University Honors Program Theses

2019

A multi-scale investigation of habitat selectivity in Coastal Plain stream fishes

Hayley A. Robinson
Georgia Southern University

A multi-scale investigation of habitat selectivity in Coastal Plain stream fishes

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Biology

By Hayley A. Robinson

Under the mentorship of Dr. James H. Roberts

Abstract

Studying the habitat use of Coastal Plain fishes enables us to develop a deeper understanding of how fishes thrive in this highly variable environment. Based on previous research by Dr. Roberts and his students, Coastal Plain fishes seem to sort into two groups: (1) species selecting stream reaches that continue to flow throughout the summer (i.e., fluvial species [F]) and (2) species occurring in streams that may stop flowing in late summer (i.e., nonfluvial species [NF]). For this study, I took a detailed look at eight of these species, spanning the F-NF gradient, and asked which environmental variables (e.g., water quality, stream size, adjacent land use) most influence species occurrence at the spatial scales of stream reaches and microhabitats. Habitat availability and use data came from electrofishing and habitat surveys of 25 sites sampled in summer 2016 and 12 sites re-sampled in summer 2018. At the reach scale, Random forest models indicated that F species consistently selected sites with higher dissolved oxygen, pH, and conductivity, whereas NF species tended to show the opposite pattern. Neither group showed consistent selectivity for stream-size, physical-habitat, or land-use variables. At the microhabitat scale, F species specialized on coarser substrate and higher velocity but showed no preference for large woody debris (LWD). In contrast, NF species specialized on low-velocity and high-LWD microhabitat configurations but showed no substrate selectivity. These findings suggest that habitat selection of Coastal Plain fishes is scale-dependent, and potentially interacts with morphology, feeding strategy, and water-quality tolerance.

Thesis Mentor: _________________________

Dr. James Roberts

Honors Director: _________________________

Dr. Steven Engel

November 2019

Department of Biology
University Honors Program
Georgia Southern University

Introduction

Understanding the habitat needs of different fishes is an important but understudied area of biology. Every species is unique in how it interacts with its environment. Habitat needs of fish are often inferred by studying patterns of habitat use in relation to habitat availability (Meffe and Sheldon 1988). Along a stretch of stream, vast amounts of spaces exist in which a fish can occupy. Each spot in the water will have unique measurements for a range of physical and water quality related variables (depth, temperature, dissolved oxygen, velocity, substrate, etc.). When a fish disproportionately uses microhabitat differently from the observed distribution in the stream, there is an indication of selectivity for those conditions. Selectivity is difficult to observe in the field because extraneous factors (competition, predation risk, availability) are not controlled (Rosendfeld 2003). True habitat requirement (in terms of growth and survival) can more clearly be established once extraneous factors are controlled, like in an experimental setting.

Obtaining a general understanding of habitat ecology is complicated by the fact that many fish species' habitat needs and preferences are context-dependent (Meffe and Sheldon 1988). Selectivity changes with different aspects such as life-stage, hydrologic conditions, and habitat availability. For example, at younger life stages, the Roanoke logperch prefers low-velocity waters and pools, but at later stages, flowing run and riffle habitat over gravel bottoms is more suitable (Rosenberger 2003). Some studies have already been conducted to test the transferability of habitat use models. The consistency and accuracy of these models depends on many factors like species, ontogeny, and scale to which they are being applied (Dunn and Angermeier 2016). In the case of the tangerine darter, a study in Virginia correctly predicted the occurrence of the fish using local and regional models in 64% and 78% of sampled habitat-units respectively (Leftwich 1997). Because there are many possible factors that contribute to why fish occupy a space (feeding success, life stage, spawning), habitat models sometimes have limited transferability between time and space, and in such cases must be tailored to specific situations.

Georgia Coastal Plain streams exhibit some of the most dynamic habitat conditions of any freshwater ecosystem in North America. There is much variation in stream conditions between seasons in this region. During, summer, many streams shrink up in the staggering heat, and dissolved oxygen levels drop from the lack of flow. The water can get very warm, and pH drops low in many areas (Table 3) (Marion et al 2015). Other streams in the region are characterized by their tendency to exhibit continuous flow year-round, which have higher means of dissolved oxygen and lower temperatures. Such harsh and dynamic conditions could lead to the idea that Coastal Plain fishes might need to be tolerant habitat generalists. This means one could expect habitat preferences to be weak for most species, because they would need to tolerate a range of conditions throughout the year.

Streams can typically be thought of as incorporating multiple levels, ranging from stream system to the smallest unit, microhabitat (Frissell 1986). In this study, I focus on two separate levels: the reach system and microhabitat system. Reach systems are defined as linearly existing on a 10^1 -meter scale, while microhabitat exists on the 10^{-1}-meter scale. Contrary to expectations of species being tolerant of water quality conditions and differences in habitat in this region, significant variation exists in occurrence of Georgia Coastal Plain fishes on multiple scales. This coincides with geographic variation in whether a reach experiences continuous flow throughout the summer (fluvial sites) or not (nonfluvial sites) (Marion et al 2015). This gradient that exists seasonally in stream-type for this region suggests that at least some of the Coastal Plains fishes exhibit habitat selectivity for conditions during the summer, and therefore separate into assemblages typically in either the fluvial or nonfluvial sites (Scott 2018). To an extent, it has been shown that certain microhabitat variables (i.e. depth and velocity) and reach-scale variables (i.e. land use and stream size) can be good predictors of overall fish assemblage in blackwater systems (Meffe and Sheldon 1988, Marion et al 2015). However, habitat preferences of most individual Coastal Plain species are poorly studied, so it is not clear which habitat variables are being selected for. Even when selectivity is observed, many variables (i.e. dissolved oxygen, substrate coarseness, pH) are strongly correlated with each other, making it difficult to establish how influential each one is individually. Another issue is whether these relationships are consistent spatially, temporally, or across assemblage type.

The goal of this study is to improve our understanding of why Coastal Plain fishes select the habitats they occupy, at scales ranging from microhabitats to stream reaches, and to assess the consistency of these habitat choices across locations and across ecologically similar species. My expectations are that differences in selectivity will be observed across the F-NF gradient for multiple scales. On the reach-scale, there are important differences in a fluvial vs. nonfluvial site in relation to flow and water quality conditions, and different species will likely select sites with certain characteristics fitting to their specific life habit. At the microhabitat scale, I expect to see selectivity based on the differential utilization of spaces like physical habitat and flow regime. My multiscale study used field sampling and a prior study on predictors of fish structure to analyze selectivity of common species along the observed F-NF gradient within a group of study sites in the Coastal Plain.

