Georgia Journal of Science

Volume 80 *No. 2 Scholarly Contributions from the Membership and Others*

Article 11

2022

Effect of Bridges on Low Order Stream Fish Assemblages, South Georgia, USA

David L. Bechler Dr.
Valdosta State University, dbechler@valdosta.edu

Recommended Citation

Bechler, David L. Dr. (2022) "Effect of Bridges on Low Order Stream Fish Assemblages, South Georgia, USA," *Georgia Journal of Science*, Vol. 80, No. 2, Article 11.
Available at: https://digitalcommons.gaacademy.org/gjs/vol80/iss2/11

Effect of Bridges on Low Order Stream Fish Assemblages, South Georgia, USA

Acknowledgements

No Acknowledgements

Effect of Bridges on Low Order Stream Fish Assemblages, South Georgia, USA

Charles W. Wright[1]
David L Bechler[2]*
[1]Georgia Military College, Natural Sciences Department,
4201 North Forrest Street, Valdosta, Georgia 31605, USA
[2]Department of Biology, Valdosta State University (Retired),
525 Tatum Mining Rd. Menlo, Georgia 30731, USA
*Corresponding author: dbechler@valdosta.edu

ASTRACT

Anthropogenic impacts such as bridge sites can greatly alter established streambed morphology, associated ecology and flora and fauna. At bridge sites, streams are often channelized approaching the site and deep pools are created at the bridge site causing ecological alterations of faunal assemblages. However, restoring channels and reducing negative construction practices allows the return of natural habitats that are likely to include more sensitive species. Recent conservation studies have suggested that anthropogenic sites may serve as potential habitats for reestablishment of populations following a drought event. This study examined the impact of bridges on fish assemblages at first through fourth order streams in the Suwannee River Basin of South Georgia. Collections were made at bridge, upstream and downstream sites via seining and setting of gill nets. Assemblage structure at bridge sites was compared to bridge structure, biological and physiochemical factors at fourteen bridge sites. Fish assemblages were least diverse upstream of bridge sites, most diverse at bridge sites, and intermediate in diversity downstream of bridge sites. The results suggest that bridge sites, if properly engineered, can serve as valuable refuges for reestablishing fish assemblages up and down stream after events such as the severe drought that impacted South Georgia in 2011.

Keywords: fish assemblages, low order streams, bridges, South Georgia

INTRODUCTION

Anthropogenic disturbance is any relatively discrete event in time that disrupts an ecosystem, community, or population structure changing resources such as availability of substratum or the physical environment (Resh et al. 1988). However, at recovered or naturalized bridge sites, the development of riffle and run habitats, similar to the natural stream pattern, may reestablish community structure as well as sensitive species (Lau et al. 2006). Naturalized bridge sites may have influences on erosion, sediment loads, riparian zones, substrate, and removal of accumulated natural debris decreased through time (Lau et al. 2006). Disturbances from bridge construction can be further mitigated if normal water flow is maintained despite the blocking effect of embankments and bridge piers. This objective can be achieved through designs that favor short ramps, long spans, hydraulically shaped piers, and streamlined artificial islands (Larson 1993). One factor that can affect fish species, but is not controlled for, is the sound produced by vehicular traffic passing over a bridge (Covo, et al. 2015; Holt and Johnston, 2015).

Highway and bridge construction cause negative perturbations on stream structure, associated algal communities, macroinvertebrates, and fish assemblages (Cline et al. 1982; Larsen 1993; Tiemann, 2004, Blettler et al. 2005; Chadwick et al. 2006; Lau et al. 2006). Physical impacts on aquatic habitat includes bridge pillars, dredging, alteration of embankments, and highway construction (Larsen 1993). Channelization, and deep pool formation constitute an ecological disturbance for aquatic fauna assemblages present (Cline et al. 1982; Resh et al. 1988). Positive effects of bridges on riparian ecosystems do not occur initially following construction but should be considered following a period of habitat naturalization (Death, 1996). Research has demonstrated that r-strategist species assemblages related to sandy unstable sediments can colonize the habitats successfully less than one year after disturbances (Blettler and Marchese 2005; Death 1996). Upstream bridges sites with silt-clayed sediments have demonstrated higher species richness and higher levels of benthic biomass than bridge and downstream sites (Blettler and Marchese 2005).

Negative effects of bridge construction on riparian ecosystems have been well documented in fifth and higher order streams supporting the importance of medium and large streams for macroinvertebrates, game fish, and vegetation (Vannote et al. 1980; Blettler and Marchese 2005). Some studies have considered macroinvertebrate and fish assemblages following a period of recovery or naturalization at fifth and higher order bridge sites. A few studies have considered macroinvertebrate assemblages on fourth and lower order streams following a period of naturalization, but rarely have studies considered the effects on fish assemblages at fourth and lower order stream bridge sites (Joy and Death 2000; Blettler and Marchese 2005).

Natural streams characteristically display greater substrate size heterogeneity, while anthropogenic affected sites characteristically display greater substrate size homogeneity (Lau et al. 2006). Variation in substrate type can affect feeding and reproductive behaviors in organisms leading to changes in assemblage from having both sensitive and tolerant species present to just tolerant species. Sparse to moderate instream cover and overhanging vegetation is present in natural streams and often absent at bridge sites, which decreases the number of potential niches available (Lau et al. 2006). Research on the impact of canopy cover on aquatic fawn and vegetation have been studied extensively (Arimoro et al. 2012, Casatti 2009, Kaluza et al. 2020, Wallace and Eggert 2009).

The purpose of the original research was to appraise the impact of naturalized bridge sites at first through fourth order streams in the Suwannee River basin of South Georgia, USA, as related to macroinvertebrate and fish assemblages (Wright 2015). The work presented here examines the fish communities above, at and below 14 bridges sites. Variations in physiochemical and biological factors were assessed for their effects on the fish assemblage structures to determine the overall level of anthropogenic effect bridges have on species diversity and biotic potential. This has allowed the development of an understanding of the difference between bridge site and natural site assemblages, while determining bridge sites might be a source of wetland species and assemblage diversity following stochastic drought events.

The significance of this research was that it addressed the absence of research on fish species found at bridge sites at first through fourth order streams. The research was accentuated by the severe drought in the Southern United States during the summer of 2011 (Wisniewski et al. 2013). Additional concerns for the health of rivers and streams have been brought to bear considering increases of combined investment by all levels of government in highway and bridge infrastructure. Bridges averaged 40 years old with half built before 1964 with the possibility that 26.7% of all bridges structurally deficient or functionally obsolete in the study area (Peters, 2006). Present day fauna are the result of geology, human habitation, and distance from species source populations (Joy and Death 2000).

MATERIALS and METHODS

Field-site Description

During a drought in the southeastern United States, 14 bridge sites in south-central Georgia in the Suwannee River drainage basin were assessed for anthropogenically generated affects upon fish and macroinvertebrate assemblages (Wright 2015) of which the impact on fish is presented here. The sites were predominantly below baseflow for much of the year and at some sites flow was completely interrupted for an extended time-period. Latitude and longitude were calculated for each site with a Garmin Handheld Global Positioning System (GPS) using World Geodetic System (WGS) 84. Global Positions were cross verified using Google Earth set to Garmin GPS WGS 84 (Google Inc., 2012), and converted to decimal degrees expediting the geo-location of each site in the Geographic Information System Arc Map edition 10 from Environmental Systems Research Institute (Esri 2014, 2016, 2017). The conversion to decimal degrees facilitated the assessment of each site using PASSaGE 2 statistical software (Rosenberg and Anderson 1998).

Collection Sites and Protocol

Research sites were in the Tifton Upland and Okefenokee Plains regions of Georgia (Table I, Figure 1) (Griffith et al. 2001). Streams in these regions are dominated by agricultural land use, which is predominately coniferous sylvan culture. Third and fourth order streams were in the Tifton Upland and first and second order streams were in the Okefenokee Plains region (Wright 2015). Sites were divided into upstream (U), bridge (B) and downstream (D) subsites producing 42 data sets. Upstream subsites were defined as areas above pools and runs associated with bridges and served as controls against which the bridge and downstream subsites were compared. Upstream habitats were often complex with many areas of roots and braided stream morphology through shallow flatwoods blackwater habitats. Downstream habitats were often shallow runs with modest riffles and large woody debris and often extended into woodlands. Some upstream and downstream sites shared morphological features or similar levels of desiccation due to the drought. All bridge subsites had a deep run morphology generating a thalweg for the riparian system and most had aquatic macrophytes. The first and second order streams had shallow flatwoods systems entering the bridge run from braided morphology and exiting to braided morphology. The third and fourth order streams had flatwoods systems entering the bridge run from winding channel morphology and exiting to winding channel morphology.

Fish species were collected during riverine base flow or lower to provide the maximum accuracy for assessing fish species assemblage diversity (Lau et al. 2006; Chadwick et al. 2006). Fish were collected through seining of all subsites while at upstream and downstream subsites, repeated seine hauls were made in all habitat sites with ten seine hauls being made after the last new species was collected. Collections from each subsite were preserved and stored in separate containers. Seining of unique habitats was performed for each subsite to obtain samples of narrow niche species. Two seines used were a 170 cm width x 120 cm high with a 0.5 cm mesh, and a 450 cm width x 125 cm high with a 0.25 cm mesh with the net used dependent on the habitat being seined. Prior to collecting of fish, physicochemical data was collected and a monofilament gill net (30.48 m long x 1.83 m high with 7.62 cm mesh) for open water fish was set-up in runs and pools at the bridge subsites, upstream subsites, and downstream subsites that were too deep to seine. Due to drought conditions, very few runs of a depth requiring the use of a gill net were found upstream or downstream.

