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# FISH ASSEMBLAGES OF THE WITHLACOOCHEE RIVER BASIN IN SOUTH GEORGIA, USA

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# FISH ASSEMBLAGES OF THE WITHLACOOCHEE RIVER BASIN IN SOUTH GEORGIA, USA

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## ABSTRACT

An ichthyofauna review of literature prior to 2008 on the Withlacoochee River basin in south-central Georgia, USA, indicated that the fish fauna was underrepresented compared to other rivers, such as the Suwannee River basin in Florida of which the Withlacoochee River is a tributary. A survey would provide the state of Georgia potentially valuable additional information on the fish fauna within the basin. A 45-site survey within the eastern portion of the Withlacoochee River basin was conducted from 2008 to 2010 employing seines, gill nets, fish traps, trotlines, and creel surveys. Surveys yielded 51 fish species of which three, the Alabama Shad (*Alosa alabamae*), the Suwannee Bass (*Micropterus notius*), and the Metallic Shiner (*Pteronotropis metallicus*), are species of concern in Georgia with the Alabama Shad and the Suwannee Bass listed on the International Union for Conservation of Nature threatened species red list. Three species not previously recorded in the Withlacoochee River basin, the Brown Darter (*Etheostoma edwini)*, the Gulf Coast Pygmy Sunfish (*Elassoma gilberti*), and the Hogchoker (*Trinectes maculatus*) were collected. The first known sympatric populations of the Everglades Pygmy Sunfish (*Elassoma evergladei*) and the Gulf Coast Pygmy Sunfish were found at one location. Invasive species found in the drainage basin were the Green Sunfish (*Lepomis cyanellus*) and two specimens of Pacu (*Piaractus brachypomus*). A Friedman cumulative comparison indicated that fish assemblage distribution patterns, as related the stream orders of the river and the potential impact of a sinkhole plain in the river, did not vary from other published surveys on distribution patterns. However, a cluster analysis comparing species composition by collection site showed some similarities among river orders.

***Keywords:*** fish survey, species richness, distribution patterns, assemblage structures, species of concern

## INTRODUCTION

The objectives of this research were to conduct a detailed ichthyofaunal survey of the eastern portion of the Withlacoochee River basin from its headwaters to the Georgia/Florida state line to assess species present and fish assemblage structure throughout the watershed. The Georgia Atlas of Freshwater Fishes compiled by Straight et al. (2009) indicates the Withlacoochee River basin is underrepresented regarding fish known to inhabit the system. Besides examining fish biodiversity and assemblage structure, the current research identified variations in the hydrology and geomorphology as they related to variations in ichthyofauna. The current survey documents range extensions for both invasive species and species of concern listed for the state of Georgia. These results show the importance of the understudied river systems of southern Georgia with respect to their biodiversity and hydrology. This research, like all surveys, provides baseline information for future studies done in the Withlacoochee River system and elsewhere.

## The Withlacoochee River System

The eastern headwaters of the Withlacoochee River Basin (Figure 1) start out as Little Creek and the New River in and southeast of Tifton, Georgia (Mobile Water Data 2015). After being joined by Young Mill Creek at Georgia Highway 37, the river is referred to as the Withlacoochee, which lies to the east of New River. From there, the eastern portion of the basin drains southward into Florida where it flows into the Suwannee River. These rivers have a high degree of hydrologic and geomorphic diversity throughout the length of the system. The headwaters of the system are composed of small, slow-moving streams and interconnected wetlands. The first few kilometers of the system are shallow waterways with sandy bottoms lying predominantly in flatwoods habitats (Barnett et al. 2007; Riggs et al. 2010). Downstream, the river is a faster flowing rocky-bottom river with high sand and clay banks and a sinkhole plain. The karst topography of the region is evident in the structure of the river as it approaches the Georgia/Florida state line, where the geomorphology changes again, and the river becomes a slow-moving river with limestone banks, long runs, and greater depths.

The Little River, which is not completely depicted in Figure 1, originates in Turner County, Georgia, flows south into western Tift County, and joins the Withlacoochee River south of Hwy 133 west of Valdosta, Lowndes County. Other smaller streams include Okapilco Creek, which starts in Worth County and flows through Colquitt and Brooks Counties before flowing into the Withlacoochee River, and Piscola Creek, which starts in Thomas County, flows into Brooks County and joins Okapilco Creek before flowing into the Withlacoochee River southeast of Quitman, Georgia.

The Withlacoochee River basin has been understudied with respect to its vertebrate fauna and factors influencing the faunal diversity and distribution of species. Several species of fish have a documented range that covers many rivers in north Florida, but the northern boundary of those ranges has previously been depicted to end at the Georgia state line (Lee et al. 1980). This indicates that survey work has been more extensive in Florida, but is lacking or unreported in south Georgia. As a tributary to the Suwannee River, the Withlacoochee may be inhabited by much of the same ichthyofauna, and surveying the river should provide documented range extensions for species whose known ranges currently end at the state line.

One potentially important attribute of the Withlacoochee river system is a sinkhole plain northwest of Valdosta, Georgia, where, during low water periods, the river drains completely underground through multiple sinkholes (McConnell and Hacke 1993; Bechler and Salter 2014) contributing an average 3.17 $m^3/s$ to the Floridan aquifer (Krause 1979). The recharge into the upper Floridan aquifer (Plummer et al. 1998) begins to return to the surface with increased volume through springs south of the sinkhole bed. This section of the river is of interest because it raises questions concerning fish assemblage structure north and south of the sinkhole plain. Environmental variability such as this and other factors (e.g. floods) can restrict or prevent the formation of persistent assemblages (Kuehne 1966; Capone and Kushlan 1991). A key hypothesis that can be examined is whether the ephemeral surface flow north of the sinkhole plain and the presence of the sinkhole plain are effectively dividing the river system into two separate ichthyofaunal systems that are significantly different in fish fauna structure or does the fish fauna maintain a continuous degree of change from the headwaters to the lower end of the river, as has been observed by others (Kuehne 1966; Sheldon 1968)?

Brett Albanese (personal communication) identified seven species of concern that the Georgia Department of Natural Resources is seeking distribution data on in the Withlacoochee River: (1) Spotted Bullhead (*Ameiurus serracanthus*), (2) Alabama Shad (*Alosa alabamae*), (3) Metallic Shiner (*Pteronotropis metallicus*), (4) Suwannee Bass (*Micropterus notius*), (5) Blackbanded Sunfish (*Enneacanthus chaetodon*), (6) Gulf Sturgeon (*Acipenser oxyrinchus desotoi*), and (7) Gulf Coast Pygmy Sunfish (*Elassoma gilberti*). *Acipenser oxyrinchus desotoi* is federally listed as a threatened species through the Endangered Species Act (ESA 2019). Assessing the presence or absence of these species of concern would lead to future conservation efforts to help protect the aquatic habitats and surrounding environment.

This survey also assessed the presence of invasive fish species in the watershed. Invasive species in other local rivers are the Flathead Catfish (*Pylodictis olivaris*) and the Common Carp (*Cyprinus carpio*), among others. Flathead catfish attain a large body size and have a widespread and increasing range. As a piscivorous top predator, it is thought to be responsible for the top-down restructuring of fish fauna in many of the habitats it invades (Vokoun and Rabeni 2006). The presence of invasive species in the Withlacoochee River could cause assemblage restructuring throughout the system and could cause significant changes to the population dynamics of native fish in the area.

As more detailed survey work takes place in the remainder of the Withlacoochee Basin and other aquatic systems in Georgia and Florida, analyses of similarities in fish fauna structure between rivers and basins can be carried out in greater detail. Examples of such studies include Winemiller (1995), Cook et al. (2004), Hoeinghaus et al. (2007), and Olden et al. (2010). In addition, data presented in this study will allow indexes of biological integrity to be assessed should changes in the habitat structure of the Withlacoochee River and its associated basin take place (Angermeier and Karr 1986). Over time, local and regional influences impacting the hydrology of the system, the introduction of invasive species (Fausch et al. 1990; Angermeier and Winston 1998), and the effects on vulnerable species (Angermeier 1995) can be better assessed.