Experimental Design

My data set for examining reach-scale habitat selection was collected in summer 2016 by Becky Scott as part of her M.S. thesis work in Dr. Roberts' lab (Scott 2018). She selected 25 sites for study, in wadable freshwater streams within the Altamaha, Ogeechee, and Savannah river basins in the vicinity of Statesboro (Figure 2). Each site was a 150-m-long reach of stream, sampled using methods described below. To characterize microhabitat-scale habitat selection, I re-sampled 12 of these sites in 2018. These were the only 12 sites the field crew were able to sample for logistical and accessibility reasons. A summary of candidate habitat variables for this study and their origins can be found in Table 2.

Fish Sampling- On each sampling occasion, each site's fish community was characterized by backpack electrofishing in upstream direction, using two Halltech direct-current backpack electrofishers and four dipnets (Figure 1). The abundance of each species was recorded, resulting in presence/absence records for all species at each site. In 2018, I also marked the specific capture location of each individual of my focal species using an orange flag. Focal species included American eel (*Anguilla rostrata)*, blackbanded darter (*Percina nigrofasciata)*, flier (*Centrarchus macropterus*, largemouth bass (*Micropterus salmoides*), mud sunfish (*Acantharchus pomotis)*, speckled madtom (*Noturus leptacanthus)*, spotted sucker (*Minytrema melanops)*, and warmouth (*Lepomis gulosus)*. These species were selected because they are common and abundant enough to provide sufficient data points, as well as their representation of species across the postulated F-NF gradient. They are present at a range of sites and are not considered habitat generalists (Table 1).

Microhabitat measurement- Following fish sampling, to measure the overall microhabitat availability within the site, we began at the downstream starting point, with every 10 meters of stream being analyzed as a transect perpendicular to the banks. Starting on either bank, depth, velocity, and substrate were measured every 1 meter across the width of the stream for overall habitat availability measurements. Depth was measured with a meter stick, and velocity was measured with a Swoffer flow meter at 0.6x depth for accurate measurement of highest flow within the water column. Substrate type (detritus, mud, sand, or gravel) was noted at each spot. Large woody debris (LWD) items were counted within each 10-meter-long section of the site. For comparison with microhabitat availability, I characterized habitat use for each captured individual of focal species by measuring these same habitat attributes at each orange flag. Depth, velocity, and substrate were recorded at the exact spot of the flag, whereas LWD was measured for the entire 10-m section in which that flag occurred.

Reach Measurement- Temperature, dissolved oxygen, and conductivity were measured at the downstream starting point of each site following fish sampling using a YSI Pro2030 meter, and pH was measured using a Eutech Instruments pHTestr. Because all three of my water quality variables (dissolved oxygen, pH, and conductivity) were strongly positively correlated with each other, I chose not to use them as individual predictor variables in models. Rather, I performed a principal component analysis (PCA) to reduce water-quality variation among sites to a single dimension capturing the majority of the variation. Data were centered and standardized prior to analysis and a PCA was run in R. I then used the first principal component resulting from the PCA as an index of water quality in downstream analyses. Other reach-scale habitat data (mean depth, mean width, mean LWD, average bank height, and % sand gravel) came from field measurements taken by Scott (2018). Data points taken post hoc include % development, % agriculture, sinuosity, gradient, drainage area. Sinuosity and gradient were calculated using ArGIS 10.4. Variables related to land cover (% development and % agriculture) were determined by analysis of Coastal Change Analysis Program Land Cover Atlas (C-CAP) land raster data as well as integration of ArcGIS 10.4.

Data analysis – Data from in-stream microhabitat measurements were compiled into separate histograms for each variable and focal species which analyzed use compared to overall habitat availability across all sites in which a species occurred. Spotted suckers were not analyzed for microhabitat preference due to small sample size. Furthermore, largemouth bass were split into juvenile and adult stages for analysis due to observed differences in selection based on life stage. To assess microhabitat-scale selectivity, I binned each microhabitat variable into 4-13 bins (depending on the variable) and then, for each variable for each species, used a chi-square test to evaluate whether the distribution of habitat use differed significantly from the distribution of habitat availability (both characterized as percent frequency). Tests with $P < 0.05$ were taken as evidence for non-random selection of habitat. Only sites where a given species was captured were used for this analysis. Spotted suckers were excluded from this analysis due to small sample size. To assess reach-scale selectivity, I used random forest (RF) regression models in the random Forest package for R to model the presence or absence of each focal species among sites as a function of the reach-scale habitat variables. RF is a nonlinear regression technique that seeks to split up the 25 sites into homogeneous groups – in this case groups of sites with consistently high or low species presence – based on splitting rules developed from the independent variables. By systematically building splitting rules based on randomly-drawn subsets of the independent variables, and evaluating how well different variables discriminate present vs. absent sites, RF provides a measure of the relative importance of each variable for predicting that species' occurrence. Namely, importance is measured as the % decrease in model prediction accuracy when that variable is removed from models; thus greater decreases in accuracy indicate greater importance of that variable. I considered all variables with importance scores ≥ 10 to merit interpretation. In addition to the importance of each habitat variable, for each species I also recorded the percent accuracy of the model for correctly predicting species presence at occupied sites (sensitivity) and for correctly predicting absence at unoccupied sites (specificity). All else being equal, a completely uninformative model would exhibit a sensitivity of p and a specificity of $1-p$, where p is the proportion of sites occupied by that species (i.e., the model does no better than random). Classification rates higher than this indicate a model that predicts presence/absence better than random.

Results

Microhabitat scale

For depth, all species showed nonrandom selection for shallower depths across sites (Figure 4). Similarly, all species showed nonrandom selection for velocity, but there was a clear separation in assemblage preference. F species tended to select faster velocities, and NF species selected slower velocities (Figure 5). LWD selectivity was not as defined between assemblages. Four species showed nonrandom selectivity for LWD: mud sunfish, warmouth, flier, and speckled madtom (Figure 6). These species all showed selectivity for higher counts of LWD within a site. Two F species (American eel and blackbanded darter) showed nonrandom preference for substrate type, selecting gravel over other types (Figure 7). All associations are reflected by significant P-values determined during Chi-square tests, listed under each panel in the figures.

