigated influenced fish species richness independent of stream flow, while some environmental predictors were also correlated with native fish species richness as interactive terms with stream flow. These results indicated that environmental predictors act on fish species richness both independently of and in conjunction with an increase in flow. Similarly, results of similar analyses on average values of environmental predictors indicated that native fish species richness was related similarly to both measures for some environmental predictors whereas other predictors show associations unique to each measure. These results indicate that ascribing the species-area relationship to a simple linear increase in heterogeneity with an increase in area may be too simplistic a model and that similar to a patch dynamics perspective both the variety of and the variability in stream habitat are important drivers of fish species richness. In my 3rd chapter I investigated whether there were fish assemblage and environmental differences between blackwater and clearwater streams of coastal Alabama. My results suggest that streams of coastal Alabama exhibit 2 unique types, blackwater and clearwater, each with unique environmental parameters and fish assemblage compositions. In my 4th chapter, I calculated weight-length regression (WLR) parameters for 17 species (9 families) of Alabama coastal stream fishes, most of which were non-game species with previously unpublished parameters. I also investigated whether season, stream type, environmental variables, or species traits influenced WLR parameters. My results suggested WLR parameters were affected by season with higher WLR slope (*b*) in fall-collected fish than in spring and summer collections. My results also suggested that the slope of log length was lower in clearwater stream populations and that stream water pH and invertebrate density influenced fish weight. Species closely associated with clearwater stream assemblages and surface-water column feeders demonstrated a shift into a juvenile life stage at a smaller size. My results suggested that correlating WLR parameters with both abiotic and biotic factors and life history characteristics can provide further insight into contrasting patterns of season, stream type fish assemblage membership, environmental variables, and species traits.

Overall, my dissertation explores ecological questions using stream fish from the theory level in investigating potential mechanisms underlying species-area relationships, at the landscape level addressing whether and how stream fish assemblages varied by stream type, and at the population level using WLR data to investigate ecological questions with a variety of temporal, environmental, and trait predictors. My dissertation highlights the utility of using stream fishes across a range ecological investigations. My work also adds to our understanding of the species-area relationship by expressing how the contribution of different environmental predictors may need to better understood independently or in conjunction with an increase in stream flow (as a proxy for area). Additionally, it appears that the relative magnitude of some environmental predictors are important correlates of fish species richness whereas for other predictors variability might have a greater influence on fish species richness. This dissertation also provides a more definitive identification of separate stream types in coastal Alabama complete with unique fish assemblages which should better aid conservation and management objectives in this region. Finally, WLR approaches are underutilized for ecological applications and this dissertation serves as a means of broadening those investigations. My work demonstrates that more ecological information can be gleaned about the effects of seasonal variation, environmental conditions, and traits on fish populations using weight-length data.

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Chapter 1. Introduction to utilizing fishes for species richness pursuits and related investigations.

1.1 Introduction

Identification of biodiversity and its conservation in the face of current levels of rapid species loss from environmental change are essential goals in preserving quality of life for all societies (Angermeier and Winston 1999, Odum and Barrett 2005, Noble et al. 2007). To achieve these goals scientists must identify the biotic and abiotic components that collectively constitute unique ecosystems (Ehrlich and Wilson 1991, Angermeier and Winston 1999, Odum and Barrett 2005). Further understanding of more familiar and lesser-known ecosystems and their component species can contribute to conservation of biotic diversity by allowing for preventative rather than reactive approaches to preserving ecosystem health (Doak and Mills 1994, Angermeier and Winston 1999, Barrett 2001, Odum and Barrett 2005). Of particular concern to many is the rapid decline in North American native fish species over the last half century (Fausch et al. 2002). Relevant criticisms of stream ecological research address the lack of research efforts pursuing phenomenon over multiple scales, the understanding of which might better guide stream management and conservation efforts (Fausch et al. 2002).

One of the most fundamental and long-standing relationships in ecology and stream fish ecology is the species-area (S-A) relationship where the number of species increases with area (Gleason 1922, MacArthur and Wilson 1967, Oberdorff et al. 1995). The S-A relationship has provided insight for important ecological theories such as island-biogeograhpy (MacArthur and Wilson 1967) and is often considered close to a rule in ecology (Schoener 1976). Lotic systems can act as insular environments within the terrestrial environment and can serve as model systems for further research exploring the dynamics of the S-A relationships (MacArthur and

Wilson 1967). Stream fish richness and assemblage membership is influenced at multiple scales (Fausch et al. 2002). At the landscape level, water body type may have a strong influence over fish assemblage membership, though this is a lesser studied phenomenon especially in the southeast (Winemiller et al. 2008).

Indeed, much of the research that led to developing classification systems for streams has been based on high-gradient mountainous regions (Frissell et al. 1986, Seelbach et al. 2006, Melles et al. 2012). Additionally, much of the previous work aimed at determining what structures stream fish assemblages has primarily examined differences along a gradient of stream size or between and not within regions (Whiteside and McNatt 1972, Barila et al. 1981, Angermeier and Schlosser 1989). More work is needed to address determinants of fish assemblage variability across similarly-sized streams within a region.

Little work has been done to investigate faunal or environmental differences between stream types in the southeastern (SE) US. The low elevation coastal terrace streams of Alabama are understudied and recent efforts to create a state-wide IBI have suggested this region may require unique scoring criteria (O'Neil and Shepard 2007). A comparison of blackwater and clearwater stream fish assemblages in this region offers a model system to allow an investigation of which environmental and biological parameters may result in strong stream type associations in SE coastal plain streams. Classification systems such as Poff's (1997) hierarchical landscape filters does not allow for disparate stream types and associated assemblages within a small geographic area. Although disparate stream types and associated assemblages have indeed been found in other systems (Winemiller et al. 2008). Further study of what mechanisms elucidate these patterns would benefit our understanding of these unique systems and aid erudition of stream ecological theory.

At the population level techniques such as weight-length regressions have long been used as descriptors of populations in different water bodies but there has been less application of these techniques to ecological questions (Froese 2006). Weight-length relationships (WLRs) have been described as foundational elements in fisheries research (Anderson and Neumann 1996). In addition, WLR parameters have been used to study the condition of fish populations exposed to a suite of biotic and abiotic conditions (Stucky and Klaassen 1971, Wiener and Hanneman 1982, Bolger and Connolly 1989, Cone 1989, Morato et al. 2001, Cade et al. 2011, Moradinasab et al. 2012). Most often used to assess condition of fishes of sport or commercial interest recent investigations emphasize the potential value of WLRs in examining the effects of water body type, habitat type, and further exploring ecological implications of these data across seasons (Froese 2006, Ogle and Winfield 2009). Additionally, examining how WLR parameters vary with species' biological traits such as feeding strategy, longevity, and fecundity may help to identify potential life history trade-offs (in these traits and growth) in variable aquatic environments (Frimpong and Angermeier 2010).

The broad goals of my dissertation are to examine what variables influence fish species richness and assemblage composition at multiple scales from across ecoregions to between populations using a variety of parametric and non-parametric tools. The primary objectives of this dissertation are:

1) to assess whether variability in environmental parameters influences native fish species richness independently or interactively with stream flow

2) to determine whether fish assemblages differed between blackwater and clearwater streams and to identify also what environmental parameters differed between these two stream types and correlate these environmental parameters to potential differences in fish assemblages

3) to calculate weight-length regression parameters for stream fishes of blackwater and clearwater streams of coastal Alabama and determine if these parameters differed by environmental parameters or by species trait

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Chapter 2. Examining fish species richness response to both variability in stream heterogeneity parameters and variability and stream size interactions

2.1 Introduction

One of the most fundamental and long-standing relationships in ecology is the species-area (S-A) relationship where the number of species increases with area (Gleason 1922, MacArthur and Wilson 1967, Martin 1981, Blake and Karr 1987, Lomolino and Weiser 2001). The S-A relationship has provided insight for important ecological theories such as island-biogeograhpy (MacArthur and Wilson 1967) and is often considered close to a rule in ecology (Schoener 1976). Most researchers attribute contributions to this pattern either largely to immigration and emigration rates (MacArthur and Wilson 1967) or to the increase in habitat heterogeneity and therefore niche space with an increase in area (Williams 1964).

MacArthur and Wilson's (1967) theory of island biogeography predicts that species richness largely depends on island size, with larger islands being better able to support larger more stable populations. Even prior to the publication of their Island Biogeography theory, researchers posited that larger habitat patches contain a greater number of niches and thus more species (Williams 1964). Island biogeography theory did recognize that large islands also have more variable topography, soils, and other attributes that influence colonization and extinction rates. In fact, the authors stated that their 'ultimate' theory may not even mention area because area itself rarely has a direct effect on species; instead, a larger area would include more habitats (i.e., increased heterogeneity), which is responsible for increased species presence (MacArthur and Wilson 1967).

Though considered separate concepts, it is still widely held that area (island size) and habitat heterogeneity often are coupled (Williams 1964, Eadie 1986). That is, as sampling area increases so too does habitat heterogeneity and the area-heterogeneity relationship is largely responsible for the shape of the species-area (S-A) curve (MacArthur and Wilson 1967, Allan 1995, Cooper et al. 1998, Scheiner et al. 2000). Although some research has concluded that species richness can largely be determined by area alone (Simberloff 1976).

Previous S-A research has been conducted in a diverse array of system types including: oceanic islands, mountaintops, forests, woodlots, shelter belts (tree and shrub rows along fields), individual plants, stones in streams, streams and rivers, and other artificial and natural systems (Arrhenius 1921, Gleason 1922, MacArthur and Wilson 1967, Brown 1971, Simberloff 1976, Martin 1981, Livingstone et al. 1982, Riebesell 1982, Ambuel and Temple 1983, Eadie et al. 1986, Blake and Karr 1987, Angermeier and Schlosser 1989, Oberdorff et al. 1995). MacArthur and Wilson (1967) emphasized the potential for lotic systems as models to investigate these relationships, as these systems can act as insular systems within a terrestrial environment. Typically stream fish abundances and species richness can be accurately estimated and habitat parameters can be clearly defined especially in smaller lotic systems (Angermeier and Schlosser 1989). S-A research in these systems has provided additional insight into species richness and area relationships and identified additional predictor variables potentially responsible for these patterns (Oberdorff et al. 1995).

Across a range of stream sizes, Oberdorff et al. (1995) found that global fish species richness increased with both basin area and stream discharge. In addition, by including system net primary productivity into predictive models they could explain most of the variation in species richness. These findings suggest that system size (after accounting for energy differences, Currie 1991) is responsible for much of the patterns in species richness as predicted by island biogeography (Oberdorff et al. 1995). Other studies have found additional measures of stream size to be good predictors of fish species richness, such as stream length, surface area, and distance from source (Livingstone et al. 1982, Eadie et al. 1986, Oberdorff et al. 1993).

Many factors influence fish species richness and assemblage composition, with level of influence often related to spatial scale (Oberdorff et al. 1995, Jackson et al. 2000). At large scales, stream/river size has been found to be an important predictor of richness (Livingstone et al. 1982, Oberdorff et al. 1995). Examining patterns of fish species richness across stream sizes will allow us to better understand the influence of both stream size and heterogeneity variables. To explore these patterns, we addressed the following questions:

1. What factors provide the predominant influences on stream fish species richness?
2. Do stream size and heterogeneity variables influence stream fish richness independently or do heterogeneity variables and stream size interact to influence stream fish richness?

2.2 Methods

2.2.1 Dataset

We used the Mid-Atlantic Integrated Assessment (MAIA) Environmental Monitoring and Assessment Program (EMAP) dataset in this study (Stoddard et al. 2006). The MAIA data were collected in summer (1997 – 1998) in the Mid-Atlantic Highlands as well as in coastal and piedmont ecoregions. The MAIA dataset included 1st – 7th order (Strahler 1952) streams. We selected parameters that represented native fish species richness, benthic invertebrate species total richness and density, stream physical habitat, water chemistry, discharge, periphyton/chlorophyll levels, watershed land use/cover, and additional geographic factors, thus allowing for incorporation of a wide range of putative factors affecting fish richness (Table 1). Sample reaches consisted of a stream length 40x the wetted width of the stream with a minimum reach length of 150 m (Stoddard et al. 2006).

2.2.2 Statistical Methods

Some sites in MAIA were sampled more then once; in these cases, data from repeat sampling trips were deleted prior to analysis. Variables with more than 10% of values missing from variables of interest were also not included as were samples with zero flow. Several measures of invertebrate species richness and density and periphyton were reported for both pools and riffles per each site visit, thus each site visit was represented by 2 rows of data whereas fish species richness data and other parameters were represented by a single row of data per site visit. When both the riffle and pool habitats were sampled at a single site we averaged the data between habitats for both periphyton and invertebrate density and richness. When multiple samples were present for the same site we chose the sample with earliest complete record for all variables. Variables that were averaged between pool and riffle samples included: total macroinvertebrate density (number/m^2), total macroinvertebrate distinct taxa richness, periphyton ash free dry mass/m^2 (AFDM), chlorophyll a (mg) per m^2 of stream bed, and ratio of chlorophyll a (mg) to periphyton AFDM (Stoddard et al. 2006)).

To reduce the number of variables prior to model building we used random forest regression trees to assess variable importance in predicting native species richness (Breiman 2001, Cutler et al. 2007). Random forests are a collection of classification trees for continuous variables (Breiman 2001, Crawley 2007). Each tree is generated from a bootstrapped data set independent of other trees (Breiman 2001). Bootstrapped samples are a selection from the dataset, with each data point used about 64% of the time in a given sample; the rest of the samples are considered out-of-bag (OOB) or unused in that particular bootstrap (Liaw and Wiener 2002, Cutler et al. 2007). A tree is fit to an independent bootstrap sample and then used to predict OOB samples (Cutler et al. 2007). A large number of trees is generated (in our case

2000) which combine predictions from all trees (Breiman 2001, Cutler et al. 2007). The importance of predictor variables is assessed by calculating the increase in mean square error (MSE) on the OOB portion of the data and after permuting (randomly shuffling) each predictor variable (Liaw and Wiener 2002). The difference between the MSE on the OOB data and after permutation of predictor variables is then averaged over all trees and normalized by standard deviation of the differences and multiplied by 100 to put in terms of a percent; the higher the value the more important the variable (Liaw and Wiener 2002). The second measure of importance of predictor variables, node purities, is measured by the Gini index, which measures impurity of the outputs after a split. It is based on the total decrease in node impurities from splitting on the predictor variable averaged over all trees and measured by residual sums of squares (Liaw and Wiener 2002). Random forests were conducted with R package randomForest (Liaw and Wiener 2002).

After variable selection to quantify the degree of heterogeneity across stream sizes, we sorted streams by discharge and calculated both the average native species richness and discharge across every 3 streams. We selected 3 streams for grouping to both preserve sample size while avoiding pooling samples that varied widely in size. We then calculated coefficients of variation (CVs) for the predictor variables of interest as identified by random forest across the corresponding three streams. Calculating coefficients of variation allowed us to use these measures as a proxy for heterogeneity in predictor variables within similar stream sizes. Coefficients of variation are dimensionless and provide an expression of the relationship between mean and dispersion (Brown 1998). Using measures of variance in predictor variables over time or space (as in our study) is expected to yield new understanding in stream process (Palmer et al. 1997).

To select the predictor variables most strongly associated with native species richness, environmental predictors, and independently and potentially related to an increase or decrease in log Q (used as a proxy for stream area) we used 4 distinct techniques: forward stepwise, all-subsets, AICc model selection methods and the lasso shrinkage method. We selected a stepwise procedure in part because it is one of the oldest algorithms for selecting predictor variables and despite criticism it continues to be used in research efforts and has found continued support for its value in a recent comparison of regression techniques (Whittingham et al. 2006, Bernot et al. 2008, Murtaugh 2009, Mundry and Nunn 2009). In stepwise procedures, regression models are compared with and without a specific predictor. Comparisons continue with sequential addition and potential deletion of further predictor variables and AIC values are used to assess the best model (Venables and Ripley 2002, Murtaugh 2009). We performed forward stepwise selection procedures in package Mass in R (Venables and Ripley 2002).

We also performed all-subsets regression or exhaustive variable selection which compares all possible groupings of predictor variables and subsets of these possible sets are selected based on supplied criterion (Murtaugh 2009). Many ecologists have favored all-subsets regression over stepwise procedures but both have been recently criticized for the potential likelihood of overfitting (Quinn and Keough 2002, Dahlgren 2010). We performed all-subsets regression with package Leaps in R which performs an exhaustive search for the best predictors of the dependent variable using an efficient branch-and-bound algorithm (Venables and Ripley 2002). The algorithm returns a best model of each size; therefore, results do not depend upon a penalty for model size (i.e. AIC, Venables and Ripley 2002). The branch-and-bound algorithm is a structured search of all possible solutions by partitioning the space of all feasible solutions into smaller and smaller subsets; a lower bound is calculated for the cost of solution within each of these subsets and subsets whose cost exceeds that of another known solution are discarded and excluded from further consideration (Lawler and Wood 1966). This continues until the solution with the least cost is found (Lawler and Wood 1966).

We also preformed model selection with Akaike's Information Criterion corrected for small sample bias (AIC_c) in package MuMIn in R (Akaike 1973, Burnham and Anderson 2002, Bartoń 2016). AIC is a commonly used method of comparing multiple models as it is considered to employ a good balance of parsimony and accuracy (Wagenmakers and Farrell 2004, Arnold 2010). AIC_c penalizes the variance of a model for the number of predictor variables (Quinn and Keough 2002). Models with low AICs are considered to have the best fit and of the models with similarly low AICs, the one with the fewest parameters should be selected (Quinn and Keough 2002).

Finally, we performed lasso, a regression shrinkage method that uses penalized estimation to avoid overestimation when working with few degrees of freedom (Witten and Tibshirani 2009). Lasso is a potentially more reliable tool than the best or all-subsets method for variable selection, particularly when the analysis includes a large number of parameters and a small sample size (Dahlgren 2010). The lasso algorithm maximizes model fit with an 'L1 penalization', this is essentially placing a cap on the sum of the absolute values of all coefficients in the model, thus with this technique, coefficients can be shrunk to near zero (Dahlgren 2010, Nicolè et al. 2011). We conducted lasso in package GLMnet in R (Friedman et al. 2010). Optimal shrinkage was calculated by cross validation, which is used to determine whether a data set has good predictive ability (Friedman et al. 2010, Nicolè et al. 2011).

Collinearity among predictor variables can result in large variances for some regression slopes in a model; this can result in these variables being excluded from the final model regardless of their strength of relationship to the response variable (Quinn and Keough 2002). To examine these potential hazards, we assessed collinearity in our models prior to variable selection by calculating the variance inflation factor (vif) in package car in R (Quinn and Keough 2002, Fox and Weisberg 2011). Post variable selection multiple linear regressions (MLRs) were performed on selected model parameters, allowing assessment of the relationship between response and predictor variables while adjusting for all other predictor variables (Quinn and Keough 2002). All data were centered prior to analyses to convert predictor variables to a similar scale (Quinn and Keough 2002).

To better interpret results of models based on coefficients of variation for each predictor variable we performed an additional MLR based on averages. We calculated the average of each predictor variable across the same 3 streams as previously grouped by discharge ranking. We included variables as selected by all-subsets regression. Data were gain centered prior to analyses to convert predictor variables to a similar scale (Quinn and Keough 2002).

The MAIA dataset additionally included two predictors that are indicative of instream channel complexity: SDWXD (standard deviation of width*depth product [m^2]), and SDDEPTH (standard deviation of thalweg depth [cm]). These variables were standard deviations calculated across all depth * widths and across all depths taken at each transect along the stream reach (Kaufmann and Robison 1998). We examined how these measures of heterogeneity of channel form influence native species richness with MLRs. For these investigations we included the singular terms: log Q, SDWXD, SDDEPTH, and the interaction terms log Q* SDWXD and log Q * SDDEPTH, where the singular terms in the model indicate when SDWXD and SDDEPTH are important predictors of native spp. richness after taking into account log Q. Significance of the interaction terms indicates when these terms influence native spp. richness along a gradient of stream flow. These data were centered prior to analyses (Quinn and Keough 2002). All analyses were conducted in R version 3.2.1.

2.3 Results

2.3.1 Random Forest Variable Selection

We selected heterogeneity predictors from the top 20 variables ranked by random forest with a % increase in MSE of > 5% (Table 1). We built a full model with the response variable native species richness, predictors that included average log Q and the CVs of DOC (dissolved organic carbon (mg/L)), XCDENMID (mean mid-channel canopy density (%)), XCDENBK (mean bank canopy density (%)), XFC_NAT (fish cover - natural types (sum areal proportion), COND (conductivity (uS), and CHL_M2 (chlorophyll a (mg) per m^2 of stream bed). We also added interaction terms to our model which consisted of each singular predictor x log Q. Significance of singular terms indicates a relationship between the CVs of these terms (i.e. not log Q) and native species richness after taking log Q into account. Significance of interaction terms indicates that CVs of these predictor variables increase or decrease in conjunction with stream flow and that this relationship is correlated with native species richness.