## MATERIAL & METHODS

### Study Sites

Fish were surveyed at 45 sites within the Georgia stretch of the Withlacoochee River basin from its headwaters near Tifton, Georgia, to south Lowndes and southeastern Brooks Counties, where the river flows into Florida (Appendix Table I, Figure 1). Sites were identified by alphanumeric codes such that 1 to 4 indicates 1$^{st}$ through 4$^{th}$ order stream sites and a character (A through S) indicates individual sites on each stream

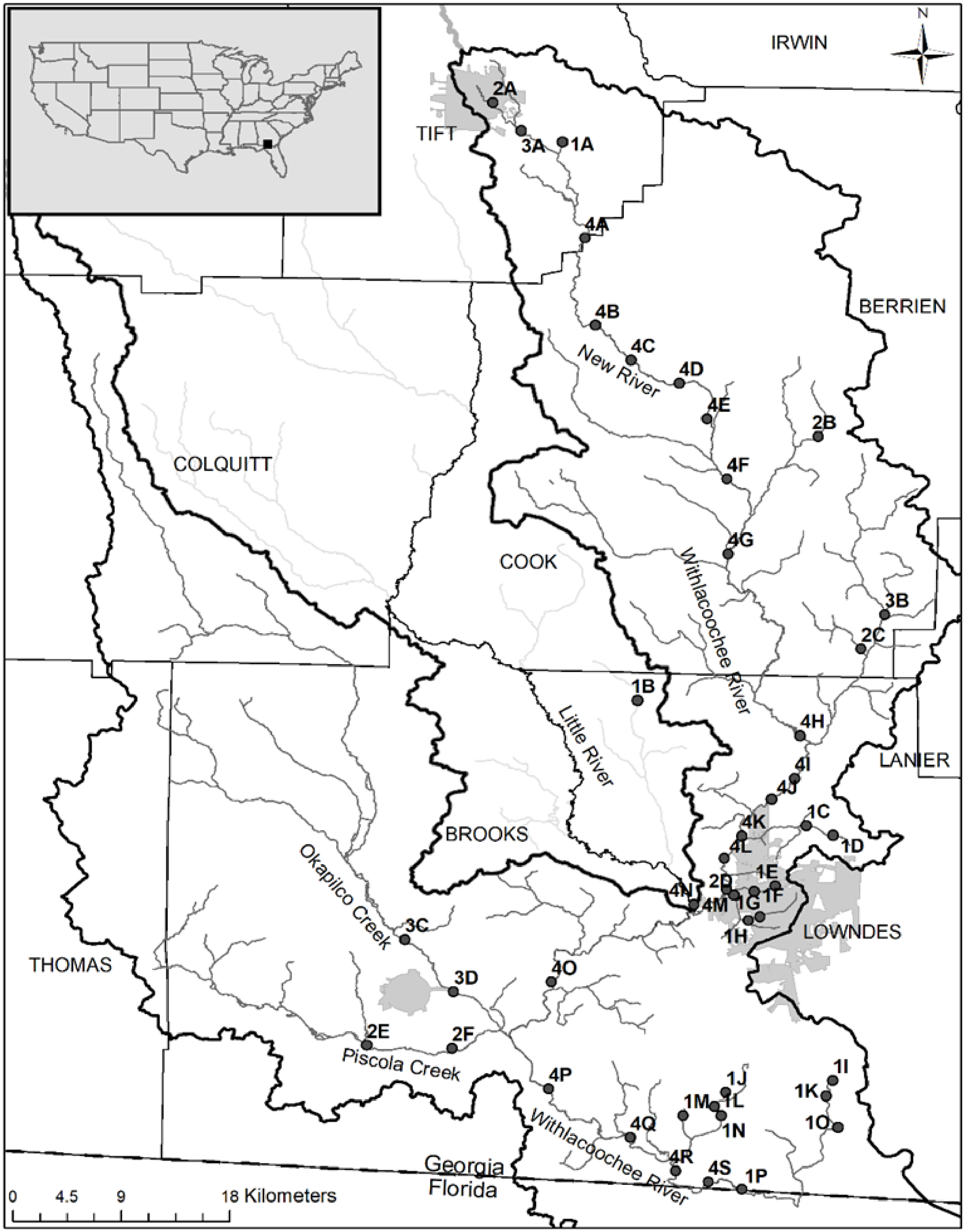


***Figure 1.*** Withlacoochee River basin, Georgia, USA. Alphanumeric codes represent collection sites with numeric values 1 through 4 representing river streams and letters representing sites sequentially from north to south. Names composed of all capital letters indicate county names. The gray area above 'TIFT' shows Tifton, Georgia, and the gray area above and west of 'LOWNDES' indicates Valdosta, Georgia. The dark square on USA map, in the upper-left corner, shows the location in the state of Georgia. Georgia map courtesy of the U.S. Geological Survey.

order starting from north to south. An alphanumeric code was employed to more efficiently identify the structure of collecting sites (the stream order and the north-to-south location) as they relate to Figure 1 and tables as well as the text of the publication. Collecting primarily occurred when weather permitted in the spring, summer, and fall months, with most collections made in the summer and fall. An inhibiting factor was high waters in the spring months.

Dahlberg and Scott (1971a) reviewed the fishes of the Withlacoochee River basin at 10 sites using collection data from the University of Georgia, Tulane University, and the University of Michigan. Selected sites were sampled during Ichthyology classes, offered by the Department of Biology at Valdosta State University. Lots from these collections were included as part of this study as well as collections from Auburn University, Tulane University, Cornell University, the Florida Museum of Natural History, the University of Michigan, and the Georgia Department of Natural Resources Nongame Conservation Section.

## Field Collections and Methods

For conservation, a maximum of five voucher specimens per species per site were collected, and all other fish that could be accurately identified in the field were recorded and released. Specimens retained were euthanized in the field with tricaine methyl sulfonate (MS-222) in accordance with Valdosta State University's Institutional Animal Care and Use Committee regulations, which are based on the American Society of Ichthyologists and Herpetologists' Use of Fish in Research guidelines. Voucher specimens were fixed in 10% formalin for 24 hours, soaked eight or more hours in low volume continuous water drip, and preserved in 55% isopropyl alcohol in accordance with Georgia Department of Natural Resources (Brett Albanese, personal communication). Multiple seines were used as the primary collection method (seine dimensions: 235 cm W × 135 cm H × 0.25 cm mesh; 170 cm W × 120 cm H × 1 cm mesh; and 450 cm W × 125 cm H × 0.25 cm mesh) dependent on site structure. At each collection site, seining occurred until all habitat types (e.g., riffles, pools, fallen limbs, aquatic plants, hollow logs) were sampled resulting in variable reaches of each site being seined. Seining stopped after 10 consecutive hauls returned no new species. Two gill net types (monofilament and twine) each of three different dimensions (sizes: 30.48 m W × 1.83 m H × 7.62 cm, mesh; 45.7 m W × 1.83 m H × 7.62 cm mesh; and 30.48 m W × 1.83 m H × 2.54 cm mesh) were set to catch fish not easily captured by seining. Gill nets were placed across the river during the spring months to catch anadromous fish from the Gulf of Mexico; however, high water during the spring of both years often prevented setting gill nets spanning the river. Gill nets were set parallel to one bank or were set in backwaters, such as springs and sloughs. Trotlines were made with nylon cord and had treble hooks attached to 30 cm leads that hung below the main line with hook lines positioned 1.0 to 1.3 m apart along the main line, which varied based on where each end could be attached to a tree limb overhanging the water. One to two trotlines were set out at sites that could be visited the next morning. Bait for the trotlines alternated between artificial "stink bait" and bait recommended by fishermen. Fish traps (1.37 m long cylinders, 2.5 cm mesh with inverted cone entrances) were baited with canned cat food, cotton-seed cake, and sardines. Four fish traps were set at sites that could be visited the next morning. Both gill nets and traps were set in the late afternoon and checked early the following morning. When possible, creel surveys of local fishermen were made with four occurring.

The Peterson Field Guide to Freshwater Fishes (Page and Burr 1991), the Atlas of North American Freshwater Fishes (Lee 1980), Fishes of Alabama (Boschung and Mayden 2004), and The Fishes of Tennessee (Etnier and Starnes 1994), as well as select publications on new species (Snelson et al. 2009), were used to identify species. Data collected was recorded as present or absent (detection = 1, no detection = 0) binary data. Hydrological watershed maps developed by the Georgia Department of Natural Resources (Environmental Protection Agency Division) and the U.S. Geological Survey, were used in ArcGIS 9.3 (ESRI Inc. 2008) to georeference collection sites. Data and figures were organized using Microsoft Excel 2007 and imported into StatsDirect (StatsDirect Ltd 2007) and PRIMER v6.1 (Clarke and Gorley 2006) for similarity (Bray-Curtis similarity option), dendrogram, and cluster analysis. Due to the low sample sizes ($n$ = 10) of 2$^{nd}$ and 3$^{rd}$ order streams, and because they were hydrologically very similar to 1$^{st}$ order streams, these two orders were combined for some analyses.

## Data Analyses

Because only representative specimens of each species were kept at collection sites, data are nonparametric, and analyses are based on presence or absence. Cluster analysis was used to compare the similarities in species composition among collection sites (Clarke and Gorley 2006). Specific analyses involved the following: (1) comparisons of fish assemblage above and below the sinkhole plain (site 4K), (2) analyses of fish assemblages by and between stream orders, and (3) a systematic analysis of species presence as related to the contribution of various species based on steam order, presence of species of concern, and invasive species.

