

Fish Assemblage Structure in Lentic Vegetated Microhabitat in South Georgia

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John Shannon Salter, Jr.

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This thesis, "Fish Assemblage Structure in Lentic Vegetated Microhabitat in South Georgia," by John Shannon Salter, Jr., is approved by:

Thesis Chair

David L. Bechler, Ph.D.
Professor of Biology

Committee
Member

John F. Elder, Ph.D.
Professor of Biology

Matthew N. Waters, Ph.D.
Assistant Professor of Biology

Section

Brett W. Albanese, Ph.D.
Georgia Dept. of Natural Resources, Nongame Conservation

Interim Dean of the
Graduate School

James T. LaPlant
Assistant Vice President for Research

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ABSTRACT

Aquatic vegetation provides multiple resources such as shelter, food, and breeding habitats for many fish species. Fishes that occupy habitats with similar ecological characteristics are described as fish assemblages. However, not all vegetation offers the same set of resources. Therefore, I hypothesize not all fish assemblages that occupy aquatic vegetation are identical. Based on vegetated structure complexity in the water column, I predicted that submergent vegetation would contain the most fish diversity. This study involved an analysis of fish assemblages at 18 vegetated lentic sites in south Georgia. Total area, percent vegetated surface area coverage, water volume, and major plant species as well as other physicochemical data were recorded for each locality. Comparative analysis of each location was conducted using, one-way ANOVA, Freidman test, Principal Component Analysis (PCA) and linear regression analyses. Thirty-two fish species were collected across all sites, and significant differences in fish assemblages existed between sites. No defining factors related to assemblage structure were identified. PCA identified *Gambusia holbrooki*, *Leptolucania ommata*, *Elassoma okefenokee*, and *Lepomis macrochirus* as principal species defining fish assemblage structure. From these results, three fish subguilds of aquatic vegetation were identified.

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Chapter I

INTRODUCTION

Microhabitat selection is habitat selection on a more finite scale, and as such a few species have evolved to occupy very specific niches. Microhabitats are defined as being composed of environmental variables that affect individual behavior (Morris 1987; Jorgensen 2004). First and foremost, microhabitats are inherently spatial (based on physical structure) because there may be any suite of acceptable microhabitats in a given location but only one that is optimal (McIvor 1988; Morris 1987; Jorgensen 2004). As an example, bird species that live within the low shrub habitat built nests within particular vegetation types in a non-random pattern (Martin 1998). Secondly, the temporal circumstances within the individual's lifetime play a significant role in microhabitat choice. Both life stages of an animal and seasonality can affect microhabitat choice of an individual. Small and juvenile age classes of some species typically choose more complex habitats where more refugia are available because they are subject to higher levels of predators (Leber 1985; Bellows et al. 2001; Rozas et al 1988; Main 1987). Because of the universal position and orientation of the globe, predictable fluctuations of environmental factors are abundant. Therefore, microhabitats of individuals shift with these variables (McIvor 1988; Adolph 1980; Stephenson 1994). In some cases, the microhabitat shift, in this case macrohabitat shift, is so large that it is

hypothesized as the origin of large scale migration (Alerstam 2003; Pulido 2007; Dingle 2007). When the seasonality and/or physical structure are relatively stable, especially in the tropical rain forests near the equator, species do not need to shift microhabitats very often and can evolve to become highly specialized (Pianka 1966). For example in Amphibia, the glass frogs or Centrolenidae of the tropics have translucent skin to aid in camouflage within the dense tropical rain forest (Jacobson 1985). The resources available within a microhabitat determine the species, common or rare, that can live within it.

The Role of Aquatic Vegetation

The density of aquatic macrophytes plays a direct role in the potential ichthyofauna structure because of the area occupied by the structure of the vegetation. The surface area to volume ratio has potential to be very large for some plant species and subsequently allows for colonization of epiphyton which contributes to the base of the food web and ultimately fish diversity (Grenouillet 2002; Kelly & Hawes 2005; Warfe and Barmuta 2006; Thomaz & Cuna 2010). However, as structural complexity and food resources increase, the mobility and therefore ability of predatory species to successfully capture prey is reduced (Grenouillet 2002; Savino et al. 1992; Warfe & Barmuta 2006; Lillie & Budd 1992). The density or complexity of aquatic vegetation can play a distinct role in the fish species diversity. Complexity can increase by the presence of multiple species or increase density of a single species. However, certain types of vegetation create more complex habitats because of morphology. For example, floating vegetation typically has a simple stem within the water column but submergent vegetation has a complex stem with several filamentous leaves which creates a more complex aquatic

environment. It is intuitive to hypothesize that higher ichthyofaunal species richness would correlate with intermediate macrophyte densities where both predator and prey species can flourish (Grenouillet 2002; Savino et al. 1992; Warfe & Barmuta 2006; Valley et al. 2004; Wiley et al. 1984; Mittelbach 2001). Another way of describing the intermediate density hypothesis is that both small and large niches exist in terms of resources available. However, within a defined area, only a certain amount of space is allocated for niches. In other words, either there can be several small niches within highly complex systems, few large niches, or a mix of the two as explained in the intermediate density hypothesis (Thomaz & Cuna 2010; Lillie & Budd 1992; Shmida & Wilson 1985). There is potential for more species richness to be observed in more structurally complex macrophyte habitats because a large number of small niches would be available (Shmida & Wilson 1985; Thomaz & Cuna 2010; Lillie & Budd 1992). However, some research has provided quantifiable evidence that structurally complex systems do not have an impact on predation success or predator growth rates which makes it difficult to predict the effect of vegetation complexity on fish diversity (Warfe & Barmuta 2006; Savino et al. 1992; Kovalenko 2009).

The basis of any aquatic food web within a microhabitat is the primary producers that utilize sunlight via chlorophyll to grow, reproduce, and most importantly supply a food source for consumers. Although macrophytes play an important role in chlorophyll production, algae are responsible for the majority of carbon and energy to consumers such as aquatic invertebrates and fishes (Vadeboncoeur et al. 2006). First and foremost, photosynthesis requires the presence of sunlight which is limited by the presence of obstructions vertically in the water column including phytoplankton, dissolved organic

carbon, turbidity and macrophytes themselves therefore limiting potential algae growth to the upper water column (Jones et al. 2003; Rooney et al. 2003; Binzer 2006). However, if water depth is too great or lacks clarity, the presence of thick and complex vegetation will increase the potential surface area available for attachment. Algae abundance and diversity are positively correlated with the abundance and diversity of invertebrates present. The plants would be deprived of critical resources by the phytoplankton in the upper water column without the invertebrates present to consume to algae (Fuller et al. 1986; Declerck et al. 2007; Jones et al. 2003). Subsequently, the fish fauna mediates the abundance and diversity of the invertebrates (Grenouillet et al. 2002; Jones et al. 2003). Ultimately, the abundance and diversity of microhabitat characteristics play a role in the potential fish fauna.

The reason for varying hypotheses is partially based on the experiments being conducted on different vegetation types that could conflict in structure within the water column. There are three categories for classification of aquatic macrophytes consisting of emergent, floating, and submergent (McDermid & Naiman 1983). In defining emergent vegetation, approximately half of the plant and the majority of foliage are out of the water column. The exact opposite of emergent vegetation would be submergent where most of the plant including foliage is within the water column. Floating vegetation is a median of the two previously mentioned because the majority of the plant can be above or below water but the leaves are confined to the water surface. Because each type of vegetation has its own unique environmental variables associated with it, each vegetation type is associated with a different level of structural complexity (Grenouillet 2002). Of the three types of macrophytes, submergent vegetation is the most structurally complex for aquatic

life because its leaves are whorled around the stem and within the water column (McDermid & Naiman 1983; Barnett & Schneider 1973; Warfe & Barmuta 2006). Previous research has principally focused on submergent vegetation in relation to ichthyofaunal success but with mixed results and rarely compares all three different vegetation types

Other Factors' Effects on Fish Fauna

Freshwater aquatic communities of the Coastal Plain of the southeastern United States are predominantly described as blackwater ecosystems (Mallin et al. 2004). The geographic variables of the Southeast are the primary reason for the establishment of the unique aquatic ecosystem present and can play a role in defining a fish assemblage. On a large scale, elevation above sea level is low and relatively uniform throughout the Coastal Plain (Paller 1994). Elevation limits the species present because it is correlated with past glaciation, water temperature, water current, dissolved oxygen, and other factors (Amarasinghe et al. 2001; Cook et al. 2004; Fu et al. 2004; Zhao et al. 2006). In a global study, species richness increased with decreasing altitude but this was largely a result of the extension of Rapoport's Rule (Amarasinghe et al. 2001). However, other studies have verified this conclusion on a regional scale in both China and Virginia, United States (Cook et al. 2004; Fu et al. 2004; Zhao et al. 2006). These results are largely a result of the extension of Rapoport's Rule to altitude and defined as species at lower elevations have more limiting home ranges than those at higher elevation (Fu et al. 2004; Stevens 1992). Because dispersal is attributed to home range size and fish assemblage similarities from different locations, the hydrological connectivity can play a significant role in the ichthyofauna. Because the main river is the principle sink of a watershed, the proximity

of streams and lakes to the main river is an important factor. Within stream communities, the ichthyofauna close to the mainstem of a watershed shares greater species richness than those that are more distant (Argent et al. 2009; Hitt et al. 2008). Lakes share the same phenomena in relation to connectivity with greater isolated lakes having less similarity with less isolated lakes (Olden et al. 2001). The principal reasoning behind the various patterns of connectivity is largely a result of the small scale geographic patterns (Olden et al. 2001; Argent et al. 2009; Hitt et al. 2008).

On the scale of individual water bodies such as wetlands, lakes, and tributaries of rivers, several innate characteristics of the location could play a role in the fish assemblage structure. The most obvious component of lake ecosystems is size which consists of surface area and depth. When first examining a water body, the area covered by water is the initial characteristic observed. A large water body will most likely have a larger amount of species diversity than a smaller water body of similar ecological characteristics because of the larger abundance of various habitats (Emmrich et al. 2011). In terms of refugia, pelagic deep-water habitat provides very little shelter and therefore little refuge for prey species so large predatory species are typically found here (Emmrich et al. 2011; Harvey & Stewart 1991). Littoral shallow water, on the other hand, may provide copious amounts of vegetation for shelter and therefore prey species are abundant there. Because maximum lake depth is a function of the change in depth from the shore, it is necessary to consider the bed slope as an important factor shaping species diversity. A steep slope will provide little refuge habitat for prey species but a large amount for predator species, but a shallow slope may provide a large amount of shelter with vegetation, but little depth for predators to maintain adequate mobility (Duarte & Kalff

1986). All of the local geographic variables ultimately contribute to the various local habitats which are comprised of biological and chemical characteristics.

