An Assessment of the Anthropogenic Affect of Bridges on Fish and Macroinvertebrate Assemblages

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ABSTRACT

Anthropogenic impacts such as bridge sites can greatly alter established streambed morphology and associated ecology. At bridge sites, streams are often channelized approaching the site and deep pools are created at the bridge site causing ecological disturbances of fish and invertebrate 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 sites of anthropogenic origin may serve as potential habitats for reestablishment of populations following a drought event. This study examined fish and macroinvertebrate assemblages, and physiochemical factors associated with these assemblages at 14 bridge sites involving first through fourth order streams in the Suwannee River Basin of south Georgia. Fish assemblages were least diverse upstream of bridge sites, most diverse at bridge sites, and intermediate downstream of bridge sites. Macroinvertebrate assemblages did not exhibit as distinctive a pattern as did fish assemblages. Upstream macroinvertebrate assemblages were less diverse than bridge site and downstream assemblages, a pattern that was disrupted for the bridge site by third order stream data. The results from this study suggest that bridge sites, if properly engineered, can serve as valuable refuges for reestablishing fish and macroinvertebrate assemblages up and down stream after events such as the severe drought that impacted south Georgia in 2011.

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Chapter I

INTRODUCTION

Bridge Construction

Highway and bridge construction have been shown to cause negative perturbations in the benthic community structure by disturbing natural stream conditions (Cline et al., 1982; Larsen, 1993). Bridges can impact aquatic habitat with pillars, dredging, embankments, and highway construction (Larsen, 1993). Streams are often channelized during construction, and deep runs are created under the bridge (Cline et al., 1982). The channelization, and deep pool formation constitute an ecological disturbance for fish and macroinvertebrate assemblages present (Resh et al., 1988). Positive effects of bridges on riparian ecosystems does not occur initially following construction but should be considered following a period of 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 & Marchese, 2005; Death, 1996). Sites upstream from the bridges with silt-clayed sediments demonstrate higher species richness and higher levels of benthic biomass than bridge and downstream sites (Blettler & Marchese, 2005).

Research supports the use of invertebrates as indicators of stream health, but the close association of benthic invertebrates to sediment grain size, and current velocity supports consideration of their use as indicators of anthropogenic disturbances in riparian systems (Death, 1996). 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 & Marchese, 2005). Some studies have considered macroinvertebrate and fish assemblages following a period of 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 & Death, 2000; Blettler & Marchese, 2005).

Disturbance

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 “naturalized” bridge sites, riffle and run habitats (the natural stream pattern) may reestablish as well as sensitive species (Lau et al., 2006). Naturalized bridge sites will have other influences such as erosion, sediment loads, destruction of riparian zones, alteration of substrate, and removal of accumulated debris decreased by the progression of time (Lau et al., 2006). Disturbances from bridge construction can be further mitigated if normal water flow is maintained in spite of 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).

Natural Streams

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 in-stream cover and overhanging vegetation is present in natural streams and often absent in bridge sites, which decreases the number of niches available (Lau et al., 2006).

Purpose and Significance

The purpose of this thesis is to appraise the impact of naturalized bridge sites along fourth and lower order streams in the Suwannee River basin of south Georgia as it relates to macroinvertebrate and fish assemblages. Bridges create environments that often differ from undisturbed stream environments with respect to many physiochemical and biological properties. Variations in physiochemical and biological factors were assessed for their effects on the assemblage structures so as 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 if naturalized bridge sites might be a source of wetland species and assemblage diversity following stochastic drought events.

Significance of this research was that it addressed the absence of research on the fish species found at bridge sites along 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 in light of increases of combined investment by all levels of government in highway and bridge infrastructure. Bridges are averaging 40 years old, half were built before 1964 with 26.7% of all bridges structurally deficient or functionally obsolete (Peters, 2006). Further, it must be kept in mind that, present day fauna are the result of geology, amount of human habitation, and distance from species source populations (Joy & Death, 2000).

Chapter II

MATERIALS AND METHODS

Study Sites

During a drought in the southeastern United States, 14 bridge sites in the south central region of Georgia along the Suwannee River drainage basin were assessed for anthropogenically generated affects upon fish and macroinvertebrate assemblages. The sites were predominantly below baseflow for much of the year and at some sites flow was completely interrupted for an extended period of time. Latitude and longitude were determined 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). The conversion to decimal degrees facilitated the assessment of each site using PASSaGE 2 statistical software (Rosenberg & Anderson, 1998).