# RESULTS

A total of 51 fish species (Table I, Appendix Tables II, III, and IV) were collected across all sites. Four species were found only in 1$^{st}$ order streams with an occurrence of 6.3–12.5%; while 13 (25.5%) species were found only in 4$^{th}$ order streams with an occurrence of 5.2–42.1% (Table II). Twenty-five species (49.0%) were found in all stream orders. No species was collected at every site; however, *L. macrochirus* and *G. holbrooki* were each collected at 86.7% of all sites and in all stream orders (Table II).

To examine relationships between stream orders and potential influencing factors, null and alternate hypothesis were developed and examined. The null hypothesis was collection sites cluster (discussed below) based on their stream order such that a cluster consists predominantly of a specific stream order. Supplementary to the null hypothesis is that closely related stream orders (example: 3$^{rd}$ and 4$^{th}$ orders) show some clustering due to transitional habitats within each stream order. The alternate hypothesis is that variations in species composition, and therefore habitat types, result in stream orders not closely related showing some clustering.

## Cluster Analysis

To examine similarities in species composition among collection sites, a cluster analysis was run producing a dendrogram (Figure 2). All sites with a minimum similarity of 48% were considered part of a single group with six groups occurring. The 48% cutoff was chosen because it most closely reflected natural groupings such that at least half of all

***Table I***. Phylogenetic listing of families and alphabetical listing of species within families in the Withlacoochee River study area. Phylogenetic order is based on Moyle and Cech (2004), Page and Burr (2011), and FishBase (2015).

| Family | Species | Family | Species |
|---|---|---|---|
| Lepisostidae | *Lepisosteus osseus* | Fundulidae | *Fundulus chrysotus* |
| | *Lepisosteus platyrhincus* | | *Fundulus cingulatus* |
| | | | *Fundulus lineolatus* |
| Amiidae | *Amia calva* | Poeciliidae | *Gambusia holbrooki* |
| Anguillidae | *Anguilla rostrata* | | *Heterandria formosa* |
| Clupeidae | *Alosa alabamae* | | *Leptolucania ommatta* |
| Umbridae | *Umbra pygmaea* | Achiridae | *Trinectes maculatus* |
| Esocidae | *Esox americanus vermiculatus* | Antherinopsidae | *Labidesthes sicculus* |
| | | Elassomatidae | *Elassoma evergladei* |
| | *Esox niger* | | *Elassoma gilberti* |
| Cyprinidae | *Cyprinella venusta* | | *Elassoma okefenokee* |
| | *Notemigonus crysoleucas* | Centrarchidae | *Centrarchus macropterus* |
| | | | *Elassoma zonatum* |
| | *Notropis maculatus* | | *Enneacanthus gloriosus* |
| | *Notropis petersoni* | | *Enneacanthus obesus* |
| | *Notropis texanus* | | *Lepomis auritus* |
| | *Opsopoeodus e. emiliae* | | *Lepomis cyanellus* |
| | *Pteronotropis metallicus* | | *Lepomis gulosus* |
| | | | *Lepomis macrochirus* |
| Catastomidae | *Erimyzon sucetta* | | *Lepomis marginatus* |
| | *Minytrema melanops* | | *Lepomis microlophus* |
| | | | *Lepomis punctatus* |
| Ictaluridae | *Ameiurus natalis* | | *Micropterus notius* |
| | *Ameiurus nebulosus* | | *Micropterus salmoides* |
| | *Ictalurus punctatus* | | *Pomoxis nigromaculatus* |
| | *Noturus gyrinus* | Percidae | *Etheostoma edwini* |
| | *Noturus leptacanthus* | | *Etheostoma fusiforme* |
| Aphredoderidae | *Aphredoderus sayanus* | | *Percina nigrofasciata* |

sites were represented by the same or consecutive stream orders (example: group 2 is composed of six sites and contains four 1st and 2nd order streams). Species within each group that occurred in 50% or more of all sites within the group were examined as to habitat preferences using Page and Burr (2011) and FishBase (2015). The value of 50% occurrence was chosen for two reasons: (1) greater occurrence most strongly influence the Bray-Curtis dissimilarity index that the cluster diagram (Figure 2) is based on (Clarke and Warwick, 2001), and (2) fish with the greatest occurrence across multiple sites should also reflect the most abundant or primary habitat types at sites thus giving insight into factors influencing assemblage structure in each group. Three sites, 4S, 4R, and 1M, fell outside of the 48% similarity minimum value and showed very limited similarity to each other or any group of sites and are not analyzed as part of any group.

Group 1 consisted of sites 1E, 1H, 1K, 1N, 2A, and 4M. Of the 10 species in this group, the most abundant ($n = 5$) were dominated by the Centrarchidae and included *M. salmoides, C. macropterus, L. macrochirus,* and *L. auritus*. Dominant species, based on 50% occurrence, included *L. macrochirus, L. auritus, N. texanus*, and *G. holbrooki.*

***Table II.*** Occurrence by stream order. Stream orders are 1st, 2nd and 3rd, and 4th. Due to low sample sizes and physical similarities, 2nd and 3rd order streams are combined. Percentages, the numbers under the stream orders, are rounded up to the nearest 0.1%. Sample sizes for stream order is as follows: 1st order = 16, 2nd and 3rd orders = 10, and 4th order = 19.

| Species name | 1st | 2nd, 3rd | 4th | Species name | 1st | 2nd, 3rd | 4th |
|---|---|---|---|---|---|---|---|
| *L. marginatus* | 6.3 | 10.0 | 5.3 | *P. nigromaculatus* | 37.5 | 30.0 | 42.1 |
| *L. microlophus* | 6.3 | 10.0 | 10.5 | *E. a. vermiculatus* | 43.8 | 50.0 | 47.4 |
| *L. ommatta* | 6.3 | 10.0 | 0.0 | *P. nigrofasciata* | 43.8 | 40.0 | 63.2 |
| *F. lineolatus* | 6.3 | 0.0 | 5.3 | *L. punctatus* | 43.8 | 30.0 | 79.0 |
| *L. cyanellus* | 6.3 | 0.0 | 5.3 | *L. auritus* | 50.0 | 40.0 | 52.6 |
| *E. zonatum* | 6.3 | 0.0 | 10.5 | *L. gulosus* | 56.3 | 20.0 | 36.8 |
| *P. metallicus* | 6.3 | 0.0 | 15.8 | *N. crysoleucas* | 62.5 | 20.0 | 42.1 |
| *U. pygmaea* | 6.3 | 0.0 | 0 .0 | *C. macropterus* | 68.8 | 30.0 | 36.8 |
| *F. cingulatus* | 6.3 | 0.0 | 0.0 | *L. macrochirus* | 81.3 | 100.0 | 84.2 |
| *E. obesus* | 6.3 | 0.0 | 0.0 | *G. holbrooki* | 87.5 | 80.0 | 89.5 |
| *L. platyrhincus* | 12.5 | 10.0 | 5.3 | *N. petersoni* | 0.0 | 10.0 | 52.6 |
| *A. nebulosus* | 12.5 | 10.0 | 5.3 | *A. calva* | 0.0 | 10.0 | 5.3 |
| *F. chrysotus* | 12.5 | 10.0 | 10.5 | *N. maculatus* | 0.0 | 0.0 | 5.3 |
| *E. gilberti* | 12.5 | 0.0 | 0 .0 | *I. punctatus* | 0.0 | 0.0 | 5.3 |
| *N. leptacanthus* | 18.8 | 0.0 | 21.1 | *N. gyrinus* | 0.0 | 0.0 | 5.3 |
| *E. okefenokee* | 18.8 | 10.0 | 0 .0 | *E. evergladei* | 0.0 | 0.0 | 5.3 |
| *E. gloriosus* | 25.0 | 20.0 | 5.3 | *T. maculatus* | 0.0 | 0.0 | 5.3 |
| *E. niger* | 25.0 | 40.0 | 15.8 | *A. rostrata* | 0.0 | 0.0 | 10.5 |
| *H. formosa* | 25.0 | 10.0 | 15.8 | *M. notius* | 0.0 | 0.0 | 10.5 |
| *A. natalis* | 25.0 | 20.0 | 21.1 | *E. edwini* | 0.0 | 0.0 | 10.5 |
| *A. sayanus* | 25.0 | 40.0 | 73.7 | *A. alabamae* | 0.0 | 0.0 | 15.8 |
| *N. texanus* | 25.0 | 30.0 | 73.7 | *O. e. emiliae* | 0.0 | 0.0 | 15.8 |
| *E. sucetta* | 31.3 | 10.0 | 10.5 | *M. melanops* | 0.0 | 0.0 | 15.8 |
| *E. fusiforme* | 31.3 | 50.0 | 63.2 | *L. osseus* | 0.0 | 0.0 | 21.1 |
| *M. salmoides* | 31.3 | 30.0 | 68.4 | *C. venusta* | 0.0 | 0.0 | 42.1 |
| *L. sicculus* | 31.3 | 40.0 | 79.0 | | | | |