Reach scale

Using random forest regression analysis, I looked at the relationship each reach-scale variable has to the presence or absence of each of my focal species (Table 6). The variables ranged in importance for each of the focal species as well as positively and negatively in influence. The variable that was most important overall in influencing species occurrence was water quality PCA axis 1 (WQPCA), for which higher values indicate greater dissolved oxygen, pH, and conductivity. This variable was positively associated with site occupancy for 4 F species (spotted sucker, American eel, blackbanded darter, and speckled madtom), but negatively associated with occurrence for 2 NF species (warmouth and flier). For largemouth bass, the relationship with WQPCA was complex (i.e., not uniformly positive or negative, but multimodal). Sand/gravel was an important variable for 2 species from each assemblage; positively for F species (blackbanded darter and speckled madtom) and highly negative for NF species (flier and warmouth). Drainage area was another variable that was determined to be important to several species. Three F species were positively affected by drainage area (spotted sucker, American eel, largemouth bass). One NF species, fliers, had a negative relationship with drainage area, but the importance factor was not high. Sinuosity had slightly negative effects on 2 F species; American eels and blackbanded darters. Gradient was determined to have a complex relationship in occurrence of speckled madtoms, of which direction was not able to be found. Mean depth positively influenced largemouth bass occurrence while negatively influencing warmouth occurrence. Two land use variables (% development and % agriculture) had slightly negative effects on largemouth bass and blackbanded darters, respectively. Warmouths had a slightly negative relationship with average bank height. Mean LWD positively influenced blackbanded darter occurrence while having a complex relationship to American eel. It also negatively influenced mud sunfish occurrence. Mean width and basin did not demonstrate importance values for any species.

This reach-scale analysis was also used to test classification accuracy for each species regarding prediction of presence or absence at a site. The bottom of Table 6 shows a comparison of number of sites which a species occurred overall and the accuracy at which the model predicts occurrence based on filters determined in the random forest model. The model worked best for blackbanded darters (highest percent classification accuracy for presence and absence). It also predicted presence or absence of most other F species well, but not both concurrently. For NF species, classification accuracy was highly predicted for presence of warmouth and absence of mud sunfish but did not provide good accuracy for either aspect of flier occurrence. Accuracy of the model coincides with trends in number of sites of occurrence. The model better predicted presence in species that occurred at a high number of sites (warmouth, largemouth bass, American eel, and blackbanded darter) and absence of species that occurred at few sites (speckled madtom, spotted sucker, and mud sunfish).

Discussion

My results suggest that fishes within Georgia's Coastal Plain select habitat based on multiple variables that are scale dependent. The assemblages (fluvial and nonfluvial) in which our species separate into reflect certain selected patterns in habitat use. Fluvial species selected sites with higher water quality, faster velocities, and coarser substrate, which is reflective of water bodies that exhibit a fluvial nature. Our nonfluvial species selected slower velocities, lower water quality, and finer substrates, all of which are indicative of water bodies that are nonfluvial. When understanding why these patterns have emerged between assemblages, it is important to consider the ecology and biology of each species. The fluvial species in this study all have similar body types: elongated and tube-like or vertically depressed. Their selectivity is likely in association with their morphology; they are adapted for flowing systems which have coarser substrate. This body plan allows them to use a feeding strategy in which they feed on invertebrates drifting down stream. Being able to successfully utilize certain microhabitats with fluvial characteristics is likely helpful for individual fitness. It is also important to note that the nonfluvial species in this study were all laterally compressed centrarchids, which may indicate adaptation to nonfluvial water bodies in a similar way. Laterally compressed fish have body plans built for staying in place, not flowing waters. The morphology and habitat selectivity of this group reflects life habits indicative of fish that are predators who use bursts of speed to snatch food out of the water column, and may be more successful in habitats where they can lie in wait and not expend energy. Overall, the trends in selectivity seem to coincide with the ecology of each species. It was surprising that all species indicated selection for shallower depth, which could be related to food availability and feeding success.

It is important to note possible sources of bias encountered during this study. For the microhabitat study, most sites were within the Ogeechee River watershed. Habitat use was not assessed fairly for populations residing in the Altamaha and Savannah basins, which could have yielded different use patterns on the microhabitat scale for each species than those in other watersheds. Also, there is always human error to be associated with field sampling. Efficiency can always be improved, and it is relevant to consider the possibility of missing individuals when shocking in the field. Another aspect to consider is this study was mostly reflective of adult individuals. This is due to the team attempting consistent fish identification in the field, therefore focusing mostly on adult specimens which are easier to identify. Largemouth bass is the only species to be separated by life stage in this study. Considering the different resource needs for different life stages, separating species into juvenile and adult habitat use could have yielded new patterns in selection.

On the reach-scale, the occurrence of the fluvial assemblage of species was most influenced by water quality. Better water quality is a good indication of occurrence for our selected species from this study. This coincides with other factors related to sites we studied being fluvial in nature that were found to influence the occurrence of the F species assemblage. Sand/gravel substrate and drainage area positively influenced occurrence of F species, while sinuosity had a negative influence. These reach-scale variables are indicative of water bodies that exhibit continuous flow within the Coastal Plain. The reach-scale selection my focal species have exhibited reinforces our idea of selection based on a fluvial-nonfluvial gradient overall within the Coastal Plain and give insight to specific to the mechanisms which drive occurrence.

The reach-scale classification accuracy analysis worked better overall for fluvial species. Some models for individual species, like mud sunfish, had very low accuracy in predicting occurrence, but almost perfect accuracy in predicting absence. Mud sunfish only occurred at 4 sites overall, so understandably the model tries to predict that the species *isn't* there when analyzing for different sites. When applying classification analysis to other situations, one can think about its importance from a management standpoint. If the goal is to locate a rare species, then it is more important for the model to accurately predict or even overpredict presence, not absence. It is important to consider that habitat variables may not be significant at the population level (Dunn and Angermeier 2016) and therefore models applied to management aspects must take that into account. The results from this study show there are most likely more levels to habitat selection than accounted for in the percent classification analysis, and further studies can be done to assess the factors allowing for accurate prediction of presence of species.

There is a possibility that the type of models used in this study will not be transferable over space and time, but establishing selectivity patterns is still fundamental to understanding the true habitat requirements for different species. The success of transferability can be dependent on life stages, resource needs, difference in habitat availability, or saturation of sites by individuals (Mattingly and Galat 2004). Different models have been proven to be specific to certain life stages, especially in their transferability, like the comparison for juvenile and adult rainbow trout which yielded success in application to adults but not to juveniles (Thomas and Bovee 1993).