Water chemistry variables, which can be influenced by geologic, biotic and anthropogenic sources, play a role in fish species diversity of water bodies. As the pivotal component of aerobic respiration used by all animals including fishes, dissolved O₂ concentration within the water column is pivotal for fish species diversity (Slack 1971; Tonn & Magnuson 1982). More derived species, such as Centrarchidae, have difficulty coping with reduced oxygen concentrations so species with the ability to tolerate lower oxygen concentrations, such as Ictaluridae, dominate the water column (Tonn & Magnuson 1982). Acidity is another important factor in blackwater systems. As acidity increases, typically the fish fauna decreases in species richness because only non-natives and certain resilient natives can tolerate it (Schofield & Driscoll 1987; Henderson & Crampton 1997). Eutrophication is an excellent example of the relationship between water quality parameters and resulting fish species richness. When a large amount of nutrients enters into a freshwater body from a natural or anthropogenic source, it causes an increase in primary producer abundance at the water surface (Sawyer 1966; Pilati et al. 2009). Subsequently, the alga reduces the photic zone and aquatic vegetation dies causing a loss of habitat. Dead vegetation and animals are decomposed which requires oxygen thus reducing the O₂ concentration and pH (Sobczynski & Joniak 2013). Because of the acidity, lack of food and habitat, and O₂ concentration only a few species of fish can survive (Sawyer 1966).

Significance

From the perspective of the United States Fish and Wildlife Service (USFWS), aquatic vegetation serves a significant purpose in aquatic ecosystems by providing essential elements or variables for a healthy habitat (Dahl 2011). However, the total acreage of wetlands in the United States has decreased over the last 50 years (Dahl 2011). Maintenance of wetlands and the vegetation within them is important in maintaining species diversity. Because of the importance of wetlands in maintaining species diversity, it is necessary to know the value of vegetation in preserving species diversity.

The problem with previous research examining the relationship of multiple variables comparing species richness is that it does not include both local and regional factors. Some research has only examined local factors and fewer still with consideration for aquatic vegetation corresponding to species diversity (Gorman & Karr 1978; Rahel 1984; Kovalenko et al. 2009; Main et al. 2007; Powers et al. 2003). Other research has focused only on regional factors (Oberdorff 1995). What research has compared local and regional variables does not account for vegetation structure and has mixed results (Taylor et al. 2006; Rathert et al. 1999; Cook et al. 2004; Angermeier & Winston 1998). This research provides an innovative and more complete analysis of the importance of vegetation to fish species diversity.

Species of Interest

In cooperation with Georgia Department of Natural Resources (DNR) (Bechler 2011), there are several species which are of principle concern. In the family Centrarchidae, *Enneacanthus chaetodon*, the blackbanded sunfish, is a small predatory sunfish typified by its 5-6 black vertical bars along the side with the first passing through

the eye (Cooke 2009; Laerm 1986). In Georgia, the species is listed as S1 critically endangered (Darden 2000). Historically, the species has been discovered regularly in the Okeefenokee Swamp and sparsely across the Coastal Plain of south Georgia (Darden 2000). Unfortunately, the most recent surveys in Georgia have not detected the species (Darden 2000; Tate 2005). However, Tanya Darden's samples were collected during a drought year and half of her collections were under low water levels (Darden 2000).

Other species of interest were of concern with the Georgia DNR because of inconclusive range distributions and lack of historical information from surveys. Within the family Fundulidae, *Fundulus lineolatus*, *F. rubrifrons*, *F. chrysotus*, and *F. cingulatus* are distributed broadly throughout south Georgia. The distribution and genetic relationship of these species is not completely understood. *Elassoma gilberti* was recently described with incomplete distribution knowledge for south Georgia (Snelson 2009). Some invasive species that are of interest include the *Pomacea insularum* and *Hydrilla verticillata*.

Chapter II

METHODS

Field Data

Twenty sampling locations were selected from 2012 satellite imagery as having large amounts of vegetation and proximity to Valdosta, Georgia using Google Earth® and historic locations where *Enneacanthus chaetodon* had been discovered. At each sample site, vegetated microhabitat sites were identified that were adjacent to the bank and a minimum of approximately 30 m in length to attain a sufficient sample size for each plot. A transect line was staked out on the bank dividing the entire plot into 4 meter subplots. Each subplot was not sampled any further than 3 meters from the bank. In order to minimize habitat disturbance, vegetation data and fish species were only collected from half of each subplot, i.e., a 2 m wide seine haul was made through each subplot.

First, vegetation data were collected before it would be heavily disturbed by the fish sampling procedure. Because of warm temperatures that cause an extended growing season typical of south Georgia, the growth of foliage is limited to the surface (Lillie & Budd 1992). As such, the surface area coverage of each subplot was determined with a 1 meter grid separated into 16 equal quadrats 25 x 25 cm squared (0.0625 m²). The percentage of vegetation surface area coverage was recorded on a 25% interval scale (0 = no vegetation, 25 = 1-25%, 50 = 26-50%, 75 = 51-75%, 100 = 76-100%) for each 0.0625 m² or quadrant of each square meter. In statistical analysis, the plant density of each plot

was the mean of all surface area percentages for each subplot. The depth of each subplot was measured where the water met the bank, middle, and outer edge of each subplot. Because of the variability of the bed of the water bodies, the slope of the bed of half the length of each subplot was calculated as follows:

$$\frac{d_2 - d_1}{1/2 l}$$

$d_2 - d_1$ where d_2 is the depth measured furthest from the bank, d_1 is the depth measured closest to the bank, and l represents the length of the subplot. Slopes for each subplot were converted to mean bed slope. The dominant vegetation type of each subplot was based on Grenouillet (2000) model of dominant vegetations at 75% of an entire plot. If the dominant vegetation could not be identified on location, a specimen was taken and identified later in the lab. The dominant vegetation of the entire plot was based on the number of subplots dominated by it.

After attaining the aquatic macrophyte data, depth measurements, and water chemistry data, fish species were collected by seining half the width of each subplot. Seines used were 2 m high by 2 m wide with a 0.08 cm mesh or a 2 x 3 m height by width with a 0.25 cm mesh. The opening of the latter net was maintained at 2 m with a piece of rope 2 m long tied between the poles. Seine hauls were pulled from 0.5 m outside of the outer edge of the subplot to the edge of the bank. Fish specimens were collected under scientific collecting permit #CN:_9134 and those from the Okefenokee were collected under the National Wildlife Refuge System Research and Monitoring Special Use Permit #41590-12-024. All fish species captured within the vegetated plot were euthanized in MS222 according to AUP-00039-2011 as set forth by Valdosta State

University (see Appendix B), fixed in 10% formalin, and preserved in 55% isopropanol after washing 24 hours in tap water. If the specimens were needed for genetic analysis as part of other studies, they were immediately preserved in more than 70% ethanol after euthanasia. All specimens were determined to species and counted.

Water Chemistry

Water chemical samples and variables were measured at three locations along the outer edge of the vegetation of the plot in the middle and at both ends. Dissolved oxygen concentration, temperature, conductivity, and pH were measured on site using WTW Cond 340i, Fisher Scientific AP85A Waterproof pH/Cond Meter, and YSI DO200. The mean of the three separate measurements of all water chemistry variables were utilized in statistical analysis. Samples that required further analysis within the laboratory were collected from the middle of the plot within the vegetation. To prevent any possible degradation of organic materials, the water samples were immediately stored on ice in a closed cooler after collection.

Chlorophyll A and B were extracted and quantified using the methods described in Jeffrey and Humphrey (1975). Because of the high amount of suspended solids that frequently prevented proper filtration, only 100 mL of water sample was vacuum filtered through 47 mm paper. The filter and organic constituents, separated from the water, were combined with 3 mL of 90% acetone, manually broke the filter with glass rod in solution, and stored for 24 hours. After 5 minutes of centrifuging, the solution was separated from the precipitate and analyzed with a wavelength scan with a Beckman DU[®] 640 spectrophotometer. Quantification was accomplished by the equations from Jeffrey and

Humphrey (1975). In addition, the tannic content was measured at the 609 wavelength with the filtered water sample (Wang & Hsieh 2001).

Additional data collected using Google Earth® included: (1) elevation, (2) distance to the main river along the stream channel draining a plot site, and (3) slope along the stream channel (change in elevation along the stream channel from the plot to the main river).

Statistical Methods

All data were organized in Microsoft Excel 2007. Fish species abundance indices were analyzed using the Friedman's test followed by a Connor's multiple pairwise comparison (Stats Direct Ltd., 2007). Bray-Curtis similarity analysis, multi-dimensional scaling (MDS), and principle component analysis (PCA) were developed using Primer v6 (Clarke & Gorley 2006).

Post-hoc comparisons were made between fish assemblages using abundance, species diversity was quantified for all further statistical analyses. Comparisons between species richness of specific vegetation types as well as figures were made using Microsoft Excel 2007. Maps for geographical representation of vegetated plots were created using ArcGIS 10.1 (ESRI Inc. 2008)

Prior to running regression analyses, independent variables were tested for normality using Shapiro-Wilks normality tests (StatsDirect Ltd., 2007). If the distribution of a variable was not normal, it was transformed using \log_{10} , natural log, square root, and x^2 and retested for normality. The transformation with the highest test for normality was then used. Linear regression models were produced with fish species diversity as the dependent variable.

Chapter III

RESULTS

Although most of Georgia is typified by slow-flowing blackwater, the area was in the middle of a serious drought during the summer and fall of 2011 when data collection occurred. Because of the drought and species of interest, locations selected were predominantly shallow aquatic ecosystems. Originally, 20 locations were sampled; however, two locations were eliminated from statistical analysis. One location did not have a complete data set (Bevel Creek) and at another site (Linton Lake) the sampling conditions involving depth within the plot and excessive amounts of peat were so poor that fish sampling efficiency was extremely low when seining took place (Table 1). The remaining 18 locations were from five different watersheds (Figure 1). Two locations were sampled twice or contributed two plots and data sets to the study. AL4 and AL5 were both from Lake Charles and AL2 and BBS were from Fletcher's Pond (Figure 1, Table 1). Deviating the most from the other sites in terms of location, Guest Mill Pond is part of the headwaters of the Satilla River and drains to the Atlantic Ocean whereas all other locations drain into the Gulf of Mexico (Figure 1).