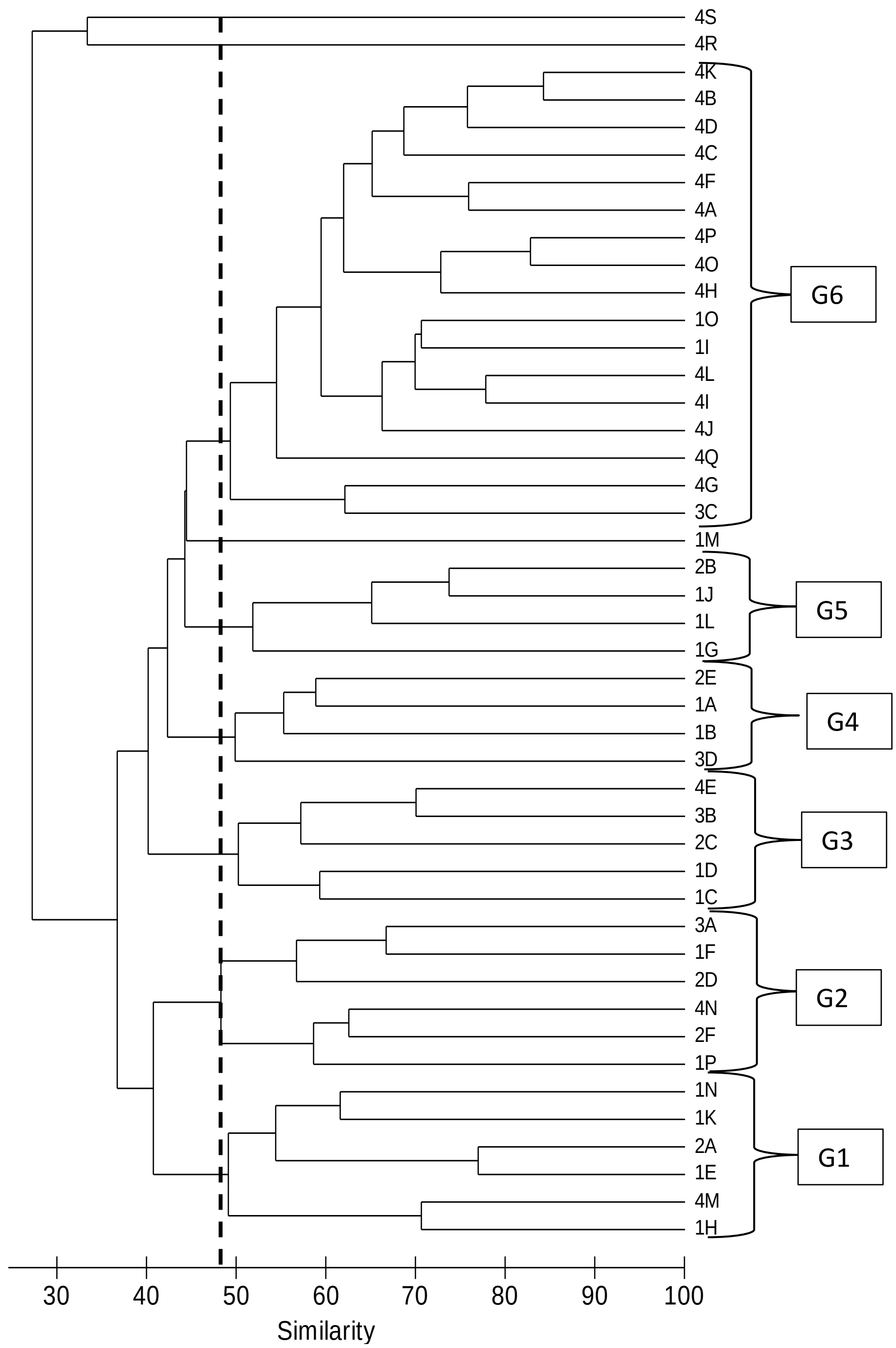


***Figure 2.*** Species assemblage similarity dendrogram. Collecting sites follow the alphanumeric code in Table I in the appendix, Figure 1, and Canister (2009). The letter *G* plus a number indicates groupings that represent 48% similarity. The dashed line represents the 48% break point for similarities in grouped sites described in the main text.

Group 2 included sites 1F, 1P, 2D, 2F, 3A, and 4N. Of the 17 species in this group, five had an occupancy of 50% or greater. These are *P. nigrofaciata, L. auritus, L. punctatus, L. macrochirus*, and *G. holbrooki.*

Group 3 included sites 1C, 1D, 2C, 3B, and 4E. A total of 22 species occurred in this group with eight having an occupancy of 50% or greater. Dominant fish species included *E. okefenokee, E. niger, E. americanus, E fusiforme, A. sayanus, C. macropterus, L. macrochirus*, and *G. holbrooki*.

Group 4 included sites 1A, 1B, 2E, and 3D. A total of 11 species occurred in this group with eight having an occupancy of 50% or greater. Dominant fish species were *L. platyrhincus, E. niger, A. sayanus, C. macropterus, L. macrochirus, G. holbrooki*, and *L. sicculus*.

Group 5 included sites 1G, 1J, 1L, and 2B. A total of 19 species occurred in this group with 10 having an occupancy of 50% or greater. Dominant species included *E. gloriosus, E. sucetta, A, natalis, L. gulosus, N. crysoleucas, C. macropterus, L. macrochirus, L. punctatus, L. auritus,* and *G. holbrooki*.

Group 6 included 16 sites with only three (1I, 1O, 3C) not being 4$^{th}$ order sites. A total of 42 species occurred in this group with 14 having an occupancy of 50% or greater. These are *N. petersoni, L. gulosus, L. macrochirus, L. punctatus, P. nigromaculatus, M. salmoides, E. americanus, N. crysoleucas, P. nigrofasciata, E. fusiformi, A. sayanus, L. sicculus, N. texanus*, and *G. holbrooki*.

Out of all 4$^{th}$ order sites ($n$ = 19), 14 clustered in group 6 (Figure 2) with similarity values of 50.0% or greater. Exceptions to this were sites 4S and 4R, which breakout at a similarity value of 25.0%, and sites 4E, 4N and 4M, which are interspersed with 1$^{st}$ through 3$^{rd}$ order streams with similarity values ranging from 47.0 to 70.6%. Less distinct patterns of association are seen for 1$^{st}$ order streams ($n$ = 16) with eight showing the strongest similarities to other 1$^{st}$ order streams, three showing the strongest similarities to 2$^{nd}$ order streams, and the remainder showing a mix of similarities to 3$^{rd}$ and 4$^{th}$ order streams.

## Sinkhole Plain Impact

Site 4K (Figure 1) represents the sinkhole plane and recharge zone of the Floridan aquifer (Krause 1979; Plummer et al. 1998). Sites north of the sinkhole plane experience considerable desiccation during droughts with only bridge sites and limited reaches of streams composed of deeper runs and channels retaining water (Wright 2013). To test the hypothesis that sites north and south of the sinkhole plane differ in assemblage structure, the occurrence of all species based on presence and absence at all 1$^{st}$ through 4$^{th}$ order sites north of the sinkhole plain was compared to all such sites south of the sinkhole plain. A two-sided Kendall tau test was highly significant ($P < 0.0001$) for the nonparametric ordinal ranking of species north verses south of the sinkhole plain indicating a strong positive similarity in rank order. Since the hydrologic structure of the Withlacoochee River is considerably different below the sinkhole plain, especially below its junction with the Little River, additional comparisons of species rank order were conducted comparing 1$^{st}$ order, 2$^{nd}$ plus 3$^{rd}$ order, and 4$^{th}$ order sites above and below the sinkhole plain. All three comparisons were highly significant (two-sided Kendall tau test: 1$^{st}$ order comparisons, $P < 0.012$; 2$^{nd}$ plus 3$^{rd}$ order comparisons, $P < 0.002$, 4$^{th}$ order comparisons, $P < 0.001$) indicating that the occurrence and rankings of species within each stream order are similar above and below the sinkhole plain.

## Stream Order Comparisons and Influences

To assess the influence of stream order on the total species count ($n$ = 51), a species accumulation plot by stream order collection sites was produced (Figure 3). First order sites contributed the greatest number of species ($n$ = 37) to the overall count with 13 of the 16 sites adding one to 10 new species. Of the 10 combined 2nd and 3rd order sites, only two sites (2D and 2E) increased the total species count by adding two new species. Of the nineteen 4th order sites, nine sites added one or more new species to the total species count.