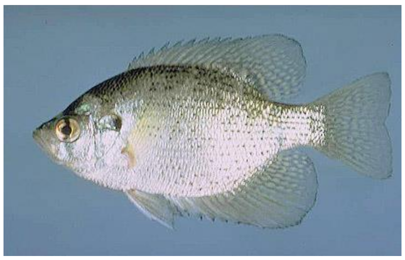
Focal Species

1. *Anguilla rostrata*

(American eel)

2. *Percina nigrofasciata*

(Blackbanded darter)



3. *Centrarchus macropterus*

(Flier)

4. *Micropterus salmoides*

(Largemouth bass)

5. *Acantharchus pomotis*

(Mud sunfish)

6. *Noturus leptacanthus*

(Speckled madtom)

7. *Minytrema melanops*

(Spotted sucker)

8. *Lepomis gulosus*

(Warmouth)

Image Sources

Images 3, 4, 5, 8: https://www.efish.fishwild.vt.edu/

Image 6, 7: https://www.outdooralabama.com/

Image 2: https://alchetron.com/Blackbanded-darter

Image 1: http://www.ncfishes.com/

Table 1. Focal species presence/absence by site. "1" indicates presence at a site, and "0" indicates absence.

Stream	Site	Mud sunfish	Flier	Warmouth	Largemouth bass	Spotted sucker	American eel	Speckled madtom	Blackbanded darter
Ohoopee River	A1D	0	0	1	1	1	1	0	1
Ohoopee River	A1U	0	1	1	1	1	1	0	1
Little Ohoopee River	A2D	0	1	1	1	0	1	1	1
Little Ohoopee River	A2U	0	0	1	1	0	0	0	0
Pendleton Creek	A3D	0	0	0	0	1	1	0	1
Pendleton Creek	A3U	0	0	1	1	1	0	0	1
Buckhead Creek	O1D	0	0	0	0	1	1	1	1
Buckhead Creek	O1U	0	0	0	1	0	1	1	1
Williamson Swamp Creek	O2D	0	0	1	1	1	1	1	1
Williamson Swamp Creek	O2U	0	1	1	1	0	1	1	1
Ogeechee Creek	O3D	1	1	1	1	0	1	0	1
Mill Creek	O4D	0	0	0	1	0	0	1	1
Mill Creek	O4U	1	1	1	0	0	0	0	0
Black Creek	O5D	1	1	1	1	0	1	0	1
Black Creek	O5U	1	1	1	1	0	0	0	0
Canoochee River	O6D	0	0	1	1	1	1	1	1
Canoochee River	O6U	0	0	1	0	0	1	0	0
Fifteenmile Creek	O7D	0	0	1	1	0	1	0	0
Fifteenmile Creek	O7U	0	1	1	0	0	0	0	0
Lotts Creek	O8D	0	0	0	1	0	1	1	1
Lotts Creek	O8U	0	1	1	1	0	1	0	0
Beaverdam Creek	S1D	0	0	1	1	1	1	1	1
Beaverdam Creek	S1U	0	0	1	1	1	1	0	1
Ebenezer Creek	S2D	0	1	1	0	0	0	0	1
Ebenezer Creek	S2U	0	1	1	0	0	0	0	1

Table 2. Summary of candidate habitat variables.

Scale	Variable	Unit	Source
Micro	Depth	cm	Measured at 1-m intervals across transects spaced at 10-m intervals along stream
	Velocity	cm/s	
	Substrate	categorical	
	LWD	number/10 m	Counted between transects
Reach	Water Quality PCA	unitless	PCA analysis used to reduce water quality variation to a single dimension
	Watershed Area	km^2	Calculated using USGS StreamStats software
	Stream Gradient	m	Obtained from Digital Elevation Model data from USGS National Map
	Bank Height	m	Measured in field at every 10 meter transect
	Sinuosity	unitless	Calculated using ArcGIS 10.4
	Mean Depth	m	Calculated from field measurements
	Mean Width	m	Calculated from field measurements
	% Sand/Gravel	%	Calculated from substrate observations in field
	% Development	%	C-CAP classes 2-4 and ArcGIS 10.4
	% Agriculture	%	C-CAP classes 6-7 and ArcGIS 10.4

Table 3. Water quality variables for each site. For analysis, these variables are summarized into a single dimension (water quality PCA or WQPCA) to capture the range of conditions and account for correlation to each other.

Stream	Temperature	pH	DO	Specific Conductivity
Buckhead Creek	26.50	8.30	6.77	156.10
Pendleton Creek	26.70	7.70	4.77	118.00
Buckhead Creek	25.30	7.40	4.50	236.80
Williamson Swamp Creek	26.10	7.40	5.35	90.10
Williamson Swamp Creek	26.60	7.00	5.80	52.90
Mill Creek	27.10	7.00	4.36	75.20
Beaverdam Creek	25.50	7.20	3.47	171.00
Lotts Creek	26.40	7.10	4.05	88.10
Beaverdam Creek	26.60	7.10	2.31	140.50
Ogeechee Creek	26.30	7.30	2.21	64.60
Canoochee River	26.00	6.80	2.29	160.90
Ohoopee River	25.50	7.10	2.01	103.70
Pendleton Creek	24.60	6.50	4.50	160.90
Fifteenmile Creek	25.40	7.10	3.17	62.10
Ebenezer Creek	26.20	7.10	0.78	72.50
Little Ohoopee River	25.50	7.00	0.88	101.60
Ohoopee River	25.70	6.90	1.87	65.40
Black Creek	25.80	6.60	1.75	65.80
Ebenezer Creek	26.10	6.50	0.92	64.90
Lotts Creek	24.20	6.60	3.26	70.20
Black Creek	26.10	6.40	0.73	73.00
Little Ohoopee River	24.00	6.80	1.46	102.70
Fifteenmile Creek	24.70	6.50	1.02	86.30
Mill Creek	24.50	6.30	0.19	90.90
Canoochee River	23.60	6.30	1.97	52.20

Table 4. Summarization of habitat variables measured in-stream for each site.