Table I. Bridge sites sampled. Site codes (column 1) are listed alphanumerically with a number indicating stream order and a letter indicating individual sites. Descriptions (column 2) provide the stream names and highways (Hwy)/roads (Rd.) of bridge sites. Latitude (column 3) and Longitude (column 4) provide geographic coordinate data for locations. Date (column 5) indicates collection date in 2011.

Sites	Descriptions	Latitude	Longitude	Date
1A	Grand Bay Cr. At Hwy 221	83.1300	30.9516	11-May
1B	Mud Cr. at Perimeter Rd.	83.2351	30.8048	13-May
1C	Suwanoochee Cr. at Hwy 94	82.5821	30.6833	7-Aug
2A	Grand Bay Cr. at Hwy 84	83.0934	30.9025	25-May
2B	Grand Bay Cr. at Hwy 94	83.1354	30.7686	6-Jul
2C	Mud Cr. at Vann Rd.	83.1800	30.7779	3-Jun
3A	Alapahoochee R. at Hwy 376	83.1213	30.7037	6-Jun
3B	Alapahoochee R. at Hwy 135	83.0881	30.6287	4-Jun
3C	Little R. at Hwy 122	83.4569	31.0005	20-Aug
4A	New R. at Hwy 125	83.4283	31.3610	30-May
4B	New R. at CR 252	83.4206	31.2944	1-Jul
4C	Withlacoochee R. at Hwy 37	83.3217	31.1204	18-Jun
4D	Withlacoochee R. at Hwy 122	83.3019	31.0139	25-Jun
4E	Withlacoochee R. at Staten Rd.	83.2890	30.9330	2-Sep

Fish were euthanized in the field using buffered tricaine methyl sulfonate (MS222) at a concentration of 500 mg/L in accordance IACUC with the American Fisheries Society (AFS 2014) and the American Society of Ichthyologists and Herpetologists (ASIH 2013). Specimens were fixed in 10% formalin for 24 hours, soaked in water for 24 hours, and preserved in 55% isopropyl alcohol. Collections were made under the Georgia Department of Natural Resources scientific collection permit CN:9134. Specimens were deposited at the Georgia Museum of Natural History-Athens (GMNH 2016).

Independent variable data sets initially included all variables listed in Table II (construction parameters, physical, chemical, and biological). Substrate samples of 0.25 L were collected during the summer at each subsite, homogenized, and oven dried at 60^{O}C for 3 days. A 10 ml sample was oven heated at 550 ^{O}C for 4 hours to eliminate all organic material and then weighted. Organic content was based on the original weight minus the final weight. A 50.0 ml sample was sifted through substrate sieves and the resultant volumes collected in each sieve were measured to assess substrate ratios for each subsite.

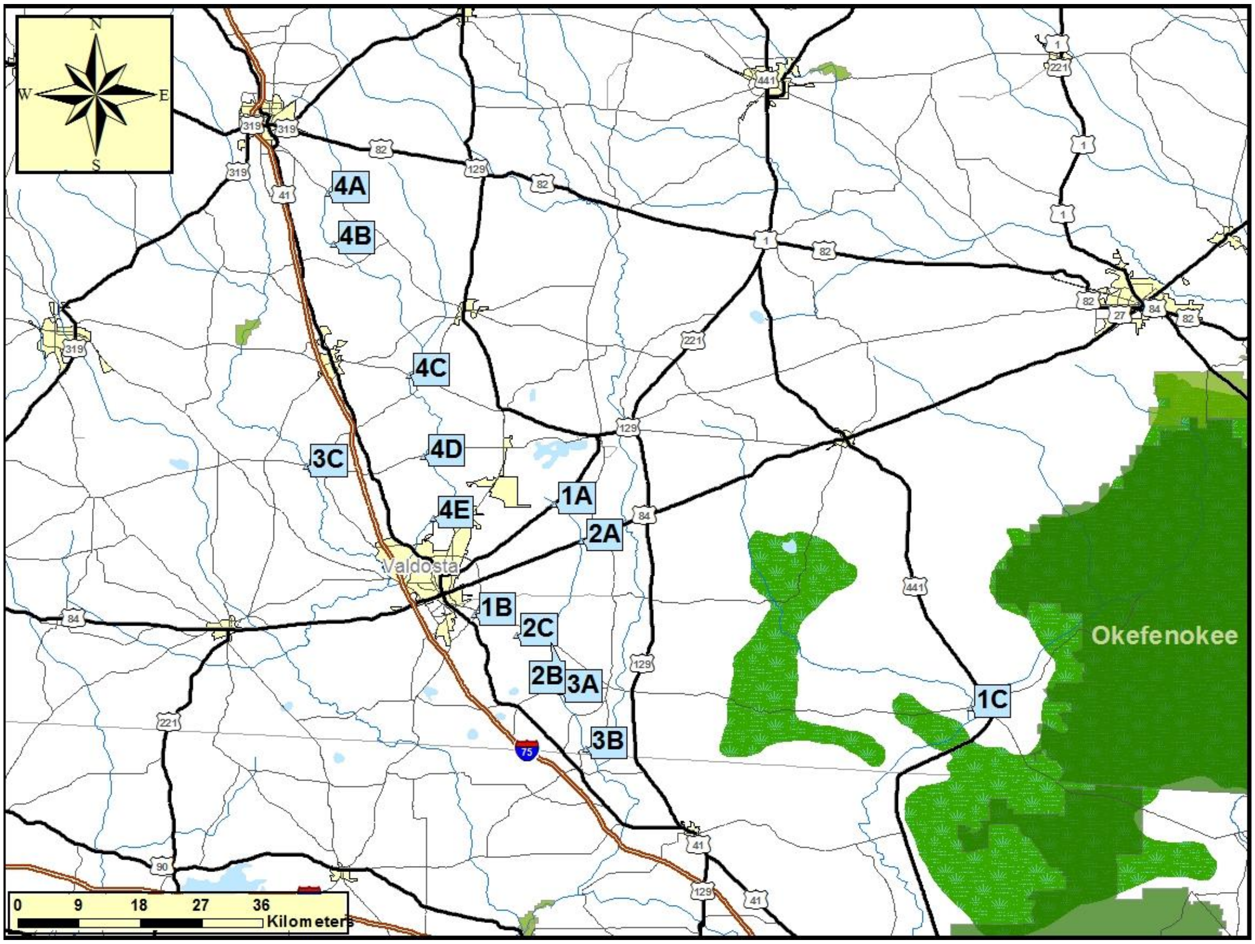

Figure 1. Collections sites in south central Georgia, USA. Sites lie within the Tifton Upland and Okefenokee Plains regions and identified in blue squares. Site descriptions are in Table I. Blue lines and images represent streams and water bodies. The Okefenokee swamp is indicated in green. Thick dark/black lines with numeric values indicate major roads and narrow lines indicate side roads. Interstate I-75 is represented by a dark orange line. Map was developed from ArcMap (2012).

Temperature, chemical properties (oxygen content, pH, and conductivity), and flow were collected May to September 2011, and January to February 2012 for each subsite. Temperature and oxygen were measured using an YSIDO200 meter, pH was measured using a Fisher Scientific AP85A Waterproof pH/Conductivity meter, and conductivity was measured using a WTW Cond 340i meter. Physical properties, quantitative infrared (IR) samples, and vegetation coverage were collected once during the summer from May to September. Physical properties involving the size of water bodies included evenly spaced transect lines set at 5 m apart across the bridge site width, a bisecting line for the bridge site run length, and depth measurements. Depth measurements were measured from the center of the stream with one in the open area of the bridge pool, one under the bridge, one upstream, and one downstream from the bridge. The physical property of surface area was calculated using Google Earth measurement applications (Google Inc. 2012). Vegetation surface area was calculated by multiplying the mean width of all vegetation measurements at a site times the length of the main pool where vegetation ran the length of the pool.

Quantitative infrared samples were collected using a 0.25 L scoop and the resulting slurry was placed in a 1.25 L Zip-lock freezer bag and stored at -60°C. Samples were thawed, decanted onto filter paper, and rinsed to dissolve limestone-based ions and minerals (i.e., $CaCO_3$, $CaSO_4$ etc.) present. Samples were dried on the filter paper in a fume hood at room temperature, 25 g samples were extracted and soaked in 10 ml of methanol for 48 hours. The resulting solution was filtered using 0.20 um filter paper and added one drop at a time to 3 M Polyethylene Type 61-100-12 IR Cards (Manning et al. 2004). The dried cards were tested using a Mattison FTIR spectrophotometer, Mattison Instruments 2013.

Specimen Identification

Baseline data for fish species most likely to be found at collecting sites was retrieved from Barnett et al. (2007), Canister (2007) and Canister and Bechler (2019). Fish were identified using the Peterson Field Guide to Freshwater Fishes and other sources (Page and Burr 1991; Fishbase 2012; Darden 2008; Lazara 2002; Ghedotti and Grose 1997; Gilbert et al. 1992; Rivas 1966; Brown 1956; Wiley 1986; Brown 1958; Wiley and Hall 1975; Snelson et al. 2009; Rider and Schell 2012; Fishes of Georgia 2022) and personal communication with Dr. Brett Albanese 2012 (Georgia Department of Natural Resources). Fish assemblages were defined as all fish collected at each subsite and were divided into guilds based on species use of environmental resources (Page and Burr 1991, Simberloff 1991). Guild categories were: (1) benthic-near or on the bottom, (2) open water-mid to upper water column, (3) near vegetation-near or slightly in vegetation, (4) vegetation-lives in vegetation, and (5) open water-lives at the top of the water column.