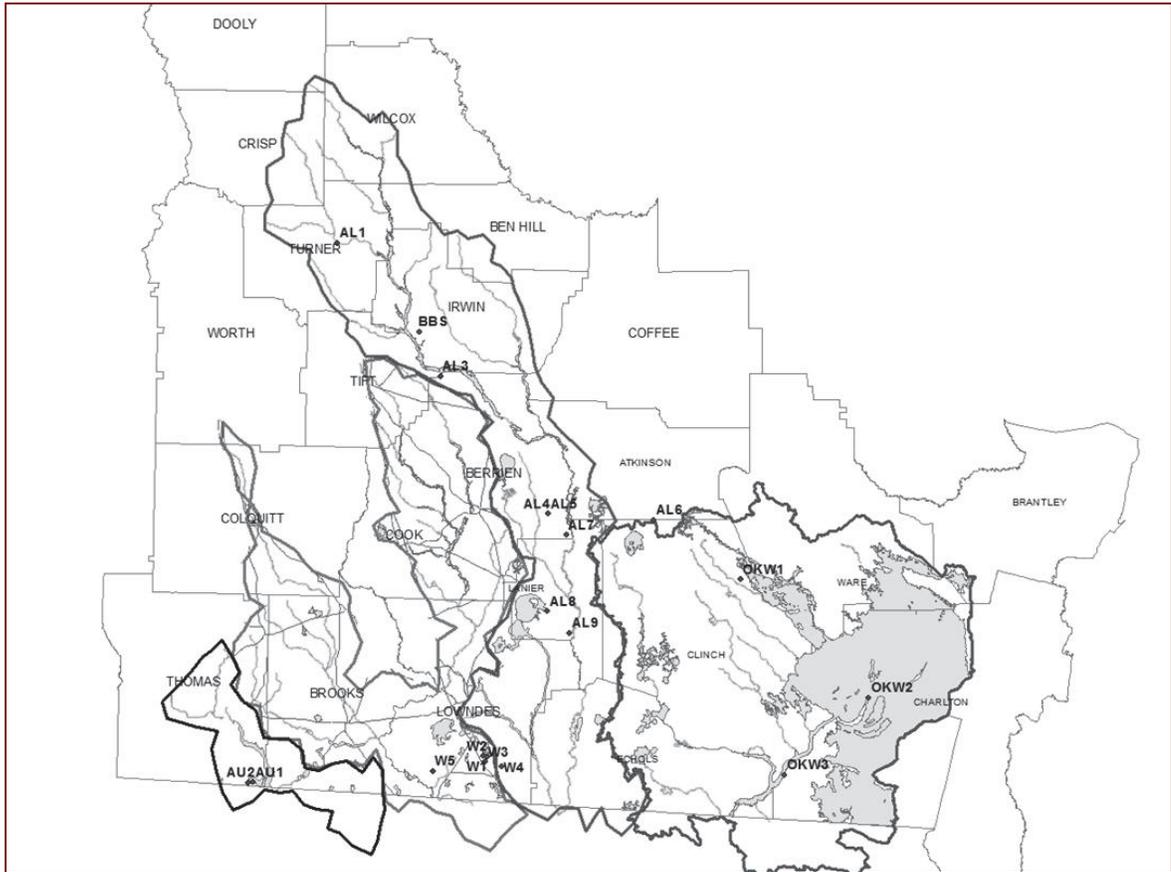


Figure 1. Map of the sampling area within Georgia, USA, and the locations of each sample plot. From West to East, the major watersheds are outlined as the Aucilla, Withlacoochee, Alapaha, and Suwannee Rivers. AL6 lies in the Satilla basin which is not outlined. A description of each site is presented in Table 1.

Table 1. Location descriptions and coding used for each wetland from which a vegetated plot was selected. See Figure 1 for a graphical representation of plot distributions.

<u>Code</u>	<u>Name</u>	<u>Nearest Road</u>	<u>Latitude</u>	<u>Longitude</u>
AL3	Barnes Pond	Enigma-Turner Church Rd	31.46733	-83.34158
AL1	Deep Creek Swamp	SR 112/107	31.73009	-83.57954
AL4	Lake Charles	Highway 135	31.1959	-83.0937
AL5	Lake Charles Barrow Pit	Highway 135	31.19597	-83.0937
AL2	Fletcher Lake	Sam Caldwell Lane	31.55505	-83.38921667
BBS	Fletcher Lake	Sam Caldwell Lane	31.55505	-83.38921667
AL7	Smith Large Wetland	Highway 64	31.15389167	-83.05232778
AL8	Banks Lake	Highway 122	31.003455	-83.09661667
AL9	Moore Carolina Bay	Good Hope Rd	30.96008333	-83.04566667
AL6	Guest Mill Pond	Hwy 89	31.18197778	-82.84986111
AU2	Connell Creek	Oak Grove Rd	30.66631667	-83.78321667
AU1	Linton Lake	Mitchell Rd	30.66683333	-83.7711
W1	Bevel Creek	Loch Laurel Rd	30.71849444	-83.24340278
W5	Clyattville Pond	Clyattville-Nankin Rd	30.68976667	-83.3605
W4	Lake Balboa	Coral Gables Drive	30.6977	-83.20306667
W2	Brown's Pond	Pine Bluff Rd	30.71756667	-83.23525
W3	Lloyd Turner Wetland	Corinth Church Rd	30.707084	-83.239374
OKW3	Suwannee River Barrow Pit	Highway 94	30.67725	-82.55625
OKW1	Rayonier Pond	Swamp Rd	31.0641	-82.652
OKW2	Fargo Canoe Trail	Highway 17	30.82860278	-82.36086667

The greatest species richness was observed in Connell Creek and the least in Lake Charles Barrow Pit. The most abundant species was *Gambusia holbrooki*, the mosquitofish, followed by *Leptolucania ommata*, the pygmy killifish (Table 2). Other relatively common species that were not insectivores included *Lepomis macrochirus* and *Centrarchus macropterus*. Uncommon species included the large predatory species *Amia calva*, *Micropterus salmoides*, and *Esox americanus*. Of all the ichthyofauna collected during the study, a few species are of particular importance. *Elassoma gilberti* was collected in large numbers from Connell Creek, Southwest Georgia (Table 2). *Fundulus*

cingulatus was collected from two locations, Smith Large Wetland and Brown's Pond. The rarest species of all those collected, the endangered blackbanded sunfish, *Enneacanthus chaetodon*, (Freeman et al 2009) was collected from the BBS plot (Table 2). Because the BBS location was previously unknown as a location containing *E. chaetodon*, it was a significant find.

Other species of significance are those that shape the assemblage structure most significantly. Using PCA analysis, the most abundant species were typically the smallest and also the most critical in delineating the fish assemblages of each plot (Table 2 and Figure 2). PC1 and PC2 account for 86.8% of the cumulative variation of the entire data set. *Gambusia holbrooki* and *Leptolucania ommata* contribute 96% and 73% to PC1 and PC2 respectively. Other important species according to PCA include *Enneacanthus gloriosus* and *Aphredoderus sayanus*; however, the latter was most likely identified as significant in the analysis because it was captured in very large numbers from Connell Creek (Figure 2 and Table 2).

Species	AL7	W4	AL8	AL6	W2	OK W2	AL9	AL2	AL4	W5	AL5	AL1	BBS	OK W3	AU2	OK W1	AL3	W3
<i>A. nebulosus</i>												3			7			
<i>A. calva</i>															1			
<i>A. sayanus</i>															264			
<i>C. macropterus</i>				2		6		7	14	1		53	8		6	9		
<i>E. evergladei</i>				4														
<i>E. gilberti</i>															22			
<i>E. okefenokee</i>			2	12			73							153		3		
<i>E. zonatum</i>															71			
<i>E. chaetodon</i>													10					
<i>E. gloriosus</i>			75	8	190	1	4					2		35	2	5	10	
<i>E. obesus</i>																		1
<i>E. sucetta</i>				2												8		
<i>E. americanus</i>				1					1						4			
<i>E. niger</i>		1	10	3	1								1		1	2		
<i>E. fusiforme</i>		1	2		22		1						1	5		1	8	16
<i>F. chrysotus</i>	52	4	18	7	50		12	2					18			6		118
<i>F. cingulatus</i>	2				2													
<i>F. lineolatus</i>	8			8	7								2			7		
<i>G. holbrooki</i>	4	56	5	12	57	119	43	7	22	37	9	127	2	26	136	905	507	506
<i>H. formosa</i>	33	12			59		37								6			258
<i>L. siculus</i>														1			1	
<i>L. platyrhincus</i>															1			
<i>L. gulosus</i>		2			7							2					2	2
<i>L. macrochirus</i>	54	77	2	17	11	23	83						2	23		25	1	74
<i>L. ommata</i>	25		42	35	254	2	10							42		323	1	136
<i>M. dolomieu</i>	1																	
<i>M. salmoides</i>		2	1			5	1	2	1					4				1
<i>N. crysoleucas</i>								1	1			27			1			
<i>N. maculatus</i>							4											
<i>N. gyrinus</i>																		
<i>P. nigromaculatus</i>	1	4										26		18				
<i>U. pygmaea</i>															1			
Unable to Identify							130											

Table 2. Species counts from each vegetated plot. For complete species list including families, see Figure A7.
*Unidentified specimens were Centrarchids of approximately 1 cm in length.

In support of the PCA results, the cluster diagram in Figure 3 consisted of three major branches or clades breaking out at approximately 20% similarity. The three branches were analyzed separately via PCA to delineate influential species for each branch (Figure 4, 5, and 6). Within the upper or top most branch, the terminal clades (W3, AL3, OKW1, AL1, OKW2, and AU2) are defined by the abundance of *G. holbrooki* with between 100-500 *G. holbrooki* in each (Figure 3\4 and Table 2). The two subclades within the uppermost branch are separated by the abundance of *G. holbrooki*. The top three sites have more than 500 specimens while AL1, OKW2, and AU2 in the lower subclade had between 119 and 136 individual *G. holbrooki*. In the lower most primary branch, terminal clades AL5, AL2, W5, and AL4 all had low species diversity and 3 of the 4 plots had less than 14 *G. holbrooki* (Table 2 and Figure 3, 6). The central branch, AL6, AL8, OKW3, AL9, W4, and AL7 are dominated by *L. ommata* with 35-42 specimens, another small species, and *Lepomis macrochirus*, a predatory sunfish (Figure 3). The BBS plot is quite unique because it is greatly distinguished from all other plots because the most dominant species within the plot was *Fundulus chrysotus* (Table 2). Another common species influential to all branches of the cladogram was *Enneacanthus gloriosus* (Figure 4, 5 and 6). Additionally, the plot AL2 and BBS were sampled from the same wetland and within approximately 50 meters of each other but not with similar species assemblages collected (Figure 3).