VIFs for all terms in the model were ≤ 6 for all 13 terms in the full model, suggesting that terms fell within bounds for strong collinearity (Quinn and Keough 2002). Eight of the 12 predictor variables had VIFs <2. Forward stepwise procedures selected the full model without removing any terms as the final model with the lowest AIC score. After variable selection, MLR results indicated this selection technique included 3 non-significant terms (Table 2). All-subsets procedures dropped 1 term from the full model: log Q * XFC_NAT. MLR results on these model parameters selected by all-subsets included 2 close-to but non significant terms (Table 2). AIC_c model selection dropped 2 singular (XCDENBK and XCDENMID) and 4 interaction terms (log Q * COND, log Q * DOC, log Q * XCDENBK, log Q * XCDENMID) from the top model although each parameter was included in at least several high ranking models as determined by Δ AICc <2 from the top model (Table 3). MLR results on the top ranking AICc model indicated that COND and log Q * COND were marginally significant predictors (Table 2). Lasso regression shrinkage procedures shrunk to zero the singular terms XCDENMID and XCDENBK as well as all the interaction terms, indicating little effect of those terms on native species richness. Results of the lasso MLR indicated that the reduction in the number of predictor variables did result in a loss of potential predictive power indicated by a slightly lesser value of adjusted R^2 in comparison to fuller models (Table 2). Non-significant intercepts (close to 0) were present in each of the MLR models as expected as these data were centered prior to analyses (Quinn and Keough 2002).

2.3.2 MLR Results

MLR results on average values of predictor variables selected from the all-subsets model on coefficients of variation values of predictors indicated that the singular terms DOC ($\beta = -0.28$, S.E. = 0.14, $P = 0.06$), XCDENMID ($\beta = -1.07$, S.E. = 0.35, $P = 0.004$), and XCDENBK ($\beta = 0.57$, S.E. = 0.19, $P = 0.004$) were related and COND ($\beta = -0.20$, S.E. = 0.11, $P = 0.08$) was marginally related to native species richness (Table 4). Whereas the singular terms log Q ($\beta = -0.03$, S.E. = 0.23, $P = 0.88$), XFC_NAT ($\beta = -0.09$, S.E. = 0.11, $P = 0.46$), and CHL_M2 ($\beta = 0.12$, S.E. = 0.14, $P = 0.38$) were not related to native species richness. The interactive terms log Q * XCDENMID ($\beta = 0.72$, S.E. = 0.21, $P = 0.001$) and log Q * XCDENBK ($\beta = -0.34$, S.E. = 0.17, $P = 0.06$) were related to native fish species richness and log Q * DOC ($\beta = -0.18$, S.E. = 0.14, $P = 0.07$) was marginally related. Whereas the interactive terms log Q * COND ($\beta = 0.08$, S.E. = 0.20, *P* = 0.69) and log Q * CHL_M2 (β = 0.02, S.E. = 0.14, *P* = 0.9) were not related to native fish species richness.

Within stream MLR analyses indicated that SDWXD and SDDEPTH were highly collinear as indicated by VIFs > 10 (Quinn and Keough 2002). Therefore, we split these investigations into 2 MLRs which both included native species richness as the response variable. The first included the terms: log Q, SDWXD, and log Q * SDWXD. Log Q in this model was not significantly related to native species richness (β = -0.007, S.E. = 0.09, *P* = 0.94). Both SDWXD (β = 1.24, S.E. = 0.2, *P* = <0.0001) and the interaction term log Q * SDWXD (β = -0.67, S.E. = 0.1, *P* = <0.0001) were highly correlated with native species richness. The model was significant (*P* = <0.0001) and had an adjusted R-squared of 0.29. Although VIFs close to 10 for the interaction term and 11 for the singular term SDWXD indicate relatively high collinearity in this model even after centering the data (Quinn and Keough 2002).

The second within-stream model included the terms: log Q, SDDEPTH, and log Q * SDDEPTH. Log Q in this model was significantly related to native species richness (β = -0.15, S.E. = 0.08, *P* = 0.05). Both SDDEPTH (β = 0.36, S.E. = 0.0.08, *P* = <0.0001) and the interaction term log Q * SDDEPTH (β = -0.33, S.E. = 0.07, *P* = <0.0001) were highly correlated with native species richness. The model was significant (*P* = <0.0001) and had an adjusted R-squared of 0.24. VIF’s were less than 2 for each term in this model indicating low levels of collinearity (Quinn and Keough 2002).

2.4 Discussion

Results across models indicated that the variability of predictor variables and their relationship to native species richness was stronger independent of the flow variable versus as an interactive term with flow. Our results are perhaps counter to some research which concluded that species richness was largely determined by area alone (Simberloff 1976). However, results of the MLRs as selected by both stepwise and all-subsets procedures indicate that the relationship between variability of predictor variables and the interaction with flow are indeed important predictors most especially with dissolved organic carbon, mid-channel and bank canopy, and chlorophyll, which suggests the influence of these parameters is not wholly independent of streams size. These results indicate that the relationship between log Q and variability in environmental parameters does influence native species richness.

The interactive relationship between chlorophyll and discharge appears to be potentially the most important of the interaction terms as this one was also selected in the top model using AIC_c. These results tend to support conclusions by Oberdorff et al. (1995) that stream area and energy are important predictors of species richness. However, the results of our study also suggest that the interaction between discharge and the variability in energy is also an important predictor of species richness.

These results tend to both support but also broaden the assertion by MacArthur and Wilson (1967) that a larger area would include more habitats (i.e., increased heterogeneity), which is responsible for increased species presence. Our results suggest that variability in habitat parameters can both be positively and negatively related to native species richness. Our results also support (in part) their assertion relative to the significance of interaction terms, in that the variability of these terms and the interaction with discharge is related to species richness.

Within-stream analyses of stream channel complexity indicated that variability in channel width and depth is more important than an increase in log Q when predicting native fish species richness. Not surprisingly however, the interaction of this term with log Q was highly significant indicating that variability in this term is related to a gradient of stream size. Stream depth and flow were also both independently and interactively related to native species richness, indicating complexity in the relationship between these terms that may not be measured by reporting each independently.

Results of the MLR regression using predictor averages instead of coefficients of variation indicate that the relationship between log Q and the relative magnitude of a predictor variable may not be the same as the relationship between log Q and the variability in a predictor variable. In the analyses using averages, log Q was no longer a significant variable indicating that when log Q is taken into account, the value of other predictor variables become more important predictor of native fish species richness. Whereas in the analysis examining the relationship between native species richness, log Q, and variability in predictor variables log Q remained an important predictor. The difference in the strength of these relationships may be informative in that the overall adjusted r^2 was slightly higher in the model including coefficients of variation rather than averages. Also interesting was according to our results native fish species richness was related to the variability in chlorophyll and the interaction of chlorophyll variability and log Q but not to the average values of chlorophyll. Similarly, native species richness appears to be more related to the variability in native fish cover and less so the average values. Likewise, the relationship between the variability in conductivity and the interaction with log Q was more strongly related to native fish species richness than the same interaction represented by average values. These results support a patch dynamics perspective in that both the variety of and the variability in stream habitat are important driver of fish species richness (Townsend 1989, Hildrew and Giller 1994, Allan 2004).

Recent disagreements in the literature about appropriate application of regression selection methods gives researchers pause about the best tools to apply to their questions of interests (Murtaugh 2009, Dahlgren 2010). Our application of 4 currently used methods allowed us to draw interpretations across methods. Although some methods were less restrictive in allowing predictors to be included in the final model (stepwise) while some were more restrictive (lasso), across methods it is apparent that the variability in environmental predictors was important independent of stream flow. The selection of the interaction terms for some of these variables with log Q also indicates that variability in environmental predictors in relation to an increase or decrease in the magnitude of stream flow can also be an important predictor of stream fish richness. Understanding which environmental predictors respond interactively and which independently would enhance our understanding of the species richness and area relationship. These results indicate that ascribing the species-area relationship to a simple linear increase in heterogeneity with an increase in area may be too simplistic a model. Many environmental variables are also likely related independently to native fish species richness. Finally, the relationship between these predictors and native species richness appears variable when measured by coefficients of variation or average values this may indicate that fishes may be constrained by the levels or some variables (i.e. food) whereas response to the variability in other parameters may be more similar to that expressed by the intermediate disturbance hypothesis (Connell 1978).

2.5 References

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TABLE 1. MAIA variables included in random forest analysis. Predictor variables selected for inclusion in across stream assessment of the influence of varibility in heterogeneity and the interaction with area are denoted with a *. Predictor variables selected for inclusion in within stream assessments of the affect of variability in stream channel complexity and stream area regression analyses indicated with a **. Watershed area, although indicated as an important predictor of native species richness in the random forest analysis was not important in any other forms of preliminary analyses with this dataset and FLOW_CFS was selected as a measure of area. Prop. is an abbreviation for proportion.

MAIA dataset abbreviation	Variable definition	% MSE	Node purity	Included in further analyses?
AREAWSHA	watershed area (hectares)	27.5798374	848.122189	
XWIDTH	mean wetted width	24.9003522	685.870096	
SDWXD	std. dev. of width*depth product (m^2)	22.7084384	680.245644	**
XDEPTH	thalweg mean depth (cm)	20.3369559	526.425202	
KM_SEA	straight-line distance to ocean (km)	12.5129416	117.347719	
LON_DD	longitude at mid-reach	12.2099679	147.615114	
DOC	dissolved organic carbon (mg/L)	12.0264864	146.100295	*
SDDEPTH	std. dev. of thalweg depth (cm)	11.7433664	233.164111	**
XCDENMID	mean mid-channel canopy density (%)	10.6262663	119.729169	*
ELEV	elevation at mid-reach	8.7546956	89.8864782	
FLOW_CFS	instantaneous discharge (ft^3/sec)	8.5052873	153.603131	*,**
XFC_NAT	fish cover-natural types (sum areal prop.)	7.9602958	81.0976461	*
COND	conductivity (uS)	7.0173218	140.793623	*
XCDENBK	mean bank canopy density (%)	6.2113804	53.9523288	*
AG_TOT	% watershed agricultural land	5.9856656	63.7561221	
XFC_ALL	fish cover-all types (sum areal prop.)	5.3119414	76.2108702	
CHL_M2	chlorophyll a (mg) per m^2 of stream bed	5.2604028	85.8572702	*
LAT_DD	latitude at mid-reach	4.7741067	70.9570046	
PHSTVL	closed headspace pH	4.1831318	78.1734219	
URB_TOT	% watershed urban	3.8288843	56.9972568	
SO4	sulfate (ueq/L)	3.7573794	76.7862948	
PCT_SAFN	substrate sand & fines <2 mm (%)	3.6302383	33.8487635	
XBK_A	bank angle-mean (degrees)	3.6257719	65.7940736	
XFC_BIG	fish cover-LWD,RCK,UCBorHUM(sum areal prop.)	3.3669063	56.7843157	
XFC_RCK	fish covr-boulders (areal prop.)	3.0328255	29.9983158	
XFC_BRS	fish covr-brush&small debris (areal prop.)	2.921007	68.0682882	
SINU	channel sinuosity (m/m)	2.8130229	67.4573676	
MG	magnesium (ueq/L)	2.7958403	74.505763	
FOR_TOT	% watershed as forest (NLCD-41,42,43)	2.6493443	42.1284777	
TOTLRICH	total distinct taxa richness	2.5126221	68.943694	
XFC_AQM	fish covr-aq. macrophytes (areal prop.)	2.5050411	54.5442557	
XC	riparian veg. canopy cover	2.4525692	47.2830373	
PCT_BIGR	substrate >= coarse gravel (>16 mm) (%)	2.4015429	20.7676027	
CHL_MASS	ratio of chlorophyll a (mg): periphyton AFDM	2.3764877	57.4499249	
PCT_SFGF	substrate <= fine gravel (<=16 mm) (%)	2.3690792	21.3091978	
PCT_SA	substrate sand - .06-2 mm (%)	2.179556	37.8932723	
XBKF_H	bank angle - mean (degrees)	2.0890377	53.3595557	
SLOPMEAN	mean watershed slope (%)	1.9951933	39.8457442	
PCT_RC	substrate concrete (%)	1.9428332	3.0139099	
PCT_SLOW	slow water habitat (% glide & pool)	1.9028433	49.8880133	
PCT_FN	substrate Fines - silt/clay/muck (%)	1.7657371	31.8268254	
LSUB_DMM	Log10 est. substrate geom. mean diam. (mm)	1.7280001	59.5048051	
CL	chloride (ueq/L)	1.5767324	49.7168888	
PTL	total phosphorous (ug/L)	1.5709109	57.5091905	
PCT_DRS	dry channel or subsurface flow (%)	1.5040957	0.4281329	
PCT_FA	falls (% of reach)	1.4773851	1.9588946	
NTL	total nitrogen (ug/L)	1.4290438	39.8816384	
PCT_POOL	pools - all types (% of reach)	1.2911829	29.698651	
XFC_UCB	fish cover - undercut banks (areal prop.)	1.2154182	25.5948165	
ANC	gran ANC (ueq/L)	0.9519071	69.3917953	
XEMBED	mean embeddedness - channel+margin (%)	0.8568182	25.3934638	
XFC_HUM	fish cover - artif. structs. (areal prop.)	0.5682662	15.1854529	
AFDM_M2	periphyton ash free dry mass (biomass)/m^2	0.3751558	48.3071775	
PCT_BDRK	substrate bedrock (%)	0.3666104	47.7274135	
PCT_FAST	fast water habitat (% riffle & faster)	0.2027625	42.2731541	
NO3	nitrate (ueq/L)	0.1649041	41.6643311	
PRECIP_M	annual precipitation (m)	0.1129581	29.4569976	
XFC_OHV	fish cover - overhang veg. (areal prop.)	-0.1913682	37.1881691	
CA	calcium (ueq/L)	-0.6675554	58.1270303	
PCT_ORG	substrate wood or detritus (%)	-0.9028218	6.8931251	
TOTLDENS	macroinvertebrate density (number/m^2)	-1.6523774	35.7873032	
XFC_LWD	fish cover - large woody debris (areal prop.)	-2.5189245	45.3990804	
PCT_HP	substrate hardpan (%)	-2.8396962	3.5878522	

TABLE 2. Multiple linear regression results of models selected by forward stepwise, all-subsets and AICc selection and lasso regression shrinkage methods. Predictor variables were centered prior to analysis.

Method and variable	β	SE	t	P	adj. r^2
Forward stepwise				<0.0001	0.56
Intercept	0.13	0.11	1.23	0.22	
log Q	0.27	0.13	2.02	**0.05**	
DOC	-0.36	0.10	-3.46	**0.001**	
XCDENMID	0.61	0.21	2.96	**0.005**	
XFC_NAT	-0.19	0.09	-2.02	**0.05**	
COND	-0.16	0.09	-1.78	0.08	
XCDENBK	-0.32	0.16	-2.06	**0.05**	
CHL_M2	-0.27	0.10	-2.72	**0.009**	
log Q * DOC	-0.20	0.10	-2.11	**0.04**	
log Q * XCDENMID	-0.51	0.16	-3.15	**0.003**	
log Q * XFC_NAT	-0.02	0.13	-0.17	0.87	
log Q * COND	-0.18	0.12	-1.53	0.13	
log Q * XCDENBK	0.42	0.15	2.77	**0.008**	
log Q * CHL_M2	-0.26	0.10	-2.63	**0.01**	
All-subsets				<0.0001	0.57
Intercept	0.14	0.11	1.28	0.2	
log Q	0.26	0.13	2.09	**0.04**	
DOC	-0.36	0.10	-3.51	**0.001**	
XCDENMID	0.62	0.20	3.05	**0.004**	
XFC_NAT	-0.19	0.09	-2.07	**0.04**	
COND	-0.16	0.09	-1.79	0.08	
XCDENBK	-0.33	0.15	-2.18	**0.03**	
CHL_M2	-0.27	0.10	-2.77	**0.008**	
log Q * DOC	-0.20	0.09	-2.19	**0.03**	
log Q * XCDENMID	-0.51	0.15	-3.30	**0.002**	
log Q * COND	-0.17	0.10	-1.63	0.1	
log Q * XCDENBK	0.42	0.15	2.83	**0.007**	
log Q * CHL_M2	-0.26	0.09	-2.74	**0.009**	
AIC$_c$				<0.0001	0.49
Intercept	-0.03	0.09	-0.37	0.7	
log Q	0.46	0.10	4.69	**<0.0001**	
DOC	-0.34	0.09	-3.57	**0.0007**	
XFC_NAT	-0.26	0.09	-2.77	**0.008**	
COND	-0.16	0.09	-1.77	0.08	
CHL_M2	-0.29	0.09	-3.09	**0.003**	
log Q * CHL_M2	-0.19	0.10	-1.95	0.06	
Lasso				<0.0001	0.47
Intercept	0.00	0.09	0.00	1	
log Q	0.42	0.10	4.27	**<0.0001**	
DOC	-0.36	0.10	-3.68	**0.0005**	
XFC_NAT	-0.27	0.09	-2.87	**0.006**	
COND	-0.17	0.09	-1.83	0.07	
CHL_M2	-0.26	0.10	-2.72	**0.009**	

TABLE 3. Summary of multiple regression models and predictor coefficient estimates for explaining native species richness within Δ < 2 of (and including) the top model based on AIC$_c$ scores. Variables abbreviated according to TABLE 1.

Rank	Intercept	CHL_M2	COND	DOC	log Q	XCDENBK	XCDENMID	XFC_NAT	log Q * CHL_M2	log Q * COND	log Q * DOC	log Q * XCDENBK	log Q * XCDENMID	df	logLik	AICc	ΔAICc	weight
1	-0.03	-0.29	-0.16	-0.34	0.46			-0.26	-0.19					8	-64.206	147.1	0	0.088
2	-0.07	-0.29	-0.16	-0.40	0.48			-0.24	-0.21		-0.13			9	-63.104	147.6	0.53	0.068
3	0.12	-0.26	-0.15	-0.34	0.33	-0.31	0.57	-0.17	-0.25		-0.19	0.39	-0.49	13	-57.103	147.6	0.56	0.067
4	0.14	-0.27	-0.16	-0.36	0.26	-0.33	0.62	-0.19	-0.26	-0.17	-0.20	0.42	-0.51	14	-55.477	147.7	0.63	0.064
5	-0.04	-0.32		-0.35	0.44			-0.25	-0.20					7	-65.917	147.9	0.79	0.059
6	0.12	-0.30		-0.36	0.29	-0.29	0.61	-0.18	-0.27		-0.19	0.40	-0.48	12	-58.841	147.9	0.84	0.058
7	-0.04	-0.35		-0.37	0.36		0.16	-0.26	-0.22					8	-64.69	148	0.97	0.054
8	-0.04	-0.32	-0.14	-0.36	0.39		0.13	-0.26	-0.21					9	-63.506	148.4	1.33	0.045
9	-0.03	-0.30	-0.17	-0.35	0.42			-0.27	-0.19	-0.12				9	-63.524	148.4	1.37	0.045
10	-0.07	-0.31		-0.41	0.47			-0.24	-0.22		-0.13			8	-64.897	148.5	1.38	0.044
11	0.00	-0.26	-0.17	-0.36	0.42			-0.27						7	-66.275	148.6	1.51	0.041
12	0.15	-0.23	-0.16	-0.31	0.32	-0.38	0.65		-0.26		-0.21	0.42	-0.56	12	-59.248	148.7	1.66	0.038
13	0.03	-0.28	-0.15	-0.35	0.41		0.18	-0.23	-0.19				-0.14	10	-62.278	148.8	1.71	0.038
14	0.02	-0.31		-0.37	0.38		0.21	-0.23	-0.21				-0.13	9	-63.697	148.8	1.71	0.037
15	-0.06	-0.29	-0.17	-0.41	0.45			-0.25	-0.21	-0.12	-0.13			10	-62.364	149	1.88	0.034

TABLE 4. Multiple linear regression results of average values of predictor variables selected by the all-subsets model on coefficients of variation values of the same predictors. Predictor variables were centered prior to analysis.