Stream	Used for Micro?	Basin	Mean Width	Mean Depth	Average Bank Height	Mean LWD	Sub Sand/Gravel
Buckhead Creek	No	Ogeechee	7.200	0.690	1.552	2.667	0.876
Pendleton Creek	No	Altamaha	8.047	0.787	1.733	3.067	0.882
Buckhead Creek	No	Ogeechee	6.067	0.630	0.500	5.333	0.687
Williamson Swamp Creek	Yes	Ogeechee	9.747	0.575	0.655	6.067	0.852
Williamson Swamp Creek	Yes	Ogeechee	8.393	0.281	0.698	3.467	0.600
Mill Creek	Yes	Ogeechee	3.893	0.402	1.550	3.800	0.980
Beaverdam Creek	Yes	Savannah	5.703	0.405	0.174	2.333	0.658
Lotts Creek	Yes	Ogeechee	7.357	0.717	1.545	3.714	0.839
Beaverdam Creek	Yes	Savannah	5.067	0.361	0.571	4.800	0.500
Ogeechee Creek	No	Ogeechee	5.593	0.388	0.691	2.400	0.684
Canoochee River	Yes	Ogeechee	8.393	0.629	1.637	5.533	0.922
Ohoopee River	No	Altamaha	12.167	0.554	0.480	3.067	0.377
Pendleton Creek	No	Altamaha	7.603	0.645	1.377	4.267	0.598
Fifteenmile Creek	No	Ogeechee	6.380	0.319	0.530	4.733	0.593
Ebenezer Creek	No	Savannah	9.307	0.745	1.616	5.800	0.492
Little Ohoopee River	No	Altamaha	7.420	0.330	0.553	5.267	0.113
Ohoopee River	No	Altamaha	9.080	0.413	1.182	4.267	0.726
Black Creek	Yes	Ogeechee	7.247	0.389	0.793	2.933	0.485
Ebenezer Creek	No	Savannah	3.973	0.617	1.193	4.333	0.686
Lotts Creek	Yes	Ogeechee	6.207	0.600	0.563	4.000	0.396
Black Creek	Yes	Ogeechee	6.863	0.440	0.497	2.200	0.571
Little Ohoopee River	No	Altamaha	8.960	0.505	0.632	2.200	0.600
Fifteenmile Creek	No	Ogeechee	7.380	1.343	0.707	2.600	0.489
Mill Creek	Yes	Ogeechee	3.607	0.376	0.412	1.600	0.196
Canoochee River	Yes	Ogeechee	4.173	0.298	0.705	3.733	0.547

Table 5. Summarization of reach-scale habitat variables analyzed through outside sources for each site.

Stream	Used for Micro?	Gradient	Sinuosity	% development	% agriculture
Buckhead Creek	No	0	1.019	1.421	19.801
Pendleton Creek	No	1	1.125	5.394	27.203
Buckhead Creek	No	0	1.042	1.142	30.572
Williamson Swamp Creek	Yes	3	1.365	0.606	25.964
Williamson Swamp Creek	Yes	0	1.000	0.251	8.913
Mill Creek	Yes	0	1.412	3.281	37.243
Beaverdam Creek	Yes	0	1.055	1.346	16.419
Lotts Creek	Yes	0	1.157	3.219	31.930
Beaverdam Creek	Yes	1	1.347	1.024	12.782
Ogeechee Creek	No	0	1.077	2.209	23.694
Canoochee River	Yes	1	1.286	3.600	28.680
Ohoopee River	No	1	1.076	0.587	10.101
Pendleton Creek	No	2	1.284	3.675	30.959
Fifteenmile Creek	No	2	1.104	2.687	24.642
Ebenezer Creek	No	1	1.102	0.881	13.931
Little Ohoopee River	No	0	1.130	0.939	7.245
Ohoopee River	No	1	1.064	1.035	6.956
Black Creek	Yes	0	1.000	2.183	15.501
Ebenezer Creek	No	1	1.195	6.998	9.206
Lotts Creek	Yes	1	1.183	1.812	24.250
Black Creek	Yes	1	1.275	1.856	29.458
Little Ohoopee River	No	0	1.735	0.477	19.177
Fifteenmile Creek	No	2	1.297	1.945	32.295
Mill Creek	Yes	1	1.296	9.958	23.896
Canoochee River	Yes	0	1.871	3.010	29.784

Table 6. Random forest regression analysis of reach-scale variables for focal species. Importance scores for variables with scores >= 10. Positive relationships are in blue; negative in red; complex in italics.

	Mud sunfish	**Flier**	**Warmouth**	**Largemouth bass**	**Spotted sucker**	**American eel**	**Speckled madtom**	**Blackbanded darter**
Basin								
Drainage area		**11**		**13**	**18**	**10**		
Gradient							*10*	
Sinuosity						**14**		**11**
Mean width								
Mean depth			**10**	**16**				
Average bank height			**15**					
Mean LWD	**20**					*14*		**10**
% sand gravel		**34**	**21**				**19**	**16**
Water quality PCA		**13**	**27**	*14*	**12**	**11**	**36**	**46**
% Dev				**18**				
% agriculture								**15**
# of sites occupied	4	11	20	18	9	17	9	18
% Classification accuracy (presences / absences)	25 / 95	64 / 57	95 / 40	100 / 14	33 / 81	88 / 25	67 / 87	94 / 71

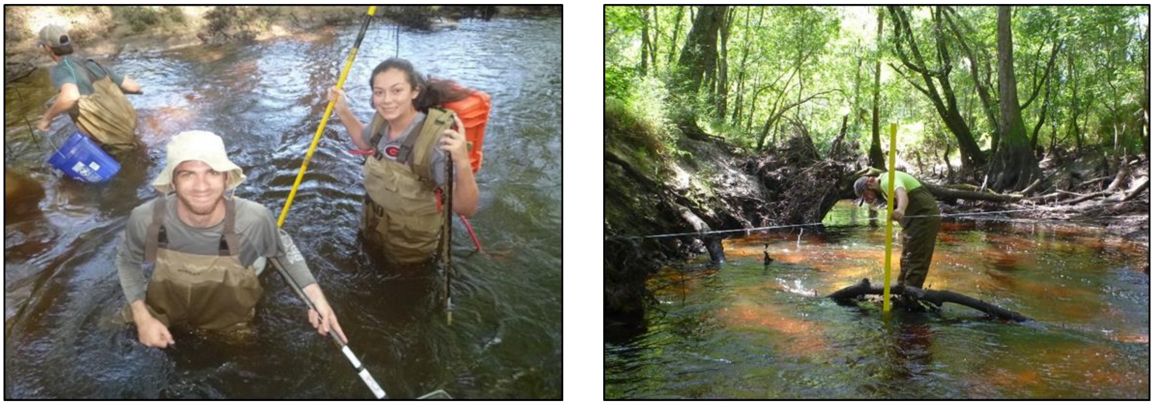


Figure 1. In-stream fish and microhabitat sampling methods, respectively.