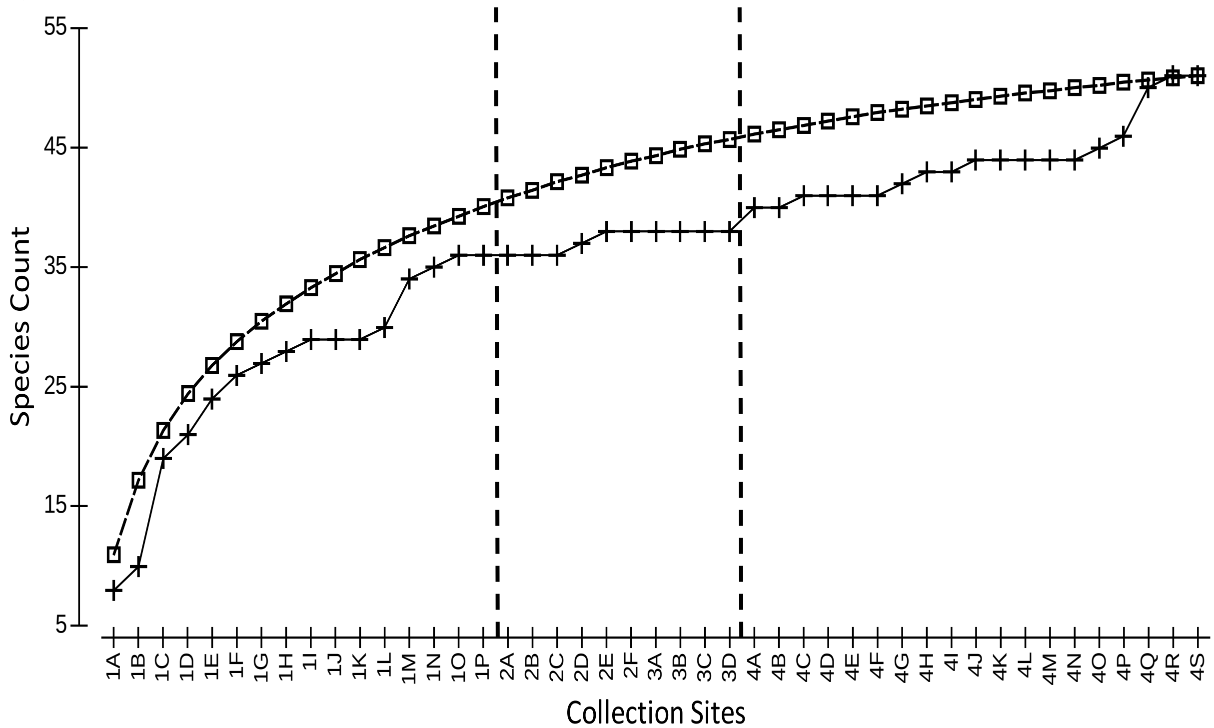


***Figure 3.*** Species accumulation plot by stream order collection site. Square (□) symbols represent the UGE (Primer 6) accumulation values. Plus (+) symbols represent the Sobs index for species accumulation. Dashed lines demark 1st order, 2nd plus 3rd order, and 4th order sites. Collecting sites follow the alphanumeric code in Table I.

To compare assemblages among stream orders, sites were pooled by stream order except for 2nd and 3rd order streams, which were combined as one group. Each species was assigned a value based on its rate of occurrence or sum across all sites within the stream order from which the species was collected. We found no difference in overall assemblage structure above and below the sinkhole plain (Friedman cumulative comparison, $P = 0.0571$; binary comparison, $P = 0.4722$) reaffirming the results of the Kendal tau test. However, all possible pair-wise comparisons based on stream order did yield significant differences indicating differences based on stream order (cumulative comparison, $P = 0.0001$; squared ranks approximate equality of variance/chi square, $df = 2$, $P = 0.0001$). This analysis was followed by pair-wise comparisons (1st vs 2nd and 3rd, $P = 0.0051$; 1st vs 4th, $P = 0.0004$; 2nd and 3rd vs 4th, $P = 0.0001$) indicating significant differences in species composition between stream orders.

**Species Systematic Analysis**

The Cypriniformes were represented by nine species (Table I; Appendix Table I) with the most abundant being *Notropis texanus* (Cyprinidae) present at 21 sites. Specimens of *Cyprinella venusta* matched the subspecies *C. v. cercostigma* based on specific morphometric traits (*n* = 11 specimens) that included: (1) pharyngeal tooth count of 1,4—4,1, (2) lateral line scales *r* = 37—40, (3) scales around the body 27—31, and (4) a dark spot in the more posterior portion of the dorsal fin. An exception to the basic pattern found in *C. v. cercostigma* was that some specimens possessed a weak dark line extending into the spot on the base of the caudal fin and the spot was not very distinguishable causing these specimens to superficially look like *Cyprinella leedsi*. No other *Cyprinella* species were collected that might be hybridizing with *C. venusta*. Because color variation may exist due to environmental factors, the pharyngeal tooth counts and other morphometric traits were considered stronger distinguishing features, and samples were recorded as *C. venusta*.

Cyprinodontiformes were represented by six species including *G. holbrooki,* which was present at 39 sites, all stream orders, and all habitats. Thirty species of Perciformes were present with the greatest number (*n* = 14) represented in the Centrarchidae. *Lepomis macrochirus* was present at 39 sites and with no observed preference for any specific habitat type. Many less common species (*A. calva, A. rostrata, E. evergladei, E. gilberti, E. obesus, E. edwini, F. cingulatus, F. lineolatus, I. punctatus, L. cyanellus, L. ommata, M. notius, N. maculatus, N. gyrinus, T. maculatus,* and *U. pygmaea*) were only found at one or two sites during the survey.

Three Georgia species of concern (*M. notius*, *A. alabamae*, and *P. metallicus*) were collected. *Micropterus notius* was found at sites 4R and 4S (Figure 1), which are part of the main river channel in the southern end of the survey area. *Alosa alabamae* was found at sites 4Q and 4S in the southern portion of the survey area and was also found in north Lowndes County at site 4J north of the sinkhole plain. *Pteronotropis metallicus* was found in the main river channel at sites 4G, 4I, and 4L in the upper portions of the system and in an unnamed creek (site 1N) near the Florida state line.

The invasive *L. cyanellus* was found in a drainage ditch associated with an unnamed creek (site 1L) and man-made pond in the southern end of the drainage basin. It was also found at Staten Road site 4J, which is 26 km (in a straight line) from site 1L. After completion of the study, a local fisherman brought in two specimens of *Piaractus brachypomus* (synonym *Colossoma bidens*) and a Pacu in the Serrasalmidae (formerly Characidae; Orti et al. 2008) caught at site 4L. *Piaractus brachypomus* specimens have not since then been collected at site 4L and were not included in any analyses reported above.

## DISCUSSION

The primary purpose of this study was to gain information about the composition of species within the study area. Thirty-nine of the 51 species found in the Withlacoochee River basin were on the anticipated list developed by Cannister (2010). Species in this group were expected to be present in the Withlacoochee for one of two reasons: (1) the Withlacoochee River basin falls within the current documented range for the species, or (2) current species distribution maps are constrained by state boundaries rather than environmental barriers. The latter is made evident by the fact that some species inhabiting the Suwannee River do not have distribution records portrayed in Lee et al.

(1980) that extend north of the Georgia/Florida state line, as well as in the Review of Georgia Fishes by Dahlberg and Scott (1971a). Because no obvious barriers occur in this portion of the Withlacoochee River, it stands to reason that species ranges within South Georgia are underrepresented.

While it is reasonable to anticipate the presence of many species in this area, this survey and the institutional collections reviewed provide support for the system's species richness. The Georgia Atlas of Freshwater Fishes compiled by Straight et al. (2009) includes a substantial number of records within the state; however, species found during the survey, but not listed, include *E. edwini*, *E. gilberti*, *F. cingulatus*, *F. chrysotus*, *T. maculatus*, and *U. pygmaea*.

*Elassoma gilberti* is morphologically very similar to *E. okefenokee*. Snelson et al. (2009) showed only *E. okefenokee* to be present in the Withlacoochee drainage. The present survey found both species present within the drainage and documented them occurring sympatrically at one collection site (1C). A more recent survey (Bechler and Salter 2014) found *E. gilberti* at site 1M in the southern end of Lowndes County.

*Heterandria formosa*, the Least Killifish, was recorded north of the Florida state line (Chaney and Bechler 2006) in a survey that extended the range into central Lowndes County in flatwoods habitats. Based on unpublished laboratory tests, the authors predicted that *H. formosa* may not inhabit the Withlacoochee further north due to its inability or unwillingness to move upstream against minimal currents (Bechler, personal communication); however, *H. formosa* was found at site 4G on the Withlacoochee River in Berrien County, doubling its range north on the river. This increased distribution could have occurred during periods of extremely low water and slow currents. Other possibilities exist: (1) *H. formosa* could have been flushed into the Withlacoochee River basin via flooding of a nearby basin such as that of the Alapaha River (Chapman and Kramer 1991), or (2) during interglacial periods with higher sea levels, the species could have migrated from nearby coastal regions into its current sites (Siddall et al. 2003).

Two species listed as state-threatened or rare in Georgia (Georgia DNR 2015) were collected. A*losa alabamae* was previously listed (Straight et al. 2009) as present in the Withlacoochee River basin near the Florida state line and was collected during this study at two locations near the Florida state line. The third collection site in the northern reaches of Lowndes County provides evidence of this species' greater migration activities within Georgia.