Sites were divided into upstream (U), bridge (B) and downstream (D) subsites, which produced 42 data sets. Upstream subsites served as controls against which the bridge and downstream subsites were compared. Upstream habitats were often complex with many roots and braided (intertwined channels) stream morphology through a shallow flatwoods black water system. Downstream habitats were often shallow runs with modest riffles and large woody debris. Some upstream and downstream sites shared morphological features or similar levels of desiccation. All bridge subsites had a deep run morphology generating a thalweg for the riparian system and most had macrophytes. First and second order streams (small streams) had shallow flatwoods systems entering the bridge run from braided morphology and exiting to braided morphology. Third and fourth order streams (slightly larger streams) had flatwoods systems entering the bridge run from winding channel morphology and exiting to winding channel morphology.

Collection Protocol

Collection of fish and macroinvertebrate samples occurred from May to September 2011 within the guidelines of the Georgia Department of Natural Resources (DNR) scientific collecting permit #1934 issued to Dr. David L. Bechler of Valdosta State University. At bridge subsites, fish were collected through extensive seining of all habitat types, 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. Fish and macroinvertebrate 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. Seines used were a 170 cm W x 120 cm H x 0.5 cm mesh, and a 450 cm W x 125 cm H x 0.25 cm mesh with the particular net used dependent on the habitat being seined. Prior to collecting of fish, physicochemical data, and macroinvertebrates, a gill net for large 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. The gill net possessed a monofilament mesh which measured 30.48 m W x 1.83 m H x 7.62 cm, and was set along the center and length of the run or pool. Due to drought conditions, very few runs of a depth requiring the use of a gill net were found upstream or downstream. A D-frame kick net was used to sample the macroinvertebrates using three one meter passes of every type of unique habitat (submerged roots, rocky substrate, sandy substrate, leaf litter, large woody debris, macrophytes, and other unique habitats) located at the bridge site, upstream, and downstream (Barnett et al., 2007).

Fish were euthanized in the field using buffered tricaine methyl sulfonate (MS222) at a concentration of 500 mg/L in accordance with American Veterinarian Medical Association (AVMA) guidelines for the euthanasia of animals. Following, AVMA guidelines for the euthanasia of animals is standard operating procedure (SOP) for compliance with the Institutional Animal Care and Use Committee (IACUC) of Valdosta State University (Appendix C), and in accordance with the American Society of Ichthyologists and Herpetologists (ASIH, http://www. asih.org/). All research was in compliance with the SOP 002, 003, 010, 011, and 013 for the IACUC of Valdosta State University. Specimens were fixed in 10% formalin for 24 hours, soaked in water for 24 hours, and preserved in 55% isopropyl alcohol. Macroinvertebrates and debris were stored in 2 liter bottles in an 80% ethanol solution with rose Bengal dye. Macroinvertebrates collected in seine nets were placed in MS222 solution until collecting was completed and were then transferred to macroinvertebrate collection bottles of 80% ethanol solution with rose Bengal dye.

A 0.25 L substrate sample was collected once during the summer from the bridge, upstream, and downstream subsites. Substrate samples were homogenized and dried in an oven at 60 degrees for 3 days. A 10 ml sample was used to assess the organic content of the sample and a 50 ml sample was sifted through substrate sieves and the resultant volumes collected in each sieve were measured to assess substrate ratios for each subsite.

The dried 10 ml sample was weighted and then heated in an oven to 550 ^{O}C for 4 hours to eliminate all organic material and then weighted. The original weight minus the resulting weight provided the organic content weight of the sample.

Chemical properties and flow were collected twice for each subsite, once between May and September 2011, and later between January and February 2012. The chemical properties measured for each site were temperature, oxygen content, pH, and conductivity. 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 across the bridge site width, a bisecting line for the bridge site run length, and depth measurements. The 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. The additional physical property of surface area was calculated using Google Earth measurement applications (Google Inc., 2012).