Statistical Methods

Data were organized using Microsoft Excel (Microsoft 2010, 2021); and for parametric analyses, fish data was standardized using hectometers for the main bridge pool length prior to statistical analyses. Friedman's test, one-way analysis of variance (ANOVA), and Scheffé multiple comparisons test were performed in StatsDirect (StatsDirect Ltd. 2007). Shapiro-Wilkes tests in Statistica (StatSoft Inc. 2012) were used to test for normalization of data sets prior to regression analyses and modeling. Variables that were not normal were transformed using log normal (ln x), log to the 10th (log10 x), squared (x^2), and square-root ($\sqrt{x}$) values. Transformed variables were tested for normality and the strongest P value ≤ 0.05 was chosen to replace the original data. Following, normalization of data sets Primer v6 (Clarke and Gorley 2006), StatsDirect (StatsDirect Ltd. 2007), Sigma Plot (Systat Software 2012), and Statistica (StatSoft Inc. 2012) were used to conduct Principal Components Analyses (PCA), regression analyses and pair-wise multiple comparisons modeling.

RESULTS

Scientific and common names of fish species collected are in Appendix Table I and Table II. A total of 7,056 specimens and 43 species (Table II, Appendix Table I) were collected across all subsites. A range of 23 to 33 species were collected across individual sites and a range of nine to 29 species across all subsites. The seven most common species were *Aphredoderus sayanus* (n = 319), *Micropterus salmoides* (n = 335), *Centrarchus macropterus* (n = 356), *Notropis petersoni* (n = 415), *Lepomis macrochirus* l (n = 766), *Gambusia holbrooki* (n = 1,049), and *Labidesthes sicculus* (n = 2,495). The seven least common species were *Amia calva* (n = 1), *Ameiurus brunneus* (n = 1), *Ameiurus nebulosus* (n = 1), *Elassoma evergladei* (n = 1), *Lepisosteus osseus* (n = 2), *Umbra pygmaea* (n = 2), and *Acantharchus pomotis* (n = 2). Seven species collected within only one subsite and one stream order included: first order sites, *Enneacanthus obesus*, second order sites, *Amia calva*; and fourth order sites, *Lepisosteus platyrhincus*, *Fundulus chrysotus*, *Elassoma evergladei*, *Etheostoma edwini* and *Acantharchus pomotis*. Appendix Table I provides subsites information for all species. No species were unique to third order streams. Species listed in Appendix Table I show that within subsites no species were unique to only upstream subsites, one species was unique to downstream sites and 11 species were found at only bridge subsites. One species was only found at upstream and bridge subsites, three species only at bridge and downstream subsites, and 25 species were found at all subsites. A Chi Square Goodness of Fit test, assuming a random distribution of 3.583 species per subsite and combined subsites, was significant (Species n = 43, DF = 6, Chi-square = 51.561, P < 0.0001).

Table II. Fish families and number of species collected at 14 bridge sites. Families are listed in phylogenetic order by columns from top to bottom and left to right. Number of species collected per each family are listed after the family name.

Bridge Study Site Fish Families and Species Numbers

Lepisosteidae 2
Amiidae 1
Aphredoderidae 1
Umbridae 1
Esocidae 2
Cyprinidae 6
Catastomidae 2
Ictaluridae 4
Atherinopsidae 1
Fundulidae 3
Poeciliidae 2
Centrarchidae 12
Elassomatidae 3
Percidae 3

A Friedman's test on fish species assemblages for all subsites by stream order was significant (T^2 [F] = 5.5242, df = 1763, Critical *t* = 1.9613, and *P* < 0.0001). Significant results were found in 40.65% of the 861 comparisons with 4.00% of significant comparisons occurring within subsites from the same stream order and 36.65% of significant differences occurring between different stream orders and subsites. A Friedman pair-wise multiple comparison test provided percentage dissimilarities by stream order by site. Comparison of dissimilarities of fish species composition by stream orders (Figure 2) produced a general pattern such that an increase in dissimilarities occurred from first to fourth order sites. Comparisons of same stream order sites resulted in lower dissimilarity vales than comparisons of different stream order sites.

Fish species totals comparing all subsites were entered into a one-way analysis of variance (ANOVA) followed by a Scheffé multiple comparisons test. The one-way ANOVA was significant (F [variance ratio] = 6.4638, P = 0.0038), and the Scheffé multiple comparisons test identified only bridge subsites as being significantly different from upstream subsites (critical value = 2.5448; B vs. U, P = 0.004; D vs. U, P = 0.1176; and B vs. D, P = 0.3609). The mean number of species in fish assemblages by stream order and subsites (Figure 3) shows two key patterns. Pattern 1, bridge sites possessed the greatest mean diversity of fish species followed by downstream subsites with lowest mean diversity occurring at upstream sites. Pattern 2 showed that the mean number of species at each subsite increases from first order to fourth order. Bridge sites at third order streams showed minor variation in mean species numbers.

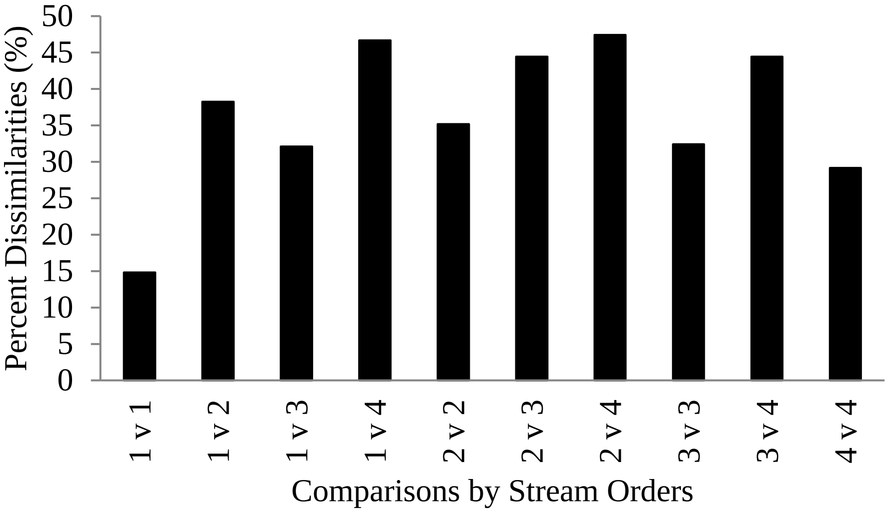

Figure 2. Percent dissimilarities within and between stream orders. Dissimilarities are based on all species collected at all subsites by stream order. The letter 'v' stands for 'verses' such that 1 v 2 indicates all first order sites compared to all second order sites.

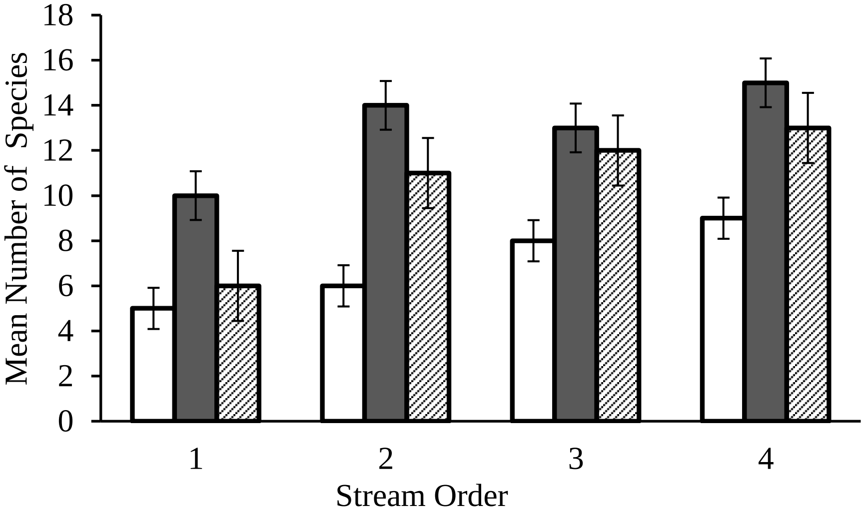

Figure 3 Mean number of fish species per subsites per stream order. Open bars represent upstream subsites, sold bars represent bridge subsites and slash bars represent downstream subsites.

Fish species numbers organized by habitat guilds (Figure 4) were entered into a one-way ANOVA followed by a Scheffé multiple comparisons test. The one-way ANOVA was significant (F [variance ratio] = 11.366859, $P < 0.0001$), and the Scheffé multiple comparisons test supported the use of guilds to identifying variations by habitats within sites and the number of species associated with habitats (Critical Value = 4.93954, $P < 0.0001$). The greatest species diversity in habitat guilds occurred at bridge subsites, then downstream subsites, and lowest at upstream subsites. The debris guild at each subsite was substantially less diverse than other guilds, which were more similar in mean numbers and pattern.