We expected *Micropterus notius* to be limited in its distribution, based on habitat requirements (Koppelman and Garrett 2002). Specimens were caught at two locations, McIntyre and Arnold Springs (Sites 4R and 4S), which are located in close proximity to each other in the main river near the Florida state line (Figure 1). This species avoids acidic waters (Koppelman and Garrett 2002) and, therefore, the springs were anticipated to be ideal habitats within the Withlacoochee River basin due to alkaline water introduced by the springs.

One of the invasive species documented in this survey was *L. cyanellus,* a generalist. The native range of the Green Sunfish was only recorded as far east as the Chattahoochee drainage in South Georgia (Dahlberg and Scott 1971b; Page and Burr 2011). One site was at the base of a spillway that drains a recently impounded pond, which suggests stocking of the pond may have been the point of introduction (Dahlberg and Scott 1971b). The Green Sunfish was also collected from the Withlacoochee River in north Lowndes County at site 4J, Staten Road. This species is more aggressive than other *Lepomis* species (Moyle

1976), allowing the Green Sunfish to outcompete native species. The Green Sunfish has also been known to hybridize with other *Lepomis* species (Sigler and Sigler 1987). They do well in a wide range of habitats, and their large mouth allows them to feed on a wide variety of prey items (McKechnie and Tharratt 1966).

The six dendrogram groups (Figure 2) defined via clustering analysis comprised 42 of the 45 collection sites except for sites 1M, 4R, and 4S, which lie outside of the groups. Site 1M, located west of Clyattville, Lowndes County, is a shallow, 1st order tributary passing under a roadbed with a low current and habitat composed of limbs, logs, stumps, and other debris. Data for this location came from the Valdosta State University ichthyology collection. The closest similarity (46%) for 1M was group 6, which is composed of nearly all of the main 4th order river channel sites. Examination of the collection site revealed strong channelization where the stream passes under a road via a large corrugated pipe. A short distance upstream, the creek returns to a shallow tributary. The creek is also joined to a small man-made pond downstream off the deeper portion of the channel. Wright (2013) has found that low order stream bridge sites often have fish assemblages more similar to higher order streams and this, in combination with the pond, could account for the fact that the collection site is most similar, but weakly, to many 4th order sites in Group 6.

While not listed within a specific group, sites 4R (McIntyre Spring) and 4S (Arnold Spring) possessed a similarity of 29% and 34%, respectively, with the rest of the system. Both sites lie within 3.0 km of each other and are located within the Withlacoochee River's main channel in southern Lowndes County. Both sites were surveyed during the study, and 4R was supplemented by Valdosta State University's collection data. Low similarity values for both sites were due to the presence of species uncommon in the Withlacoochee system. Site 4R had a low species count ($n$ = 8) and two species, *E. edwini* and *M. notius*, were rarely collected elsewhere in the system. The 4S collection had 10 species and six of those were found at less than five other collection sites.

Collection sites in group 1 were primarily 1st order tributaries, except for site 4M. Species found at five of the six sites within this group were *G. holbrooki*, *L. macrochirus*, *L. auritus*, and *N. texanus* and comprised a consistent assemblage across group 1 sites. Site 4M is located at the confluence of three creeks (One-Mile, Two-Mile, and Three-Mile Creeks) that drain runoff from the central part of Valdosta, Georgia. While this site was a 4th order stream by definition, the depth and water volume at this site were more consistent with 2nd or 3rd order streams, possibly because of the large quantity of detritus, mostly sand, washed in via the creeks.

Group 2 consisted of sites along tributaries of the Withlacoochee River that are dissimilar in hydrologic factors and habitat but possessed many of the more common species such as *G. holbrooki*, *L. macrochirus*, *L. auritus*, and *P. nigrofasciata*, and no uncommon species that would affect their similarities. Group 2 shared the first three most common species with group 1, except for *N. texanus* that was only found at site 4N suggesting that it may be in that location due to factors not revealed in this study. The reverse is also true with *P. nigrofasciata,* which was present at all sites in group 2 but at only one site in group 1. Group 3 site's composition was similar to that of group 2 as there was very little in common with the sites in terms of habitat and structure. These sites were grouped due to the consistent presence of species common to the basin and the absence of uncommon species.

Groups 4 and 5 consisted of a total of eight sites. Group 4 involved small tributaries whose site-specific species richness ranged from six to nine different species. Three of these species, *G. holbrooki, L. macrochirus,* and *L. sicculus*, were present at all locations. Group 5 was composed of three 1st order sites and one 2nd order site located from the northern portion of the survey area in Nashville (site 2B) through Valdosta (site 1G) to the southern portion of Lowndes County (sites 1J and 1L). Four species were present at all sites, *G. holbrooki, L. punctatus, N. crysoleucas,* and *E. sucetta*, and another four species (*L. macrochirus, L. gulosus, C. macropterus,* and *A. natalis*) were present at 75% of the sites in this group. The commonality of species composition within each group shows how species composition influences cluster analyses and permits the identification of similarities between sites.

Group 6 contained the greatest number of sites ($n$ = 17) and all but three sites were located along the New River and main channels in the Withlacoochee River. Sites within this group also had high species richness. Two sites had nine different species while 22 species were recorded from another site. This difference in richness between sites was partly due to an inability to collect within deeper waters of some sites due to theft of gill nets and fish traps stolen during this survey. Sites 1I and 1O were anomalies in group 6 because they lie at two sites along the 1st order tributary Bevel Creek, northwest of Lake Park, Georgia. The creek drains Brown's Pond, a large, dammed, shallow Carolina Bay and Lake Louise, a 0.056 $km^2$ (5.6 ha) sinkhole lake (Riggs et al. 2010). Much of the species richness in Bevel Creek may come from the stocking of Brown's Pond and the native fish community of Lake Louise as well as other bodies of water feeding into Bevel Creek. Site 3C also appears as an anomaly in group 6. This site was located on Okapilco Creek, a mid-sized tributary that drains much of the western portion of the Withlacoochee River basin, including the city of Quitman, Georgia. Though not part of a main river channel, site 3C is a tributary draining into the Withlacoochee River. Its size and the fact that it also involves a bridge site may be strongly influencing species richness (Wright, 2013).

Collection sites within the above six groups had a degree of species similarity sufficient to draw logical conclusions regarding assemblage structure. Two species, *G. holbrooki* and *L. macrochirus*, were present in small tributaries as well as the main river channel. *Gambusia holbrooki* showed no preference among these locations due to its broad environmental tolerance (Schweizer and Matlack 2005) and *L. macrochirus* may have similar tolerances. Because of their success in many different habitats within the drainage, these species formed part of the assemblage at nearly all collection sites.

As stated in the results, the greatest number of species occurred in 1st order streams followed by 4th order streams. Both 2nd and 3rd order streams contributed minimally to species richness increase as depicted in Figure 3. This latter point could be due to two factors: (1) fewer 2nd and 3rd order sites were sampled, and (2) these sites often have habitat structures that resembles those observed in 1st order sites and, as such, most likely did not contribute greater habitat diversity that might be associated with increasing fish richness.

The large open waterways of 4th order sites had species relatively unique to them such that an increase in the species count occurred due to the presence of *C. venusta*, *L. osseus*, *A. alabamae*, *M. melanops*, *O. e. emiliae*, *A. rostrata*, *E. edwini*, *M. notius*, *E. evergladei*, *I. punctatus*, *N. maculatus*, *N. gyrinus*, and *T. maculatus* that were all found within the main river channel and not in smaller tributaries. In contrast, 2nd and 3rd order streams did not yield any species that might be considered specific to these streams. The nature of these streams, being transitional between the confluence of smaller streams and larger main channels, is reflected by the inhabitants of these streams also being found in smaller streams, the main channel, or both. Because no fish were unique to these streams, they did not contribute to a marked increase in species richness. Kuehne (1962) and Sheldon (1968), working on stream systems in Kentucky and New York respectively, found that there was a trend for increasing species richness that followed a longitudinal pattern such that species richness increased from the headwaters to the lower reaches of the system they sampled. While data was not specifically collected to compare their results to Withlacoochee River fish assemblages, the general pattern of increasing richness for $1^{st}$ to $4^{th}$ orders sites is supportive of their studies. Additionally, studies have shown significant trends and correlations between hydrological factors (e.g., stream depth, width, flow rate, cover, and debris) and assemblage structure (Marchetti and Moyle 2001; Poff and Allan 1995).