Variable	β	SE	t	P	adj. r^2
All-subsets				<0.0001	0.49
Intercept	0.41	0.14	2.91	**0.005**	
log Q	-0.03	0.23	-0.15	0.88	
DOC	-0.28	0.14	-1.96	**0.06**	
XCDENMID	-1.07	0.35	-3.02	**0.004**	
XFC_NAT	-0.09	0.11	-0.75	0.46	
COND	-0.20	0.11	-1.80	**0.08**	
XCDENBK	0.57	0.19	3.05	**0.004**	
CHL_M2	0.12	0.14	0.88	0.38	
log Q * DOC	-0.18	0.10	-1.87	**0.07**	
log Q * XCDENMID	0.72	0.21	3.37	**0.001**	
log Q * COND	0.08	0.20	0.40	0.69	
log Q * XCDENBK	-0.34	0.17	-1.95	**0.06**	
log Q * CHL_M2	0.02	0.14	0.13	0.9	

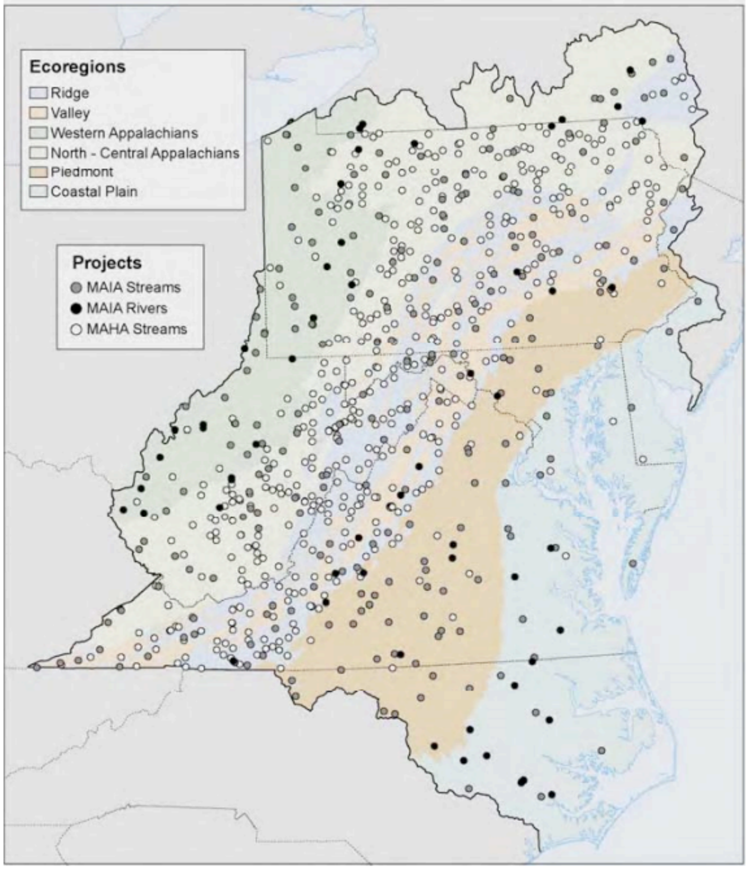

Figure 1. The Mid-Atlantic Integrated Assessment (MAIA) and Mid-Atlantic Highlands Assessment (MAHA) streams (Stoddard et al. 2006). Background colors indicate ecoregions sampled.

Chapter 3. Environmental and fish assemblage differences in blackwater and clearwater streams of coastal Alabama

3.1 Introduction

Biodiversity conservation in the face of current levels of species loss from environmental change is an essential goal in preserving quality of life for all societies (Angermeier and Winston 1999, Odum and Barrett 2005, Noble et al. 2007). To achieve these goals scientists must identify the biotic and abiotic components that collectively constitute unique ecosystems (Ehrlich and Wilson 1991, Angermeier and Winston 1999, Odum and Barrett 2005). Further understanding of both familiar and lesser-known ecosystems can contribute to conservation of biotic diversity by allowing preventative rather than reactive approaches to preserving ecosystem health (Doak and Mills 1994, Angermeier and Winston 1999, Barrett 2001, Odum and Barrett 2005).

To develop an understanding of community composition it is necessary to identify all co-occurring species, although more typically communities are defined based on fewer but well-studied species with high economic or societal importance (Angermeier and Winston 1999). Dominant vegetation historically has been used to classify terrestrial ecosystems (sensu Whittaker 1966), but may not be as appropriate or useful in characterizing aquatic ecosystems with the possible exception of some coastal and estuarine zones (Ackleson and Klemas 1987, Angermeier and Winston 1999). In freshwater systems fishes often are used for classifying communities and they are reliable indicators for assessing community integrity (Karr 1981, Moyle 1995, Noble et al. 2007).

Stream fish assemblages reflect a combination of historical, biogeographical, local environmental and biological processes (Winemiller 2010). However, identifying which factors are responsible for structuring assemblages remains a challenge for fish ecologists (Gido and Jackson 2010). For example, all fish species that occur in a region can presumably occupy a given stream, although actual residence depends on an ability to tolerate environmental conditions and biotic constraints (Tonn et al. 1990, Quist et al. 2005) such as predation and to some extent competition, especially for closely-related species such as within centrarchid assemblages (Werner 1984, Werner and Hall 1988, Jackson et al. 2001).

One of the more predominant and influential models for illustrating how fish assemblages are structured is the concept of a hierarchical series of selective pressures or filters from broad scale climatic and geological influences to fine scale biotic processes (Smith and Powell 1971, Jackson and Harvey 1993, Tonn 1990, Poff 1997, Jackson et al 2001). Termed 'landscape filters' (Poff 1997), this concept tends to minimize the amount of variability in abiotic conditions predicted to occur at streams located together within smaller hierarchical levels (i.e., within region, within basin). Although different authors have incorporated or stressed the importance of various filters, many studies have found relationships between fish assemblage membership and environmental parameters thus providing support for the nonrandom structure of fish assemblages (Jackson et al. 2001).

Much of the theory driving stream classification has been based on high-gradient mountainous regions (Frissell et al. 1986, Seelbach et al. 2006, Melles et al. 2012; but see Ward and Stanford 1983, Resh et al. 1988, Junk et al. 1989). Additionally, much of the previous work aimed at determining what structures stream fish assemblages primarily has examined differences along a gradient of stream size or between (and not within) regions (Whiteside and McNatt 1972, Barila et al. 1981, Angermeier and Schlosser

1989). More work is needed to address determinants of fish assemblage variability across similarly-sized streams within a region.

Lesser-studied regions such as the SE US coastal plain, present unique opportunities to explore differences in environmental variables and assemblages across stream types. Southeastern US coastal plain streams stretch from Louisiana and far eastern Texas to southeastern Virginia (Smock and Gilinsky 1992, Patrick 1996). They receive substantial allochthonous inputs from seasonally-inundated flood streams and are highly variable in pH, acidity, size, extent and connectivity to floodplain, and diversity of habitat types (Smock and Gilinsky 1992, Patrick 1996). These low-gradient systems may have greater variability in ecological parameters such as temperature and dissolved oxygen than their high-gradient counterparts (Benke and Wallace 1990). Typically, drainage is slower than in mountain and piedmont systems and extensive floodplains can occur even alongside headwater streams (Smock and Gilinksy 1992). In addition, these 1st-3rd order coastal streams can experience greatly reduced or even no flow in summer and are inhabited by biota that differ greatly from systems with more permanent flow (Resh et al. 1988).

Blackwater and clearwater stream types have a worldwide distribution from the tundra to the tropics, at times occurring within the same basin (Winemiller et al. 2008, O'Donnell et al. 2010). Previous studies in blackwater/clearwater systems have demonstrated faunal differences between them (Winemiller et al. 2008, Gonçalves and Braga 2012). Indeed, different stream types with corresponding differences in aquatic fauna were recognized as early as the mid-19th century in South America (Wallace 1853). Most assemblage comparisons between stream types have taken place in South American streams where many species have lesser known life histories, making environmental and biotic comparisons between stream types more difficult than might be in systems with more well-studied species (Winemiller et al. 2008, Gonçalves and Braga 2012). Difficulties making faunal or physical comparisons between blackwater and clearwater streams also arise due to differences in basin position; clearwater streams typically occur at higher elevations or further upstream within a basin, whereas blackwater streams typically occur at lower elevations and lower positions in a basin (Winemiller et al. 2008, Gonçalves and Braga 2012).

Little work has been done to investigate faunal or environmental differences between blackwater and clearwater stream types in the southeastern (SE) US. Coastal Alabama streams feature both blackwater and clearwater types, co-occurring within basins and both occupying either low or middle orders within a stream network with little difference in elevation between upper and lower portions of the basin (Meyer 1990, Winemiller et al. 2008, O'Donnell et al. 2010). The low elevation southernmost coastal plain streams of Alabama are understudied and recent efforts to create a state-wide index of biotic integrity (IBI) have suggested this region may require unique scoring criteria, potentially because the fauna of this region is lesser known (O'Neil and Shepard 2007). A comparison across stream types in this region may reveal strong stream type associations in SE coastal plain streams, thus indicating a potential need for inclusion of a stream type parameter within the IBI.

Blackwater streams are the most common stream type of the SE US Atlantic and Gulf Coastal Plain (Smock and Gilinsky 1992). In general, blackwater streams are characterized by tea-colored water, low pH, and high dissolved organic carbon (DOC, Benke et al. 1985, Meyer 1990). Typically, these streams drain sandy soils that cannot retain DOC, which quickly enters the stream channel and provides a dark color (Meyer 1990). Southeastern US blackwater streams also typically show high seasonal variation in environmental parameters, in part due to seasonal fluctuations in flow (Smock and Gilinsky 1992). Woody debris in blackwater streams provides stable structure in shifting sand substrates and food and habitat resources supporting benthic invertebrate and fish assemblages (Benke et al. 1985, Smock et al. 1985, Smock and Roeding 1986, Hauer and Benke 1987, Meyer 1992).

In contrast, clearwater streams typically are highly transparent, contain less tannins, and are slightly less acidic than blackwater streams (Winemiller et al. 2008, Duncan and Fernandes 2010). In the SE US, clearwater streams have garnered less research attention than blackwater streams, perhaps due to the greater prevalence of blackwater systems (Smock and Gilinsky 1992). In coastal Alabama, blackwater and clearwater streams occur in close proximity, with some heterogeneous basins including both stream types. Because of their proximity, these stream types presumably share access to the same regional species pool (Poff 1997). Yet, differences in environmental and biotic habitat parameters among stream types should result in differences in assemblages (Tonn and Magnuson 1982, Quist and Hubert 2005).

Coastal streams in Alabama provide an opportunity to examine fish assemblage differences between stream types and to identify factors influencing assemblage composition. We compare environmental and biotic parameters between blackwater and clearwater streams. First we quantify whether there are environmental and non-fish biotic differences between stream types. Second we compare fish assemblage differences between stream types. Finally, we quantify relationships between any environmental differences between stream types and representative fish assemblages.

3.2 Methods

3.2.1 Study Area

We studied several $1^{st} – 4^{th}$ order streams in the Fish River, Bon Secour River, Magnolia River, Wolf Bay, and nearby smaller basins of Baldwin County, Alabama (Table 1). Sites were selected across basins in wadeable stream reaches (Figure 1). This region experiences a subtropical climate and is predominately < 25 m above sea level. These low-gradient streams drain southern pine hill and coastal lowland physiographic regions emptying into the Gulf Coast (Boshung and Mayden 2004). These streams are influenced by a high-flow period that begins with the onset of winter rains in November/December, and a summer/fall low flow period that occurs because of low rainfall and high evapotranspiration, which often leads to low levels of dissolved oxygen (DO) (Metzler and Smock 1990, Meyer 1992, Smock and Gilinsky 1992). Lower-order streams can be more rainfall dependent and thus more variable in environmental parameters such as DO and temperature than higher-order streams, although the latter also can fluctuate dramatically in higher order streams (Whiteside and McNatt 1972).

The soils of the area are largely sandy entisols with wedges of inceptisols that lack organic material or clays and are typical of subhumid regions (Soils Survey Staff, NRCS, 2014). Study streams were characterized by shifting sand and woody substrates. Habitat included pools, predominately scour pools and undercut banks, separated by glides with some riffle sequences. Wetted stream widths at summer baseflow ranged from ~ 1.5 to 5.5 m and depths from 0.1 to 0.4 m.

Most stream reaches were heavily shaded by a mixture of longleaf pine *Pinus palustris*, swamp tupelo *Nyssa biflora*, Atlantic white cedar *Chamaecyperis thyoides*, pond and bald cypress *Taxodium ascendens* and *distichum*, sweetgum *Liquidambar styraciflua*, sweet bay *Magnolia virginiana*, tulip poplar *Liriodendron tulipifera*, and water oak *Quercus nigra* in the overstory. Understory trees included red maple *Acer rubrum*, black titi *Cliftonia monophylla*, redbay *Persea palustris*, and the invasive Chinese tallow *Triadica sebifera*. Wax myrtle *Morella cerifera*, hollies *Ilex* spp., groundsel *Baccharus halimifolia*, buttonbush *Cephalanthus occidentalis*, swamp titi *Cyrilla racemiflora*, river cane *Arundinaria gigantea*, blueberry *Vaccinium corymbosum* and invasive Chinese privet *Ligustrum sinense* were the predominant shrubs in the riparian area (S. Phipps, Weeks Bay Foundation, personal communication).

Land use in the area was largely row crop agriculture. Particularly peanuts, cotton, corn, legumes, and other vegetables along with pasture land (USDA 2015). Impervious surface levels vary by basin but were 5% on average, though levels in some more urban basins indicated a potential for adverse effects on stream hydrology and biotic response (Schueler et al. 2009).

3.2.2 Environmental Data

Blackwater and clearwater streams were initially selected for comparison based on water color and habitat criteria. Identified clearwater streams were highly transparent, while blackwater streams were typically stained with tannins (Winemiller et al. 2008, Duncan and Fernandes 2010). Initial observations indicated many of the smaller blackwater streams were slow, sluggish streams while smaller clearwater streams had swift currents in comparison. Blackwater streams also typically had more woody and detritus substrates, while clearwater streams typically had sandy bottoms with pockets of woody debris.

Data used to discriminate between stream type included streamwater physicochemistry and habitat parameters. Instream physicochemical parameters monitored included: temperature (°C), salinity (ppt), DO (mg/L), pH, total dissolved solids (g/L). These parameters were monitored at each stream visit with a YSI 556 MPS (YSI Inc., Yellow Springs, OH, USA).

Also measured were amount of coarse woody debris (CWD) in the active channel, wetted width, and 5 cross-sectional depths. We quantified all dead, live, and buried (within upper 10 cm of substrate) CWD >2.5 cm in diameter within a half meter upstream or downstream of the transect (Maloney et al. 2005). Transects were typically spaced every 10 m along the study reach using a modified transect method, with the first transect placed at the start of the reach and every 10 m thereafter (Wallace and Benke 1984). CWD data were converted to planar area by multiplying the diameter by length of CWD then dividing by the total area sampled within each transect (m^2 of CWD per m^2 of stream bed) to estimate woody debris per m^2 along entire reach length (Maloney et al 2005). Percent substrate was also estimated for each transect and averages were calculated to represent an entire reach. Substrates were classified as bedrock, gravel, sand, or silt based on a modified Wentworth scale (Wentworth 1922).

Water grab samples were collected in June 2013 for 13 sampling sites to provide data on anions (NO_3^- mg/L) and dissolved organic C (DOC mg/L); DOC data were used as a parameter distinguishing between blackwater and clearwater stream types (Benke et al. 1985, Meyer 1990). Samples were collected in pre-washed polypropylene bottles, rinsed with stream water prior to collection at mid-depth in the water column to ensure a representative sample, stored at 4 C prior to analysis, and analyzed within 2 d of collection at the Edward A. Hauss Elemental Analysis Laboratory, School of Forestry and Wildlife Sciences, Auburn University. Anions were analyzed using a Dionex ICS – 1500 Ion Chromatography System (Dionex, Sunnyvale, CA). DOC analysis was done using a Shimadzu TOC-V series total organic C analyzer (Tokyo, Japan). Additional grab samples for DOC and NO_3- were collected spring 2010 through spring 2011 at pre-2013 sampling sites to be used to obtain average values across the study period (B. Schneid, Auburn University, unpublished data). DOC values included in analyses were an average DOC value calculated from 11-12 visits taken from October 2009 – June 2010 and an additional sample taken in June 2013. DOC for 4 streams sampled once only in 2013 represented that single season.

Four sites were added to the 2013 field season to increase sample size. These sites are represented solely by on-site measures taken during 2013 sampling events (mentioned above) including water chemistry, discharge, substrate, CWD, and land use variables. Sites sampled prior to (and including) 2013 also included habitat data such as stream width, bankfull width and height (annual high water estimate), and incision height (height to first terrace from water surface) which were measured using standard EMAP physical survey techniques (Kaufmann and Robison 1998). These measures were taken at established transects spaced 1/10 of the sample reach length, equidistant along the sample reach; reaches were 40 x the average channel width but not less than 150 m (e.g., see Kaufmann and Robison 1998). These reaches overlapped with reaches used for fish sampling.

Benthic invertebrates were collected during fall 2008 and spring 2009 with a Surber sampler (250 µm). Three composite samples were taken per stream reach in glides/runs when available, and 3 Surber samples were collected per sample. Identifications were taken to genus level for most organisms (except oligochaetes). Invertebrate data were then used in analyses as a representation of benthic food availability in these streams (B. Schneid, Auburn University, unpublished data).

3.2.3 Hydrologic variables

To investigate potential relationships between hydrological variables and fish assemblage structure by stream type, we calculated several hydrological metrics incorporating short and longer-term data. Stream discharge (Q) was quantified at each sampling using the velocity-area method (Gore 1996) with a Marsh-McBirney Model 2000 Flo-Mate (Marsh-McBirney, Inc., Frederick, MD). In addition, we recorded water stage (height above fixed datum relative to stream bed) and water temperature with Solinst pressure transducers (Levelogger Gold, model 3001, 15-min intervals) from February 2009 to March 2010. Transducers were housed in a perforated PVC pipe and installed near the downstream end of each stream reach. Discharge (Q) was estimated at a range of stage levels during the study using the mid-section method (Raghunath 2007). Rating curves were developed to convert stage to Q for each site using observed Q values and estimated values for high flow with Manning's equation (Leonard et al. 2000, Thomas & Nisbet 2007, Helms et al. 2009). A base flow index (BFI) was calculated as the ratio of baseflow to total flow; baseflow was determined using a 3-pass recursive digital filter, a forwards, backwards, and forwards process of filtering out peak flows and estimating base flows (Nathan & McMahon 1990). Calculating base flow allowed us to estimate storm event frequency; which was characterized as the frequency with which discharge exceeded 5x the median discharge (Q_fre5, Helms et al. 2009). We also included minimum discharge values recorded for each stream as a measure of site-specific hydrologic variation over the study.

3.2.4 Land Use/land Cover

We used land use/land cover (LULC) data to determine how land use parameters may differ between stream types. For this investigation we included the following parameters: % agriculture (Ag), % forest, and % impervious surface, each calculated for each stream watershed. Data from 2009 aerial photographs of Baldwin County with a resolution of 0.15 m were used to delineate LULC. Additionally, elevation data were obtained from the 1/3 Arc Second (10 m) National Dataset, USGS (http://viewer.nationalmap.gov/viewer/). A vector file with infrastructure and hazard areas was obtained through Alabama Comprehensive GIS Inventory of Coastal Resources (http://www.gsa.state.al.us/gsa/coastal/gis_rs2.html) developed by the Geological Survey of Alabama and the Emergency Management Agency, and 2005 LULC designations made by Baldwin County were used as reference data for roads and streams. ArcGIS was used to delineate LULC categories and ArcHydro was used to define subwatersheds draining each study stream. Additionally LULC parameters for 4 sites sampled in June 2013 were taken from Morrison (2010), which were generated from a Landsat Thematic Mapper (TM) image (March 2008 flyover) from USGS Earth Resource Observation and Science (EROS) and geo-referenced to Digital Ortho Quarter Quads (DOQQ) corresponding to a Geodetic Reference System (GRS) spheroid, a North American Datum of 1983 (NAD 83) datum, and a Universal Transverse Mercator (UTM) projection with a Root Mean Square Error (RMSE) of < 0.5 pixels. LULC parameters for the 4 additional sites sampled in June 2013 were not included in environmental analyses.

3.2.5 Fish Sampling

Fishes were sampled in fall, spring, and summer beginning in fall 2008 through summer 2010 with an additional summer sample collected in 2013. Study reaches for fishes (usually 30x wetted width but not less than 60 m, Rahel and Hubert 1991) were block-netted at the upstream and downstream terminus of the sampling reach, and sampled with a backpack electrofisher (Smith-Root LR-24) with 3 passes per reach. Reaches at sites were mostly consistent from season to season and shifted up or downstream only slightly between visits when necessary. All available habitats were sampled. Fish were anesthetized in MS-222, field-preserved in 10% formalin, and then returned to the laboratory. After fixation for 2 weeks, fish were transferred to 70% ethanol. Fish were identified using keys in Boschung and Mayden (2004) and standard length (mm) and total mass (g) were measured. After processing, fish were deposited into the Auburn University Museum of Natural History fish collection.