When comparing each plot's fish assemblages on a one-to-one basis, the Friedman analysis results indicated that only those plots from the tree major branches of the Cluster diagram (Figure 3) described above, and which was based on a Bray-Curtis similarity analysis, were significantly different (Table 3). The only exception to this

observation was OKW2 vs AU2, which are both in the upper most branch of the Cluster diagram. Of all the plots included in the study, BBS proves to be the most unique in fish assemblage because it is the only plot dominated by *Fundulus chrysotus* and was also low in the total number of fish collected (N = 44). Most notably, the Cluster diagram similarity values of every relationship was less than 70 and the overall Friedman analysis had a significance value of $P = 0.0002$ indicating highly significant differences between most plots in the ranking of species similarities. Therefore, the data set was not compiled of only densely or sparsely vegetated sites. Rather, PCA verifies that in terms of vegetation surface coverage the data was randomly selected.

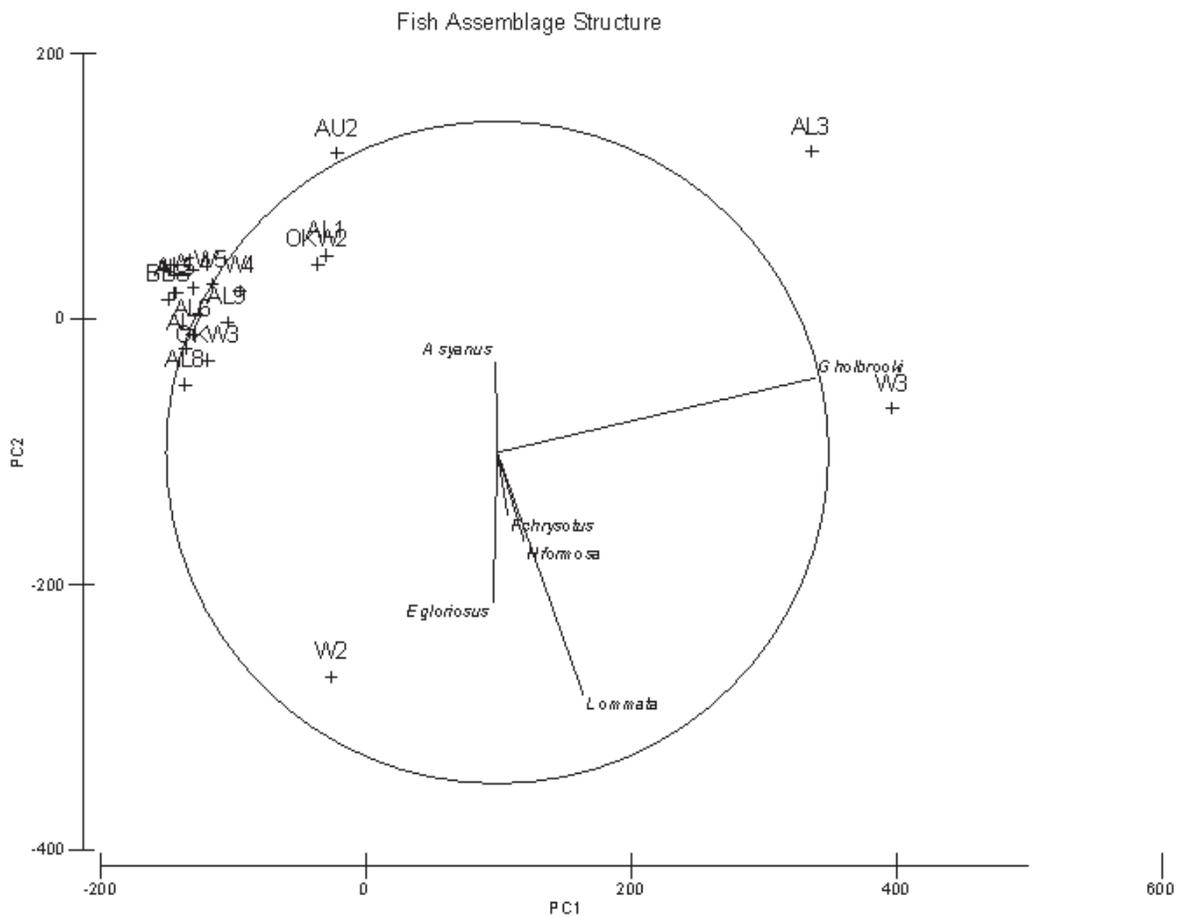


Figure 2. Principal Component Analysis of the species composition of the fish assemblage within all vegetated plots.

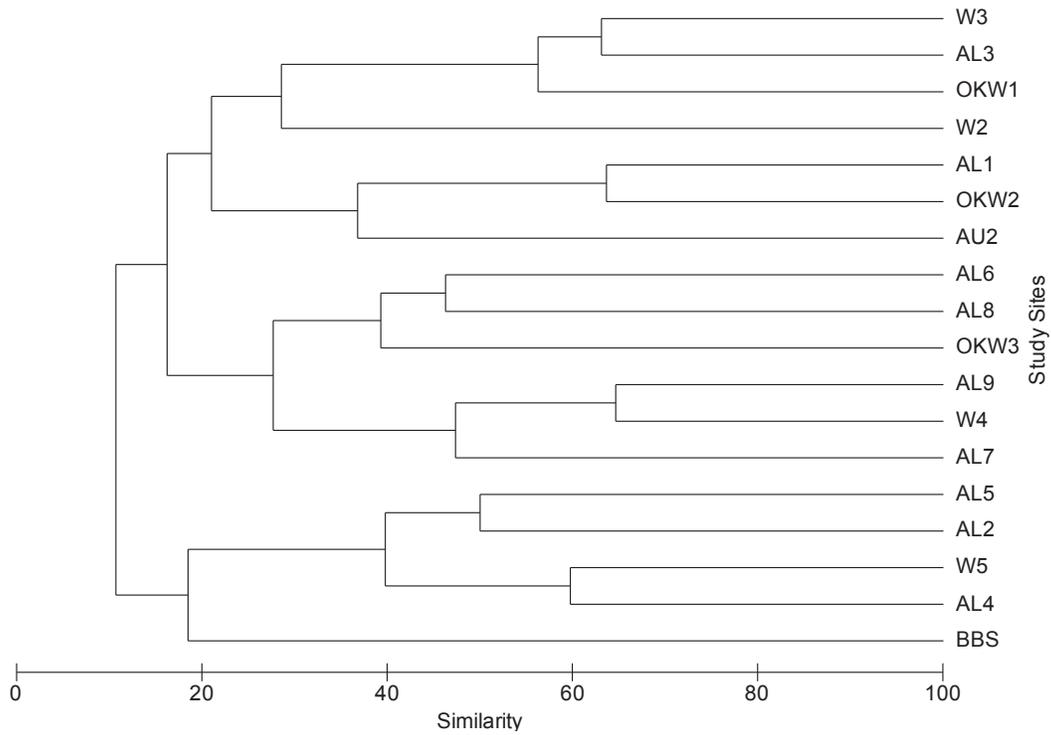


Figure 3. Cluster diagram of study plots. Based on Bray-Curtis Similarity indices of all vegetated plots comparing fish species and developed with Primer6.

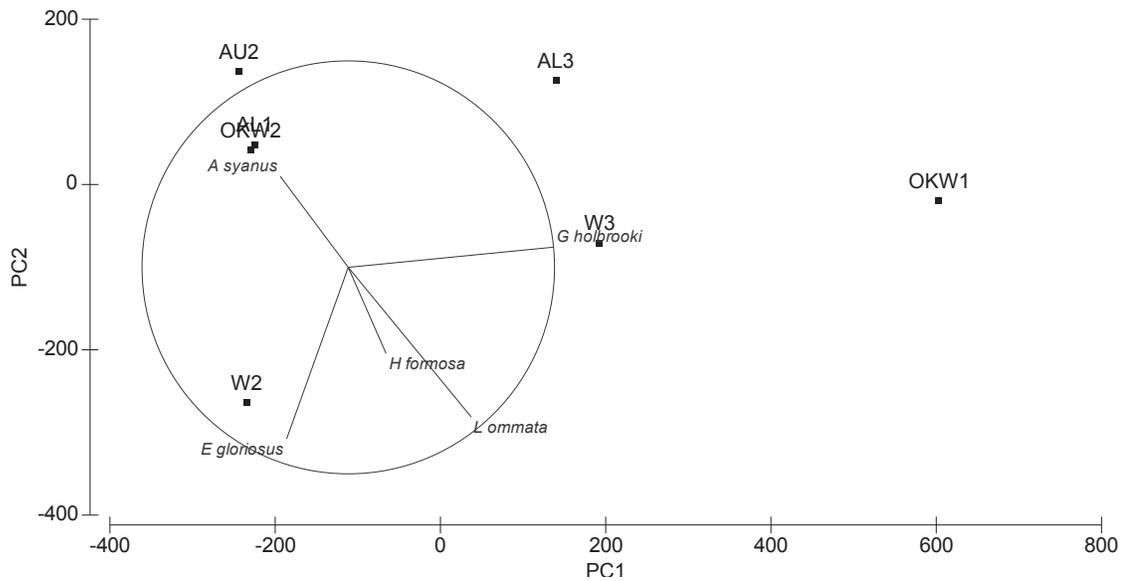


Figure 4. PCA analysis of Branch 1 from Figure 3.