The Friedman test results showed significance differences between the species composition of the varying stream orders. These results support studies that have shown positive correlations between stream order and species richness (Hynes 1970; McNeely 1986). Much of the species richness was due to reoccurring species (e.g., *G. holbrooki, L. macrochirus,* and *L. auritus*). The uncommon species that were recorded formed an assemblage that was a nested subset of the common species assemblages (Taylor and Warren 2001). This nesting is generally stronger on a small spatial scale than it would be on a regional scale due to biotic and abiotic factors that can form localized areas of ideal habitat within a system (Cook et al. 2004). One factor that can greatly influence habitat is anthropogenic activities (Morgan and Cushman 2005, Powers et al. 2003). During the study, sampling that took place in the channelized areas in the immediate proximity of bridges often appeared to yield greater species richness than was found further upstream or downstream from the bridge; however, these implied results were not quantified in this study, but were by Wright (2013) in a follow-up study.

## SUMMARY

This survey provides insight into the ichthyofuana of a substantial portion of the Withlacoochee River, and has helped identify additional areas of needed research. Such research includes but is not limited to the following: (1) The Little River, which enters the Withlacoochee River on the southwest corner of Valdosta and contributes a greater volume of water at this point than does the Withlacoochee River itself. The Little River also extends north of Tifton, Georgia, the site of the headwaters of the Withlacoochee River, up to Ashburn, Georgia, in Turner County. In addition, the Little River flows through uplands areas with limited areas of flatwoods that typify the headwaters of the Withlacoochee on the east side of Tifton. As such, a detailed survey of the ichthyofuana of the Little River needs to be conducted to ascertain the contribution it is making to the entire river system. (2) Members of the family Ictaluridae were underrepresented in the collections, and no Gulf Sturgeons were collected. It may be that their absence in the collections is due to their being less common or absent in the river (Sulak and Clugston 1999). However, theft of collecting equipment, extensive flooding during both spring collecting periods, and habitat use unique to these fish may have limited their capture. Additional collecting or creel surveys need to be carried out to get a better understanding of the presence or absence of these species. (3) Wright (2013) developed an experimental protocol and executed a study that supports the contention that anthropomorphic activities contributed to assemblage structure in the Withlacoochee Basin as well as sites outside of it. (4) Future resurveying of the Withlacoochee River and more detailed surveys of rivers in southern Georgia and northern Florida would allow comparative analysis as carried out by Angermeier and Karr (1986), Angermeier (1995), Winemiller (1995), Fausch et al. (1990), Angermeier and Winston (1998), Cook et al. (2002), Hoeinghaus et al. (2007), and Olden et al. (2010).

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As the editor-in-chief of the Georgia Journal of Science, upon submission of this manuscript, I, David L. Bechler, have recused myself of all aspects of the review process and assigned the associate editor all aspects of the review process to include acceptance or rejection. Sampling occurred May 2008 to May 2010 under Georgia Department of Natural Resources scientific collection permit CN:9134. Lots of fish have been transferred from the Valdosta State University collection to the Georgia Museum of Natural History.

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## Appendix

***Table I.*** A description of collection site locations. All sites are in South Georgia. Latitude and longitude are provided for georeferencing. Site alphanumeric terms correspond the those in Figure 1 in the main text. Municipalities or nearby cities are given at the end of the descriptions. *On private farm* indicates that access to the public is denied.

| Site | Brief description | Longitude | Latitude |
|---|---|---|---|
| 1A | Hardy Creek at its intersection with Hwy 82 | -83.4450 | 31.43423 |
| 1B | Frank's Creek at John David Rd | -83.3891 | 31.00832 |
| 1C | Cherry Creek at Skipper Bridge Rd | -83.2633 | 30.91282 |
| 1D | Cherry Creek at Freedom Park, Valdosta | -83.2430 | 30.90549 |
| 1E | 2-Mile Creek at Valdosta State University, North Campus | -83.2863 | 30.86679 |
| 1F | 2-mile Creek at Berkley Dr, Valdosta | -83.3019 | 30.86276 |
| 1G | 1-Mile C, Valdosta State University, Recreation Center | -83.2977 | 30.84335 |
| 1H | 1-Mile Creek at Gordon St | -83.3064 | 30.84023 |
| 1I | Bevel Creek at Loch Laurel Rd | -83.2432 | 30.71834 |
| 1J | Unnamed Creek at Ousley and Old Clyattville Rd | -83.3231 | 30.70937 |
| 1K | Bevel Creek at I-75 | -83.2482 | 30.70674 |
| 1L | Creek draining Pond, ~4950 Old Ousley Rd, Clyattville | -83.3317 | 30.69865 |
| 1M | Creek at Nankin Rd east of Dove Rd. | -83.3550 | 30.69173 |
| 1N | Creek 0.40 km at Nankin Rd west of Clyattville | -83.3267 | 30.69168 |
| 1O | Bevel Creek rat Hwy 376 | -83.2396 | 30.68268 |
| 1P | Withlacoochee River at Hwy 31 | -83.3113 | 30.63537 |
| 2A | New River at Prince Avenue, Tifton | -83.4970 | 31.46425 |
| 2B | Drainage ditch, Hwy 125 and Dogwood Dr, Nashville | -83.2541 | 31.20960 |
| 2C | Possum Creek at Possum Creek Rd | -83.2221 | 31.04750 |
| 2D | Creekside Tavern Creek, 1405 Gornto Rd, Valdosta | -83.3172 | 30.85972 |
| 2E | Piscola Creek at Greenville Rd | -83.5909 | 30.74526 |
| 2F | Piscola Creek at Old Madison Rd | -83.5275 | 30.74309 |
| 3A | New River at Hwy 82, Tifton | -83.4758 | 31.44282 |
| 3B | Cat Creek at Hwy 37 | -83.2047 | 31.07385 |
| 3C | Okapilco Creek at Hwy 333 | -83.5626 | 30.82587 |
| 3D | Okapilco Creek at Hwy 84 | -83.5261 | 30.78619 |
| 4A | New River at Hwy 125, Tift and Cook County lines | -83.4283 | 31.36094 |
| 4B | New River at County Rd 228, Berrien County | -83.4205 | 31.29436 |
| 4C | Hardy Creek and Withlacoochee River at Rd 354, Berrien County | -83.3936 | 31.26773 |
| 4D | New River at County Rd 120, Berrien County | -83.3580 | 31.25024 |
| 4E | Withlacoochee River at Hwy 76 | -83.2719 | 31.19730 |
| 4F | New River at Hwy 76 | -83.3223 | 31.17719 |
| 4G | Withlacoochee River at Hwy 37 | -83.3214 | 31.12013 |

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***Table I* (continued)**

| Site | Brief description | Longitude | Latitude |
|---|---|---|---|
| 4H | Withlacoochee River at Franklinville Rd | -83.2676 | 30.98130 |
| 4I | Withlacoochee River at Skipper Bridge Rd | -83.2718 | 30.94895 |
| 4J | Withlacoochee River at Staten Rd | -83.2892 | 30.93288 |
| 4K | Sinkhole Plane at Riverchase Subdivision, Valdosta | -83.3114 | 30.90490 |
| 4L | Withlacoochee River at Langdale Park | -83.3242 | 30.88797 |
| 4M | Junction of 1-Mile, 2-Mile Creek and Withlacoochee River, Valdosta | -83.3224 | 30.86400 |
| 4N | Little River at Hwy 133 | -83.3467 | 30.85272 |
| 4O | Withlacoochee River at Hwy 84 | -83.4533 | 30.79364 |
| 4P | Boat Ramp, Withlacoochee River at Knights Ferry Rd, Quitman | -83.4554 | 30.71200 |
| 4Q | Withlacoochee River at Nankin Rd | -83.3943 | 30.67489 |
| 4R | Macintyre Spring, on private farm | -83.3606 | 30.64942 |
| 4S | Arnold Spring, on private farm | -83.3363 | 30.64093 |

***Table II.*** Species presence at the 1st order sites. Letters under *1st order sites* indicate sites as presented in Table I of the appendix and Figure 1. The letter *X* indicates presence at a site.

| | | 1st order sites | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Family | Species name | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P |
| Lepisosteidae | *L. platyrhincus* | X | | | | | | | | X | | | | | | | |
| Umbradiae | *U. pygmaea* | | | | | | | | | | | | | | | X | |
| Esocidae | *E. vermiculatus* | | | X | X | | | | | X | | | X | X | | X | X |
| | *E. niger* | | | X | | | | | | X | | | X | | | X | |
| Cyprinidae | *N. crysoleucas* | | | X | | X | | X | | X | X | X | X | X | X | X | |
| | *N. texanus* | | | | | X | | X | X | | | | | | X | | |
| | *P. metallicus* | | | | | | | | | | | | | | X | | |
| Catastomidae | *E. sucetta* | | | | | | | X | | | X | | X | X | X | | |
| Ictaluridae | *A. natalis* | | | | X | | | X | | X | | | X | | | | |
| | *A. nebulosus* | | | | | | | | | X | | | X | | | | |
| | *N. leptacanthus* | | | | | | | | X | | | | | | X | X | |
| Aphredoderidae | *A. sayanus* | | X | | X | | | | | X | | | | | | X | |
| Fundulidae | *F. chrysotus* | | | X | X | | | | | | | | | | | | |
| | *F. cingulatus* | | | X | | | | | | | | | | | | | |
| | *F. lineolatus* | | | | | | | | | | | | | X | | | |
| | *L. ommatta* | | | X | | | | | | | | | | | | | |
| Poecilidae | *G. holbrooki* | X | X | X | X | X | | X | X | X | X | X | X | | X | X | X |
| | *H. formosa* | | | X | | | | X | X | X | | | | | | | |
| Atherinopsidae | *L. sicculus* | X | X | | | | | | | X | | | | X | | X | |
| Elassomatidae | *E. gilberti* | | | X | X | | | | | | | | | | | | |
| | *E. okefenokee* | | | X | X | | | | | X | | | | | | | |
| | *E. zonatum* | | | | X | | | | | | | | | | | | |
| Centrarchidae | *C. macropterus* | X | X | X | X | | | | X | | X | X | X | X | X | X | |