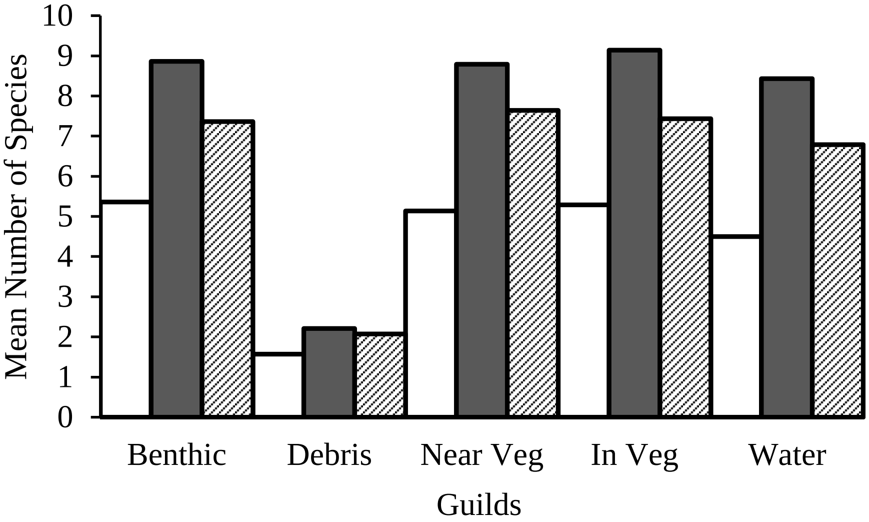

Figure 4. Mean number of fish species in guild assemblages by subsites. Open bars represent upstream subsites, sold bars represent bridge subsites and slash bars present downstream subsites.

Fish assemblage data for each subsite by stream order was run in Primer 6 (Clarke and Gorley 2006) generating eigenvector values and a Cluster Analysis (Figure 5). At each subsite, five species with the highest eigenvector values (Table IV) and positions in the Cluster Analysis (Wright 2013) were used to assess species with the greatest variability. Eight species with the highest levels of variation across subsites were: *C. macropterus* (n = 9 subsites), *G. holbrooki* (n = 8 subsites), *E. americanus vermiculatus* (n = 5 subsites), *L. sicculus* (n = 4 subsites) and *L. gulosus*, *M. salmoides*, *N. texanus* and *P. nigrofasciata* (n = 3 subsites each). The other 15 species occurred at only one or two subsites. No *C. macropterus* were collected at third order sites but were at all other stream orders. *Gambusia holbrooki* were collected within all stream orders and predominately at upstream and downstream sites. *Esox americanus vermiculatus* were collected at only first and second stream order sites with specimens collected at all subsites. *Labidesthes sicculus* were collected at second, third and fourth order downstream subsites and third order upstream subsites.

To assess similarities at bridge subsites, fish assemblages at all bridge subsites were compared with a cluster diagram (Figure 5) developed from a Curtis-Bray similarity analysis using Primer 6 (Clarke and Gorley 2006). Two patterns of branching occurred within the cluster diagram: Pattern 1 consisted of paired subsites (n = 4 pairs, n = 8 subsites), and Pattern 2 consisted of individual subsites (n = 6 subsites). Paired subsites (mean = 58.13) branched off with a wider range of dissimilarity values (minimum and maximum = 22.7642 – 64.4509, R = 41.6866) than unpaired subsites (mean = 37.4756) which had a narrower range of similarity values (minimum and maximum = 21.4286 – 50.8333, R = 29.4048). Two paired sites involved the same stream orders (first and third) and the other two involved different stream orders (first and fourth, second and fourth). Unpaired sites included only three stream orders with fourth order sites accounting for 50.00% (n = 3). Assuming unequal variances between paired and unpaired subsites, an unpaired-t test (Excel, Microsoft, 2021) was computed and was not significant (Two-sided P = 0.4174). Among the four paired subsites, two pairs involved the same stream orders 28.57% (n pairs = 2, n sites = 4), two involved different stream orders 28.57% (n pairs = 2, n sites = 4), and the six unpaired sites accounted for 42.86% (n = 6).

Table IV. Five species with highest level of variance per subsites by stream order (first, second, third, fourth). Species are ranked from highest to lowest variance based on maximum Eigen Vector values (Vect.). U = upstream subsites, B = bridge subsites, and D = downstream subsites. Full genus and species names are provided in Appendix Table I.

First Order	**Vect.**	**2nd Order**	**Vect.**	**Third Order**	**Vect.**
U		**B**		**D**	
C. macropterus	0.929	*C. macropterus*	0.986	*G. holbrooki*	0.936
E. americanus	0.550	*N. petersoni*	-0.562	*N. texanus*	0.851
A. sayanus	0.488	*M. melanops*	0.420	*P. nigrofasciata*	0.370
G. holbrooki	0.115	*F. lineolatus*	0.392	*L. sicculus*	0.290
E. gloriosus	0.071	*M. salmoides*	0.364	*H. formosa*	0.215
B		**D**		**Fourth Order**	
C. macropterus	0.929	*G. holbrooki*	-0.764	**U**	
L. omatta	-0.540	*E. americanus*	-0.550	*M. salmoides*	-0.977
E. americanus	0.488	*C. macropterus*	0.550	*E. fusiforme*	0.904
L. macrochirus	-0.421	*L. macrochirus*	-0.305	*E. zonatum*	0.176
L. gulosus	0.366	*L. sicculus*	-0.251	*C. macropterus*	-0.141
D		**Third Order**		*O. emiliae*	-0.136
C. macropterus	0.987	**U**		**B**	
G. holbrooki	0.903	*G. holbrooki*	0.936	*M. salmoides*	-0.825
E. americanus	0.204	*N. texanus*	0.851	*C. macropterus*	-0.800
A. sayanus	0.184	*P. nigrofasciata*	0.370	*E. fusiforme*	0.100
L. gulosus	0.162	*L. sicculus*	0.290	*L. omatta*	-0.048
Second Order		*N. petersoni*	0.126	*E. okefonokee*	-0.038
U		**B**		**D**	
G. holbrooki	-0.764	*C. venusta*	0.863	*L. sicculus*	-0.999
L. gulosus	0.580	*N. texanus*	0.213	*C. macropterus*	-0.664
E. americanus	0.555	*G. holbrooki*	0.323	*G. holbrooki*	-0.473
C. macropterus	0.555	*P. nigrofasciata*	-0.330	*L. punctatus*	-0.400
L. macrochirus	-0.305	*L. auritus*	0.368	*L. auritus*	-0.256

A primary objective of this study was to analyze factors at bridge subsites that influenced species diversity. Bridge subsites possessed the highest numbers of species (n = 40) and habitat dissimilar to up steam and down steam subsites. Eight species collected at only bridge sites where *L. platyrhincus*, *A. calva, U. pygmaea, N. crysoleucas*, *F. chrysotus*, *L. ommata*, *E. gloriosus* and *E. evergladei*. Three species that were collected at upstream and downstream subsites but not bridge subsites were the

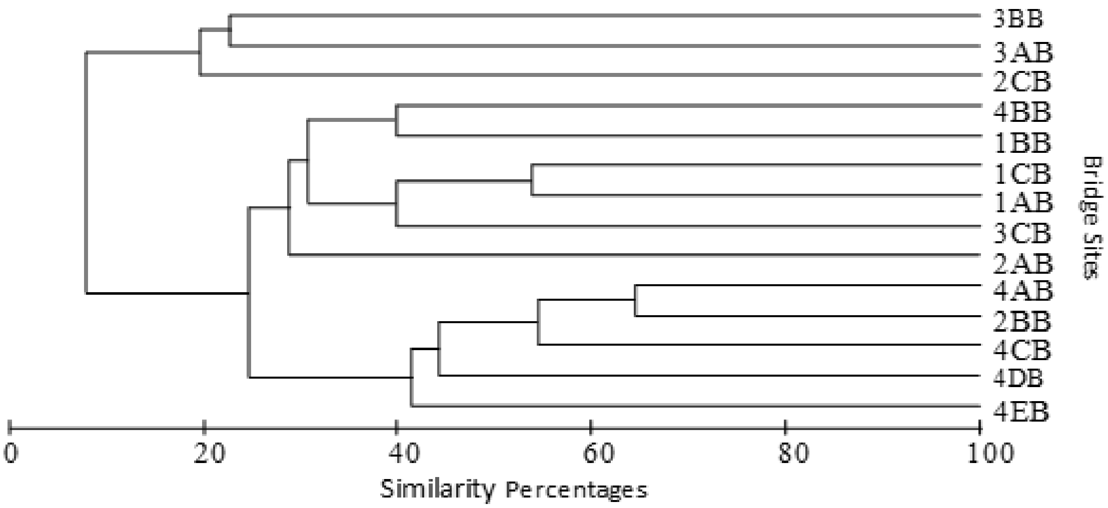

Figure 5. Cluster Analysis of fish species assemblages at bridge subsites. Numbers 1-4 represent stream orders. First alpha values (A, B, C, D, E) represents individual sites within stream orders. Second alpha value (B) indicates bridge sites.

hybrid *Pteronotropis sp. cf. hypselopterus metallicus*, and *A. brunneus* and *L. marginatus*. To access factors influencing the structure of fish assemblages at bridge subsites, regression analyses were conducted. Three nonparametric linear regression analyses were computed comparing the number of fish species to: (1) stream order, (2) number of side pools, and (3) age of the bridge (range years since built = 3 - 64), and a multiple linear regression was conducted on bridge construction variables (bridge length, bridge width, and length + width). The three nonparametric linear regression analyses were none significant (Stream order: tau b = 0.201, Two-sided P = 0.389; Side Pool Numbers: tau b = 0.215, Two-sided P = 0.368; Bridge Age, tau b = 0, Two-sided P = 0.9558) The multiple linear regression on bridge construction variables, including the combined variables of bridge length plus bridge width, which was dropped from the model due to high a correlation with the uncombined variables, was not significant (F = 1.241645, P = 0.3264, R^2 = 0.184175). A best subset regression analysis identified bridge width as the most likely predictor, but it was not significant and the estimation of variance was low (t = 1.137435, P = 0.2776, R^2 = 0.097321).