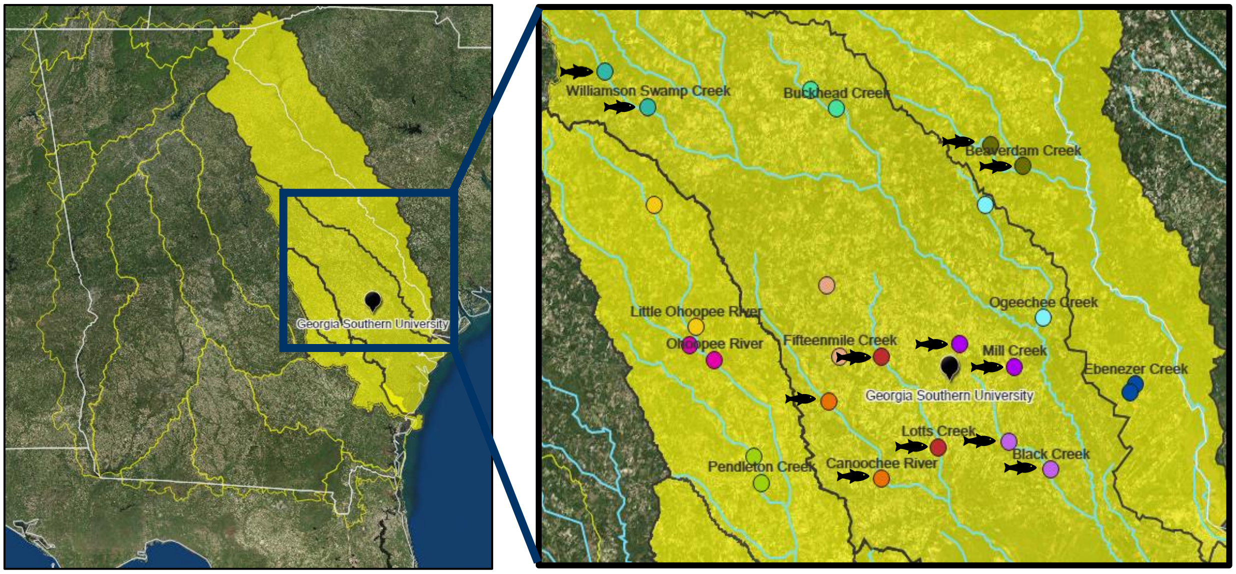


Figure 2. Map of 25 study sites (colored dots) within Georgia's Coastal Plain. Each site was sampled for reach-scale occupancy in 2016. Sites sampled for microhabitat in 2018 are marked with a .

Figure 3. Examples of (A) a fluvial site, Little Ohoopee River and (B) a nonfluvial site, Lotts Creek. Note the woody debris in both sites and difference in velocities.

Depth Associations Across Species

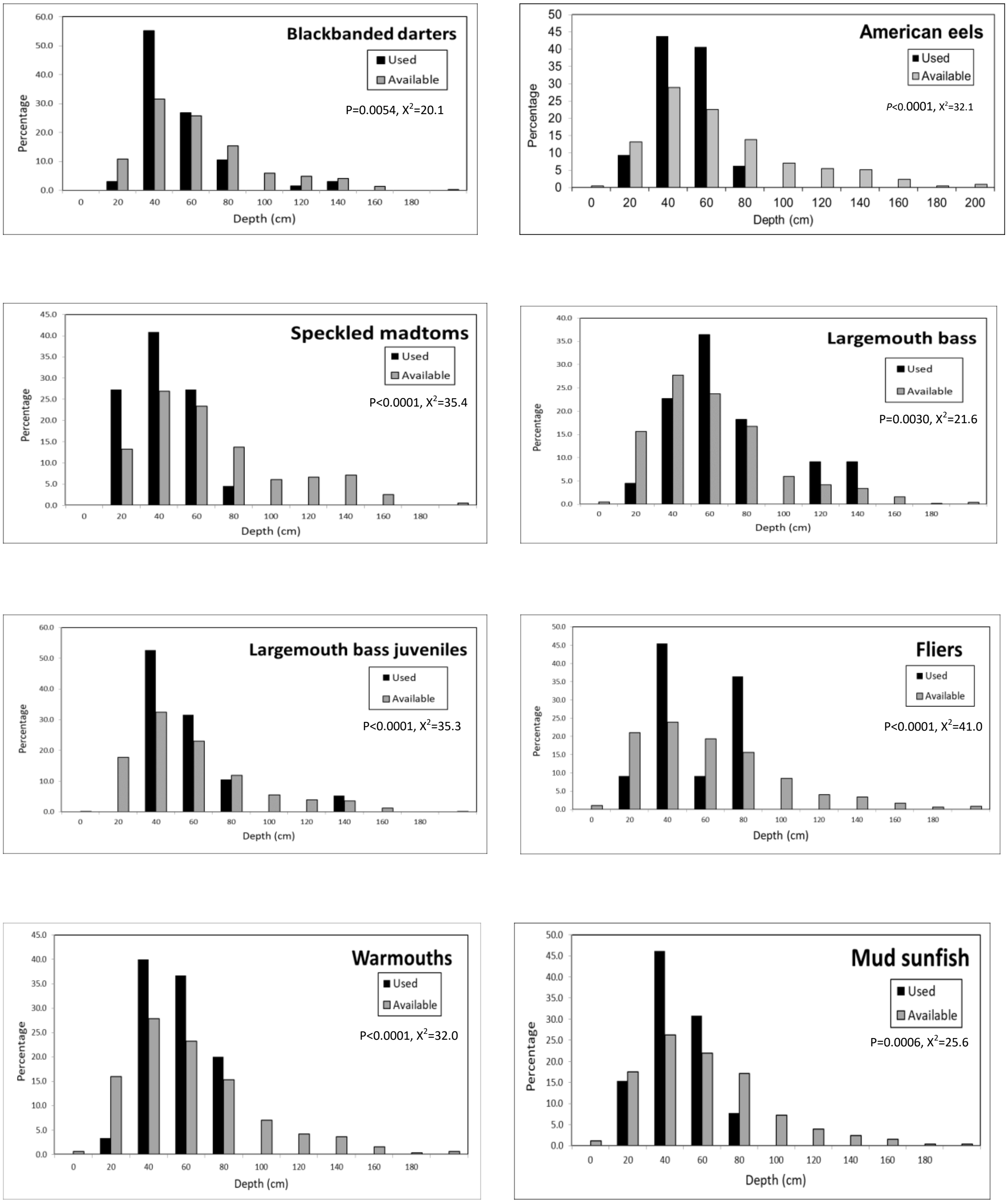


Figure 4. Panel of figures indicating overall species selectivity, if any, for depth across sites they were observed. P-values indicating significance and chi-square values are stated below each panel.