3.2.6 Statistical Analyses

To determine whether blackwater and clearwater streams were distinguishable based on environmental parameters, we used principal components analysis (PCA) to identify linear combination of variables summarizing the data (Quinn and Keough 2002, Borcard et al. 2011). PCA scores allow for reduction of collinear variables and also identify subsets of scores representing the larger environmental dataset with fewer orthogonal composite variables (McCune and Grace 2002, Kwak and Peterson 2007, Borcard et al. 2011). Variables were scaled and centered prior to analysis which sets variances equal to 1 and centers averages on 0; this overcomes difficulties inherent in combining data measured in different units and having different variances (Crawley 2007, Borcard et al. 2011).

We then used PCA scores from the first three axes, as the eigenvalues from the first component explain the most variance and subsequent components are listed in a descending order of variance explanation, for discriminant function analysis (DFA) (McCune and Grace 2002, Borcard et al. 2011). Group assignment was assessed based on PCA scores to determine if environmental data effectively distinguish predetermined blackwater and clearwater streams (McCune and Grace 2002). We assessed classification accuracy of stream types using percent of correct classification (Poff and Allan 1995), and Pearson's chi-square test with Yates' correction to test if DFA-predicted stream type differed from assigned classifications. Pearson X^2 is commonly used to compare observed and expected frequencies; Yates' continuity correction was developed to improve accuracy of X^2 for 2 x 2 tables with small frequencies (Quinn and Keough 2002).

We used nonmetric multidimensional scaling (NMDS) to describe fish assemblage patterns by predetermined stream type. NMDS is an optimal method for displaying structure in data due to species differences by ordinating sites in species space based on a calculated dissimilarity matrix (McCune and Grace 2002). NMDS is especially applicable to ecological data because it effectively accommodates non-normal data, missing data, and does not require assumptions of linearity (McCune and Grace 2002). Fish abundance data were square root-transformed with a Wisconsin double standardization where species are standardized by species maxima and later relativized by site totals, which reduces influence of rare and common species (Bray & Curtis 1957, McCune and Grace 2002, Oksanen et al. 2013).

To test for differences in fish assemblages between blackwater and clearwater streams we used nonparametric multivariate analysis of variance (NPMANOVA, Anderson 2001). To examine overall differences, we pooled the 2 fall samples (October 2008 and October 2009), the 2 spring samples (April 2009 and Spring 2010), and the 3 summer samples (July 2009, August 2010, and June 2013) and used stream type and season as fixed and blocking variables, respectively (McCune and Grace 2008, Brewer 2008. Type differences were examined within each individual sampling season with the NPMANOVA (Anderson 2001). Significance of each NPMANOVA was assessed with 1000 permutations (Anderson 2001).

We also conducted an indicator species analysis (ISA, Hill et al. 1975) based on NMDS scores to determine which fish species best characterized blackwater and clearwater stream assemblages (Dufrêne and Legendre 1997, McCune and Grace 2002). ISA describes the degree to which each species diverges among previously defined groups (McCune and Grace 2002, Borcard et al. 2011), and thus aids in describing assemblage types (McCune and Grace 2002). Individual species can be used to indicate different habitat types or changes in the environment or examine the null hypothesis of no difference in species between stream types (McCune and Grace 2002, De Cáceres et al. 2010).

Last, to quantify how fish assemblage structure was related to measured environmental variables we used canonical correspondence analysis (CCA, ter Braak 1986, 1994, 1995; Palmer 1993, Legendre and Legendre 2012) a multivariate direct gradient analysis method that ordinates sample units and relates them to environmental variation (McCune and Grace 2002, Winemiller et al. 2008). CCA cannot assess assemblage structure unrelated to measured environmental variables (McCune and Grace 2002); however, in our case we used it to assess how fish assemblage structure was related to measured environmental and habitat variables and, thus could differentiate blackwater and clearwater stream types based on PCA and DFA.

The initial CCA contained environmental and fish data from 50 of 74 site visits sampled from 2008 to 2013, with inclusive environmental variables being the same 15 variables included in the initial PCA. Twenty-four site visits were excluded from the analysis due to missing data. Significance of environmental vectors in the initial CCA model were assessed using permutation of environmental variables where the shuffled data values are as extreme as the test statistic for the actual observed data, the P value is the tail probability of the null distribution of the test statistic from the random sample of possible permutations (Oksanen et al. 2013). The final (reduced) CCA model included only those variables significant at $P = 0.05$. In turn, fish species included in both the initial and final CCA were those associated with blackwater or clearwater streams as determined by ISA.

All statistical analyses were conducted in R 3.1.0 GUI (R Development Core Team, 2014). NMDS, NPMANOVA, and CCA were conducted with package vegan (Oksanen et al. 2013). ISA was conducted using the package indicspecies (De Cáceres and Legendre 2009), and PCA and DFA were done with the package MASS (Venables and Ripley 2002). Significance was accepted at an alpha level of ≤ 0.05.

3.3 Results

3.3.1 Stream Type Classification

The PCA included a total of 15 environmental and biological variables: benthic invertebrate density (m^2), maximum depth, Q at time of site visit, minimum Q, Q_fre5, NO_3^-, average DOC, incision depth, % Ag, % Forest, large woody debris, % sandy substrate, TDS, DO (mg/L), and pH (Table 2). Impervious surface was removed from the analysis due to its high correlation with % Forest ($r = 0.57$, $P < 0.0001$). The PCA was based on data from 5 of 8 clearwater and 5 of 7 blackwater sites visited 4-7 times for a total of 50 site visits out of 74 total visits from 2008 to 2013. Sites that were not included in the analysis were excluded due to missing parameters.

The 1st principal component (PC-1) of the PCA explained 32.4% of the variance in the environmental variables, which was interpreted as an overall clearwater - blackwater stream axis as indicated by higher negative loadings (> 0.300) from % sandy substrate, depth, and DO (mg/L) and higher positive loadings from benthic invertebrates (Figure 2). In turn, PC-2 (16.1% of variation), was interpreted as an axis primarily separating larger and smaller clearwater streams, as indicated by high negative loadings from Q_fre5, Q, TDS, and pH all of which were more commonly associated with larger clearwater streams, and high positive loadings from NO_3^- and % Ag, higher values of which were commonly associated with smaller clearwater streams (Figure 2). Higher values of % Ag on PC-2 also were shared with most blackwater streams (Figure 2). PC-3 (13.9% of variation), was interpreted as an axis for primarily larger, incised blackwater stream axis because of high loadings from DOC, incision depth, and % Forest.

DFA correctly classified clearwater and blackwater stream types based on PC 1-3 scores with 96% accuracy. Pearson's X^2 with Yates' correction indicated predictions differed from random ($X^2 = 38.99$, df = 1, $P < 0.0001$). All 24 streams predicted to be clearwater were 100% correctly classified, whereas 24 of the 26 (92.3%) streams predicted to be blackwater streams were correctly classified.

3.3.2 Fish Assemblages

We collected 31 species (5126 individuals) from 14 families over the study (Table 3). The number of individuals collected at a study site during one sampling event ranged from 38 to 281 in clearwater streams and 2 to 177 in blackwater streams, whereas species richness ranged from 7 to 17 in clearwater streams and 1 to 14 in blackwater streams. Centrarchidae and Cyprinidae were the most common families (30 and 25% of total catch, respectively). Centrarchidae was the most common family in clearwater streams (35%), with as many as 8 centrarchid species collected across streams of this type. In contrast, Centrarchidae contributed only 22% of blackwater stream catch (7 of 8 centrarchid species represented in this stream type) and, except for Redspotted Sunfish *Lepomis miniatus*, (15.5% of blackwater catch), most species were captured only rarely and never more than 7 individuals of any other centrarchid species were captured in a stream reach across 37 stream site visits. Cyprinidae was the most common family in blackwater streams (40%), which consisted mostly of Flagfin Shiner *Pteronotropis signipinnis* (35% of total blackwater catch) and Sailfin Shiner *Pteronotropis hypselopterus* (4% of total blackwater catch). *Notropis* spp. were not captured in blackwater streams. Cyprinidae contributed only 18% of the catch in clearwater streams (Table 3).

The families Atherinopsidae and Fundulidae were collected only in clearwater streams. Atherinopsidae was represented by one rarely captured species, Brook Silversides *Labidesthes sicculus* (0.3%). Fundulidae represented by the Blackspotted Topminnow *Fundulus olivaceus* also was rare (0.18%). In blackwater streams, the family Eleotridae was represented by 2 species, the Fat Sleeper *Dormitator maculatus* (0.5%) and the Largescale Spinycheek Sleeper *Eleotris amblyopsis* (0.5%) although they were captured in only 1 blackwater stream (Table 3).

Overall, Flagfin Shiner, Bluegill *Lepomis macrochirus*, Eastern Mosquitofish *Gambusia holbrooki*, Redspotted Sunfish *Lepomis miniatus*, and Blackbanded Darter *Percina nigrofasciata* were numerically the most abundant species. Overall Flagfin Shiner was the most frequently collected species (16.8% of total abundance), but was found mostly in blackwater (vs. clearwater) streams (35 vs. 7%, respectively). Bluegill was the 2nd-most collected species (13.8%), although it was more common in clearwater than blackwater streams (19 vs. 3%, respectively). Eastern Mosquitofish was the 3rd-most frequently collected species overall (10.6%), but was more common in clearwater than blackwater streams (13 vs. 5%, respectively, Table 3).

3.3.3 Assemblages by Stream Type

Fish assemblages showed distinct separation by stream type (Figure 3). NMDS ordination produced a 2-dimensional solution with a final stress of 22.5% after 3 iterations (Figure 3). Separation of fish assemblages was consistent over fall, spring, and summer seasons as indicated by results of the NPMANOVA, which showed significant differences between blackwater and clearwater stream fish assemblages ($F = 14.184$, $P < 0.001$) when all sampling events were included. Each pooled sampling season also showed significant differences between blackwater and clearwater stream fish assemblages (fall *F* = 4.17; spring *F* = 4.36; summer *F* = 6.70; all *P* < 0.001). And 4 of 7 sampling dates also showed significant differences between blackwater and clearwater stream fish assemblages. Assemblages were significantly different between stream types in fall 2009 (*F* = 2.45, *P* = 0.02), spring 2010 (*F* = 2.80, *P* = 0.007), summer 2010 (*F* = 2.32, *P* = 0.02), and summer 2013 (*F* = 3.24, *P* = 0.001).

ISA identified 39% of the 31 species captured as indicator species (Table 3). Two were significantly associated with blackwater streams, Redfin Pickerel *Esox americanus americanus* (*P* = 0.005) and Lake Chubsucker *Erimyzon succetta* (*P* = 0.025). In contrast, 10 species were associated with clearwater streams, including Bluegill (*P* = 0.005), Eastern Mosquitofish (*P* = 0.005), Blackbanded Darter (*P* = 0.005), Speckled Madtom *Noturus leptacanthus* (*P* = 0.005), Southern Brook Lamprey *Icthyomyzon gagei* (*P* = 0.005), Green Sunfish *Lepomis cyanellus* (*P* = 0.005), Largemouth Bass *Micropterus salmoides* (*P* = 0.005), Weed Shiner *Notropis texanus* (*P* = 0.005), and the rarely captured Longear Sunfish *Lepomis megalotis* (*P* = 0.04) and Gulf Darter *Etheostoma swaini* (*P* = 0.045) (Table 3).

CCA axes 1 and 2 accounted for 31.1 and 28.9%, respectively, of the species-environment variance in the data set. The final CCA consisted of 12 indicator species and 10 biological and environmental variables determined significant at *P* < 0.05 based on the initial CCA using the full data set (Figure 4). CCA environmental axis 1 (CCA-1) described an environmental/habitat gradient from clearwater streams, indicated by high negative loadings (> 0.3) of % sandy substrate, Q, maximum depth, and DO, to blackwater features, as indicated by high loadings of benthic invertebrate density, average DOC, and % of the basin as Forest (Table 4). Species with strong negative loadings on CCA-1 were the clearwater indicator species Green Sunfish, Weed Shiner, Gulf Darter, and Blackbanded Darter. In contrast, species with strong positive loadings on CCA-1 were the blackwater indicator species Lake Chubsucker and Redfin Pickerel (Figure 4).

CCA environmental axis 2 (CCA-2) described a gradient from smaller, more incised, clearwater streams, as indicated by high negative loadings of incision depth, NO_3-N, % Forest, and DO, to larger clearwater streams, as indicated by high positive loadings of Q and Q_fre5. Clearwater indicator species with high negative loadings on CCA-2 were captured in greater numbers in small clearwater streams (i.e., Southern Brook Lamprey and Eastern Mosquitofish, Table 4). Clearwater indicator species with intermediate loadings on CCA-2 were either captured more ubiquitously across stream sizes (i.e., Speckled Madtom), commonly captured in mid-sized streams (i.e., Largemouth Bass), or both (Bluegill; Table 4). Clearwater indicator species with slightly higher loadings on CCA-2 had high captures in the mid- to large-sized streams (i.e., Green Sunfish; Table 4). Clearwater indicator species with the highest loadings on CCA-2 were captured almost exclusively in large clearwater streams (i.e., Weed Shiner, Gulf Darter, Longear Sunfish; Table 4).

3.4 Discussion

3.4.1 Habitat Contrasts Between Blackwater and Clearwater Sites

Our results indicated that stream type can, in part, be explained by environmental differences as indicated by the accuracy of DFA discrimination between types based on PCA loadings of environmental data. Clearwater streams tended to be deeper, have sandier substrates, and higher levels of DO (mg/L) than blackwater streams. In contrast, blackwater streams showed higher DOC, higher invertebrate densities, and more forested basins as indicated by the PCA. The relationship between percent forested land and blackwater streams does not necessarily indicate that other land use types will result in clearwater streams as percent agricultural land also showed an association with blackwater streams. Incorporating more detail about forest composition type in future analyses may lead to a more definitive understanding of the relationship between the presence of forested land and stream type. For example, pond cypress is found along blackwater streams with lower pH and lower nutrient availability; in contrast the bald cypress is found along streams with high silt deposits and nutrient availability and neutral pH (S. Phipps, Weeks Bay Foundation, personal communication).

Our overall results in these SE US streams, corresponded with expectations based on other blackwater/clearwater systems (Benke et al. 1985, Meyer 1990, Smock and Gilinsky 1992, O'Donnell et al. 2010). Our data on pH in blackwater streams largely agreed with comparative work in South America, although our data found a smaller difference in pH between stream types (Duncan and Fernandes 2010). Our findings of higher DOC in SE US blackwater streams also corresponded with similar findings in Alaskan streams (O'Donnell et al. 2010).

PCA and CCA results indicated an environmental gradient across stream size in clearwater streams. Small clearwater streams tended to be more incised than blackwater streams, and larger clearwater streams tended to show slightly higher pH levels and higher TDS than smaller clearwater streams. Stream size patterns in environmental parameters were comparatively less evident among blackwater streams, although future studies should attempt to include a wider representation of discharge or stream width across blackwater streams.

Only one blackwater stream (Gum Branch) was misclassified by the DFA. This stream had slightly higher pH and TDS levels, and was flashier based on Q_fre5, than most other blackwater streams, but its pH and Q_fre5 values were higher than in many clearwater sites. Although not a measured parameter, one factor distinguishing this site from others was that the site traversed a clearcut. There is evidence that clearcutting can influence minimum flows, though the effect of this land use on TDS and stream pH is less certain (Rothacher 1965, Aubertin and Patric 1974, Martin et al. 1984, Ensign and Mallin 2001).

Overall, LULC variables did not appear to have strong importance in predicting either stream type or species abundance in this data set. Alternatively, although LULC levels encountered in this study did not show a strong influence on environmental data, few of these basins had levels of percent impervious surface approaching those typically considered impacted (Schueler 2009). The trend for these blackwater streams to have a higher percentage of forest in their basins may be responsible for the higher density of invertebrates in these streams (Benke et al. 1985, Harmon et al. 1986). Although, woody debris did not load highly on PC-3 (0.28), it did appear to be more associated with blackwater streams based on field observations. Additional study is needed to strengthen or refute these conclusions.

3.4.2 Fish Assemblage Contrasts Between Stream Types

Stark differences in fish assemblages between blackwater and clearwater streams were apparent even at the family level, with higher centrarchid species richness in clearwater streams. Similarly, in South American streams Winemiller et al. (2008) found stream type to be a good predictor of differences in fish assemblage, with some species occurring primarily in blackwater habitats and others clearwater habitats in Amazonian streams. Similar results were also obtained from assemblage comparisons between types in Brazilian streams (Gonçalves and Braga 2012). Here, we similarly found that stream size-related differences in species composition were more apparent in clearwater streams, even though stream size is considered to be a general driver of fish richness in lotic systems (Oberdorff et al. 1995).

In these clearwater streams, Speckled Madtoms were captured across all stream sizes, so they presumably had less of a stream size preference. Southern Brook Lamprey appeared to show a preference for smaller clearwater streams. Largemouth Bass and Green Sunfish were captured in greater numbers in mid-sized streams as were Bluegill, although the latter were captured somewhat ubiquitously across sizes. Captured almost exclusively in larger clearwater streams were the Weed Shiner, Gulf Darter, and Longear Sunfish. Differences in species composition in blackwater streams versus clearwater streams may have been more apparent if our sampling had included a greater number of larger, yet-wadeable, blackwater streams, although these were less prevalent on the landscape in this region. Nevertheless, there appeared to be a relationship between slightly more darters and cyprinids, particularly *Pteronotropis* species, and larger blackwater streams.

Cyprinids represented a large percentage of the overall catch in blackwater streams, yet this was almost entirely due to one species, the Flagfin Shiner. Endemic to the northern Gulf Coast, this species inhabits sandy-bottomed streams of clear or tannin stained waters (Boschung and Mayden 2004). In our study, they were consistently captured in higher numbers in blackwater streams indicating a potential preference for this stream type consistent with Mettee et al. (1996). As indicated by PCA results, higher captures of Flagfin Shiner in blackwater streams may be the result of higher prevalence of woody debris and benthic invertebrates in blackwater streams, which is the preferred habitat and food, respectively, for this species (Mettee et al. 1996). Other cyprinid species such as Golden Shiner *Notemigonus crysoleucas* were captured so infrequently that insight into any correlation between their occurrence in blackwater streams and habitat parameters remains unclear.

Both Flagfin Shiner and Sailfin Shiner were captured in similar numbers in clearwater streams. These species are difficult to distinguish morphologically, so it is possible that coexistence of such apparently similar species is due, in part, to the probable preference of the Flagfin Shiner for blackwater streams. In a study of several blackwater streams of the Florida panhandle, the Sailfin Shiner was captured in greater numbers than the Flagfin Shiner, so additional work is needed to determine if this reflects a type preference for these species or a geographical gradation from Florida through Alabama (Greenfield and Bart 2005).

The association of Redfin Pickerel with blackwater streams is in agreement with reported preferences of this species for acidic streams containing dense vegetation (Crossman 1962). Previous work in the SE US has found Redfin Pickerel to be common, if not dominant, in headwater blackwater streams (Pardue et al. 1975). Most blackwater streams in our study were not heavily vegetated, but the high abundance of woody debris may provide needed cover for this ambush predator (Mettee et al. 1996, Boschung and

Mayden 2004, Cain et al. 2008). Similar habitat associations have been found for Grass Pickerel *Esox americanus vermiculatus* (Cain et al. 2008). Woody debris in blackwater streams provides stable structure in shifting sand substrates as well as food and habitat resources benefitting benthic invertebrate and fish assemblages, providing prey for Redfin Pickerel across larval, juvenile, and adult life stages (Benke et al. 1985, Smock et al. 1985, Smock and Roeding 1986, Hauer and Benke 1987, Meyer 1992, Boschung and Mayden 2004). Similarly, in South American streams some species associated with woody debris such as the Pike Cichlid *Crenicichla lenticulata* and the Jaguar Catfish *Liosomadoras oncinus* were more often captured in blackwater streams than clearwater streams (Winemiller et al. 2008).

Association of Lake Chubsucker with slow, low-gradient waters and high organic debris is well documented (Smith-Vaniz 1968, Meffe and Sheldon 1988, Mettee et al. 1996). Interestingly, some studies have reported association of this species with clearwater streams (Boschung and Mayden 2004, but see Meffe and Sheldon 1988). We did not collect Lake Chubsuckers in clearwater streams, but rather found them in most of our smaller blackwater streams at low relative abundances.

Based on ISA results, 4 of 8 collected centrarchid species were indicator species for clearwater streams. This pattern may result from a general preference by most sunfishes for sand and gravel substrates used for spawning (Boschung and Mayden 2004), which in these clearwater streams sand was the predominant substrate. Sunfishes such as the Warmouth *Lepomis gulosus*, which select softer substrates (i.e., mud, organic debris, etc.), were not captured in greater abundance in either stream type (Larimore 1957).