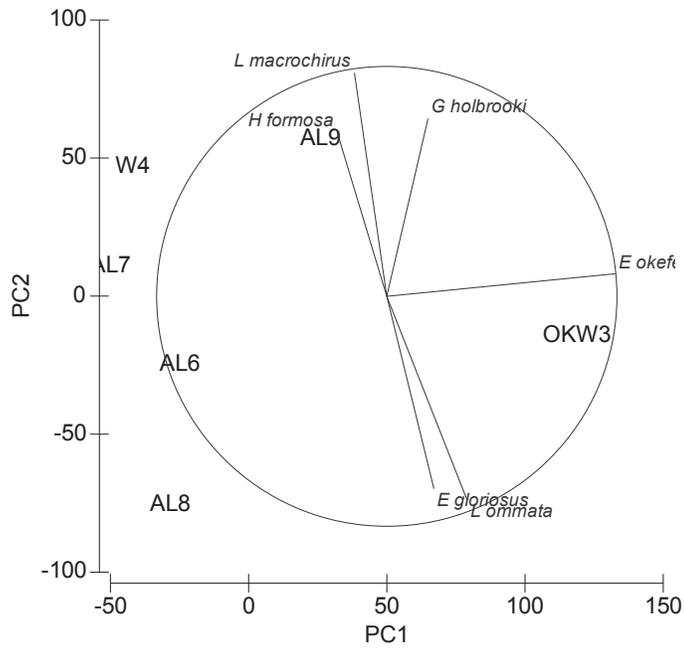


Figure 5. PCA analysis of Branch 2 from Figure 3

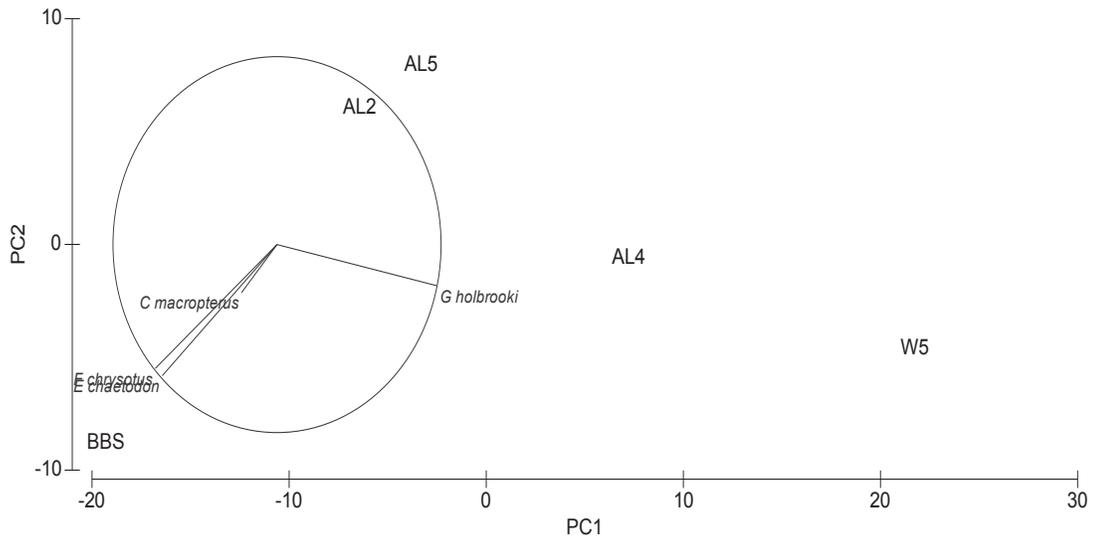


Figure 6. PCA analysis of Branch 3 from Figure 3.

Table 3. Connor's multiple pairwise comparisons on fish assemblage of all plots. Only significantly different relationships are presented here. The Relationship to the Significance Value of all comparisons is greater than 55.826876.

<u>Comparison</u>	<u>Significance</u>	<u>Comparison</u>	<u>Significance</u>
AL2 vs. OKW1	P = 0.009	OKW2 vs. OKW1	P = 0.04
AL2 vs. W3	P = 0.0216	W2 vs. AL2	P = 0.0053
AL4 vs. AU2	P = 0.0043	W2 vs. AL4	P = 0.011
AL4 vs. OKW1	P = 0.0179	W2 vs. BBS	P = 0.0368
AL4 vs. W3	P = 0.04	W2 vs. OKW2	P = 0.0259
AL5 vs. AL1	P = 0.0206	W4 vs. AL5	P = 0.01
AL5 vs. AL3	P = 0.0384	W4 vs. W5	P = 0.0236
AL6 vs. AL2	P = 0.0216	W5 vs. AL1	P = 0.0454
AL6 vs. AL4	P = 0.04	W5 vs. OKW3	P = 0.0081
AL7 vs. AL5	P = 0.0105	AL5 vs. OKW3	P = 0.0031
AL7 vs. W5	P = 0.0247	AL9 vs. AL5	P = 0.0033
AL8 vs. AL5	P = 0.0134	AL6 vs. W5	P = 0.0022
AL8 vs. W5	P = 0.0309	W5 vs. W3	P = 0.0022
AL9 vs. W5	P = 0.0086	AL2 vs. AU2	P = 0.0019
AU2 vs. AL3	P = 0.0337	AL6 vs. AL5	P = 0.0007
BBS vs. AU2	P = 0.0163	W5 vs. OKW1	P = 0.0007
OKW2 vs. AU2	P = 0.011	AL5 vs. W3	P = 0.0007

Table 4 lists fish species richness, aquatic vegetation type and percent surface coverage. The examination of aquatic vegetation surface coverage compared to fish species richness shows no trend with an R^2 value of only 0.06 indicating no true relationship (Figure 7). Because each vegetation type constitutes a different structure within the water column, it is necessary to compare the species richness and vegetation coverage of each vegetation type, respectively. The sample size of emergent vegetation is not of sufficient size for a conclusive regression comparison. However, a simple linear regression can be made between vegetation coverage and species richness within submergent aquatic vegetation, the vegetation type with the largest amount of species diversity. Submergent vegetation did not demonstrate a significant trend, again the R^2 values of 0.0037 is so weak no inferences could be made (Figure 8).

Table 4. Description of the vegetated habitat and fish diversity within each plot.

<u>Plot</u>	<u>Dominant Vegetation</u>	<u>Vegetation Type</u>	<u>Number of Fish Species</u>	<u>% Coverage</u>
AL7	<i>Panicum hemitomon</i>	Emergent	9	41.226
W4	<i>Panicum hemitomon</i>	Emergent	9	80.76
AL1	<i>Nymphaea odorata</i>	Floating	7	68.96964
AL4	<i>Nymphaea odorata</i>	Floating	5	76.84514
AL5	<i>Nymphaea odorata</i>	Floating	1	49.63942
AL2	<i>Nymphaea odorata</i>	Floating	5	61.653
W5	<i>Panicum hemitomon</i>	Emergent	2	72.5446
OKW2	<i>Nymphaea odorata</i>	Floating	6	32.244
AU2	<i>Potamogeton pedctinatus</i>	Submergent	14	62.51814
OKW3	<i>Alternanthera philoxeroides</i>	Submergent	9	86.0677
BBS	<i>Myriophyllum spicatum</i>	Submergent	8	54.764
AL3	<i>Cabomba caroliniana</i>	Submergent	7	92.4716
OKW1	<i>Potamogeton pedctinatus</i>	Submergent	11	79.4643
AL8	<i>Myriophyllum spicatum</i>	Submergent	9	87.123
AL9	<i>Alternanthera philoxeroides</i>	Submergent	10	43.802
W2	<i>Myriophyllum spicatum</i>	Submergent	11	84.283
AL6	<i>Mayaca fluviatilis</i>	Submergent	12	73.224
W3	<i>Cabomba caroliniana</i>	Submergent	9	99.262

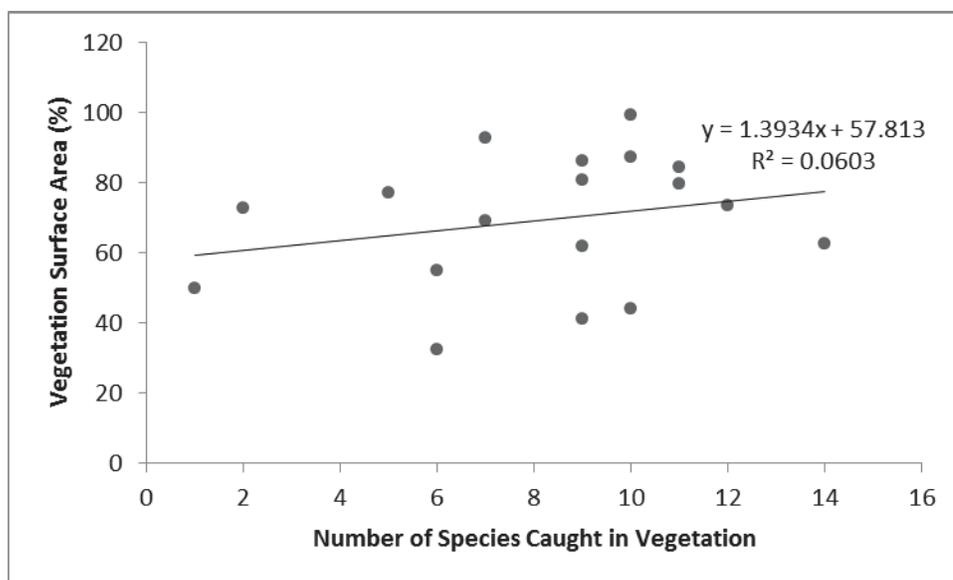


Figure 7. Fish species diversity within all vegetation types on a gradient of vegetation complexity

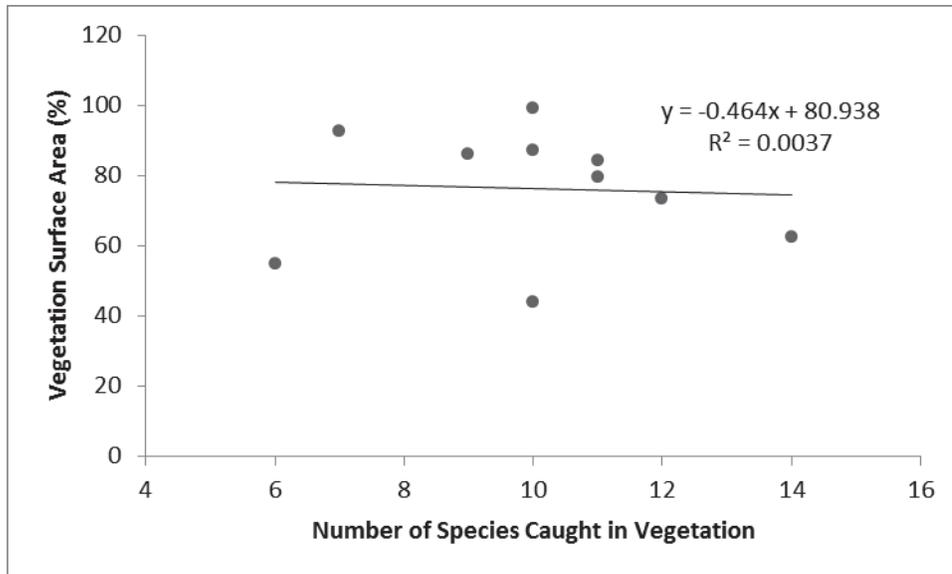


Figure 8. Fish species diversity within only submergent vegetation on a gradient of vegetation complexity.

Although a direct comparison between vegetation types and associated percent coverage and species richness is not feasible, an assessment of species richness between the differing vegetation types is possible because of the different structures associated with the respective vegetation types (Figure 9). A one-way ANOVA (analysis of variance) verifies that a comparison of mean species richness between dominant vegetation types is significant (Table 5). More specifically, a Scheffé multiple comparisons test demonstrates the mean species richness within submergent vegetation is significantly different from those occupying floating vegetation but no other relationship is significant (Table 6). A more in-depth depiction of the species diversity within the various vegetation types shows a distinction in species richness by family and the high biodiversity of submergent vegetation relative to emergent and floating vegetation (Figure 10).