Velocity Associations Across Species

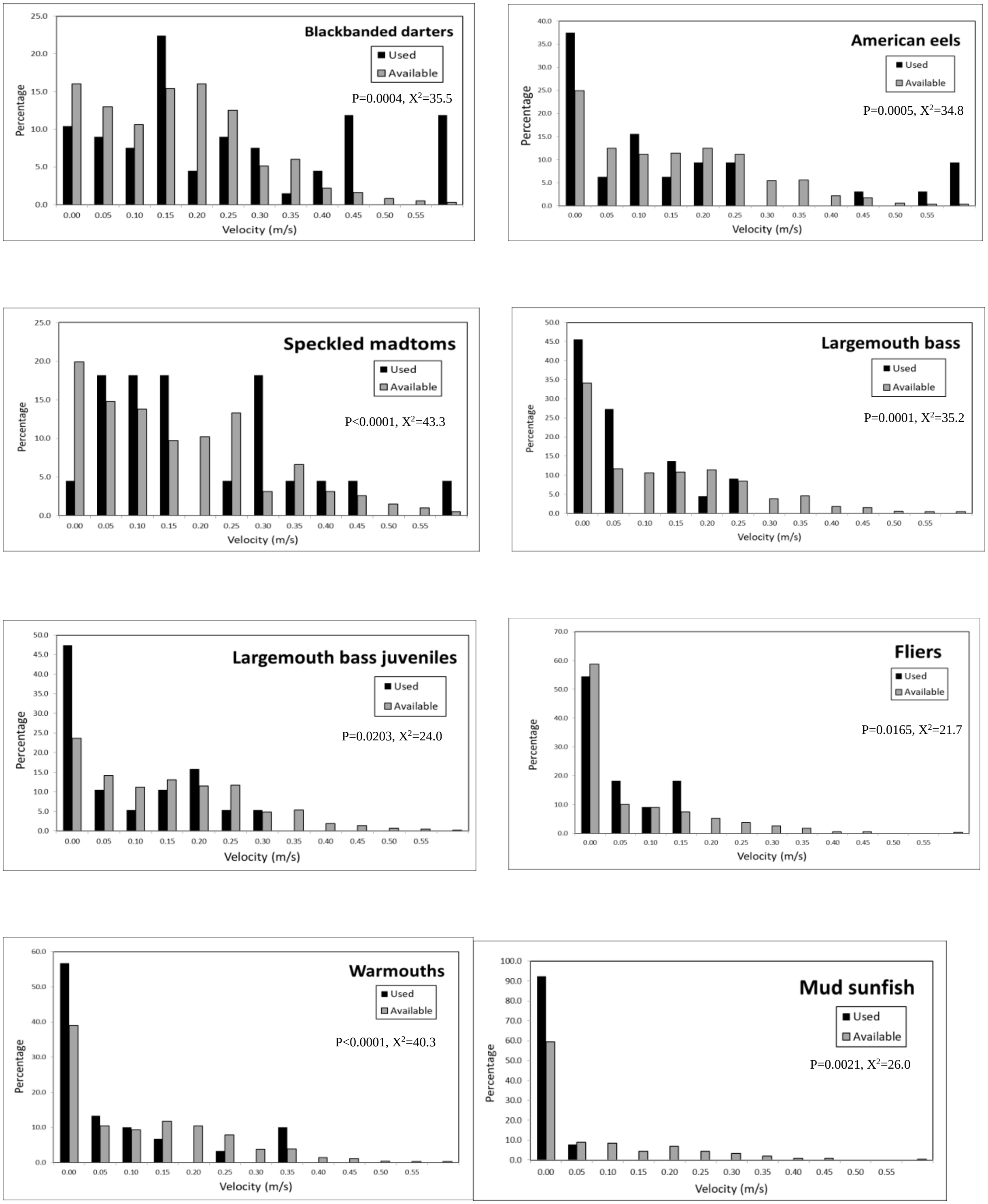


Figure 5. Panel of figures indicating overall species selectivity, if any, for velocity across sites they were observed. P-values indicating significance and chi-square values are stated below each panel.

LWD Associations Across Species

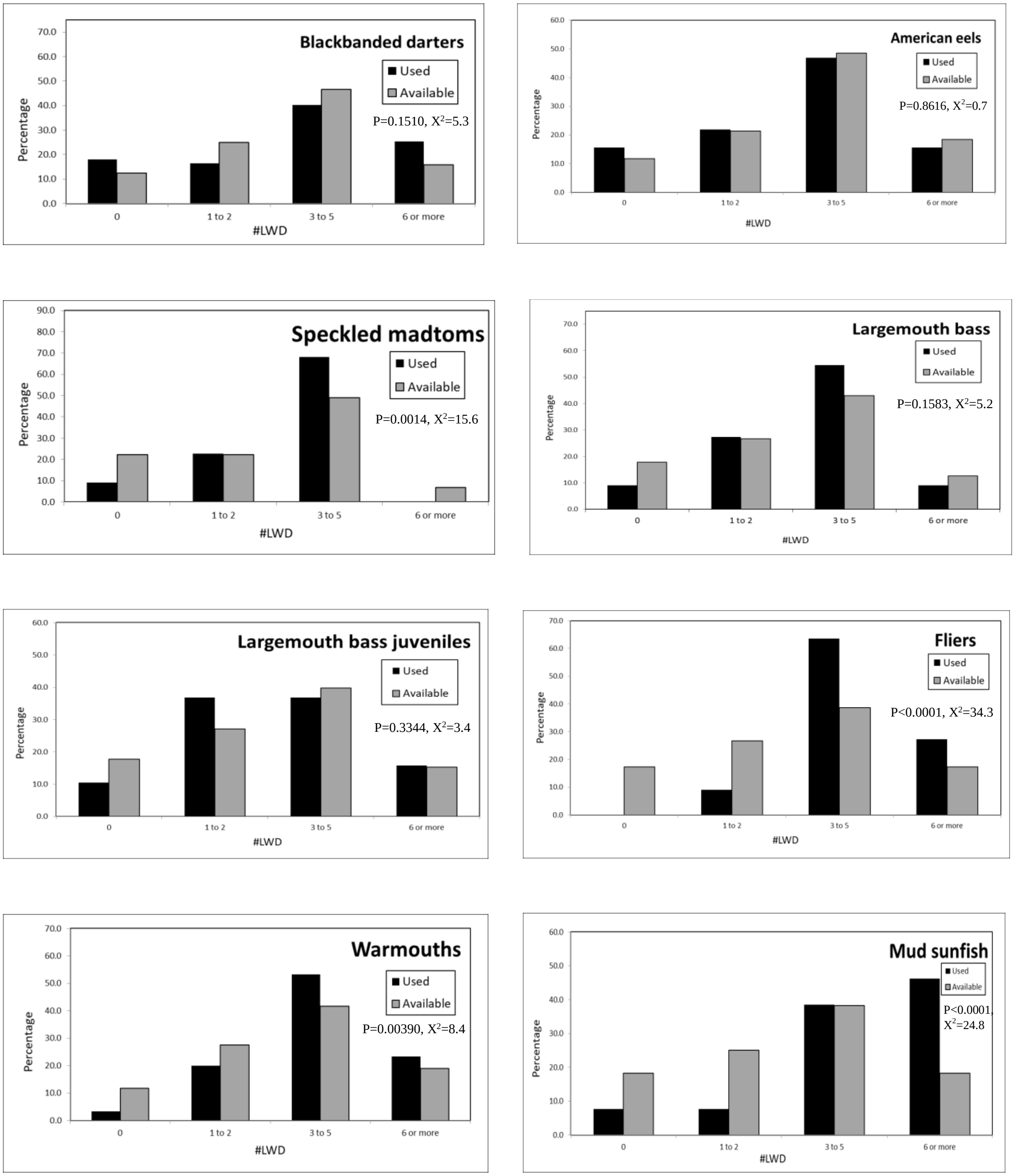


Figure 6. Panel of figures indicating overall species selectivity, if any, for LWD across sites they were observed. P-values indicating significance and chi-square values are stated below each panel.