Darter species and a single lamprey species were more commonly captured in clearwater streams. Blackbanded Darter, one of the 2 common darter species in our study, inhabits clear, small to medium-sized streams with sandy or gravel substrates (Crawford 1956, Boschung and Mayden 2004), but in the Coastal Plain are reported to inhabit turbid waters with silt and mud substrates (Boschung and Mayden 2004). However, we rarely encountered high turbidity or excessive silt and mud substrates in any of our sites. Instead, Blackbanded and Gulf darters were captured in higher abundances in clearwater streams with higher velocities and sandy substrates, habitats more similar to those reported for other inland and coastal populations (Page 1983, Meffe and Sheldon 1988, Ross 2001). Similarly, Southern Brook Lamprey was commonly captured in clearwater streams in agreement with previous studies; this nonparasitic lamprey inhabits clear sandy streams with moderate to swift current and with organic debris accumulation in slack water (Mettee et al 1996, Boschung and Mayden 2004).

3.4.3 Fish Associations and Environmental Variables

A species' life history traits such as developmental rate, body size, and feeding and reproductive strategies can result in a spectrum of fitness tradeoffs across environmental gradients (Wellborn et al. 1996). Two environmental factors in particular that varied between clearwater and blackwater streams in our study were pH and DO, both of which can influence fish physiology (Val & Almeida-Val 1995, Wilson et al. 1999, Matsuo and Val 2002, Helfman et al. 2009). Although pH was not found to be as important of a discriminator between stream types as DO in our study, previous research has found low pH to be an important descriptor of blackwater streams (Benke et al. 1985, Meyer 1990). Low pH can alter oxygen transport, digestion, and ionic balance in fishes

(Val & Almeida-Val 1995, Wilson et al. 1999, Matsuo and Val 2002, Helfman et al. 2009).

Both the Lake Chubsucker and the Redfin Pickerel have been associated with slow-water habitats and areas of low DO (Crossman 1962, O'Connor 1967, Hauer and Benke 1987, Meffe and Sheldon 1988). Dissolved oxygen tolerances of the Redfin Pickerel have been observed to be very low (Odum and Caldwell 1955). Although environmental tolerances of the Lake Chubsucker are poorly known, it has been captured in a wide range of pH values (4.3 to 9.0) and is considered to be a species tolerant of poor water quality (McLane 1955, Bennett and Childers 1966, Becker 1983, Hoyer and Canfield 1994, Hill and Cichra 2005). Indeed, according to McLane (1955) the Lake Chubsucker is one of the last fish species to perish in drying pools and ditches in Florida. In this region seasonal drying of low-order streams can result in many organisms taking refuge in small, isolated pools (Smock and Gilinsky 1992). These conditions may be responsible for the lower species richness observed in these smaller blackwater streams in comparison with clearwater streams.

Contrasts in environmental conditions and fish assemblage structure between stream types may also be a result of hydrological differences due to different primary water sources, such as deep versus shallow groundwater or surface water (Smock and Gilinsky 1992, O'Donnell et al. 2010). In clearwater streams of interior Alaska, O'Donnell et al. (2010) reported that clearwater streams received more contributions from deep groundwater sources, whereas blackwater streams were fed largely by shallower groundwater and surface sources (Smock and Gilinsky 1992). This phenomenon of differential source water is not well supported for blackwater and clearwater streams of the SE US, though differences in hydrology between stream types will likely differentially influence habitat parameters and fish assemblages (Poff and Ward 1989, Poff and Allan 1995). In this study, analyses of the hydrology and morphology of these streams found increased discharge and depth in clearwater streams. Similarly, two of our blackwater streams would dry in late summer whereas even the smallest clearwater streams did not dry thus indicating clearwater streams may indeed receive more groundwater inputs than blackwater streams. Further investigations between stream type differences should attempt to assess differential source input.

If primary water source varies between stream types, then alterations to the basin, such as urbanization, could have disparate impacts. The predicted continued increase in urbanization in the SE US is likely to result in further impact on these stream ecosystems (Nagy et al. 2011). Although most of these basins did not have levels of impervious surface cover that would be considered to be highly impacted, even a slight increase in impervious surface cover in several basins would transition their current stream quality classification from sensitive to impacted (Schueler et al. 2009). Increased urbanization could impact fish species composition, richness, and abundance and may alter or homogenize these disparate assemblages (Klein 1979, Jones et al. 1999, Helms et al. 2005, Morgan and Cushman 2005, Walsh et al. 2005). More work on the differences in the hydrology of these stream types would allow for better predictions of stream response to increased urbanization.

Considerable primary literature on stream classification systems is based on work in mountainous areas (Frissell et al. 1986, Seelbach et al. 2006, Melles et al. 2012), which does not fully incorporate the unique characteristics of low topographical areas. Coastal blackwater systems, for example, drain wetlands and swamps (Smock and Gilinsky 1992, Jones 2010). Classification systems such as Poff's (1997) hierarchical landscape filters needs refinement, as they do not allow for such disparate stream types and associated assemblages within a small geographic area. Instead of a restriction of the amount of environmental variability accounted for at smaller spatial scales, some flexibility should be allowed that accommodates and even predicts environmental and biological variability at relatively small spatial scales in systems that exhibit these phenomena. Stream ecological theories that predict or incorporate the existence of unique habitats and fish assemblage differences exhibited by these stream types in these coastal settings are necessary.

In summary, we found environmental, biotic, and fish parameters successfully discriminated between blackwater and clearwater stream types. Fish assemblage differences appear to be in part explainable by differences in environmental and biotic parameters we measured. Studies of this type can aid in the identification of environmental and biotic factors that influence fish assemblage structure and provide a further example for nonrandom structuring of fish communities (Jackson et al. 2001). Further study of what mechanisms elucidate these patterns would benefit our understanding of these unique systems and aid erudition of stream ecological theory.

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TABLE 1. Characteristics of 15 study sites. Universal Transverse Mercator (UTM) coordinates are in zone 16.

Stream	Watershed size (km^2)	Range Q (m^3/sec)	UTM North	UTM East
Bon Secour River	24.819	0.12-0.48	431989	3358457
Foley Preserve	2.52	0.001-0.03	439218	3357324
Gum Branch	6.55	0.03-0.09	444194	3361125
Hammock Creek	6.2	0.03-0.07	445928	3359735
Magnolia River	23.06	0.26-0.58	431182	3366000
Miflin Creek	8.54	0.02-0.2	443189	3364905
Miflin Creek Tributary	1.1	0.01-0.4	443189	3364905
Palmetto Creek	4.65	0.07-0.11	445499	3359236
Sandy Creek East	2.02	0.01-0.08	439887	3364097
Sandy Creek West	6.97	0.01-0.1	439975	3364145
Cowpen Creek	30.8	0.08*	421430	3372598
Baker Branch	10.5	0.03*	427931	3371783
Pensacola Branch	12.7	0.05*	422076	3377119
Perone Branch	24.8	0.33*	424385	3379504
Wolf Creek	8.44	0.15-0.28	435137	3363364

* These sites were visited once in June 2013.

TABLE 2. Land use characteristics of 15 study sites. % Impervious is total impervious surface for each watershed.

Stream	Blackwater/ Clearwater	% Agriculture	% Forest	% Impervious
Bon Secour River	C	49.41	18.44	6.37
Foley Preserve	B	45.00	38.35	2.21
Gum Branch	B	28.7	33.75	2.74
Hammock Creek	B	22.22	56.1	1.33
Magnolia River	C	38.5	20.16	7.85
Miflin Creek	B	45.1	37.2	1.77
Miflin Creek Tributary	B	52.9	24.4	0.81
Palmetto Creek	B	36.77	48.2	0.56
Sandy Creek East	C	31.4	32.03	3.74
Sandy Creek West	C	43.1	45.15	0.4
Cowpen Creek	C	29	18	16.9
Baker Branch	B	73	6	3.2
Pensacola Branch	C	33	5	6.5
Perone Branch	C	39	22	9.3
Wolf Creek	C	22.03	33.6	11.3

TABLE 3. Species captured at study sites. Indicator species annotated with BW (blackwater) or CW (clearwater) to indicate stream type association.

Family	Species	Common name	% of total catch	% Blackwater catch	% Clearwater catch	Indicator Species (Y/N)
Anguillidae	*Anguilla rostrata*	American Eel	1.7	3.60	0.800	N
Aphredoderidae	*Aphredoderus sayanus*	Pirate Perch	5.7	8.37	4.300	N
Atherinopsidae	*Labidesthes sicculus*	Brook Silverside	0.2	0	0.300	N
Catostimidae			0.6	1.1	0.3	
	Erimyzon sucetta	Lake Chubsucker	0.2	0.52	0	Y - BW
	Erimyzon tenuis	Sharpfin Chubsucker	0.4	0.58	0.324	N
Centrarchidae			30.4	21.6	34.8	
	Lepomis auritus	Redbreast Sunfish	0.04	0.10	< 0.001	N
	Lepomis cyanellus	Green Sunfish	3.6	0.30	5.200	Y - CW
	Lepomus gulosus	Warmouth	1.4	1.70	1.300	N
	Lepomis macrochirus	Bluegill	13.8	3.40	19.100	Y - CW
	Lepomis megalotis	Longear Sunfish	0.3	0.10	0.400	Y - CW
	Lepomis microlophis	Redear Sunfish	0.3	0	0.400	N
	Lepomis miniatus	Redspotted Sunfish	9.9	15.50	7.000	N
	Micropterus salmoides	Largemouth Bass	1.1	0.50	1.400	Y - CW
Cyprinidae			25.3	39.5	18.0	
	Notemigonus crysoleucas	Golden Shiner	0.02	0.06	0	N
	Notropis petersoni	Coastal Shiner	0.02	0	< 0.001	N
	Notropis texanus	Weed Shiner	2.1	0	3.183	Y - CW
	Pteronotropis hypselopteris	Sailfin Shiner	6.4	3.98	7.604	N
	Pteronotropis signipinnis	Flagfin Shiner	16.8	35.49	7.191	N
Elassomatidae			0.4	0.8	0.2	
	Elassoma evergladei	Everglades Pygmy Sunfish	0.04	0.12	0	N
	Elassoma zonatum	Banded Pygmy Sunfish	0.4	0.63	0.206	N
Eleotridae			0.3	1.0	0.0	
	Dormitator maculatus	Fat Sleeper	0.2	0.46	0	N
	Eleotris amblyopsis	Largescale Spiny Cheek	0.2	0.52	0	N
Esocidae	*Esox americanus*	Redfin Pickerel	5.1	11.25	1.945	Y - BW
Fundulidae	*Fundulus olivaceus*	Blackspotted Topminnow	0.1	0	0.177	N
Ictaluridae			6.4	3.5	7.8	
	Ameiurus natalis	Yellow Bullhead	1.3	2.31	0.766	N
	Ameiurus nebulosus	Brown Bullhead	0.1	0	0.206	N
	Noturus leptocanthus	Speckled Madtom	5.0	1.21	6.867	Y - CW
Percidae			8.9	3.6	11.5	
	Etheostoma swaini	Gulf Darter	0.1	0	0.206	Y - CW
	Percina nigrofasciata	Blackbanded Darter	8.7	3.64	11.317	Y - CW
Petromyzontidae	*Ichthyomyzon gagei*	Southern Brook Lamprey	4.4	0.75	6.278	Y - CW
Poeciliidae	*Gambusia holbrooki*	Eastern Mosquitofish	10.6	5.02	13.380	Y - CW

TABLE 4. Canonical correspondence analysis (CCA) results for biological and environmental variables and indicator species. Loadings are included only for variables indicated as significant in permutation analysis and included in final CCA.

	Axis 1	Axis 2
Eigenvalue	0.37	0.34
Percentage species-environment variance	31	29
Correlations of biological and environmental variables with axes		
Benthic invertebrates (m2)	0.85	0.07
maximum depth	-0.54	0.14
Q_fre5	-0.17	0.51
NO_3^-	0.19	-0.65
average DOC	0.56	0.26
incision depth	0.2	-0.66
% Forest	0.51	-0.63
Q	-0.48	0.71
% sandy substrate	-0.66	-0.28
DO (mg/L)	-0.32	-0.61
Correlations of indicator species		
Erimyzon succeta	2.31	0.3
Esox americanus	1.67	0.51
Etheostoma swaini	-0.49	1.67
Gambusia holbrooki	-0.03	-0.55
Icthyomyzon gagei	0.07	-0.99
Lepomis cyanellus	-0.72	1.03
Lepomis macrochirus	-0.23	-0.15
Lepomis megalotis	-0.11	1.45
Micropterus salmoides	-0.24	0.23
Notropis texanus	-0.57	1.69
Noturus leptacanthus	0.007	-0.22
Percina nigrofasciata	-0.31	0.55

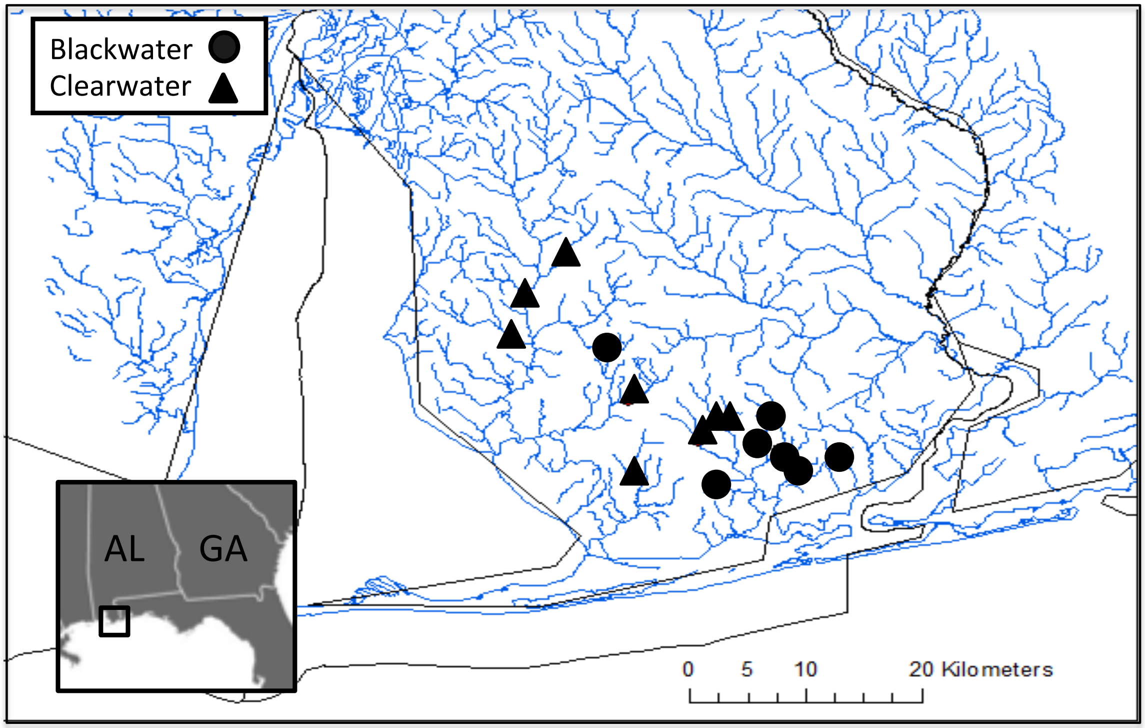

Figure 1. Sites sampled for fishes, environmental, and land use variables

(clearwater streams ▲, blackwater streams ●).

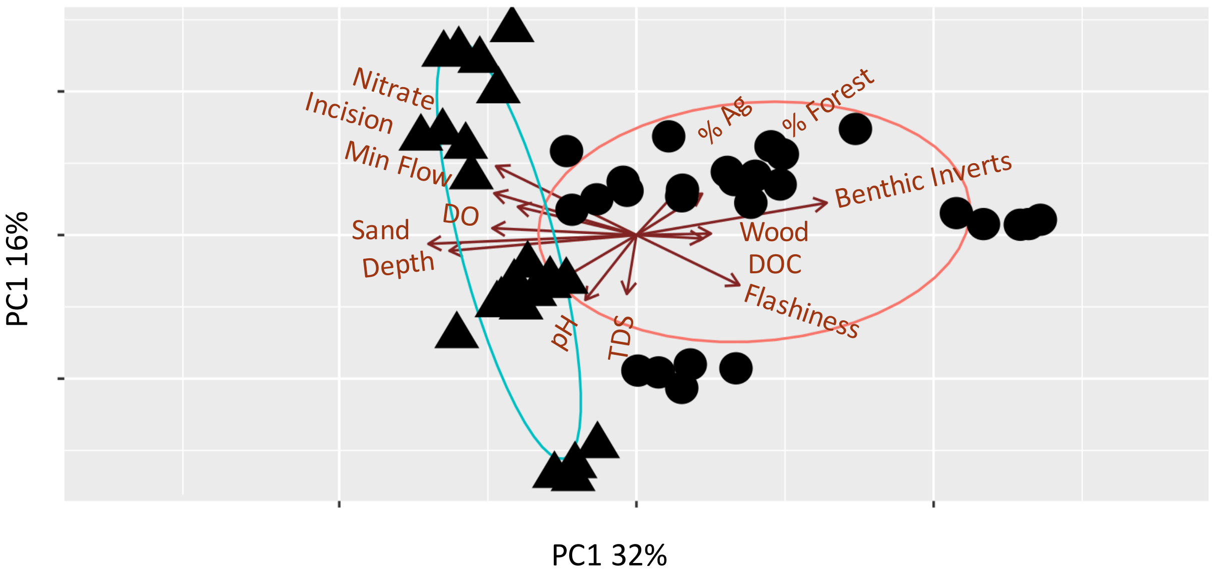

Figure 2. PCA of environmental variables (clearwater sites ▲, blackwater sites ●) with vector lengths indicating strength of association with principle components. Ellipses enclose 1 SD from the centroid.

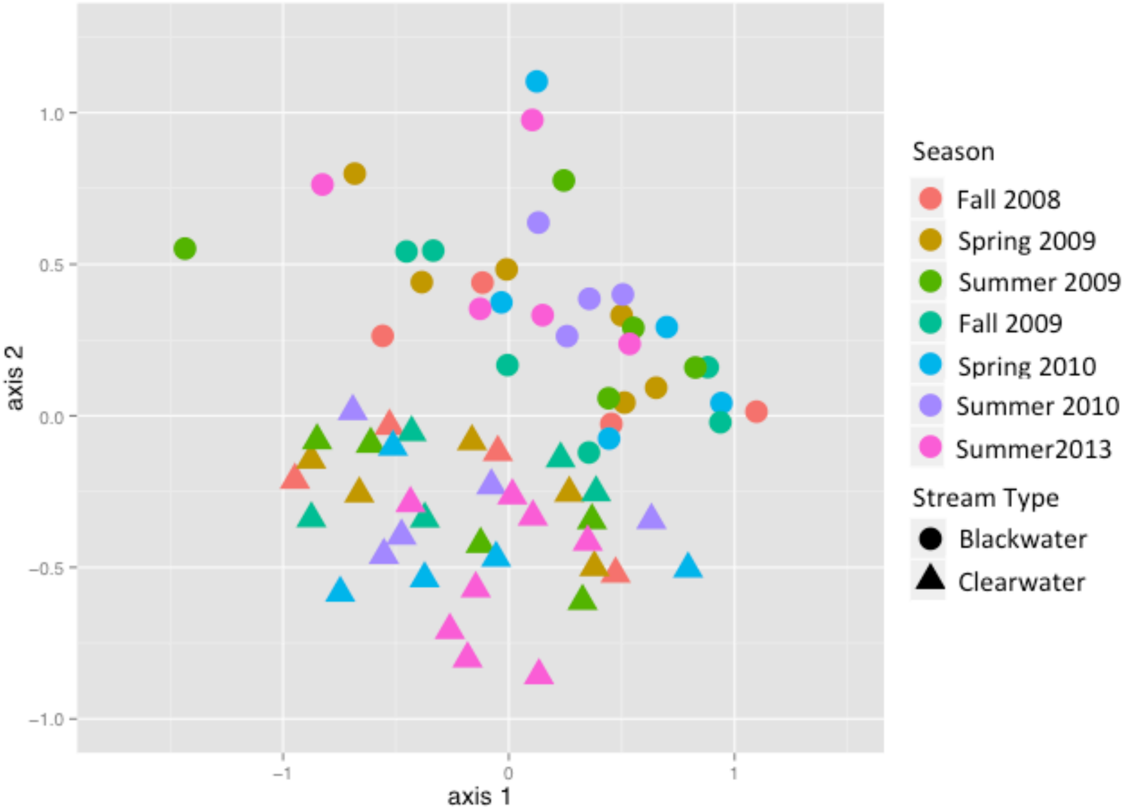

Figure 3. Non-metric multidimensional scaling (NMDS) ordination of study sites in species space (clearwater sites ▲, blackwater sites ●). Samples were taken at 15 study sites over multiple seasons as indicated by color.