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***Table II* (continued)**

| Family | Species name | 1st order sites | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P |
| | *E. gloriosus* | X | | | X | | | X | | | | | X | | | | |
| | *E. obesus* | | | | | | | | | | | | | X | | | |
| Centrarchidae | *L. auritus* | | | | | X | X | X | X | | | X | X | | X | X | |
| | *L. cyanellus* | | | | | | | | | | | | X | | | | |
| | *L. gulosus* | | X | X | X | X | | | | X | X | | X | X | | X | |
| | *L macrochirus* | X | X | X | X | X | X | | X | X | X | X | X | | | X | |
| | *L. marginatus* | | | | | | | | | | | | | X | | | |
| | *L. microlophus* | | | | | | | | | | | | | X | | | |
| | *L. punctatus* | | | | | | X | X | | X | X | | X | X | | X | |
| | *M. salmoides* | | | | | X | | | X | X | | | | X | | X | |
| | *P. nigromaculatus* | X | | | X | | | | X | X | X | | | | | X | |
| Percidae | *E. fusiforme* | X | | | X | | X | | | X | | | | X | | | |
| | *P. nigrofasciata* | | | | | | X | | X | X | | | X | X | | X | X |

***Table III.*** Species presence at 2nd and 3rd order sites. Letters under *2nd* and *3rd order sites* indicate sites as presented in Table I of the appendix and Figure 1 of the main article. The letter *X* indicates presence at a site.

| | | 2nd order sites | | | | | | 3rd order sites | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Family | Species name | A | B | C | D | E | F | A | B | C | D |
| Lepisosteidae | *L. platyrhincus* | | | | | | | | | | X |
| Amiidae | *A. calva* | | | | | X | | | | | |
| Aphredoderidae | *A. sayanus* | | X | | | X | | | X | X | |
| Esocidae | *E. a. vermiculatus* | | X | X | | | | | X | X | X |
| | *E. niger* | | | | | X | | | X | X | X |
| Cyprinidae | *N. crysoleucas* | | X | | | X | | | | | |
| | *N. petersoni* | | | | X | | | | | | |
| | *N. texanus* | X | | | | | | | X | X | |
| Catastomidae | *A. natalis* | | X | | | | | | | | |
| Catastomidae | *A. nebulosus* | | | | | | | | | X | |
| | *E. sucetta* | | X | | | | | | | | |
| Fundulidae | *F. chrysotus* | | | | | | | | | X | |
| | *L. ommatta* | | | | | | | | X | | |
| Poecilidae | *G. holbrooki* | X | X | | | X | X | X | X | X | X |
| | *H. formosa* | | | | | | | | | X | |
| Atherinopsidae | *L. sicculus* | | | | | X | | X | | X | X |
| Centrarchidae | *C. macropterus* | | X | X | | | | | X | | |
| | *E. gloriosus* | | | | | X | | | | X | |
| | *L. auritus* | X | | | X | | X | X | | | |
| | *L. gulosus* | | X | | | | X | | | | |
| | *L. microlophus* | X | | | | | | | | | |
| | *L. punctatus* | | X | | | | | X | | | X |
| | *M. salmoide* | X | | | X | | | X | | | |
| | *P. nigromaculatus* | | | | | X | | X | | X | |
| Elassomatidae | *E. okefenokee* | | | | | | | | X | | |
| Percidae | *E. fusiforme* | | X | X | | | | X | X | X | |
| | *P. nigrofasciata* | | | | X | | X | X | | X | |

***Table IV.*** Species presence at 4th order sites. Letters under *4th order sites* indicate sites as presented in Table I and Figure 1 of the main article. An asterisk indicates presence at a site.

| | | 4th order sites | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Family | Species name | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S |
| Lepisosteidae | *L. osseus* | | | X | | | | X | | | | | | | | | | X | | X |
| | *L. platyrhincus* | | | | | | | | | | | | | | | | | | | X |
| Amiidae | *A. calva* | | | | | | | | | | | | | | | | | | | X |
| Clupidae | *A. alabamae* | | | | | | | | | | X | | | | | | | X | | X |
| Anguillidae | *A. rostrata* | | | | | | | | | | | | | | | | | X | X | |
| Aphredoderidae | *A. sayanus* | X | | X | | X | X | X | X | X | X | X | X | | | X | X | X | | X |
| Esocidae | *E. a. vermiculatus* | X | | X | X | X | X | X | | X | | | | | X | X | | | | |
| | *E. niger* | | | | | X | | | | | X | | X | | | | | | | |
| Cyprinidae | *N. crysoleucas* | X | X | X | | X | | | | X | X | | | | | X | X | | | |
| | *O. e. emiliae* | X | | | | X | | | | | | | | | | | | X | | |
| | *N. petersoni* | | X | | | | X | | X | X | X | X | X | | | X | X | X | | |
| | *N. texanus* | | X | X | X | | X | | X | X | X | X | X | X | X | X | X | X | | |
| | *C. venusta* | X | | | | | | | X | X | | | | X | X | X | X | X | | |
| | *P. metallicus* | | | | | | | X | | X | | | X | | | | | | | |
| Catastomidae | *M. melanops* | | | | | | | | | | | | | | | | X | X | | X |
| | *E. sucetta* | X | | | | | X | | | | | | | | | | | | | |
| Ictaluridae | *I. punctatus* | | | | | | | | | | | | | | | | | X | | |
| | *A. natalis* | | | | | | | X | | X | X | | X | | | | | | | |
| | *A. nebulosus* | | | | | | | | | | X | | | | | | | | | |
| | *N. gyrinus* | | | | | | | | | | | | | | | | | X | | |
| | *N. leptacanthus* | X | | | | | | | | X | | | | X | | | | X | | |

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***Table IV* (continued)**

| Family | Species name | 4th order sites | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S |
| Fundulidae | *F. chrysotus* | | | | | | | X | | | X | | | | | | | | | |
| | *F. lineolatus* | | | | | | | | X | | | | | | | | | | | |
| Poecilidae | *G. holbrooki* | X | X | X | X | X | X | X | X | X | X | X | X | | X | X | X | X | X | |
| Poecilidae | *H. formosa* | | | | | | | X | | | X | | | X | | | | | | |
| Atherinopsidae | *L. sicculus* | X | X | X | X | | X | X | X | X | X | X | X | | | X | X | X | X | |
| Centrarchidae | *M. notius* | | | | | | | | | | | | | | | | | | X | X |
| | *M. salmoides* | X | X | X | X | | X | | | X | X | X | X | | | X | X | X | X | |
| | *C. macropterus* | | | X | | X | | | X | X | X | X | | | | X | | | | |
| | *L. auritus* | | | | | X | | X | X | X | X | | | X | X | X | X | X | | |
| | *L. cyanellus* | | | | | | | | | | X | | | | | | | | | |
| | *L. gulosus* | | | X | X | | | | | X | X | | X | | X | | | X | | |
| | *L. macrochirus* | X | X | X | X | X | X | | X | X | X | X | X | X | X | | X | X | | X |
| | *L. marginatus* | | | | | | | | | X | | | | | | | | | | |
| | *L. microlophus* | | | | | | | | | | X | | | | X | | | | | |
| | *L. punctatus* | X | X | | X | | X | X | X | X | | X | X | | X | X | X | X | X | X |
| Centrarchidae | *P. nigromaculatus* | X | | X | | | X | | | X | X | | X | X | | | | X | | |
| | *E. gloriosus* | | | | | | | X | | | | | | | | | | | | |
| Elassomatidae | *E. evergladei* | | | | | | | X | | | | | | | | | | | | |
| | *E. zonatum* | X | | | | | X | | | | | | | | | | | | | |
| Percidae | *P. nigrofasciata* | X | | | | | | X | X | X | X | | X | | X | X | X | X | X | X |
| | *E. edwini* | | | | | | | | | | | | | | | | | X | X | |
| | *E. fusiforme* | X | X | X | X | X | X | X | | X | | X | X | | | X | X | | | |
| Achiridae | *T. maculatus* | | | | | | | | | | | | | | | X | | | | |