Substrate Associations Across Species

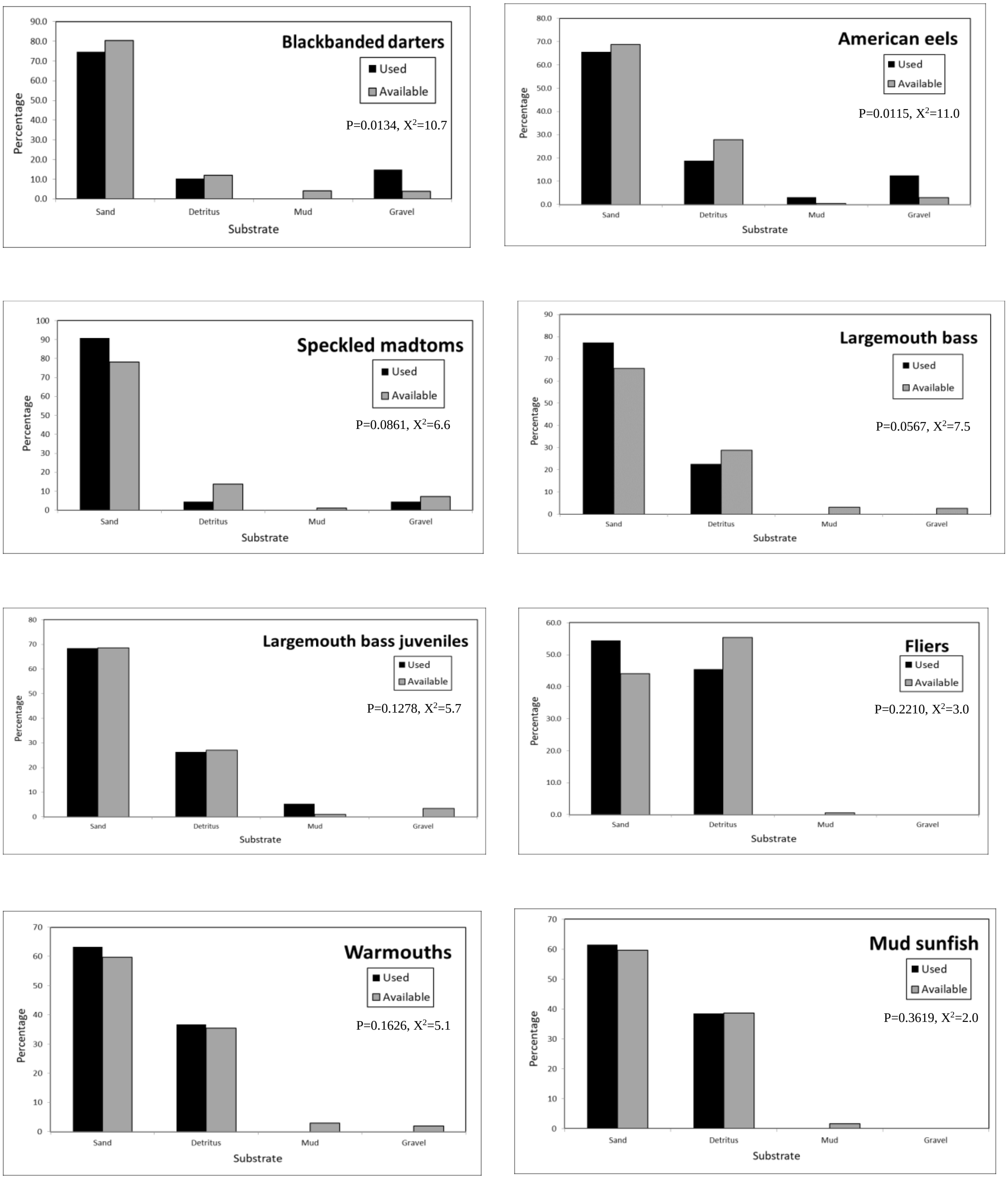


Figure 7. Panel of figures indicating overall species selectivity, if any, for substrate type across sites they were observed. P-values indicating significance and chi-square values are stated below each panel.

References

Dunn C. and Angermeier P. (2016). Development of Habitat Suitability Indices for the Candy Darter, with Cross-Scale Validation across Representative Populations. Transactions of the American Fisheries Society. 145:6, 1266-1281.

Frissell C., et al. (1986). A Heirarchial Framework for Stream Habitat Classification: Viewing Streams in a Watershed Context. Environmental Management Vol. 10, No.2. 199-21

Leftwich K., et al. (1997). Factors influencing behavior and transferability of habitat models for a benthic stream fish. Transactions of the American Fisheries Society. 126. 725-734.

Marion C., et al. (2015) Multiscale Environmental Influences on Fish Assemblage Structure of South Atlantic Coastal Plain Streams. Transactions of the American Fisheries Society. 144:5, 1040-1057.

Mattingly H. and Galat D. (2004). Predictive Performance of a Summer Microhabitat Model for the Threatened Niangua Darter, Etheostoma nianguae. Journal of Freshwater Ecology. 19:1, 109-114.

Meffe G. and Sheldon A. (1988). The Influence of Habitat on Fish Assemblage Composition in Southeastern Blackwater Systems. The American Midland Naturalist, Vol. 120, No. 2. 225-240.

Rosenberger A., et al. (2003). Ontogenetic shifts in habitat use by the endangered Roanoke logperch (*Percina rex*). Freshwater Biology. Vol. 48. 1563-1577.

Rosenfeld J. (2003). Assessing the Habitat Requirements of Stream Fishes: An Overview and Evaluation of Approaches. Transactions of the American Fisheries Society. 132:953-268

Scott R. (2018). Predictors of fish assemblage structure and dynamics in Atlantic Coastal Plain streams. Master's Thesis. Georgia Southern University, Statesboro, Georgia.

Thomas J. and Bovee K. (1993). Application and testing of a procedure to evaluate transferability of habitat suitabiity criteria. Regulated Rivers; Research & Management. Vol 8. 285-294.