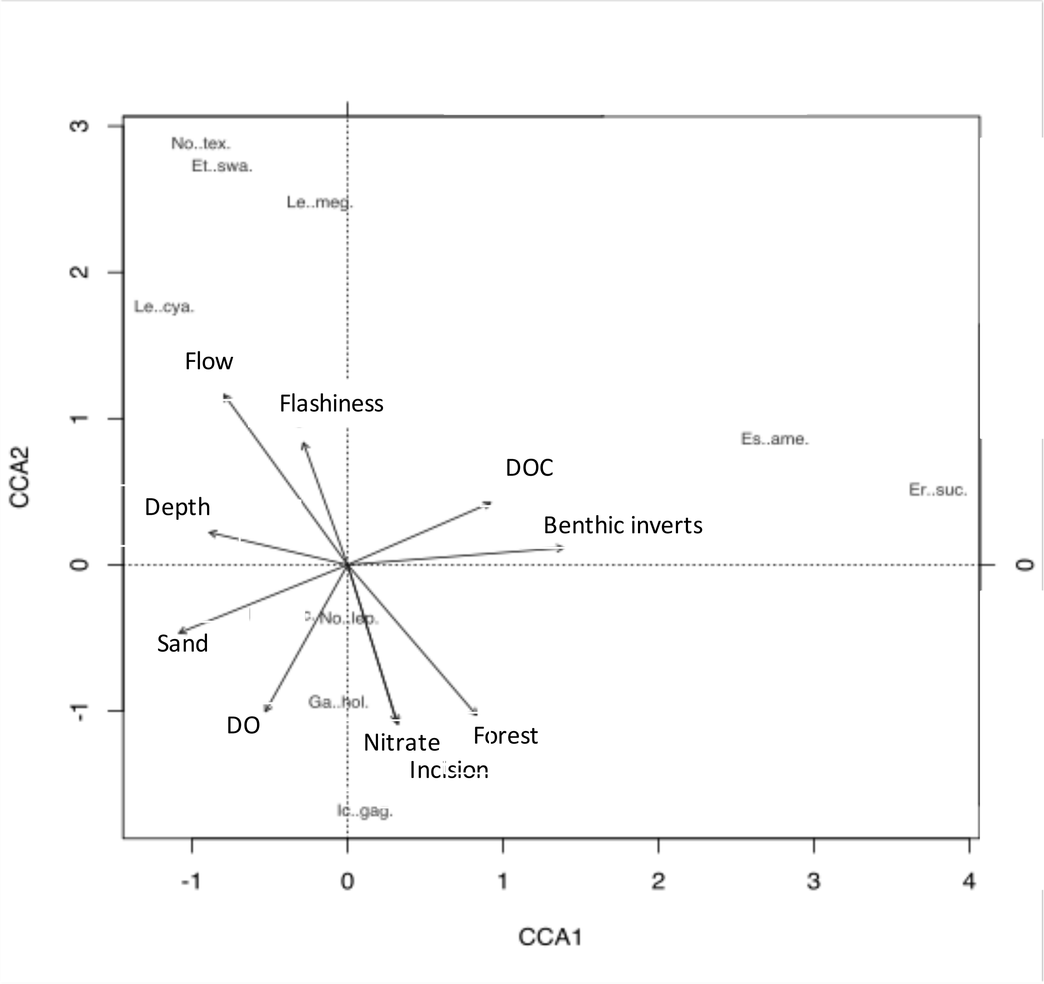

Figure 4. Plot of canonical correspondence analysis (CCA) species and environmental variable scores on CCA 1 and CCA 2 based on a matrix of indicator species assemblage composition and the matrix of 12 biological and environmental variables.

Chapter 4. Weight-length parameters of stream fishes and correlations with traits, season, stream type, and habitat.

4.1 Introduction

Weight-length relationships (WLRs) have been described as foundational elements in fisheries research (Anderson and Neumann 1996). Measures compiled from weight-length data are often used to assess condition of fishes of sport or commercial interest (Ogle and Winfield 2009). In addition, WLR parameters have been used to study the condition of fish populations exposed to a suite of biotic and abiotic conditions both within and between sites (Stucky and Klaassen 1971, Wiener and Hanneman 1982, Bolger and Connolly 1989, Cone 1989, Morato et al 2001, Cade et al. 2011, Moradinasab et al. 2012). Indeed, use of WLRs to explore effects of biotic and abiotic factors on the well-being or health of a population is an approach that continues to receive analytical refinement (Cone 1989, Froese 2006, Ranney et al. 2010). Recent investigations emphasize the potential value of WLRs in examining the effects of water body type, habitat type, and other forms of geographical variation on WLR parameters and further exploring ecological implications of these data across seasons (Froese 2006, Ogle and Winfield 2009).

The basic mathematical approach to using body size as a predictor of weight is the formula, $Y = aW^b$, where Y is the body size dimension to be predicted, W the animal's body weight and parameters a and b are empirically derived constants (Peters 1983). Similar to Galileo's 'square-cube law' the value of parameter b is typically ~3 across fishes, although this varies (Galilei 1637, Martin 1949, Froese 2006). Parameter b also changes across the life of a fish, especially during different growth stanzas as can be seen by contrasting inflection points in a weight-length plot (Martin 1949, Froese 2006). Growth stanzas typically represent different life stages of fish development such as larval, juvenile, and adult stages, but can also occur from changes in environmental conditions such as temperature, starvation, prey-switching, or environmental shifts such as from saltwater to freshwater (Martin 1949, Ricker 1979, Wootton 1998, Froese 2006). Delays in switching from one growth stanza to another can increase risk of predation and intensify competition (DeVries and Stein 1992, Hoey and McCormick 2004).

Within a species, a low value of b indicates a longer, thinner body at a given weight (Wootton 1998). This body form is typically associated with the larval life-stage, with a high surface-to-volume ratio where viscous forces predominate and cutaneous respiration is important (Wootton 1998). Later in life when inertial forces become more important, fish typically change body shape, adopting a form that improves swimming performance and feeding ability, and may reflect reproductive strategy; at this time b will typically increase although this can vary by sex (Blaxter 1969, Purcell 1977, Hunter 1981, Kamler 1992, Wootton 1998, Helfman et al. 2009, Montag et al. 2011, Hassell et al. 2012).

Within a population when $b = 3$ small individuals are thought to have the same nutritional condition and body form as larger individuals (Froese 2006). When $b > 3$ larger individuals have greater increases in height or width per unit length as ontogenetic shifts or, as commonly occurs, because larger individuals have thicker body forms (Froese 2006). When $b < 3$ larger individuals are thought to have reduced nutritional condition or elongation in form (Froese 2006). Though these relationships can also vary by sex, reproductive state, and season (Hile 1936, Tesch 1971, Froese 2006, Montag et al. 2011).

Across species, departures from $b = 3$, or isometric growth, can be ecologically informative and may be indicative of unique life history patterns or ecological niches (Wootton

1998). For example, English Sole *Pleuronectes vetulus* have been found to have a *b* = 2.7 (Gartz 2004). The English Sole is a benthic carnivorous right-eyed flatfish, born with bilateral symmetry, that undergoes a complicated metamorphosis involving eye movement to one side of the head (Helfman et al. 2009). Although they can become quite large their body shape is compressed (Helfman et al. 2009). Conversely, the Muskellunge *Esox masquinongy* has a relatively large *b* = 3.44 but is a massive ~1.4 meter fish considered to be one of the largest freshwater predators in North America (Hanson 1986, Helfman et al. 2009).

Biotic factors such as invertebrate prey abundance can be of particular importance when predicting fish growth and habitat suitability (Rosenfeld et al. 2005, Rosenfeld and Taylor 2009). Increased prey abundance can result in not only greater growth but also habitat shifts into higher velocity water, indicating that at higher prey abundances more energetically costly habitat may become metabolically profitable (Rosenfeld et al. 2005). Abiotic factors such as seasonal water flow dynamics and substrate can also have an influence on fish growth parameters (Oliva-Paterna 2003). In some systems streams with continuous flow across seasons produce fish with higher *a* parameters, indicative of better condition (Oliva-Paterna 2003). Fluctuating physical and chemical parameters from low flow conditions and overcrowding are thought to contribute to lower *a* parameters in more intermittent streams (Oliva-Paterna 2003). Additionally, examining how WLR parameters vary with species' biological traits such as feeding strategy, longevity, and fecundity may help to identify potential life history trade-offs in variable aquatic environments (Frimpong and Angermeier 2010).

Many of these investigations have thus far focused on trout species and much of that work has been limited to lentic and lotic comparisons or primarily focused on one habitat or the other (Milewski and Brown 1994, Simpkins and Hubert 1996, Kruse and Hubert 1997, Hyatt and

Hubert 2001, Pope and Kruse 2007). Kruse and Hubert (1997) found individuals in lotic cutthroat trout populations weighed more at a given length than individuals in lentic populations. The authors attributed this difference to potential evolutionary or body form differences between populations in the two habitat types (Kruse and Hubert 1997). Previously obtained weight-length information can easily be compared in a lentic vs. lotic context for many species. Comparisons of this type can contribute to current ecological investigations by first establishing whether differences in WLR parameters might be expected at a broader scale of ecological differences (Hynes 1970, Simpkins and Hubert 1996, Kruse and Hubert 1997, Pope and Kruse 2007). Further expanding these types of comparisons beyond important game species would expand our understanding of the potential variability in these parameters across habitat types (Froese 2006).

Blackwater and clearwater streams are two distinct stream types that have worldwide distributions and occasionally occur in the same basin (Meyer 1990, Winemiller et al. 2008, O'Donnell et al. 2010). These stream types co-occur in close proximity, even within a basin, and share a similar regional species pool but exhibit considerable physicochemical differences (Benke et al. 1985, Smock et al. 1985, Smock and Roeding 1986, Meyer 1990, Meyer 1992, Poff 1997). Studies in other blackwater/clearwater systems have found fish faunal differences between stream types but have not included analyses of potential WLR differences between fish species that have overlapping occurrences in both types (Winemiller et al. 2008, Gonçalves and Braga 2012).

We assessed if WLR parameters differed between fish populations in blackwater and clearwater streams and seasons. We also assessed whether differences in *b* parameters for these same species varied between lentic and lotic environments in previous studies. We hypothesized that WLR parameters will vary between stream types and that WLR parameters in blackwater streams will be more reflective of patterns seen in lentic conditions, clearwater stream WLR parameters more similar to those seen in lotic conditions.

4.2 Methods

4.2.1 Study Area

Blackwater and clearwater 1st – 4th order streams in the Fish River, Bon Secour, Magnolia, Wolf Bay, and nearby smaller basins of Baldwin County, Alabama were used as study sites (Fig. 1). Southeastern (SE) US blackwater streams, characterized by tea colored water, low pH, and high dissolved organic carbon (DOC), are the most common stream type of the SE US Atlantic and Gulf Coastal Plain (Benke et al. 1985, Meyer 1990, Smock and Gilinsky 1992). Woody debris in blackwater streams provides stable structure in shifting sand substrates and food and habitat resources benefitting benthic invertebrate and fish assemblages (Benke et al. 1985, Smock et al. 1985, Smock and Roeding 1986, Meyer 1992). In contrast, clearwater streams typically are highly transparent, less acidic, with less organic debris than blackwater streams (Winemiller et al. 2008, Duncan and Fernandes 2010). The clearwater streams of interior Alaska received more contributions from deep groundwater sources than blackwater streams which were fed largely by shallower groundwater sources (O'Donnell et al. 2010). However, this phenomenon of differential water sources is not well supported for blackwater and clearwater streams of the Southeastern, US. Nor is it known if there are differences in stream hydrology between stream types (i.e., mean minimum flows, variability across monthly flows, or mean high flows) (Olden and Poff 2003).

These subtropical low-gradient streams drain southern pine hill and coastal lowland physiographic regions emptying into the Gulf Coast (Boshung and Mayden 2004). Wetted stream widths at summer base flow ranged from ~ 1.5 to 5.5 m and depths from 0.1 to 0.4 m. Land use and land cover (LULC) data showed these basins were largely agricultural (22-49%) or covered in forest (18-33%), with some suburban and urban influence (6-11% impervious surface) (Morrison 2010, B.P. Schneid and S. Colvin, Auburn University, unpublished data). Most stream sections were heavily shaded (densiometer readings of 16 were common, S. Colvin and B. Schneid, Auburn University, unpublished data) by a mixture of longleaf pine *Pinus palustris*, swamp tupelo *Nyssa biflora*, and sweetgum *Liquidambar styraciflua*, in the overstory. (S. Phipps, Weeks Bay Foundation, personal communication).

They are influenced by a high-flow period that begins with the onset of winter rains in November/December and a summer/fall low-flow period occurring because of low rainfall and high evapotranspiration (Metzler and Smock 1990, Meyer 1992, Smock and Gilinsky 1992). Lower-order streams depend more on rainfall and thus can be more variable in environmental parameters such as dissolved-oxygen (DO), temperature, and flow than higher-order streams (in terms of complete drying), although temperature also can fluctuate dramatically in the latter (Whiteside and McNatt 1972). Stream habitat included predominately scour pools and undercut banks, largely separated by glides, with some riffle sequences.

4.2.2 Fish and Macroinvertebrate collection

Fishes were sampled in spring, summer, and fall beginning in fall 2008 through summer 2010 with an additional summer sample collected in 2013. Study reaches were electrofished with a backpack electrofisher with 3 passes using teams of 2-4 individuals with a reach length of at least 30x the wetted stream width block-netted at the upstream and downstream terminus (Rahel and Hubert 1991). Fish were sampled in 8-13 streams per season (spring, summer, or fall). Captured fish were anesthetized with MS-222 and fixed in formalin for ~ 2 weeks prior to transfer into 70% ethanol for long-term storage. Standard length (L, ± 1 mm) and total weight (W, ± 0.01 g) were measured after blotting off excess fluid. After processing fish were deposited into the Auburn University Museum of Natural History Fish Collection.

Benthic macro invertebrates were sampled with Surber samplers (mesh size = 250 μm) to estimate benthic invertebrate density for each stream sampled. These invertebrate data were then used as a representation of benthic food availability in these streams. Three samples were taken per stream reach in glides/runs when available, with 3 Surber quadrats per sample (Schneid 2015). Invertebrate samples were field-preserved in 95% EtOH, transported to the laboratory, and stored at 4 C until processed with a 2-phase method (Feminella 1996, Schneid 2015). The 2-phase method involves an initial 30 min coarse sort to remove large and rare (> 2mm) organisms and is followed by removal of several 50 mL aliquot subsamples (≥ 3 aliquots per sample) from a homogenized, 1000 mL suspension (Feminella 1996). Subsamples were picked at random microscopically until a minimum total of 300 individuals were removed for identification (Feminella 1996, Schneid 2015). Identifications were taken to the genus level for most organisms (except oligochaetes which were identified to order level; Schneid 2015). Benthic macroinvertebrate data were available only for those streams sampled prior to 2013. Invertebrate density values used were an average of density values obtained in Fall 2008 and Spring 2009 (Schneid 2015).

4.2.3 Habitat Sampling

To investigate the relationship between short- and long-term hydrological variables and fish WLR parameters we estimated several hydrologic. Stream discharge (Q) was quantified on each sampling date using the velocity-area method (Gore 1996) with a Marsh-McBirney Model 2000 Flo-Mate (Marsh-McBirney, Inc., Frederick, MD). We also estimated water stage (height above fixed datum) with Solinst pressure transducers and barologgers to estimate (and compensate for) barometric pressure (Levelogger Gold, model 3001, 15-min intervals) from February 2009 to March 2010. Transducers were housed in a perforated PVC pipe and installed near the downstream end of each stream reach. Q was estimated at a range of stage levels during the study using the mid-section method (Raghunath 2007). Rating curves were developed to convert stage to Q using observed Q values (14-19 per site) and estimated values for high flow with Manning's equation (Leonard et al. 2000, Thomas and Nisbet 2007, Helms et al. 2009). Baseflow was determined using a 3-pass recursive digital filter, a forwards, backwards, and forwards automated process of identifying and filtering out peak flows and estimating base flows (Nathan and McMahon 1990). Calculating base flow allowed an estimation of storm event frequency characterized as the frequency of exceeding 5x median discharge (Q_fre5) which we used as a measure of high flow (Helms et al. 2009). We also included minimum Q values recorded for each stream to further represent low-flow.

A single streamwater pH and temperature reading were taken during each sampling event at all sites with a YSI 556 MPS meter (YSI Inc., Yellow Springs, OH, USA). Also measured each sampling event were coarse woody debris (CWD; >2.5 cm in diameter) in the wetted channel. These measures were taken approximately every 10 m along the study reach using a modified transect method (Wallace and Benke 1984, Maloney et al. 2005). We quantified all dead, live, and buried (within upper 10 cm of substrate) CWD within 0.5 m above or below the transect (Maloney et al. 2005). Data were converted to planar area by multiplying the CWD diameter by length then dividing by the total area sampled within each transect to estimate CWD per m^2 along the entire reach length (Maloney et al 2005).

Water samples were taken in June 2013 for all 13 sampling sites primarily to measure

DOC. Blackwater streams of the SE are typified by their higher DOC content in comparison to clearwater streams which are typically highly transparent and presumably lower in DOC thus we used DOC content as an environmental determinant of stream type (Benke et al. 1985, Meyer 1990, Smock and Gilinsky 1992, Winemiller et al. 2008, Duncan and Fernandes 2010). Samples were collected at 0.6 depth in the water column in pre-washed polypropylene bottles that had been rinsed with stream water prior to sample collection, stored in a refrigerator prior to analysis, and analyzed for DOC within 2 days of collection (Schoonover et al. 2005, Morrison 2010) at the Edward A. Hauss Elemental Analysis Laboratory, School of Forestry and Wildlife Sciences, Auburn University. DOC analysis was performed using a Shimadzu TOC-V series total organic C analyzer (Tokyo, Japan). Additional DOC samples from spring 2010 through spring 2011 also were taken (B.P. Schneid, Auburn University, unpublished data).

4.2.4 Statistical analyses

A log 10 transformed length-weight regression was fit for each species and its significance tested with an *F* statistic (Froese 2006, Crawley 2007, Ogle and Winfield 2009). To assess differences in WLR slopes between populations in different stream types and seasons we performed multi-level mixed models both within and across species which allowed for assessment of both fixed and random effects (Quinn and Keough 2002). These models also are appropriate for data consisting of repeated observations on the same sampling units (Quinn and Keough 2002). Site was included as a random effect that adjusted the degrees of freedom to account for multiple observations per site. We also included interaction terms of logL*stream type and logL*season to investigate if the relationship between length and weight differed between stream types or among seasons. Overall greater or lesser *b* regardless of type or season would be indicated by the significance of type or season as simple additive parameters without interactions which were also included in the model (Quinn and Keough 2002).

To explore how WLR slopes varied with lentic or lotic habitat, we downloaded *b* parameters from FishBase (Froese and Pauly 2015) for study species and performed a multi-level mixed model regression across species with *b* as the response variable and lentic or lotic habitats as the predictor variable. This analysis allows us to determine if populations of study fishes located in faster flowing waters differ in *b* parameter from slow water populations (Hynes 1970). We included species as a random variable due to unequal number of *b* records across species. We assigned lentic or lotic habitats based on locality information provided (i.e. river=lotic and lake or reservoir = lentic) and excluded any record that did not provide information in reference to water body type. Although these *b* were generated from a mixture of fork, total, and standard length data the *b* parameter is unaffected by different measures of fish length (Froese 2006).

We also estimated correlations between logW across species and environmental parameters with multi-level mixed models with hierarchical random effects (Quinn and Keough 2002). Predictors were invertebrate density, average log Q, pH, DOC, Q_fre5, and CWD. This analysis assesses potential impact on logW by abiotic, biotic, and flow parameters some of which have shown influence on fish growth parameters in other systems (Oliva-Paterna 2003, Rosenfeld et al. 2005). Parameter inclusion in the model was first assessed with variance inflation factor (vif) on ordinary least squares models. Each parameter included in the final mixed model had a vif less than 4 (Quinn and Keough 2002). Final models were mixed models with site and species included as random effects, with species nested within site. Parameters were selected based on previous analyses conducted to determine which environmental parameters were correlated with indicator species for either blackwater or clearwater stream fish assemblages (S. Colvin, Auburn University, unpublished data). Indicator species were defined as those species strongly associated with either blackwater or clearwater fish assemblages (S. Colvin, unpublished data). Indicator species analysis describes the degree to which each species diverges among previously defined groups (McCune and Grace 2002, Borcard et al. 2011). We calculated mixed models with package nmle (Pinheiro et al. 2014) and checked vifs with package car (Fox and Weisberg 2011) in R version 3.1.1.