A multiple linear regression analysis examined the influence of chemical variables, temperature, substrate variables, current, aquatic pool dimensions and vegetation coverage on species numbers. Due to the potential interaction of total surface area (main pools and side pools) and vegetation surface area, an additional variable was developed adding total surface area plus vegetation surface area (ResearchGate 2022). Dependent and independent variables were transformed via z-score, log normal (ln), log10 (l10), squared (sq) and square root (sqrt). Original data and all transformed scores were tested for normality using Shapiro Wilk (W and V) and Shapiro Francia (W' and V') test sores (Tables in Wright, 2013). Of the 17 variables, untransformed data for pH, sand, depth, and side pool surface area tested normal with all other variables non-normal or likely not to have normal distributions (StatsDirect 2020). Standardized independent variables with the highest Shapiro Wilk and Francia scores were used in multiple linear regression analyses. The multiple linear regression run on all 17 predictor variables failed based on Givens rotations QR decomposition and singular value decomposition (SVD) analyses indicating multiple collinearities existing between variables (Daoud, 2017). These results were further supported by a Durbin-Watson test statistic (DW = 0.48989) indicating positive multiple collinearities between variables. To reduce multiple collinearities, the 17 predictor variables were divided into three sets of variables: (1) chemical and temperature, (2) substrates and current, and (3) physical factors involving main and side pool surface area, vegetation surface area and total surface area plus vegetation surface area. The three sets of variables were each run in two sets of multiple linear regressions comparing the selected original and standardized variables to species numbers per bridge subsite followed by a best subset analysis. The regression analysis on chemical variables and temperature was not significant (ANOVA, F = 2.3725, P = 0.1296). A best subsets analysis was significant (ANOVA, F = 4.86722, P = 0.031) and identified pH (P = 0.0452) and conductivity (ln, P = 0.0104), as two key predictor variables. The strength of the two variables as predictors of species number per bridge subsite was low (R^2 = 0.46948). Substrate and current multiple linear regression analysis was not significant (ANOVA, F = 0.320228, P = 0.919). The best subset analysis identified sand as the best predictive variable, but it was not significant (ANOVA, F = 0.897022, P = 0.3623), and the strength of predictivity was very low (R^2 = 0.06955). The multiple linear regression analysis on physical factors was not significant (F = 2.263404 P = 0.1546). The best subset analysis identified main pool surface area (sqrt, P = 0.0056), side pool surface area (P = 0.0118) and total surface area plus vegetation surface area as significant (P = 0.0042), with predictivity being low (R^2 = 0.318529).

The second set of regression analyses involved Principal Component Regressions (PCR) in which the three independent variable data sets listed above were first run through PCR and variables with the highest PCA1 and PCA2 scores (≥ 0.600) were used in a set of three multiple linear regression analyses on variables selected from each set of variables followed by best subsets analyses. The PCA on chemical variables plus temperature resulted in the selection of pH, O^2 ln, conductivity ln and temperature ln being selected. Conductivity ln was the only variable identified as significant (P = 0.0237) and the strength of correlation low (R^2 = 0.5132). The best subsets analysis identified pH (P = 0.0452) and conductivity (P = 0.0104) as significant. The PCA on substrates and current resulted in mud ln, gravel ln, and current ln as being selected with no variable identified as significant (P ≥ 0.622). The best subset analysis identified gravel ln as the only variable selected and it was nonsignificant (P = 0.633). The final set of variables run through PCA, pool and vegetation dimensions identified side pool surface area and vegetation surface area as key variables with neither being significant (P ≥ 0.113). The best subset analysis did not identify any variable as significant (P ≥ 0.113).

To further assess what influenced the lack of significance in the above regression models, multiple Kendall Ranked Correlations were carried out on non-transformed data comparing species numbers per bridge subsite to each of the original 17 variables. No ranking of fish species numbers verses each independent variable was significant (ranges b = -0.335243 - 0.268194; P = 0.101 - > 0.9999) indicating that no similar patterns of rank order existed between fish species number and measured variables.

DISCUSSION

A key factor impacting this research was the occurrence of moderate (n = 7), severe (n = 4) and extremely severe (n = 9) monthly drought events from January 2011 to August 2012, (Palmer Drought Index 2013), with one normal month of rain occurring after field collections were completed. The drought affected many riparian systems in South Georgia, USA, and impacted research on the status of the Blackbanded sunfish and other fish species of concern (Bechler and Salter 2013) taking place at the same time in the study area. As such, the drought provided an opportunity to assess the impact of bridges on low order streams during drought events as possible sites of refugia for fish.

Past studies have examined macroinvertebrate assemblages on fourth and lower order streams following site naturalization; and prior to this study no studies have specifically considered the effects on fish assemblages at lower order stream bridge sites (Joy and Death 2000; Blettler and Marchese 2005). This study examined the effect bridges have on fish species assemblage's and provides support for bridge sites as having potential positive effects downstream from the bridge subsite. Third order bridge sites did not have a thalweg at the bridge subsite which resulted in shallower depths, and smaller surface areas and perimeters. Afore mentioned changes helped depress silt and clay volumes and elevate sand volumes at these subsites towards ones that inhibit macroinvertebrate species diversity (Wright 2015).

Differences and similarities in fish species assemblages between upstream, bridge, and downstream subsites were supported by analyses of the data sets with upstream and downstream subsites sharing more species in common, than upstream and bridge subsites, or downstream and bridge subsites. Wellman et al. (2000) studying the impact of bridges and culverts and extended reaches above and below them found no major differences across their study sites. However, Benton et al. (2008) did find differences in Etowah Basin streams in northern Georgia, USA. Warren and Pardew (1998) also found variation based on their study of various stream crossings, with Frei (2006) noting the importance of hydraulic engineering at stream crossings and confirmed by Hotchkiss and Frei (2007).

Compared to upstream subsites, bridge and downstream subsites possessed greater species diversity with bridge subsites possessing the greatest species diversity. As such, upstream subsites could serve as controls since they were least affected by bridge subsite construction. The importance of such controls has been identified by Jackson et al. (1992). If increased fish species diversity is seen as beneficial, then factors originating at bridge subsites and downstream subsites can be seen as potentially beneficial. The difference between bridge subsite species diversity levels and the other subsite species diversity levels were greatest at the lower order streams and decreased from first to fourth order streams. This trend supports species diversity being more positively affected by bridge construction on lower order streams than higher order streams. This pattern also accounts for why research on higher order steams has found negative effects of bridge construction on fish (Cline et al. 1982; Larsen 1993; Blettler et al. 2005; Chadwick et al. 2006; Lau et al. 2006). It also suggests that from fourth to higher order streams, bridge site construction shifts from potentially positive to negative. Main and side pool surface areas and total surface area with vegetation were significant physical variables related to increased fish species diversity in the multiple linear regressions. However, these variables might decrease as significant factors as the flow and width of riparian systems increase in higher order streams (Pires, et al. 2010) leading to a mean threshold point for most systems occurring above fourth order streams.

Fish species organized into habitat guilds were used to provide an ecological measure for the affects generated by bridge construction. Persinger at al. (2011) and Spurgeon et al. (2019) discuss fish guilds as they relate to riverine habitats and preservation of such habitats. Bridge guild data matched the species assemblage data in all cases despite the guild data foci being habitat use as opposed to total species diversity. Thus, data on fish habitat by subsites matched fish species assemblage diversity data in all aforementioned trends. These results support a conceptualization of the bridge sites as just generating species diversity, but also generating habitat diversity, at least on lower order streams. Thus, converting small portions (bridge subsites) of a riparian system from a moderately productive low order stream state to a more productive medium or higher order stream with elevated levels of habitat diversity.

The multiple linear regression analysis on all 17 standardized independent variables resulted in the identification of multiple linear correlations among the independent variables with no significant variables impacting species diversity (StatsDirect 2007). Dividing the 17 independent variables into three sets of variables (chemical and temperature, substrates and current, and physical factors and vegetation surface area) and recomputing regression analyses via multiple linear regressions and principle component regressions on each set of independent variables followed by best subsets analyses resulted in the identification of pH, conductivity, main pool surface area, side pools surface area, and total surface area plus vegetation surface area as five significant variables potentially influencing fish diversity at bridge sites. The work of Pires, et al. (2010) supports the results in this study. Lower pH values at lower order bridge sites could indicate elevated levels of dissolved oxygen content (DOC) generated by concentrated levels of fulvic and humic acids in portions of blackwater systems during drought events (Meyer 1990). In the absence of sufficient macrophytes or flow, decreasing pH levels might proxy for decreased oxygen levels (Boto and Bunt 1981; Todd et al. 2009). Due to the similarities in the measurements of main pool and side pool total surface areas and total surface area plus vegetation, each may function as proxies for the other, in that they are related to habitat diversity and through that, to vegetation, pH, and oxygen. The pH during summer and main pool and side pool total surface areas of the water at the bridge subsites can support greater fish species diversity if the pH in summer is properly monitored and the surface area of the bridge subsite is maximized during bridge site construction and throughout subsequent bridge site renovation events.