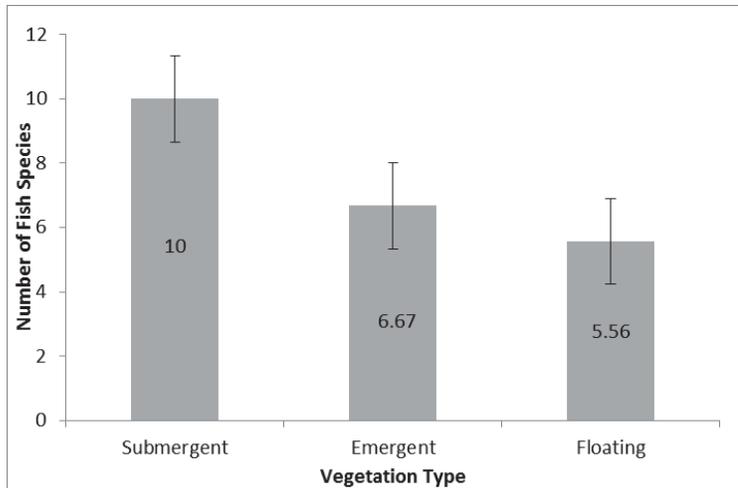


Figure 9. Fish diversity between dominant vegetation types. Error bars denote standard error.

Table 5. ANOVA of the species diversity relative to vegetation type.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square
Between Groups	73.244444	2	36.622222
Within Groups	115.866667	15	7.724444
Corrected Total	189.111111	17	
F (variance ratio) = 4.741082		P = 0.0254	

Table 6. Scheffé comparison of each vegetation type to each other. Only the comparison between floating and submergent vegetation types was statistically significant.

Comparison	Mean Difference L (95% CI)	L/SE(L)	
Floating vs Submergent	-4.4 (-8.531141 to -0.268859)	2.890403	P = 0.0361
Emergent vs Submergent	-3.333333 (-8.298347 to 1.63168)	1.82194	P = 0.2233
Emergent vs Floating	1.066667 (-4.441522 to 6.574855)	0.525528	P = 0.8721

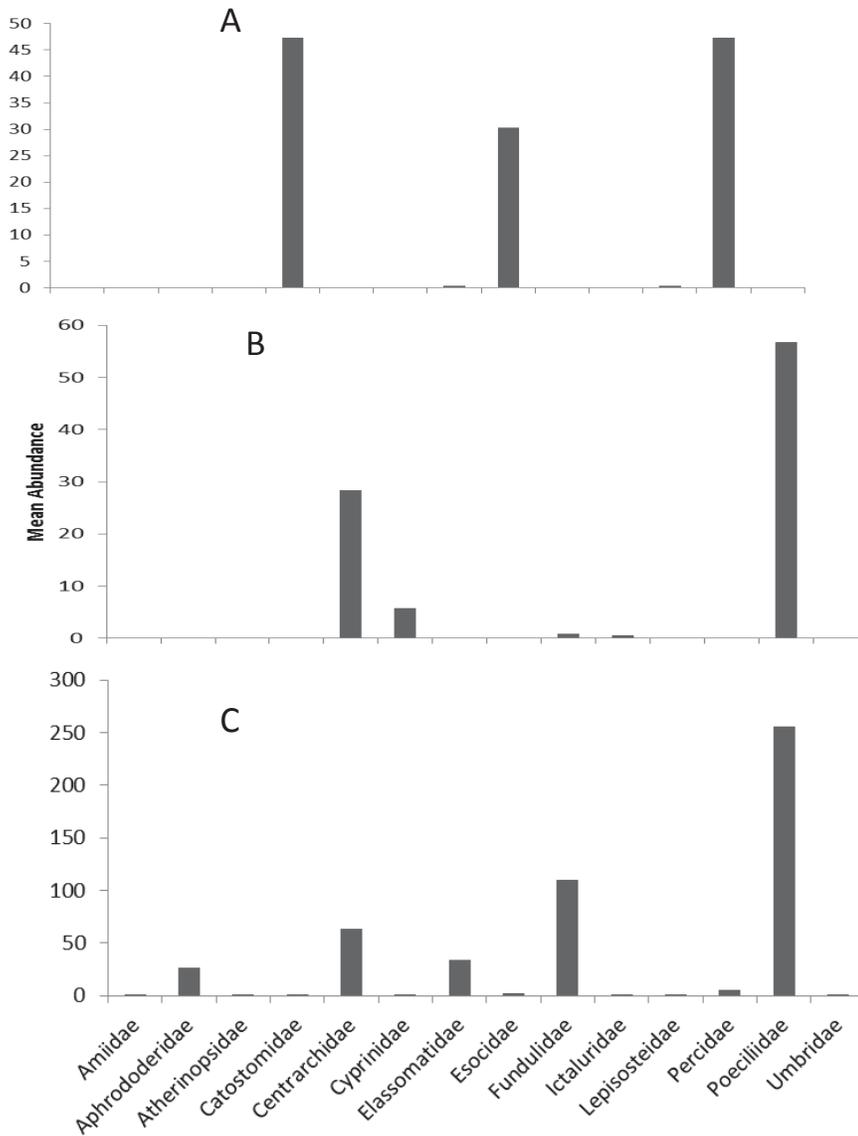


Figure 10. Species abundance classified by family within each vegetation type, respectively. A) Emergent B) Floating C) Submergent

Table 7. Shapiro-Wilks Normality on non-normally distributed variables. Transformation method and new probability values are provided in the last two columns.

<u>Variable</u>	<u>Significance</u>	<u>Transformation</u>	<u>New Significance</u>
Conductivity	P = 0.0035	Natural Log	P = 0.1600
Tannins	P = 0.0002	Square Root	P = 0.0689
Chlorophyll B	P = 0.0013	Square Root	P = 0.1414
Distance to River	P < 0.0001	Natural Log	P = 0.9904
Slope along River	P = 0.0052	Square Root	P = 0.8047
Plot Slope	P = 0.0285	Square Root	P = 0.8825

Before various regression tests were run, all variables were tested for normality and possible correlation between variables to prevent any skewed results. Six variables were found to have a non-normal distribution and were transformed (Table 7). In addition, the first and second slope were significantly correlated so the average slope of the plot was utilized in the regression analyses. The plot length was not included in the regression analyses because the total volume of each plot was included. The complete dataset for regression analyses included 13 independent variables with fish species diversity as the dependent variable (Table A1).

Table 8. Formulas and R² values of each variable against species diversity as the dependent variable.

<u>Variable</u>	<u>Formula</u>	<u>R² values</u>
Square Root Plot Slope (m)	$y = -0.0099x + 0.5345$	0.029
pH	$y = 0.0655x + 4.9305$	0.0621
Temperature (°C)	$y = 0.0956x + 27.678$	0.009
O ₂ (mg/l)	$y = 0.2181x + 0.7713$	0.1818
Natural Log Conductivity (μS)	$y = 0.0088x + 4.507$	0.0061
Square Root Tannins (mg/l)	$y = -0.0019x + 0.0914$	0.0142
Chlorophyll A (mg/l)	$y = -0.0687x + 1.1504$	0.2065
Square Root Chlorophyll B (mg/l)	$y = -0.0495x + 0.7964$	0.2508
Vegetation Coverage (%)	$y = 1.1549x + 60.031$	0.0412
Elevation (m)	$y = -2.4887x + 77.11$	0.1458
Natural Log Distance to River (km)	$y = 0.0007x + 1.7426$	4e ⁻⁶
Square Root River Slope (km)	$y = -0.2139x + 4.4916$	0.1868
Plot Volume (m ³)	$y = 0.3205x + 14.308$	0.0151

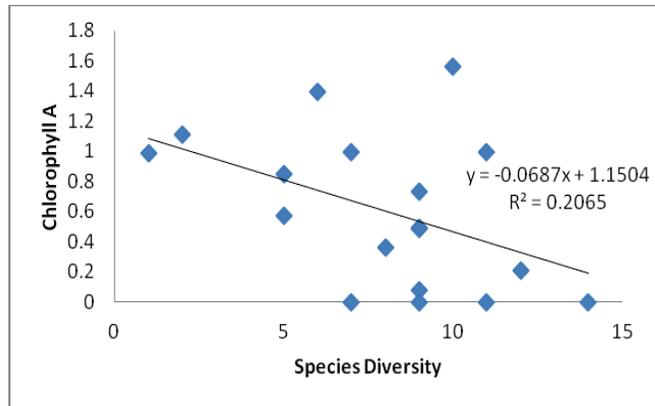


Figure 11. Correlation between Chlorophyll A and fish species diversity within each plot.

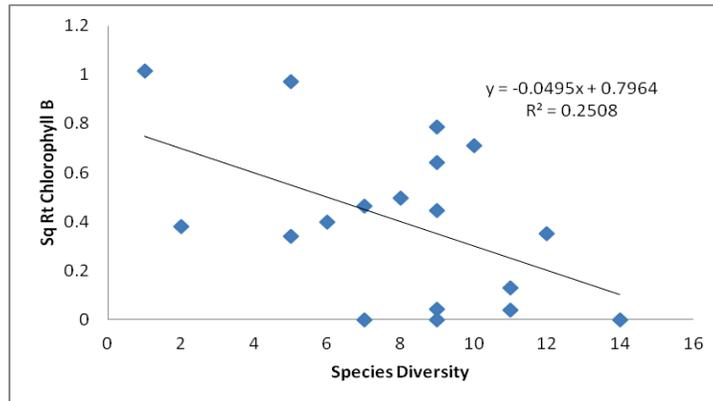


Figure 12. Correlation between chlorophyll B and fish species diversity within each plot.

None of the variables examined in the regression analyses provided a strong correlation with species diversity (Table 8). The most highly correlated variables were chlorophyll A and square root chlorophyll B. Both chlorophyll values shared the same negative correlation with species diversity (Figure 11 and 12).

Chapter IV

DISCUSSION

Impact of Drought

Throughout south Georgia, periodic droughts are expected and wetlands thick with aquatic vegetation provide refuges for several species during this time. As shallow creeks and rivers lose water, fish species are limited to the few pools and wetlands that remain. The species diversity of Connell Creek was the highest of all sites probably because it was the last length of the entire creek just upstream of a major wetland and a few months after collection the site was completely dry. A possible explanation is that the creek species were reduced to the last remaining pools of water and the wetland species could also be present at Connell Creek just upstream of a large wetland. Another explanation for the species diversity of Connell Creek was that one of the subplots along the narrow creek had very little vegetation and approximately one meter in depth allowing for predatory species to be present. Within the same plot, very shallow (< 30 cm) and heavily vegetated water provided refuge for small prey species.