Last we compared inflection points (IPs) from untransformed weight/length relationships and pre-and post-inflection slopes (slope 1 and slope 2) between populations in different stream types, whether or not species were indicator species, benthic feeders, surface and water column feeders, and across levels of longevity and fecundity. This analyses investigates whether stream type or trait variables influenced WLRs during different life stages as has been found from variability in some environmental conditions (Martin 1949, Ricker 1979, Wootton 1998, Froese 2006). Though these comparisons are more typically done within a species, trait investigations can provide additional information and generate hypotheses in fishes sharing similar traits (Frimpong and Angermeier 2010). We used multi-level mixed models with random effects (Quinn and Keough 2002). Reported parameter estimates and *P* values are from models that include stream type, indicator species status, functional feeding group, and levels of longevity and fecundity. Species was included as a random categorical variable due to two occurrences of each species in the data, one representing blackwater populations and the other clearwater populations. Traits were determined using Frimpong and Angermeier's (2009) trait database. We calculated IPs and slope 1 and slope 2 with piecewise regression with the package SiZer in R version 3.1.1 (Sonderegger 2012). We then compared IPs and slopes 1 and 2 using paired t-tests, as these IP's and slopes are essentially population parameters (Quinn and Keough 2002). All analyses were conducted in R version 3.1.1. Significance in all models was at alpha = 0.05.

4.3 Results

4.3.1 WLR Parameters

WLR parameters were calculated for 17 species from 9 families (Table 1). Sample size ranged from 17 for Banded Pygmy Sunfish *Elassoma zonatum* (Jordan 1877) to 754 for Flagfin Shiner *Pteronotropis signipinnis* (Bailey and Suttkus 1952). All relationships were highly significant (all $r^2 > 0.92$, $P < 0.0001$, Table 1). Values of b ranged from 2.63 for Southern Brook Lamprey *Ichthyomyzon gagei* (Hubbs and Trautman 1937) to 3.40 for Weed Shiner *Notropis texanus* (Girard 1856) (Table 1). Other than Brook Lamprey, all b values were > 3 with the next lowest b being 3.02 for Pirate Perch *Aphredoderus sayanus* (Gilliams 1824) (Table 1).

4.3.2 Stream Type and Season Mixed Models Results

Multi-level mixed model analyses including stream type, season, and stream type*logL and season*logL interactions indicated that logW of 9 species were correlated with at least one of these parameters (Table 2). American Eel *Anguilla rostrata* (Lesueur 1817) showed a higher b parameter in spring ($\beta = 0.61$, S.E. = 0.26, $P = 0.02$) and had a significant season*logL interaction, with b lower in the spring season ($\beta = -0.25$, S.E. = 0.11, $P = 0.03$) than in summer or fall (Table 2). Pirate Perch b was lower in clearwater streams ($\beta = -0.26$, S.E. = 0.09, $P = 0.02$), showed a significant stream type*LogL interaction with b lower in clearwater streams ($\beta = 0.13$, S.E. = 0.05, $P = 0.01$), and significantly lower in spring ($\beta = 0.39$, S.E. = 0.18, $P = 0.03$) and summer ($\beta = 0.35$, S.E. = 0.11, $P = 0.003$) than fall (Table 2). Pirate Perch b also had significant season*logL interactions lower in spring ($\beta = -0.23$, S.E. = 0.10, $P = 0.03$) and summer ($\beta = -0.21$, S.E. = 0.07, $P = 0.002$) than fall (Table 2). Banded Pygmy Sunfish also showed a season*logL interaction indicating a steeper *b* in spring (β = 0.74, S.E. = 0.28, *P* = 0.05) than other seasons (Table 2). Redfin pickerel *Esox americanus* (Gmelin 1789) *b* parameter was higher in summer (β = 0.64, S.E. = 0.24, *P* = 0.008) and also showed a season*logL interaction indicating a lower increase in logL in summer (β = -0.32, S.E. = 0.11, *P* = 0.005; Table 2). Southern Brook Lamprey had a significantly lower *b* parameter in summer (β = -0.54, S.E. = 0.27, *P* = 0.05; Table 2). Warmouth *Lepomis gulosus* (Cuvier 1829) WLR was significantly lower in clearwater streams (β = -0.44, S.E. = 0.13, *P* = 0.007) and had a significant interaction with streamtype*logL, indicating *b* lower in clearwater streams (β = 0.22, S.E. = 0.07, *P* = 0.004; Table 2). Bluegill *Lepomis macrochirus* (Rafinesque 1819) *b* was significantly less in spring (β = -0.12, S.E. = 0.06, *P* = 0.03) and summer (β = -0.32, S.E. = 0.07, *P* = <0.0001) than fall and had a significant season*logL interaction with *b* higher in spring (β = 0.09, S.E. = 0.03, *P* = 0.008) and summer (β = 0.19, S.E. = 0.04, *P* = <0.0001) than fall (Table 2). Redspotted sunfish *Lepomis miniatus* (Jordan 1877) WLR was significantly lower in clearwater streams (β = -0.21, S.E. = 0.06, *P* = 0.002; Table 2). Redspotted Sunfish also had a stream type*logL interaction with a steeper *b* in clearwater streams (β = 0.11, S.E. = 0.03, *P* = 0.0002; Table 2). Flagfin Shiner *b* parameter was higher in clearwater streams (β = 0.34, S.E. = 0.09, *P* = 0.02) than blackwater streams, there was also a significant type*LogL interaction with a lower *b* in clearwater (β = -0.25, S.E. = 0.06, *P* = <0.0001) than blackwater streams (Table 2).

All species WLR analyses indicated no difference by stream type (β = 0.11, S.E. = 0.11, *P* = 0.32; Table 2). However, there was a significant negative stream type*logL interaction indicating a lower *b* across species in clearwater streams (β = -0.12, S.E. = 0.04, *P* = 0.005; Table 2). Across species, there were also differences in *b* of both spring (β = -0.20, S.E. = 0.1, *P* = 0.04) and summer (β = -0.22, S.E. = 0.1, *P* = 0.02) compared to fall indicating lower *b* in those seasons (Table 2). Similarly, there were significant season*logL interactions with higher logL in spring (β = 0.15, S.E. = 0.06, *P* = 0.008) and summer (β = 0.13, S.E. = 0.05, *P* = 0.01) than fall (Table 2).

4.3.3 Lentic vs. Lotic Comparison

Our FishBase analyses investigating the influence of lentic vs. lotic habitat on *b* parameters of species in our study included 135 records for 7 species (Yellow Bullhead *Ameiurus natalis* (Lesueur 1819), American Eel, Pirate Perch, Green Sunfish *Lepomis cyanellus* (Rafinesque 1819), Warmouth, Bluegill, and Largemouth Bass *Micropterus salmoides* (Lacepède 1802)). Results indicated that fishes captured in lotic habitat had a smaller *b* parameter (β = -0.35, S.E. = 0.06, *P* = <0.0001) than those captured in lentic habitat. WLR parameters from Banded Pygmy Sunfish, Sharpfin Chubsucker *Erimyzon tenuis* (1855), Southern Brook Lamprey, Redspotted Sunfish, Speckled Madtom *Noturus leptacanthus* (Jordan 1877), Blackbanded Darter *Percina nigrofasciata* (Agassiz 1854), and Sailfin Shiner *Pteronotropis hypselopterus* (Putnam 1863) were not listed on FishBase and thus were not included in these analyses.

4.3.4 Environmental Mixed Models

Results of multi-level mixed models investigating the potential importance of environmental parameters with logW indicated relationships with 2 parameters. Across all species, streamwater pH was significantly positively correlated with logW (β = 0.37, S.E. = 0.15, *P* = 0.02). Invertebrate density was marginally significantly correlated with logW (β = 0.00005, S.E. = 0.00003, *P* = 0.08). CWD was removed from the model due to a vif >5 indicating moderate collinearity with other variables in the model (Quinn and Keough 2002).

4.3.5 Stream Type and Trait Mixed Models

Results of multi-level mixed models investigating the potential influence of stream type, functional groups, longevity and fecundity indicated IPs were significantly lower for clearwater indicator species (β = –65.98, S.E. = 25.28, *P* = 0.03; Table 3) versus the sole blackwater indicator species values or species that were not indicator species. IPs were also lower for surface/water column feeders (β = –39.77, S.E. = 13.18, *P* = 0.02; Table 3). IPs increased with an increase in longevity (β = 12.83, S.E. = 2.64, *P* = 0.0013) and decreased as fecundity increased (β = -0.0008, S.E. = 0.0003, *P* = 0.05; Table 3). Slope 1 was greater as longevity increased (β = 0.09, S.E. = 0.03, *P* = 0.008) and less as fecundity increased (β = -0.000009, S.E. = 0.000003, *P* = 0.03; Table 3). Slope 2 was less for surface/column feeders (β = -0.64, S.E. = 0.28, P = 0.05) and increased as longevity increased (β = 0.25, S.E. = 0.06, P = 0.002; Table 3). IPs, slope 1, and slope 2 for each individual species are shown in Table 4. Individual piecewise regression results for bluegill and redfin pickerel are shown in Figure 2. Piecewise regression results for benthic and surface water column species is shown in Figure 3. American eel was excluded from this analysis as it was not included in the traits database (Frimpong and Angermeier 2009). Weed shiners were also excluded from the analysis as there were no blackwater captures of this species.

4.4 Discussion

4.4.1 WLR Parameters

Of the 17 fish species for which we calculated WLR parameters, six had little or no previous records of WLR parameters. Seven species had WLR parameters that appear slightly to moderately outside of published estimates, although some of them were not based on species specific accounts but rather on subfamily body shape (Froese et al. 2013). Specifically, 3 cyprinid species, the Weed Shiner, Sailfin Shiner, and Flagfin Shiner, all had *b* parameters ~ 3.3 larger than the Bayesian predicted value from Froese et al. (2013). Closer to the *b* parameter of Largemouth Bass, a much deeper-bodied species. Typically, *b* is expected to increase with body depth, although the latter varies across season, year, and geographic location (Froese 2006). Seasonally it has been found that some fish species have specimens in best non-spawning condition in fall (Froese 2006). In intermittent systems seasonal-water flow dynamics can greatly influence growth parameters (Oliva-Paterna 2003). Geographic variability can indicate differences in body shape amongst populations but may also indicate variability in other factors as aquatic environments tend to be highly variable (LeCren 1951, Frimpong and Angermeier 2010). Annual environmental conditions can also fluctuate resulting in different WLR on a yearly basis (Froese 2006).

Our results also showed a broad across-species difference in the slope of log length slope by type, being lower in clearwater than blackwater streams. Indicating that for each unit gain in log length there was less of a gain in weight in clearwater streams. Typically, fish that have longer, thinner bodies have a lower exponent of *b* and larger, deeper bodied fish, especially as adults, have a larger *b* exponent (Froese 2006). However, we found a lower *b* for several species, 2 centrarchids and pirate perch, in clearwater streams coupled with positive interactions for type and log length. Indicating that although these species typically acquired more weight per unit length in clearwater streams they had a lower *b* in these populations. It should not be assumed that a lower *b* necessarily indicates a negative response to clearwater habitat as each species that had a lower *b* in this type also was an indicator species, presumably indicating an affiliation with this type. Interestingly, the only species to not share this general trend was the flagfin shiner which had a negative log length and type interaction and a lower b parameter in clearwater streams.

Differences in *b* between blackwater and clearwater populations may be due to hydrological differences resulting from different primary water sources, such as deep versus shallow groundwater or surface water (Smock and Gilinsky 1992, O'Donnell et al. 2010). Flow parameters have shown influence on fish growth parameters in other systems (Oliva-Paterna 2003). Differences in hydrology between stream types will likely differentially influence habitat parameters and fish assemblages (Poff and Ward 1989, Poff and Allan 1995). Fishes of faster flowing waters are typically more stream-lined and fishes in slow-flowing waters are typically deeper-bodied (Hynes 1970). This phenomenon of differential source water is not well supported for blackwater and clearwater streams in the SE US, although recent analyses of the hydrology and morphology of these study streams found increased discharge and depth in clearwater streams (S. Colvin and B.P. Schneid, unpublished data). Greater flow variation in clearwater streams could result in an overall more streamlined fish assemblage.

4.4.2 Seasonal Variation

These results indicate that across species, fish at a given length weighed less in spring and summer than fall, and also that the rate of gain was less in spring and summer months. Overall, WLRs were significantly lower in spring and summer than fall. This relationship is likely indicative of seasonal influences such as adult fish releasing gametes in the spring and early summer (Neumann et al. 2012). Previous studies have also shown that as fishes grow, they exhibit ontogenetic shifts in food and habitat use, often allowing young-of-year to obtain more energetically valuable food resources (Werner and Hall 1988, García-Berthou 2002, Glover et al. 2013). Taken together higher *b* in fall potentially result from a combination of young-of-year shifting into more energetically productive ontogenetic phases and adult fishes allocating resources into somatic growth (Werner and Hall 1988, Helfman et al. 2009, Neumann et al. 2012).

4.4.3 Individual Species Results

Individual fish species WLR results may provide insight into ecological differences experienced between these and populations in other ecosystems. Differences between eel b parameters in our and other southern populations and eels in northern populations may result from seasonal variation in eel life history. During winter temperatures < 5 C, eels typically burrow in the substrate and cease feeding (Walsh et al. 1983). Temperatures in our study area rarely reach < 5 C (National Weather Service 2015). Thus the higher annual and winter minimum temperatures may contribute to differences in *b* between southern and northern populations (Gray and Andrews 1971). We did not detect seasonal differences in the eel *b* as seen in Georgia eel populations of the Altamaha River which had higher *b* in fall (3.32) and summer (3.25) than in spring (3.04) (Helfman et al. 1984). And although the published WLR parameters for many species are calculated from total length and our data was derived from standard length it will only affect the *a* parameter with a greater *a* for standard than total or fork length but it does not change *b* (Froese 2006).

Our estimate of the *b* parameter for the American Eel is slightly higher than the Bayesian estimate from Froese et al. (2013) (i.e., 3.33 versus 3.14-3.24) but not appreciably higher than estimates for southern eel populations from coastal South Carolina (3.34, Harrell and Loyacano 1982) and Georgia (3.04-3.32, Helfman and Bozeman 1984). Other *b* parameters from eel populations in brackish waters (northern or southern), northern populations, or winter months are closer to 3 (Carlander 1969, Hurley 1972, Helfman and Bozeman 1984, Claro and García-

Arteaga 1994). Our results run somewhat contradictory to predictions that fish growth is high in moderately brackish waters resulting in steeper *b* at a given length (Bœuf and Payan 2001, Vøllestad et al. 2004, Glover et al. 2013). Our results found a higher *b* than those reported for brackish populations, these study streams, although coastal are freshwater streams with little estuarine influence except in rare strong storms in the lowest reaches.

Our lowest *b* for Southern Brook Lamprey (2.63) was slightly lower than that estimated by Froese et al. (2013; 3.03) based on based on WLR estimates for this subfamily and body shape. Yet it was not appreciably different than estimates for lamprey species in other systems (Sea Lamprey *Petromyzon marinus*, 2.63 (Applegate 1950), Brook Lamprey *Lampetra planeri,* 2.49 (Verreycken et al. 2011). In other systems, lamprey also have been found to have the lowest *b* parameter of the fishes measured given their elongate body shape (Froese 2006, Verreycken et al. 2011).

The *b* parameter for Largemouth Bass in our study (3.26) was slightly higher than estimated by Froese et al. (2013; 3.08) and slightly higher than the average of *b* values for Largemouth Bass given in Carlander (1977; 3.11). However, *b* parameters for Largemouth Bass in Carlander (1977) were highly variable (2.59-3.48) and derived from populations from differing water body types (pond, lake, river, reservoir), geographic locations (midwestern, western, and southern states, as well as foreign locations) and native and introduced populations. Thus indicating more data on salinity and food resources amongst other variables may be necessary to elucidate factors responsible for differences in *b* across bass populations (Glover et al. 2013).

4.4.4 Lentic vs. Lotic Populations

To corroborate or help explain results of our comparison of the *b* parameter between blackwater and clearwater streams we compared the *b* parameter between lentic and lotic populations of the same species. As our environmental data seems to indicate hydrology may be primary driver of environmental differences between these environments with clearwater streams representing the more lotic and blackwater streams the more lentic environments (S. Colvin and B.P. Schneid, unpublished data). We determined that finding similar patterns between lentic and lotic environments in other systems may aid in our understanding of the parameters influencing differences seen in our populations. Fishes captured in lotic habitats indeed had a significantly smaller *b* parameter as has been reported across many but not all species (Milewski and Brown 1994, Simpkins and Hubert 1996, Hyatt and Hubert 2001, Pope and Kruse 2007). While we did not find this result across all our species we did find lower *b* for several clearwater stream indicator species. Only one of our species, the flagfin shiner, had a higher *b* in blackwater streams.

4.4.5 Environmental Influences on Weight-Length Parameters

Our results indicated pH was significantly positively correlated to logW. Typically, clearwater streams are less acidic than blackwater streams (Winemiller et al. 2008, Duncan and Fernandes 2010). Our pH data on these study streams corroborate earlier findings, though our study did not indicate a large difference in pH between stream types (Winemiller et al. 2008, Duncan and Fernandes 2010, S. Colvin and B.P. Schneid, unpublished data). Low pH can alter oxygen transport, digestion, and ionic balance in fishes and thus be energetically influential in aquatic systems (Val and Almeida-Val 1995, Wilson et al. 1999, Matsuo and Val 2002, Helfman et al. 2009). The lowest recorded pH levels in our streams was 4.6, near the tolerance threshold for many North American minnows and other fish species in other systems indicating that pH levels in our streams may have been low enough to influence the energetics of some fish species (Laerm and Freeman 1986, McDonald et al. 1991).

An increase in invertebrate density was marginally significantly correlated with logW. Invertebrate density has been correlated with fish growth in other systems and has been indicated as an important predictor of habitat suitability (Rosenfeld et al. 2005, Rosenfeld and Taylor 2009). Our study may have further elucidated the relationship between fish WLR parameters and invertebrate density especially for fall and spring.

4.4.6 Influences of Stream Type and Fish Traits on Slopes and Inflection Points

Typically, the WLR relationship in early life stages indicates relatively slow attainment of weight with an increase in length, a rate that increases as the fish grows (Froese 2006). The transition between these two slopes can indicate a shift in diet (Stergiou and Fourtouni 1991, Werner and Hall 1988, King 2004). The shift between these two slopes, the inflection points (IPs), occurred at a smaller size for clearwater indicator species. This earlier shift could indicate that either these species have genetically fixed behaviors that result in an earlier shift or that populations of these species in clearwater streams are experiencing conditions that favor their switching to the next ontogenetic niche more quickly (Werner and Gilliam 1984, Werner and Hall 1988, Mark et al. 1989). Typically, as fish grow and shift from an early to later ontogenetic niche, mortality rates decrease (Alexander 1979, McGurk 1986, Richards and Lindeman 1987, Helfman et al. 2009). Thus an earlier shift would likely favor overall survival. Clearwater streams support a greater number of species than blackwater streams (17 vs. 14) (S. Colvin, unpublished data) the earlier shift may favor the survival for some additional species (Helfman et al. 2009).

IPs also occurred earlier for surface/water column feeders than for benthic species accounting for effects of stream type, longevity, fecundity, and status as indicator species. Metamorphosis from larval to a juvenile form is variable across fish species (Vilizzi and Walker 1999). Surface/water column feeders may need to transition to a larger more efficient swimming larval or juvenile form earlier than benthic feeders to overcome viscous forces (Purcell 1977, Hunter 1981, Helfman et al. 2009). Accuracy in feeding ability has been shown to increase with an increase in size in fishes (Blaxter 1969, Hunter 1981, Kamler 1992, Helfman et al. 2009). IPs occurred at a slightly larger size as longevity increased. Our smallest inflection point occurred in Banded Pygmy Sunfish, individuals of which infrequently live beyond 13 months and rarely exceed 30 mm in length (Boshung and Mayden 2004).

Slope 1 (prior to IP) increased with longevity and decreased with total fecundity. These results are more difficult to interpret because fecundity typically increased with longevity in these fish. However, this slope is only indicative of early larval life and a shorter larval period typically results in larvae being able to transform to juveniles more quickly whereas a longer larval period can result in longer distance dispersal (Helfman et al. 2009). More investigation is needed to determine if some longer-lived fish were spawning further from appropriate nursery grounds such that larvae would be more adapted to a longer drift period (Helfman et al. 2009). Slope 2 (post IP) was significantly less for surface/column feeders potentially indicating faster slopes in benthic fish during this phase.

Between and within populations temporal and geographic variation in temperature, salinity, hydrology, predation, competition, and diet can all influence rate of food conversion and other metabolic processes as well as feeding and locomotion strategies and ultimately morphology (Kinne 1960, Jones 1987, Brönmark and Miner 1992, Day et al. 1994, Pakkasmaa and Piironen 2001, Neves and Monteiro 2003, Rosenfeld et al. 2005, Glover et al. 2013). This approach potentially allows us to elucidate correlations between fish population WLRs and abiotic and biotic parameters not evident in a more typical comparison of species abundances across an environmental gradient. These types of analyses are lacking in stream ecological literature, and particularly in coastal streams and with non-sport species, yet they may provide ecological insight into the factors structuring the fish assemblages of these streams (Fausch et al. 1988, Oliva-Paterna 2003).