Bridges create environments that often differ from undisturbed stream environments with respect to many physiochemical and biological properties. Physiochemical and biological factors were assessed for their effects on the assemblage structures to determine the overall impact of anthropogenic effect bridges have on species diversity and biotic potential. This has allowed the development of an understanding of the difference between bridge site and natural site assemblages while determining if naturalized bridge sites might be a source of wetland species and assemblage diversity following stochastic drought events which are occurring worldwide (Keaton, et al. 2005, McCargo and Peterson 2010, Wedderburn, et al. 2012, Wedderburn, et al. 2014).

A significant point of this research was that it addressed the absence of research on the fish species found at bridge sites at first through fourth order streams in South Georgia, USA, an area predominated by flatwoods habitat (Barnett et al. 2007; Todd et al. 2009). The research was accentuated by the severe drought in the Southern United States during the summer of 2011 (Wisniewski et al. 2013). Additional concerns for the health of rivers and streams have been brought to bear considering increases of combined investment by all levels of government in highway and bridge infrastructure. Bridges in the United States are averaging 40 years old, and half were built before 1964, with 26.7% of all bridges structurally deficient or functionally obsolete (Peters 2006).

As part of the river continuum concept, first through third order streams belong to the headwater stream set, while fourth through 6th order streams belong to the medium stream order set (Vannote et al. 1980). The clearing of the bridge subsite areas of canopy, widening of the bridge subsite run, and deepening of the bridge subsite run at lower order headwater steams alters the bridge subsites and brings them closer to the physical and species state of the fourth through sixth order streams. Medium streams have the highest levels of macrophyte, fish, and macroinvertebrate species diversities (Vannote et al. 1980). In consideration of the properties and variables that have been identified for the bridge subsites, it can be consider that bridges provide an effect of elevating the river continuum measure of the first through third order streams to higher stream orders.

Future research should address the full extent of the construction shadow effect from bridge sites proceeding downstream. Identifying the distance and reduction rate of the shadow effect could help support the upstream subsites used as controls. Also, the distance of the effect could help in maximizing the full benefits of the naturalized bridge site habitat. Testing the effects of bridge sites in the current research to sites with similar morphology in areas of sharper relief or elevation could broaden the applicability of the research. While this research was carried out in flatwoods habitats, additional work on first through fourth order streams should be carried out in other habitat types to assesses the impact of such habitats. Such research also has the potential for the identification of fish species not known to inhabit such areas such as *H. formosa*, which was found further south of the study area in Lowndes County Georgia by Chaney and Bechler (2006) and was found at eight subsites during this study (Wright 2013).

REFERENCES

AFS Guidelines. 2014. Guidelines for the Use of Fishes in Research. American Fisheries Society. https://www.fullerton.edu/doresearch/resource_ library /policies/IACUC_GuidelinesforUseofFishes%202014.pdf.

ArcMap. 2012. Environmental Systems Research Institute (Esri). Web GIS Mapping Software | ArcGIS Online (esri.com). https://www.esri.com/en-us/home.

Arimoro, F.O., G.E. Obi-Iyeke, and P.J.O. Obukeni. 2012. Spatiotemporal variation of macroinvertebrates in relation to canopy cover and other environmental factors in Eriora River, Niger Delta, Nigeria. Environmental Monitoring and Assessment: 184, 6449–6461. https://doi.org/ 10.1007/s10661-011-2432-9.

ASIH. 2013. Guidelines for the Use of Fishes in Research. https: //static1. squarespace.com/static/618bf11a71fcdf5398996eda/t/618fbed1f40e6c713 dfa71ee/1636810449675/asf-guidelines-use-of-fishes-in-research-013.pdf.

Barnett, J., D.L. Bechler, C. Denizman, J. Grable, J. Nienow, J. Turco, W. Tietjen, and G.L. Wood. 2007. Watershed Restoration Action Strategy Development in the Alapahoochee River Watershed. Nonpoint Source Management Program, Section 319 Report. Submitted to Environmental Protection Division, Department of Natural Resources, Georgia, USA. 92 pp.

Bechler, D.L., and J.S. Salter. 2013. The Status of the Blackbanded Sunfish and Other Species of Concern in the State of Georgia. Final report, Non-Game Division, Georgia Department of Natural Resources, Social Circle, Georgia, Submission date 18 December 2013. 36 pp.

Benton, P.D., W.E. Ensign, and B.J. Freeman. 2008. The effect of road crossings on fish movements in small Etowah Basin streams. Southeastern Naturalist, 7(2), 301-310. https://digitalcommons.kennesaw.edu/cgi/viewcontent.cgi?article=1203andcontext=facpubs.

Blettler, C.M., and M.R. Marchese. 2005. Effects of Bridge Construction on the Benthic Invertebrates Structure in the Paran´a River Delta. Interciencia: Journal of American Science and Technology. 30(2), 60-66. http://ve.scielo.org/scielo.php?pid=S0378-44200500020004andscript=ci_art text.

Boto, K.G., and J.S. Bunt. 1981. Dissolved oxygen and pH relationships in northern Australian mangrove waterways. Limnology and Oceanography, 26(6), 1176-1178. https://aslopubs.onlinelibrary.wiley.com/doi/pdfdirect/10.4319 / lo.1981.26.6.1176

Brown, J.L. 1956. Distinguishing Characteristics of the Cyprinodont Fishes, *Fundulus cingulatus* and *Fundulus chrysotus*. Copeia. 1956(4): 251-255. https://www.jstor.org/stable/1440285.

Brown, J.L. 1958. Geographic Variation in Southern Populations of the Cyprinodont Fish *Fundulus notti* (Agassiz). American Midland Naturalist. 59(2), 477-488. https://www.jstor.org/stable/2422493.

Cannister, M.J. 2007. A Survey of the Fish Fauna of the Withlacoochee River in South Georgia. Department of Biology. Valdosta State University. Thesis, 61 pp.

Cannister, M.J., and D.L. Bechler. 2019. Fish assemblages of the Withlacoochee River basin in South Georgia, USA. Georgia Journal of Science. 77(2): 2, Article 19. Doi: https://Digitalcommons.gaacademy.org/cgi/viewcontent.cgi?article=1936andcontex ++t=gjs.

Casatti, L., C. de Paula Ferreira, and F,R. Carvalho. 2009. Grass-dominated stream sites exhibit low fish species diversity and dominance by guppies: an assessment of two tropical pasture river basins. Hydrobiologia, 632(1), 273-283. https://link.springer.com/article/10.1007/s10750-009-9849-y.

Chadwick, M.A., D.R. Dobberfuhl, A.C. Benke, A.D. Huryn, K. Suberkropp, and J.E. Thiele. 2006. Urbanization Affects Stream Ecosystem Function by Altering Hydrology, Chemistry, and Biotic Richness. Ecological Applications. 16(5), 1796-1807. https://doi.org/10.1890/1051-0761(2006)016[1796:UASEFB]2.0.CO;2.

Chaney, J.C., and D.L. Bechler. 2006. The occurrence and distribution of *Heterandria formosa* (teleostei, Poeciliidae) in Lowndes County, Georgia." Georgia Journal of Science 64(2), Article 9. https://digital commons.gaacademy.org/gjs/vol64/iss2/9/.

Clarke, K.R., and R.N. Gorley. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth. https://www.researchgate.net/publication/285668711_PRIMER_v6_user_manualtutorial_PRIMER-E_Plymouth.

Cline, L.D., R.A. Short, and J.V. Ward. 1982. The Influence of Highway Construction on the Macroinvertebrates and Epilithic Algae of a High

Mountain Stream. Hydrobiologica. 96, 149–159. http://www.ephemeroptera-galactica.com/pubs/pub_c/pubclinel 1982p149.pdf.

Crovo J.A., M.T. Mendonça, D.E. Holt, C.E. Johnston. 2015 Stress and Auditory Responses of the Otophysan Fish, *Cyprinella venusta*, to Road Traffic Noise. PLOS ONE 10(9): e0137290. https://doi.org/10.1371/journal.pone.0137290.

Darden, T.L. 2008. Phylogenetic Relationships and Historical Biogeography within the *Enneacanthus* Sunfishes (Perciformes: Centrarchidae). Copeia. 3, 630-636. https://doi.org/10.1643/CI-06-063.

Death, R.G. 1996. The Effect of Patch Disturbance on Stream Invertebrate Community Structure: The Influence of Disturbance History. Oecologia. 108(3), 567-576. https://link.springer.com/article/10.1007/BF00333735.

Daoud, J.I. 2017. Multicollinearity and Regression Analysis. Journal of Physics: Conference Series, 949, Article ID: 012009. https://doi.org/10.1088/1742-6596/949/1/012009.

Esri Inc. 2012. ArcGIS 9.3. Esri Inc. https://www.esri.com/en-us/arcgis/products/arcgis-9/overview.

Esri. 2016. http://www.esri.com/?q=esriandform=EDGSPHandmkt=en-usand httpsmsn=1andrefig=2fe7173d36d34613ad78aef05dc7adb2andsp=1andhttp%3A%2F%2Fwww. esri.com%2F=.

Esri. 2017. ARCGIS blog. Using and citing Esri data. https://www.esri.com/arcgis-blog/products/product/mapping/using-and-citing-esri-data/.

FishBase. 2012. FishBase. https://www.fishbase.de/.

Fishes of Georgia. 2022, Http://fishesofgeorgia.uga.edu/index.php?page= species pages/species_pageandkey=ptermeta.