The site with the lowest species diversity was Lake Charles Barrow Pit. The site had a steep slope, low total volume, and only six subplots of 1 m in length along the entire plot (Figure A1). The steep slope allows for few prey species to be present because predators have greater access to prey species with the greater depth availability. When considering both of these circumstances together, they provide evidence for the importance of habitat heterogeneity in maintaining species diversity (Tews et al 2004). Based on these assumptions, wetlands provide refuge for several species by providing

multiple microhabitats in close proximity relative to a river which may not have vegetation because of canopy cover and/or current.

Species Diversity

Aquatic macrophytes play a role in structuring the fish assemblages that occupy them but the extent of that role is still uncertain. None of the data supported the intermediate or high vegetation density hypotheses for species diversity. When considering species diversity compared to vegetation density regardless of vegetation type, an increasing trend was evident but too weak to allow inferences (Figure 4). The correlation between submergent vegetation density and species richness was negative and again too weak to allow inferences (Figure 5). Because no direct inferences can be made by vegetation surface coverage and species diversity, vegetation type could provide more influential results. Some research has concluded that increasing species diversity in relation to increasing vegetation complexity can be observed in submergent aquatic vegetation (Kelly & Hawes 2005; Warfe 2006). Based on the data collected, a definitive conclusion cannot be drawn on the impact of aquatic vegetation in relation to fish species diversity.

Differing vegetation types provide different habitat structure and therefore the potential for different species and numbers of each species to occupy varies. Because species composition by family was most diverse in submergent vegetation, it is possible to consider the varying structural complexities of each independent vegetation type.

Nymphaea odorata, emergent vegetation, has a simple stem in the water column whereas *Myriophyllum heterophyllum*, submergent vegetation, has a complex stem with whorled leaves in the water column; therefore, the different vegetation types provide varying

degrees of protection from predators. No significance was observed between the fish subguilds and aquatic vegetation. It can be very difficult when assuming a uniform fish assemblage to identify with vegetation. The most common species, such as *Gambusia holbrooki*, can be ubiquitous regardless of vegetation type and the rarest species, such as *Notropis maculatus*, of which only four individuals were caught, can be assumed as random chance. Those species which are relatively common and neither rare nor completely dominant could be described as occupying a specific vegetation type and guild.

A potential guild pattern can be observed amongst the entire data set regardless of vegetation type. Because of the abundance of separate species associated with differing clades or branches of the cluster diagram, potential subguilds are described (Figure 3). The three subguilds present are described by the abundance of *Gambusia holbrooki*, *Lepomis macrochirus*, *Fundulus chrysotus*, *Enneacanthus gloriosus* and *Leptolucania ommata* (Figure 4, 5, and 6). The smaller species, *G. holbrooki* and *L. ommata* shape a subguild within vegetation because they provide the large base of a trophic structure and ample food sources for higher trophic level species. In addition, high abundance of small species contributes to the passive sampling theory which basically states if you catch more fish specimens, then there is a higher likelihood of increasing species diversity (Grenouillet et al. 2002). The Centrarchids can dominate a guild by limiting the amount of basal species such as *G. holbrooki* and *L. ommata*. Because the data does not provide a single ubiquitous fish assemblage structure within aquatic vegetation, the subguild concept is presented here. Other research has associated hydrologic factors within wetlands, such as depth, being significant correlates of functional groups or subguilds

within vegetated wetlands but it does not describe the vegetation within the wetlands sampled (Main 2007; Meffe & Sheldon 1988). Within south Georgia, one aquatic vegetation fish subguilds can be described as dominantly influenced by Poeciliidae or Fundulidae. Another subguild can be described as most influenced by sunfishes of the Centrarchidae.

Macrophytes have never been examined as a predictor of species richness relative to other local and regional factors on this scale. The linear regressions indicated no variables significantly correlated with species diversity. Based on the data, the species diversity could be based on random chance. However, species diversity could also be explained by a combination of variables with chlorophyll a and b as the most influential. High amounts of food resources from algae and subsequent invertebrates, the fish species richness could potentially be explained from a bottom-up approach.

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APPENDIX A:
Supplemental Figures
Tables A1-A7

Table A1. Multivariate dataset including local and regional variables

	Square Root Plot Slope	pH	Temp (°C)	Natural Log O ₂	Natural Log Conductivity (μS)	Square Root Tannins	Chlorophyll A
AL7	0.46	5.5 4	29.3	1.26	76.87	0.007	0.74
W4	0.58	7.0 7	30.3	4.98	82.77	0.001	0.08
AL1	0.46	6.3 4	27.67	2.27	119.6	<0.001	<0.01
AL4	0.22	5.2 2	26.23	0.94	62.27	0.003	0.85
AL5	0.77	4.3 1	31.00	1.48	70.80	0.004	0.98
AL2	0.30	6.0 1	28.85	1.40	115.95	0.008	0.58
W5	0.42	5.1 3	26.20	0.30	58.60	0.007	1.11
OK W2	0.56	3.8 2	22.63	3.64	194.17	0.030	1.39
AU2	0.26	6.3 2	26.00	1.20	77.30	<0.001	0
OK W3	0.34	6.0 2	28.13	2.67	126.60	0.035	0.49
BBS	0.51	5.6 9	29.13	1.83	121.66	0.008	0.36
AL3	0.52	6.3 1	31.17	1.15	225.33	0.004	0.99
OK W1	0.06	5.5 6	33.63	6.57	111.47	0.008	0.99
AL8	0.28	5.5 0	34.43	4.41	62.53	0.006	0.50
AL9	0.59	5.0 6	27.27	1.20	114.87	0.021	1.56
W2	0.86	5.8 0	29.00	2.29	102.87	<0.001	0
AL6	0.53	4.1 67	29.96 7	4.55	81.70	0.0064	0.21
W3	0.46	4.3 06	21.06 7	3.157	76.20	0.0001	0

Table A1 (continued)

Code	Square Root Chlorophyll B	Vegetation Coverage (%)	Total Volume	Elevation (m)	Natural Log Distance to River	Square Root River Slope
AL7	0.41	41.23	15.18	53	1.97	2.02
W4	<0.01	80.76	8.75	47	15.10	3.37
AL1	0	68.97	18.06	98	16.32	2.14
AL4	0.94	76.85	19.86	66	11.71	2.64
AL5	1.03	49.64	9.98	66	11.71	2.64
AL2	0.11	61.65	6.05	88	2.74	4.65
W5	0.14	72.54	17.86	42	5.33	4.75
OKW 2	0.16	32.24	14.73	35	4.26	1.06
AU2	0	62.52	5.21	26	4.96	0.01
OKW 3	0.62	86.07	19.49	30	0.45	0
BBS	0.25	54.76	7.25	88	2.74	4.65
AL3	0.22	92.47	14.86	88	1.89	6.31
OKW 1	0.02	79.46	12.67	39	4.45	1.47
AL8	0.20	87.12	39.44	58	6.67	3.17
AL9	0.51	43.80	31.00	44	1.15	2.36
W2	<0.01	84.28	22.43	48	15.10	2.91
AL6	0.12	73.22	18.34	59	24.32	2.28
W3	0	99.26	22.56	49	78.40	3.61

Table A2. Eigenvectors of Figure 11

	PC1	PC2	PC3	PC4	PC5
Sq Rt Slope	-0.001	0.002	0.003	-0.008	-0.009
pH	-0.009	-0.013	-0.036	0.083	-0.165
Temp (°C)	-0.039	-0.027	0.034	0.961	-0.154
O2	0.018	-0.028	-0.005	0.232	0.813
LN Cond	-0.004	0.003	-0.005	-0.003	0.016
Sq Rt Tan	0.001	0.001	0.002	0.002	-0.003
Chloro A	0.004	0.012	0.019	0.015	-0.1
Sq Rt Chl B	-0.001	0.006	0.012	0.012	-0.078
% Veg	-0.094	-0.982	-0.153	-0.029	-0.015
Total Vol	0.059	-0.159	0.984	-0.032	0.003
Elevation (m)	-0.992	0.086	0.072	-0.031	0.042
Ln D2R	-0.005	-0.02	-0.004	-0.106	0.341
Sq Rt River Slope	-0.049	-0.017	0.001	-0.033	-0.392

Table A3. Eigenvectors of Figure 2

	PC1	PC2	PC3	PC4	PC5
<i>A calva</i>	0	0.001	0.002	0.003	0
<i>L platyrhincus</i>	0	0.001	0.002	0.003	0
<i>E americanus</i>	0	0.004	0.008	0.011	0.001
<i>E niger</i>	0	-0.006	0.009	-0.003	-0.01
<i>U pygmaea</i>	0	0.001	0.002	0.003	0
<i>A nebulosus</i>	0	0.009	0.014	0.018	0
<i>N crysoleucas</i>	-0.001	0.012	0	-0.01	-0.023
<i>N maculatis</i>	0	0	-0.003	0	0.011
<i>E sucetta</i>	0.006	-0.002	0.009	-0.009	0.003
<i>A syanus</i>	-0.005	0.276	0.529	0.744	0.107
<i>F chrysotus</i>	0.032	-0.193	-0.257	0.248	-0.093
<i>F cingulatus</i>	0	-0.005	0.001	0.001	-0.003
<i>F lineolatus</i>	0.003	-0.019	0.012	-0.006	-0.01
<i>L ommata</i>	0.26	-0.733	0.357	-0.012	0.046
<i>G holbrooki</i>	0.961	0.225	-0.026	-0.053	0.01
<i>H formosa</i>	0.083	-0.274	-0.599	0.563	-0.025
<i>L siculus</i>	0	0.001	0	-0.001	0.004
<i>C macropterus</i>	0.001	0.033	0.015	-0.03	-0.064
<i>E chaetodon</i>	-0.001	0.001	-0.001	-0.003	-0.008
<i>E gloriosus</i>	-0.013	-0.454	0.272	0.022	-0.076
<i>E obesus</i>	0	-0.001	-0.002	0.002	0
<i>L gulosus</i>	0.001	-0.014	0.002	0.004	-0.01
<i>L macrochirus</i>	0.018	-0.066	-0.264	0.104	0.281
<i>M dolomieu</i>	0	0	-0.001	0	0
<i>M salmoides</i>	-0.001	0.001	-0.004	-0.003	0.016
<i>P nigromaculatus</i>	-0.003	0.006	-0.002	-0.021	0.067
<i>E evergladei</i>	0	0	0	-0.001	-0.001
<i>E gilberti</i>	0	0.023	0.044	0.062	0.009
<i>E okefenokee</i>	-0.023	-0.043	-0.022	-0.081	0.939
<i>E zonatum</i>	-0.001	0.074	0.142	0.2	0.029
<i>E fusiforme</i>	0.007	-0.052	-0.012	0.03	-0.005

Table A4. Eigenvectors of Figure 4.