Quantitative infrared samples were collected using a 0.25 liter scoop and the resulting slurry was emptied into a 1.25 liter Zip-lock freezer bag that was stored at -60^{O}C. Samples were later thawed, decanted onto filter paper, and the sample was rinsed to dissolve the relatively high levels of 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 grams were measured from each sample, and the 25 grams were soaked in 10 ml of methanol for 48 hours. The solution resulting was filtered using 0.2 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 produced by Mattison Instruments in Madison, Wisconsin.

Specimen Identification

Baseline data for macroinvertebrate and fish species most likely to be found at collecting sites was retrieved from Barnett et al. (2007) and Canister (2009) respectively. Fish were identified using the Peterson Field Guide to Freshwater Fishes as well as other sources (Page & Burr, 1991; fishbase.org, 2012; naturalhistory.uga.edu, 2012; Albanese, 2012; Darden, 2008; Lazara, 2002; Ghedotti & Grose, 1997; Gilbert et al., 1992; Rivas, 1966; Brown, 1956; Wiley, 1986; Brown, 1958; Wiley & Hall, 1975; Snelson et al., 2009; Rider & Schell, 2012). Macroinvertebrate taxonomic identification varied depending on the taxon (Example: nematodes were only identified to Order) while other taxa were identified to species level (Example: crayfish, mollusks, etc.). The majority of arthropods were keyed out to family using Thompson (2004), Smith (2001), www.fws.gov 2012, Hightower (2007), McCaferty (1981), Zuellig, et al. (2011), Cushing and Allan (2001), and Epler (2001). Fish and macroinvertebrate assemblages were defined as all the fish and macroinvertebrates collected at each subsite. The macroinvertebrate assemblages were used to calculate a stream health number for each site, identify the anthropogenic effect of bridges on macroinvertebrates, and test for any correlation between macroinvertebrate assemblage diversity and fish assemblage diversity. Fish assemblages were broken into guilds based on species use of environmental resources (Simberloff, 1991). Guild categories were: (1) Benthic - stays near or on the bottom, (2) open water - stays in the mid to upper water column, (3) near vegetation - stays near or just slightly in vegetation, (4) vegetation - lives in vegetation, and (5) open water - lives at the top of the water column.

Statistical Methods

Data sets were organized using Microsoft Excel (Microsoft Inc., 2010); and where needed for parametric analyses, fish and macroinvertebrate data were 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 in StatsDirect (StatsDirect Ltd., 2007) were used to verify significance in the data sets. Shapiro-Wilkes tests in Statistica (StatSoft Inc., 2012) were used to test for normalization of data set prior to regression analyses and modeling. Variables that were not normal were transformed using log normal (ln x), log to the 10^{th} (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 variable data. Following, normalization of data sets Primer v6 (Clarke & Gorley, 2006), StatsDirect (StatsDirect Ltd., 2007), Sigma Plot (Systat Software, 2012), and Statistica (StatSoft Inc., 2012) were used to conduct regression analyses and modeling.

Regression analyses are mathematical models that predict the importance of variables in data sets. It is important to remember that regression analyses are not definitive findings, but findings suggested by arithmetic algorithms (Snodgrass et al., 1996). Conversion of these findings to a more definitive state would require concrete experiments which are difficult to generate due to the scale and fluid nature of riparian systems. Applying multiple regression analyses models (Multi Linear, Forward Stepwise, and Backward Stepwise) can provide a higher level of validity for results. A variable or variables found to be prevalent across multiple regression models, while not definitively of value, are more likely of value than a variable or variables that were selected by one model.

Chapter III

RESULTS

Descriptions and Data Sets

Research sites were in the Tifton Upland and Okefenokee Plains regions of Georgia (Griffith et al., 2001). Streams in these regions are dominated by agricultural land use, which is predominately coniferous sylvan culture. Fourth and third order streams were in the Tifton Upland of Georgia region, while first and second order streams were located in the Okefenokee Plains region (Appendix A, Table 1).