Frei, C.M. 2006. Design of fish passage at bridges and culverts: Hydraulic engineering circular-26. Doctoral dissertation. 208 pp http://www.dissertations. wsu.edu/Thesis/Fall2006/c_frei_082506.pdf.

GMNH. 2016. Georgia Museum of Natural History-Athens. https://www.naturalhistory.uga.edu/.

Ghedotti, M.J., and M.J. Grose. 1997. Phylogenetic Relationships of the *Fundulus nottii* Species Group (Fundulidae, Cyprinodontiformes) as Inferred from the Cytochrome *b* Gene. Copeia. 1997(4), 858-862. https://www.jstor.org/stable/1447306.

Gilbert, C.R., R.C. Cashner, and E.O. Wiley. 1992. Taxonomic and Nomenclatural Status of the Banded Topminnow, *Fundulus cingulatus* (Cyprinodontiformes: Cyprinodontidae). Copeia. 1992(3): 747-759. Google Inc. 2012. Google Earth 7.0.3.8542. https://doi.org/10.2307/1446151.

Google Inc. 2012. Google Earth 7.0.3.8542., http://www.google.com/earth/index.htlm.

Griffith, G.E., J.M. Omernik, J.A. Comstock, S. Lawrence, G. Martin, A. Goddard, V.J. Hulcher, and T. Foster. 2001. Ecoregions of Alabama and Georgia. Reston, Virginia, U.S. Geological Survey. https://store.usgs.gov/assets /MOD/StoreFiles/Ecoregion/112766_al_ga_front.pdf.

Holt, D.E. and C. Johnston. 2015. Traffic noise masks acoustic signals of freshwater stream fish. Biological Conservation. 187 (July 2015), 27-33. https://www.sciencedirect.com/science/article/abs/pii/S0006320715001494.

Hotchkiss, R.H., and C.M. Frei. 2007. Design for fish passage at roadway-stream crossings: synthesis report (No. FHWA-HIF-07-033). United States. Federal Highway Administration. file:///C:/Users/User/Downloads/dot_1023 _DS1%20 (1).pdf.

Jackson, D.A., K.M. Somers, and H.H. Harvey. 1992. Null models and fish communities: evidence of nonrandom patterns. The American Naturalist, 139(5), 930-951. http://jackson.eeb.utoronto.ca/files/2012/10/amnat-null-model.pdf.

Joy, M.K. and Death, R.G. 2000. Stream Invertebrate Communities of Campbell Island. Hydrobiologica. 439(1): 115-124. https://doi.org/10.1023/A:1004103815444.

Kałuża T, M. Sojka, R. Wróżyński, J. Jaskuła, S. Zaborowski, and M. Hämmerling. 2020. Modeling of River Channel Shading as a Factor for Changes in Hydromorphological Conditions of Small Lowland Rivers. Water. 12(2):527. https://doi.org/10.3390/ w1202052.

Keaton, M., D. Haney, and C.B. Andersen. 2005. Impact of drought upon fish assemblage structure in two South Carolina Piedmont streams. Hydrobiologia 545, 209–223. https://doi.org/10.1007/s10750-005-2674-z.

Larson, O.D. 1993. Denmark's Great Belt Link. Journal of Coastal Research. 9(3), 766-784. https://www.jstor.org/stable/4298128.

Lau, J.K., T.E. Lauer, and M.L. Weinman. 2006. Impacts of Channelization on Stream Habitats and Associated Fish Assemblages in East Central Indian. The American Midland Naturalist. 156, 319-330. https://doi.org/10.1674/0003-0031(2006)156[319:IOCOSH]2.0.CO;2.

Lazara, K.J. 2002. Lectotype of *Fundulus auroguttatus* (Hay) Is Designated as the Neotype of *Fundulus cingulatus* (Valenciennes) (Cyprinodontiformes: Fundulidae). Copeia. 2002(1), 227-228. https://meridian.allenpress.com/copeia/article-abstract/2002/1/227/259044/Lectotype-of-Fundulus-auroguttatus-Hay-Is?redirectedFrom=fulltext.

MacArthur R.H., and E.O. Wilson. 1967. The theory of island biogeography. Princeton, Princeton University Press. 203 pp. https://link. springer.com/article/10.1023/A:1016393430551.

Manning, T.J., M.L. Sherrill, T. Bennett, M. Land, and L. Noble. 2004. Effect of chemical matrix on humic acid aggregates. Florida Scientist 67, 266-280. Mattison Instruments. 2013. Mattison Instruments, Madison, Wisconsin. https://www.mattson.com/.

Mayden, R.L. and J. Allen. 2015. Phylogeography of Pteronotropis signipinnis, P. euryzonus, and the *P. hypselopterus* Complex (Teleostei: Cypriniformes), with Comments on Diversity and History of the Gulf and Atlantic Coastal Streams. BioMed Research International, 2015. https://www.hindawi.com/journals/bmri/2015/675260/.

McCargo, J.W. and J.T. Peterson, 2010. An Evaluation of the Influence of Seasonal Base Flow and Geomorphic Stream Characteristics on Coastal Plain Stream Fish Assemblages, Transactions of the American Fisheries Society, 139(1), 29-48, DOI: 10.1577/T09-036.1.

Meyer, J.L. 1990. A blackwater perspective on riverine ecosystems. Bioscience 40(9), 643-651. https://doi.org/10.2307/1311431.

Microsoft. 2010. Microsoft 365. https://www.microsoft.com/en-us/microsoft-365/ excel.

Microsoft. 2021. Microsoft 365. https://www.microsoft.com/en-us/microsoft-365/ excel.

Page, L.M., and B.M. Burr. 1991. A Field Guide to Freshwater Fishes of North America North of Mexico. Houghton Mifflin Company, New York, NY. 432 pp.

Palmer Drought Index. 2013. National Climatic Data Center, National Oceanic and Atmospheric Administration, USA. http://www.ncdc.noaa.gov/oa/climate/research/prelim/drought/palmer.html.

Perry, G.D. 1981. The meanings of r- and k-selection. Oecologia (Berl) 48, 260-264. https://www.researchgate.net/profile/Gregory-Parry/publication/226288387_The_meaning_of_r-_an_K-selection/links/00b7d5372c43ce156b000000/The-meaning-of-r-an-K-selection.pdf.

Persinger, J.W., D.J. Orth, and A.W. Averett. 2011. Using habitat guilds to develop habitat suitability criteria for a warmwater stream fish assemblage. River Research and Applications, 27(8), 956-966. Persinger%20Habitat%20Guilds%20River%20Res% 20Applic%202010.pdf.

Peters, M.E. 2006. Status of the nation's highways, bridges, and transit: conditions and performance. U.S. Department of Transportation. https://rosap.ntl.bts.gov/view/dot/907.

Pires, D.F., A.M. Pires, M.J. Collares-Pereira, M.F. Magalhães. 2010. Variation in fish assemblages across dry-season pools in a Mediterranean stream: effects of pool morphology, physicochemical factors and spatial context. Ecology of Freshwater Fish. 19(1) 74-86. https://doi.org/10.1111/j.1600-0633.2009.00391.x.

ResearchGate. 2022. Is it justified to combine several potential predictors into one predictor for regression analysis? https://www.researchgate.net/post/Is_it_justified_to_combine_several_potential_predictors_into_one_predictor_for_regression_analysis.

Resh, V.H., A.V. Brown, A.P. Covich, M.E. Gurtz, H.W. Li, G.W. Minshall, S.R. Reice, A.L. Sheldon, J.B. Wallace, and R.C. Wissmar. 1988. The role of disturbance in stream ecology. Journal of North American Benthological Society. 7(4), 433-455. https://www.journals.uchicago.edu/doi/abs /10.2307 /1467300.

Rider, S.J. and W. Schell. 2012. First record of *Acantharcus pomotis* (mud sunfish) from Alabama. Notes of the Southeastern Naturalist. Issue 11(1). https://bioone.org/journals/southeastern-naturalist/volume-11/issue-1/058.011.0115/First-Record-of-Acantharchus-pomotis-Mud-Sunfish-from-Alabama/10.1656/058.011.0115.short.

Rivas, L.R. 1966. The taxonomic status of the Cyprinodontid fishes *Fundulus notti* and *F. lineolatus*. Copeia. 1966(2), 353-354. https://doi.org/10.2307/1441149. https://www.jstor.org/stable/1441149.

Rosenberg, M.S., and C.D. Anderson. 1998. PASSaGE 2: Pattern Analysis, Spatial Statistics and Geographic Exegesis. version 2.0.11.6. https://passage software.net/download.php.

Shankman, D. 1996. Stream channelization and changing vegetation patterns in the U.S. coastal plain. The Geographical Review. 86(2), 216-232. https://www.jstor.org/stable/215957.

Seiyaboh, E.I., I.R. Inyang, and A.H. Gijo. 2013. Environmental impact of Tombia bridge construction across Nun River in central Niger delta, Nigeria. The International Journal of Engineering and Science, 2(11), 32-41. https: // theijes.com/papers/v2-i11/Part.2/E021102032041.pdf.

Simberloff, D. and T. Dayan. 1991. The guild concept and structure of ecological communities. Annual Review of Ecology and Systematics. 22, 115-143. https://www.tau.ac.il/lifesci/zoology/members/dayan_files/articles/Guildes.pdf.