	PC1	PC2	PC3	PC4	PC5
<i>A calva</i>	0	-0.001	0.001	0.003	0
<i>L platyrhincus</i>	0	-0.001	0.001	0.003	0
<i>E americanus</i>	0.002	-0.005	0.003	0.013	0.001
<i>E niger</i>	-0.001	0.001	0.005	0.004	-0.004
<i>U pygmaea</i>	0	-0.001	0.001	0.003	0
<i>A nebulosus</i>	0.004	-0.01	0.005	0.018	-0.017
<i>N crysoleucas</i>	0.01	-0.013	-0.001	-0.042	-0.159
<i>N maculatis</i>	0	0	0	0	0
<i>E sucetta</i>	-0.008	0.001	0.014	0.002	-0.031
<i>A syanus</i>	0.101	-0.32	0.196	0.874	0.036
<i>F chrysotus</i>	-0.023	0.19	-0.326	0.131	0.066
<i>F cingulatus</i>	0.001	0.005	0.002	0	0.007
<i>F lineolatus</i>	-0.004	0.017	0.02	0.003	-0.001
<i>L ommata</i>	-0.252	0.727	0.384	0.245	-0.414
<i>G holbrooki</i>	-0.957	-0.223	-0.006	0.013	0.159
<i>H formosa</i>	-0.054	0.29	-0.787	0.293	0.014
<i>L siculus</i>	0	-0.001	0	-0.001	0.01
<i>C macropterus</i>	0.015	-0.03	0.017	-0.078	-0.364
<i>E chaetodon</i>	0	0	0	0	0
<i>E gloriosus</i>	0.065	0.427	0.216	0.023	0.751
<i>E obesus</i>	0	0.001	-0.003	0.001	-0.001
<i>L gulosus</i>	0.002	0.014	0.001	-0.003	0.031
<i>L macrochirus</i>	-0.034	0.066	-0.193	0.045	-0.169
<i>M dolomieu</i>	0	0	0	0	0
<i>M salmoides</i>	0.002	-0.001	-0.004	-0.007	-0.015
<i>P nigromaculatus</i>	0.009	-0.011	-0.001	-0.044	-0.154
<i>E evergladei</i>	0	0	0	0	0
<i>E gilberti</i>	0.008	-0.027	0.016	0.073	0.003
<i>E okefenokee</i>	-0.003	0.001	0.005	0.001	-0.011
<i>E zonatum</i>	0.027	-0.086	0.053	0.235	0.01
<i>E fusiforme</i>	0.001	0.052	-0.028	0.009	0.14

Table A5. Eigenvectors of Figure 5

	PC1	PC2	PC3	PC4	PC5
<i>A calva</i>	0	0	0	0	0
<i>L platyrhincus</i>	0	0	0	0	0
<i>E americanus</i>	0.001	0.002	0.001	-0.019	0.029
<i>E niger</i>	0.019	0.065	0.054	0.073	0.109
<i>U pygmaea</i>	0	0	0	0	0
<i>A nebulosus</i>	0	0	0	0	0
<i>N crysoleucas</i>	0	0	0	0	0
<i>N maculatis</i>	-0.005	-0.019	-0.004	0.037	0.18
<i>E sucetta</i>	0.002	0.004	0.002	-0.039	0.058
<i>A syanus</i>	0	0	0	0	0
<i>F chrysotus</i>	0.154	0.008	-0.634	0.27	-0.523
<i>F cingulatus</i>	0.005	-0.002	-0.029	0.002	-0.047
<i>F lineolatus</i>	0.029	0.009	-0.109	-0.146	0.044
<i>L ommata</i>	-0.096	0.316	-0.203	-0.123	0.105
<i>G holbrooki</i>	-0.06	-0.338	0.54	0.084	-0.195
<i>H formosa</i>	0.058	-0.251	-0.374	0.365	0.494
<i>L siculus</i>	-0.006	0.001	-0.001	-0.003	-0.024
<i>C macropterus</i>	0.002	0.004	0.002	-0.039	0.058
<i>E chaetodon</i>	0	0	0	0	0
<i>E gloriosus</i>	-0.096	0.509	0.28	0.777	-0.018
<i>E obesus</i>	0	0	0	0	0
<i>L gulosus</i>	0.004	-0.008	0.022	-0.003	-0.064
<i>L macrochirus</i>	0.074	-0.666	0.014	0.356	-0.056
<i>M dolomieu</i>	0.002	-0.001	-0.014	0.001	-0.024
<i>M salmoides</i>	-0.019	-0.001	0.023	0.008	-0.109
<i>P nigromaculatus</i>	-0.094	0.005	0.017	-0.056	-0.578
<i>E evergladei</i>	0.005	0.008	0.003	-0.078	0.117
<i>E gilberti</i>	0	0	0	0	0
<i>E okefenokee</i>	-0.967	-0.125	-0.169	0.023	0.01
<i>E zonatum</i>	0	0	0	0	0
<i>E fusiforme</i>	-0.026	0.01	0.015	0.02	-0.095

Table A6. Eigenvectors of Figure 6.

	PC1	PC2	PC3	PC4
<i>A calva</i>	0	0	0	0
<i>L platyrhincus</i>	0	0	0	0
<i>E americanus</i>	0.008	-0.003	0.07	-0.248
<i>E niger</i>	-0.02	-0.044	-0.011	-0.039
<i>U pygmaea</i>	0	0	0	0
<i>A nebulosus</i>	0	0	0	0
<i>N crysoleucas</i>	0.001	0.027	0.086	0.261
<i>N maculatis</i>	0	0	0	0
<i>E sucetta</i>	0	0	0	0
<i>A syanus</i>	0	0	0	0
<i>F chrysotus</i>	-0.379	-0.737	-0.175	0.324
<i>F cingulatus</i>	0	0	0	0
<i>F lineolatus</i>	-0.041	-0.089	-0.023	-0.077
<i>L ommata</i>	0	0	0	0
<i>G holbrooki</i>	0.897	-0.44	-0.016	0.041
<i>H formosa</i>	0	0	0	0
<i>L siculus</i>	0	0	0	0
<i>C macropterus</i>	-0.08	-0.207	0.966	-0.076
<i>E chaetodon</i>	-0.203	-0.443	-0.114	-0.386
<i>E gloriosus</i>	0	0	0	0
<i>E obesus</i>	0	0	0	0
<i>L gulosus</i>	0	0	0	0
<i>L macrochirus</i>	-0.041	-0.089	-0.023	-0.077
<i>M dolomieu</i>	0	0	0	0
<i>M salmoides</i>	-0.006	0.058	0.101	0.771
<i>P nigromaculatus</i>	0	0	0	0
<i>E evergladei</i>	0	0	0	0
<i>E gilberti</i>	0	0	0	0
<i>E okefenokee</i>	0	0	0	0
<i>E zonatum</i>	0	0	0	0
<i>E fusiforme</i>	-0.02	-0.044	-0.011	-0.039

Table A7. Complete species list by family.

<u>Family</u>	<u>Genus species</u>
Ictaluridae	<i>Ameiurus nebulosus</i>
Amiidae	<i>Amia calva</i>
Aphrododeridae	<i>Aphrododerus syanus</i>
Centrarchidae	<i>Centrarchus macropterus</i>
Elassomatidae	<i>Elassoma evergladei</i>
Elassomatidae	<i>Elassoma gilberti</i>
Elassomatidae	<i>Elassoma okefenokee</i>
Elassomatidae	<i>Elassoma zonatum</i>
Centrarchidae	<i>Enneacanthus chaetodon</i>
Centrarchidae	<i>Enneacanthus gloriosus</i>
Centrarchidae	<i>Enneacanthus obesus</i>
Catostomidae	<i>Erimyzon sucetta</i>
Esocidae	<i>Esox americanus</i>
Esocidae	<i>Esox niger</i>
Percidae	<i>Etheostoma fusiforme</i>
Fundulidae	<i>Fundulus chrysotus</i>
Fundulidae	<i>Fundulus cingulatus</i>
Fundulidae	<i>Fundulus lineolatus</i>
Poeciliidae	<i>Gambusia holbrooki</i>
Poeciliidae	<i>Heterandria formosa</i>
Atherinopsidae	<i>Labodestes siculus</i>
Lepisosteidae	<i>Lepisosteus platyrhincus</i>
Centrarchidae	<i>Lepomis gulosus</i>
Centrarchidae	<i>Lepomis macrochirus</i>
Fundulidae	<i>Leptolucania ommata</i>
Centrarchidae	<i>Micropterus dolomieu</i>
Centrarchidae	<i>Micropterus salmoides</i>
Cyprinidae	<i>Notimagonus crysoleucas</i>
Cyprinidae	<i>Notropis maculatis</i>
Ictaluridae	<i>Noturus gyrinus</i>
Centrarchidae	<i>Pomoxis nigromaculatus</i>
Umbridae	<i>Umbra pygmaea</i>

APPENDIX B:
IACUC Approval

July 8, 2011

Dr. David Bechler
Department of Biology
Valdosta State University

RE: AUP-00039-2011
*The Status of the Black Banded
Sunfish & Other Select Species
In the State of Georgia*

Dear Dr. Bechler:

Your Animal Use Protocol referenced above has been approved by the Institutional Animal Care and Use Committee under Animal Welfare Assurance Number A4578-01. This approval is for the period of July 8, 2011 through July 7, 2014. Each year, an annual review and report request must be submitted to the IACUC to keep your protocol active. You will be contacted by the IACUC Administrator in the Office of Sponsored Programs & Research Administration approximately two months before the annual review request and report is due.

Please remember that you must obtain IACUC approval before amending or altering the scope or procedures of the protocol. You are also required to report to the Attending Veterinarian, the IACUC Chair, and/or the IACUC Administrator any unanticipated problems with the animals, which become apparent during the course, or as a result of, the research activity.

You will find the IACUC's Standard Operating Procedures and helpful resources on the Office of Sponsored Programs & Research Administration website at <http://www.valdosta.edu/ospra>. However, if you have any questions, please contact the IACUC Administrator at iacuc@valdosta.edu or 333-7837.

Sincerely,



Barbara H. Gray, Director
Office of Sponsored Programs
& Research Administration
IACUC Administrator

Cc: Dr. Phil Gunter, Institutional Official
Dr. Theresa Grove, IACUC Chair
Dr. Teresa Doscher, Attending Veterinarian
Dr. Robert Gannon, Biology Department Head