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TABLE 1. Weight-length relationships for 17 species from Alabama coastal streams. Parameters in our own study include data from both sexes.

	Assemblage characteristics				Length characteristics				WLR parameters				Range of previously published WLR parameters			
Species	*n*	*n (bw)*	*n (cw)*	IS	Mean	S.E.	Min	Max	*a*	*b*	S.E. (*b*)	r^2	*a*	*b*	Location	Source
Ameiurus natalis	57	33	24	n	141.5	4.7	28	208	0.007	3.12	0.05	0.99	0.02-0.04*	2.66-2.97*	AL, FL, WI	Carlander 1969, Murie et al. 2009, Carlander 1969
Anguilla rostrata	84	59	25	n	218.7	8.4	120	540	0.001	3.33	0.04	0.99	0.0003-0.003*	2.98-3.47*	AL, GA, NY, SC, New Brunswick and Ontario, Canada	Carlander 1969, Helfman and Bozeman 1984, Claro and García-Arteaga 1994, Harrell and Loyacano 1982, Hansen and Eversole 1984, Hurley 1972
Aphredoderus sayanus	243	109	134	n	56.4	0.98	18	91	0.01	3.02	0.03	0.98	0.02*	2.77*	NC	Sheperd and Huish 1978
Elassoma zonatum	17	11	6	n	24.8	1.2	17	34	0.008	3.19	0.15	0.96				
Erimyzon tenuis	19	10	9	n	105.4	8.5	60	195	0.006	3.17	0.16	0.96				
Esox americanus	259	194	65	y,bw	122.4	1.8	52	204	0.004	3.21	0.04	0.96	0.01*	3*	NA	Crawford 1993
Ichthyomyzon gagei	207	10	197	y,cw	83.7	1.4	30	126	0.007	2.63	0.04	0.96				
Lepomis cyanellus	157	6	151	y,cw	59.9	1.5	26	127	0.01	3.03	0.03	0.99	0.002-0.01* 0.02-0.04	3.09-3.29* 2.95-3.2	CA, IA, IL, KY, MO, OK, VT	Small 1975 (KY), Ruiz-Campos 2006 (CA), Carlander 1977 (all others)
Lepomus gulosus	55	23	32	n	78.1	3.1	25	116	0.007	3.23	0.04	0.99	0.01-0.05* 0.03	3.08-3.49* 3.05-3.14	AL, IA, IL, OK	Carlander 1977
Lepomis macrochirus	528	30	498	y,cw	57.5	1.06	21	142	0.008	3.17	0.01	0.99	0.0005-0.32* 0.004-0.06	2.07-3.6* 2.9-3.4	AL, CA, FL, IA, IL, IN, KY, MI, MO, NY, OK, PA, RI, SC, SD, TX, VA, WI	Carlander 1977
Lepomis miniatus	513	275	238	n	74.8	1.04	22	121	0.01	3.10	0.01	0.99				
Micropterus salmoides	43	6	37	y,cw	109.8	7.9	29	200	0.005	3.26	0.05	0.99	0.02-0.04* 0.01-0.03	2.77-3.48* 2.95-3.2	AL, AR, FL, IA, KY, MI, MO, NM, OK, PA, RI, TN, UT, VT, WI, Japan, Spain	Carlander 1977
Notropis texanus	216	18	198	y,cw	48.3	0.7	32	65	0.004	3.40	0.1	0.93				
Noturus leptacanthus	93	0	93	y,cw	73	2.09	18	175	0.006	3.21	0.03	0.99				
Percina nigrofasciata	225	13	212	y,cw	52.9	0.78	30	93	0.004	3.29	0.05	0.95				
Pteronotropis hypselopterus	262	57	205	n	39.7	0.4	24	56	0.005	3.29	0.06	0.92				
Pteronotropis signipinnis	754	571	183	n	36.3	0.29	15	62	0.005	3.32	0.03	0.95				

n is the sample size, *n* (bw) is the blackwater sample size, *n* (cw) is the clearwater sample size, IS indicates whether the species is an indicator species for blackwater (bw) or clearwater (cw) species, mean, S.E., min and max (minumum and maximum) are SL measures in mm, r^2 is the adjusted R-squared, all *P*-values were <0.000 , * indicates data were generated using total length, for the sake of brevity we did not include parameters generated from fork length

TABLE 2. Stream type and seasonal weight-length relationships for 9 species from coastal streams of Alabama (IS = indicator species). Type difference indicates for each unit length an increase (or decrease) in weight due to stream type (bw = blackwater, cw = clearwater). Seasonal difference indicates an increase (or decrease) in weight per unit length difference due to season from the baseline season of fall (Spring (sp), Summer(su)). Parameters include data from both sexes. Weight (gm) and length (mm) are log10 transformed. When 2 values are listed in conjunction the first is spring, the second summer.

Species	*n*	*n* bw	*n* cw	IS	Type difference	Est.	SE	*P*	Type*logL interaction	Est.	SE	*P*	Seasonal difference	Est.	SE	*P*	Season*logL interaction	Est.	SE	*P*
Anguilla rostrata	84	59	25	n	n				n				y, sp	0.61	0.26	0.02	y, sp	-0.25	0.11	0.03
Aphredoderus sayanus	243	109	134	n	y, c	####	0.09	0.02	y, c	0.13	0.05	0.01	y, sp, su	0.39, 0.35	0.18, 0.11	0.03, 0.003	y, sp, su	-0.23, -0.21	0.10, 0.07	0.03, 0.002
Elassoma zonatum	17	11	6	n	n				n				n				y, sp	0.74	0.28	0.05
Esox americanus	259	194	65	y,b	n				n				y, su	0.64	0.24	0.01	y, su	-0.32	0.11	0.005
Ichthyomyzon gagei	207	10	197	y,c	n				n				y, su	-0.54	0.27	0.05	n			
Lepomus gulosus	55	23	32	n	y, c	####	0.13	0.01	y, c	0.22	0.07	0.004	n				n			
Lepomis macrochirus	528	30	498	y,c	n				n				y, sp, su	-0.12, -0.32	0.06, 0.07	.03, <0.0001	y, sp, su	0.09, 0.19	0.03, 0.04	.008, <0.0001
Lepomis miniatus	513	275	238	n	y, c	####	0.06	####	y, c	0.11	0.03	0.0002	n				n			
Pteronotropis signipinnis	754	571	183	n	y, c	0.34	0.09	0.02	y, c	###	0.06	<0.0001	n				n			
All species	3732	1425	2307	NA	n	0.11	0.11	0.32	y, c	###	0.04	0.01	y, sp, su	-0.2, -0.22	0.1, 0.1	0.04, 0.02	y, sp, su	0.15, 0.13	0.06, 0.05	0.008, 0.01

TABLE 3. Results of comparison between piecewise regression parameters used to examine correlations between inflection points and growth slopes 1 and 2 between fish populations in blackwater and clearwater streams and by fish traits.

	Inflection points			*Slope 1*			*Slope 2*		
Group	Est.	SE	*P*	Est.	SE	*P*	Est.	SE	*P*
Intercept	88.72	28.14	0.01	-0.38	0.28	0.20	-0.20	0.61	0.74
Type (clear)	1.91	5.61	0.74	-0.03	0.07	0.72	0.10	0.15	0.49
Indicator species	-65.98	25.28	0.03	0.01	0.25	0.97	-0.69	0.55	0.24
Non-indicator	-39.57	24.51	0.15	0.13	0.25	0.60	0.12	0.53	0.83
Benthic	8.68	14.14	0.56	0.19	0.14	0.21	0.57	0.31	0.10
Surface water	-39.77	13.18	0.02	0.04	0.13	0.75	-0.64	0.28	0.05
Longevity	12.83	2.64	0.00	0.09	0.03	0.01	0.25	0.06	0.00
Fecundity	-0.001	0.00	0.05	-0.00001	0.000003	0.03	-0.00001	0.000007	0.08

TABLE 4. Individual species inflection points (IP), and growth slopes 1 and 2 with trait data from Frimpong and Angermeier (2009).

Species	IS	Population	IP	Slope1	Slope2	Benthic	Surwcol	Longevity	Fecundity
Ameiurus natalis	n	b	131.91	0.58	2.05	TRUE	TRUE	7	7000
Ameiurus natalis	n	c	136	1.11	1.78	TRUE	TRUE	7	7000
Aphredoderus sayanus	n	b	57.4	0.13	0.28	TRUE	TRUE	4	400
Aphredoderus sayanus	n	c	59.13	0.13	0.43	TRUE	TRUE	4	400
Elassoma zonatum	n	b	23	0.03	0.06	TRUE	TRUE	2	970
Elassoma zonatum	n	c	22.11	0.02	0.05	TRUE	TRUE	2	970
Erimyzon tenuis	n	b	153.35	0.65	2.59	TRUE	FALSE	8	10000
Erimyzon tenuis	n	c	125.47	0.33	2.03	TRUE	FALSE	8	10000
Esox americanus	y, b	b	121.23	0.26	0.62	FALSE	TRUE	7	4584
Esox americanus	y, b	c	151.32	0.27	1.17	FALSE	TRUE	7	4584
Ichthyomyzon gagei	y, c	b	93.93	-0.05	0.14	FALSE	FALSE	5	3264
Ichthyomyzon gagei	y, c	c	71.23	0.02	0.05	FALSE	FALSE	5	3264
Lepomis cyanellus	y, c	b	106	0.97	1.54	TRUE	TRUE	8	10000
Lepomis cyanellus	y, c	c	64.21	0.2	0.61	TRUE	TRUE	8	10000
Lepomus gulosus	n	b	81.69	0.33	1.11	TRUE	TRUE	8	63000
Lepomus gulosus	n	c	85.26	0.35	1.4	TRUE	TRUE	8	63000
Lepomis macrochirus	y, c	b	69.38	0.25	0.75	TRUE	TRUE	10	50000
Lepomis macrochirus	y, c	c	77.9	0.22	0.97	TRUE	TRUE	10	50000
Lepomis miniatus	n	b	79.71	0.39	1.16	TRUE	TRUE	5	15000
Lepomis miniatus	n	c	81.71	0.39	1.36	TRUE	TRUE	5	15000
Micropterus salmoides	y, c	b	93.94	0.35	0.51	TRUE	TRUE	16	109314
Micropterus salmoides	y, c	c	131.96	0.34	2.15	TRUE	TRUE	16	109314
Noturus leptacanthus	y, c	b	68.7	0.12	0.52	TRUE	FALSE	2.5	45
Noturus leptacanthus	y, c	c	102.45	0.25	1.02	TRUE	FALSE	2.5	45
Percina nigrofasciata	y, c	b	62.2	0.08	0.34	TRUE	FALSE	2.5	250
Percina nigrofasciata	y, c	c	58.69	0.08	0.21	TRUE	FALSE	2.5	250
Pteronotropis hypselopterus	n	b	36.56	0.03	0.08	FALSE	TRUE	2	800
Pteronotropis hypselopterus	n	c	39.88	0.04	0.09	FALSE	TRUE	2	800
Pteronotropis signipinnis	n	b	40.25	0.05	0.13	TRUE	TRUE	2	500
Pteronotropis signipinnis	n	c	40.63	0.04	0.12	TRUE	TRUE	2	500

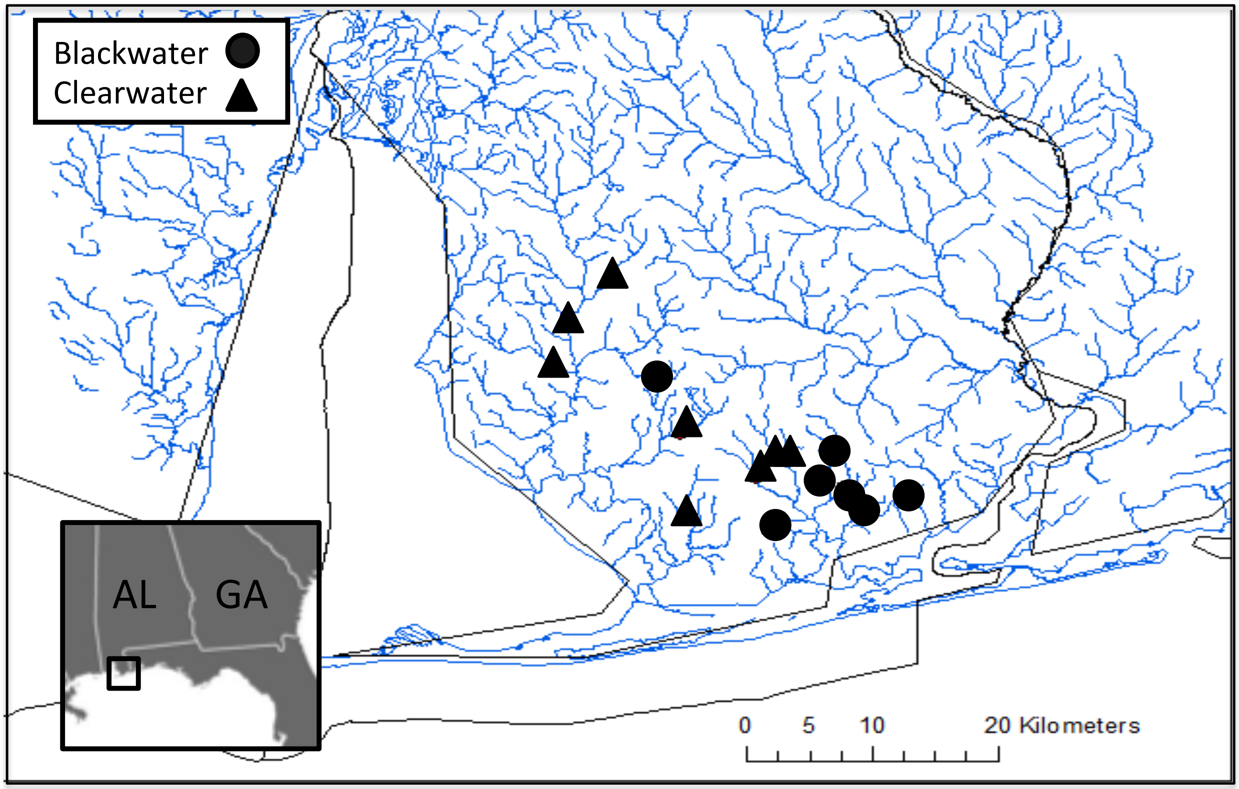

Figure 1. Sites sampled for fishes and environmental variables (clearwater sites ▲, blackwater sites ●).

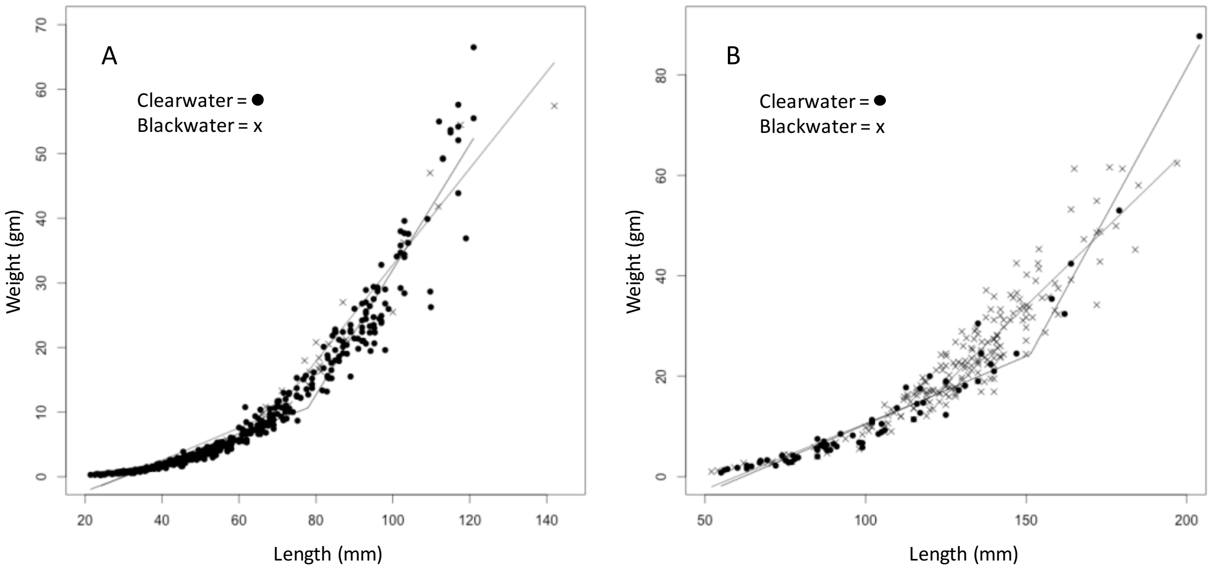

Figure 2. Plots of untransformed length x weight piecewise regression results for clearwater and blackwater populations of Bluegill (A), a clearwater indicator species, and Redfin Pickerel (B) a blackwater indicator species. Both species showed earlier inflection points in blackwater streams.

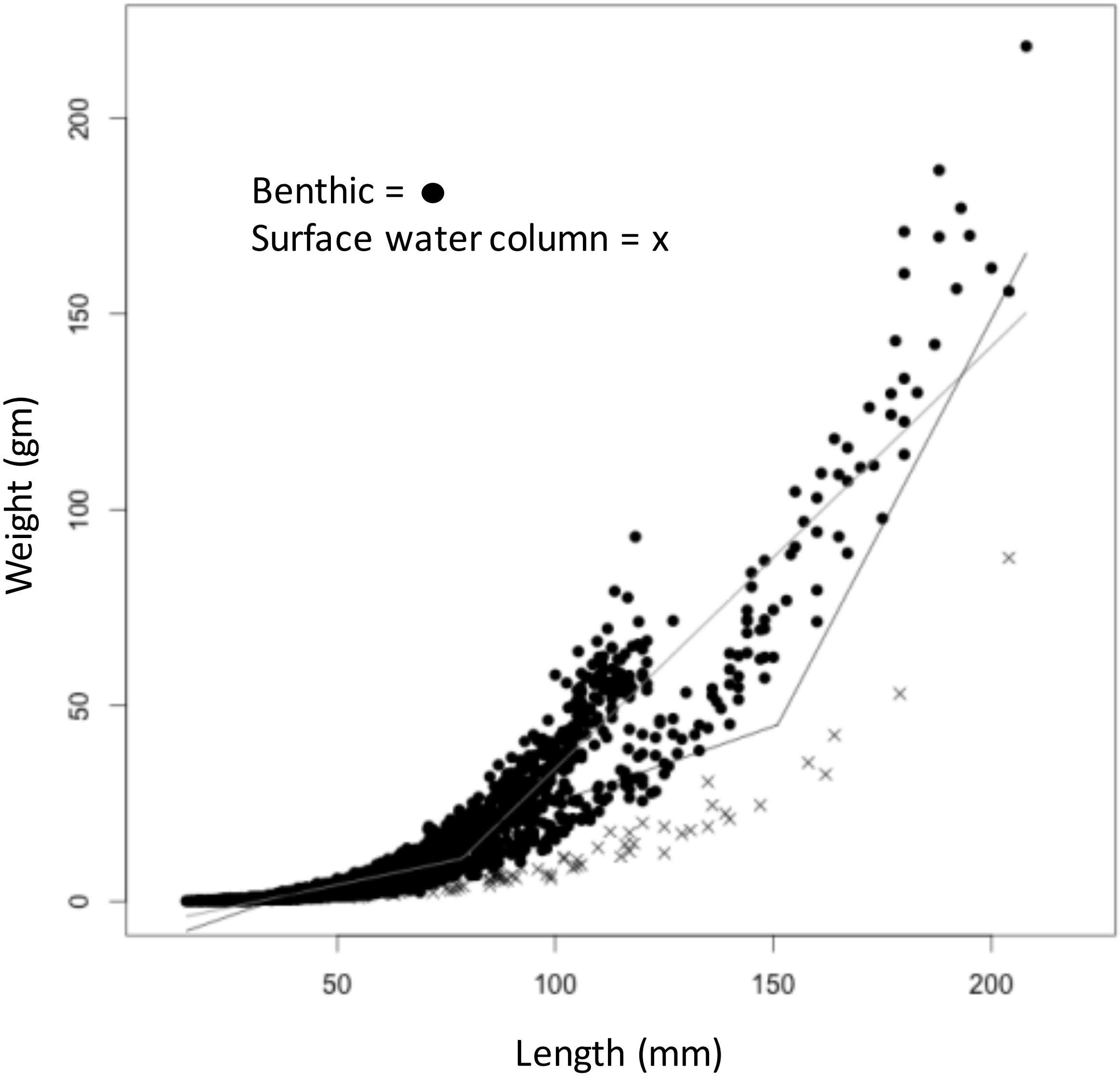

Figure 3. Plot of untransformed length x weight piecewise regression results for populations of benthic and surface water column species.