Snelson, F.F., T.J. Krabbenhoft, and J.M. Quattro. 2009. *Elassoma gilberti*, a new species of pygmy sunfish (Elassomatidae) from Florida and Georgia. Bulletin of the Florida Museum of Natural History, 48(4), 119-144. https://www.floridamuseum.ufl.edu/wp-content/uploads/sites/35/2017/03/ bulletin-vol48no4.pdf.

Spurgeon, J., M. Pegg, P. Parasiewicz. and J. Rogers. 2019. River-wide habitat availability for fish habitat guilds: implications for in-stream flow protection. Water, 11(6), p.1132. file:///C:/Users/User/Downloads/water-11-01132%20(3).pdf.

StatsDirect Ltd. 2007. StatsDirect Statistical Software. England: StatsDirect Ltd. 2007. (1990-2020 StatsDirect Limited). http://www.statsdirect.com.

StatSoft Inc. 2012. TIBCO® Data Science https://www.tibco.com/products/data-science?utm_medium=cpcandutm_source=bingandutm_content=sandutmCampaign=bng_s_DS_nam_en_brand_betaandutm_term=%2Bstatisticaand_bt=and_bm=eand_bn=oandmsclkid=97ccdd57d3de1a00186bbf5ea60ed012andutm_source=bingandutm_medium=cpcandutm_campaign=bng_s_DS_nam_en_brand_betaandutm_term=%2Bstatisticaandutm_content=statistica.

Systat Software. 2012. Systat Software, Inc. https://sigmaplot.fileplanet.com/ and https://systatsoftware.com/.

Tiemann, J.S. 2004. Short-term effects of logging and bridge construction on habitat of two Kansas intermittent streams. Transactions of the Kansas Academy of Science, 107(3), 136-142. https://www.researchgate.net/profile/Jeremy-Tiemann/publication/232684319_Short-term_effects_of_logging_and_bridge_construction_on_habitat_of_two_Kansas_intermittent_streams/links/53f37d100cf2da8797448ce8/Short-term-effects-of-logging-and-bridge-construction-on-habitat-of-two-Kansas-intermittent-streams.pdf.

Todd, M.J., G. Vellidis,, R.R. Lowrance, and C.M. Pringle. 2009. High sediment oxygen demand within an instream swamp in Southern Georgia: implications for low dissolved oxygen levels in coastal blackwater streams 1. Journal of the American Water Resources Association, 45(6), 1493-1507. DOI: 10.1111/j.1752-1688.2009.00380.x.

Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. Canadian Journal of Fishes and Aquatic Sciences. 37, 130-137. https://doi.org/10.1139/f80-017.

Wallace, J.B., S.L. Eggert. 2009. Benthic invertebrate fauna, small streams. Encyclopedia of Inland Waters, 2, 173-190. https://www.nrs.fs.fed.us/pubs/jrnl/2009/nrs_2009_wallace_001.pdf.

Warren Jr, M.L. and M.G. Pardew. 1998. Road crossings as barriers to small-stream fish movement. Transactions of the American Fisheries Society, 127(4), 637-644. https://www.srs.fs.usda.gov/pubs/ja/ja_warren006.pdf.

Wedderburn, S.D., Barnes, T.C. and Hillyard, K.A. 2014. Shifts in fish assemblages indicate failed recovery of threatened species following prolonged drought in terminating lakes of the Murray–Darling Basin, Australia. Hydrobiologia 730, 179–190. https://doi.org/10.1007/s10750-014-1836-2.

Wedderburn, S.D., Hammer, M.P. and Bice, C.M. 2012. Shifts in small-bodied fish assemblages resulting from drought-induced water level recession in terminating lakes of the Murray-Darling Basin, Australia. Hydrobiologia 691, 35–46. https://doi.org/10.1007/s10750-011-0993-9.

Wellman, J.C., D.L. Combs, and S.B. Cook. 2000. Long-term impacts of bridge and culvert construction or replacement on fish communities and sediment characteristics of streams. Journal of Freshwater Ecology, 15(3), 317-328. https://www.tandfonline.com/doi/pdf/10.1080/02705060.2000.9663750.

Wiley, E.O. 1986. A study of the evolutionary relationships of *Fundulus* topminnows (Teleostei: Fundulidae). American Zoologist, 26(1), 121-130. https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetailandidt=8799898.

Wiley, E.O. and Hall, D.D. 1975. *Fundulus blairae*, a new species of the *Fundulus nottii* complex (Teleostei, Cyprinodontidae). American Museum of Natural History. American Museum Novitates, 2577, 1-13. https://digital library.amnh.org/bitstream/handle/2246/2764/N2577.pdf?sequence=1.

Wisniewski, J.M., K.D. Bockrath, J.P. Wares, A.K. Fritts, and M.J. Hill. 2013. The mussel–fish relationship: a potential new twist in North America? Transactions of the American Fisheries Society, 142(3), 642-648. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.949.6737andrep=rep1andtype=pdf.

Wright, C.W. 2013. An assessment of the anthropogenic effect of bridges on fish and macroinvertebrate assemblages. Thesis, Valdosta State University. https://vtext.valdosta.edu/xmlui/handle/10428/1293. Thesis 93 pp.

APPENDIX

+Table I. Fish species scientific names (genus and species) collected at study sites. Families are in bold fonts. Subsites individual species were collected from are listed as: U = upstream, B = bridge, D = downstream and are in parentheses. Numbers preceding scientific names correspond to species common names listed in Appendix Table I. Note, *Pteronotropis hypselopterus* has been identified as part of species complex and is listed as P*teronotropis* sp. cf. *hypselopterus metallicus* (Mayden and Jason, 2015).

Family: Genus and Species

Lepisosteidae

1. *Lepisosteus osseus* (BD)
2. *Lepisosteus platyrhincus* (B)

Amiidae

1. *Amia calva* (B)

Aphredoderidae

1. *Aphredoderus sayanus* (UBD)

Umbridae

1. *Umbra pygmaea* (B)

Esocidae

1. *Esox americanus vermiculatus* (UBD)
2. *Esox niger* (UBD)

Cyprinidae

1. *Notemigonus crysoleucas* (B)
2. *Opsopoeodus emiliae* (UBD)
3. *Notropis petersoni* (UBD)
4. *Notropis texanus* (UBD)
5. *Cyprinella venusta* (UBD)
6. *Pteronotropis sp. cf. hypselopterus metallicus* (B)

Catostomidae

1. *Minytrema melanops* (UBD)
2. *Erimyzon sucetta* (BD)

Ictaluridae

1. *Ameiurus brunneus* (D)
2. *Ameiurus nebulosus* (B)
3. *Noturus gyrinus* (UBD)
4. *Noturus leptacanthus* (UBD)

Atherinopsidae

1. *Labidesthes sicculus* (UBD)

Family: Genus and Species

Fundulidae

1. *Fundulus chrysotus* (B)
2. *Fundulus lineolatus* (UBD)
3. *Leptolucania ommata* (B)

Poeciliidae

3. *Gambusia holbrooki* (UBD)
4. *Heterandria formosa* (UBD)

Centrarchidae

1. *Micropterus notius* (BD)
2. *Micropterus salmoides* (UBD)
3. *Centrarchus macropterus* (UBD)
4. *Lepomis auritus* (UBD)
5. *Lepomis gulosus* (UBD)
6. *Lepomis macrochirus* (UBD)
7. *Lepomis marginatus* (UD)
8. *Lepomis punctatus* (UBD)
9. *Pomoxis nigromaculatus* (UBD)
10. *Enneacanthus gloriosus* (B)
11. *Enneacanthus obesus* (B)
12. *Acantharchus pomotis* (UB)

Elassomatidae

1. *Elassoma evergladei* (B)
2. *Elassoma okefenokee* (B)
3. *Elassoma zonatum* (UBD)

Percidae

1. *Percina nigrofasciata* (UBD)
2. *Etheostoma edwini* (UBD)
3. *Etheostoma fusiforme* (UBD)

Table II. Fish species common names collected at study sites. Families are in bold fonts. Subsites species were collected from are listed in Results, Table II. Fishes of Georgia (2022) provides information on individual species.

Family: Common Names

Lepisosteidae
1. Longnose gar
2. Florida gar

Amiidae
1. Bowfin

Aphredoderidae
1. Pirate Perch

Umbridae
1. Eastern mudminnow

Esocidae
1. Redfin pickerel (Eastern)
2. Chain pickerel

Cyprinidae
1. Golden shiner6
2. Pugnose minnow
3. Coastal shiner
4. Weed shiner
5. Blacktail shiner
6. Sailfin and Metallic Shiner (Hybrid)

Catostomidae
1. Spotted sucker
2. Lake chubsucker

Ictaluridae
1. Snail bullhead
2. Brown bullhead
3. Tadpole madtom
4. Speckled madtom

Antherinopsidae
1. Brook silverside

Family: Common Names

Fundulidae
1. Golden topminnow
2. Lined topminnow
3. Pygmy killifish

Poeciliidae
1. Eastern mosquitofish
2. Least killifish

Centrarchidae
1. Suwannee bass
2. Largemouth Bass
3. Flier
4. Redbreast sunfish
5. Warmouth
6. Bluegill
7. Dollar sunfish
8. Spotted sunfish
9. Black crappie
10. Bluespotted sunfish
11. Banded sunfish
12. Mud sunfish

Elassomatidae
1. Everglades pygmy sunfish
2. Okefenokee pygmy sunfish
3. Banded pygmy sunfish

Percidae
1. Blackbanded darter
2. Brown darter
3. Swamp darter