Independent variable data sets initially included all variables listed in Appendix A, Table 2, and were organized into the categories: construction (Tables 3a,b,c), physical (Tables 4a,b), chemical (Tables 5a,b), and biological (Tables 6 and 7a,b). Graphs depicting means for substrate types by subsites (upstream, bridge and downstream) by stream order given in Appendix B and include gravel (Figure 1), sand (Figure 2), silt (Figure 3) and clay (Figure 4). In Figure 1, mean gravel volume displays depressed levels for all upstream subsites that may relate to the near absence of anthropogenically deposited allochthonous granitic material. Lower levels of gravel volumes at second order bridge subsites could result from elevated levels of clay sized siltation inundating those sites, while equally high values of third and fourth order streams could indicate an upper limit to the mobility of material from bridge subsites. Mean sand volume (Appendix B, Figure 2) levels are inversely affected by the perturbations of the other substrates. Mean silt volume (Appendix B, Figure 3) displays low to moderate levels at all subsites except at first order bridge sites, most likely due to decreased water volume and slower flow rates generating greater levels of silt sedimentation in the bridge runs. The lowest volumes of silt sediment were found at the bridge and upstream subsites of third order streams, most likely related to natural or anthropogenically generated morphology. Mean clay volume by stream order (Appendix B, Figure 4) indicated elevated clay volumes for most of the bridge and upstream subsites but drastically reduced volumes of clay for the downstream subsites, most likely due to the sequestering of clay in the bridge subsite run. An exception to this trend was seen at bridge and upstream subsites on third order streams. At these subsites clay volume was nearly nonexistent for the bridge subsites and barely measurable for the upstream subsites, most likely related to natural or anthropogenically generated morphology.

Quantitative IR results were not included in independent variable data sets due to the detection of an excessive level of carbon-hydrogen single bonds in all the samples. The net result was a presence of high levels of carbon-hydrogen single bonds for all subsites that was not unique and could not provide any data beyond validating the presence of cellulose based organic material in the black water systems of the study area.

Macroinvertebrates

Macroinvertebrates are listed in Appendix A, Table 8. Data for all macroinvertebrate subsite collections are in Appendix A, Tables 9 through 14. The Friedman's test run on macroinvertebrate assemblages for all subsites was significant (T^2 [F] = 2.3324, Critical t (1066 df) = 1.9622, and $P < 0.0001$), and significant subsite pair-wise multiple comparison results are in Appendix A, Tables 15a,b. Significant results were found in 10.4% of the 1722 possible pairwise combinations. Significant subsite pair-wise multiple comparison results were compiled by stream order and subsite and were then converted to percentage (Table 3.1, and Figure 3.1). Figure 3.1 demonstrated two trends with increasing stream order numbers. One trend was an increased difference between lower order streams and higher order streams. A second weaker trend was a difference between two of the same order streams as stream order increased. Percent values of Figure 3.1 were below 40% and only one exceeded 25%. Graphs of mean stream health number of each subsite by stream order (Appendix B, Figure 5) and mean number of species for macroinvertebrate assemblages of each subsite by stream order (Figure 3.2) were generated. The scales between Appendix B, Figure 5 and Figure 3.2 were not the same, but trends for both graphs had some similarities. The lowest stream health numbers and macroinvertebrate diversity in assemblages were found at upstream subsites. A dichotomy was displayed in both graphs between the bridge and downstream subsites of second order streams and their upstream counterparts. Exception to the trend was displayed in both graphs where values of bridge subsites for third order streams fell below the level of upstream subsites. The drop in the third order streams were most likely related to substrate differences resulting from variances in river morphology, bridge sites with riffles in the place of a run.

Macroinvertebrate assemblage data for each subsite was run in Primer 6 (Clarke & Gorley, 2006) generating Principle Components Analyses (PCA) identifying the organisms that showed the highest levels of variation across sites (Appendix B, Figures 6 through 8). PCA of upstream subsite macroinvertebrate assemblages (Appendix B, Figure 6) identified *Viviparus georgianus* (a right turning, gilled snail), and Chironomidae (midges) as the organisms with the most variation. These results could support the normally lotic nature of the subsites supporting snails' need for flow and oxygen, while drought conditions during sampling, lentic like, and nearly anaerobic detritus accumulations in eddies and along the banks support Chironomidae populations. PCA of bridge subsite macroinvertebrate assemblages (Appendix B, Figure 7) identified nematodes and Simulidae as the organisms with the most variation across subsites. These results could be a product of the mostly lentic nature of the bridge subsite run and side pools. PCA of downstream subsite macroinvertebrate assemblage (Appendix B. Figure 8) identified Dytiscidae and Simulidae as the organisms with the greatest variation across all subsites. These could result from the lotic nature of the system supporting Dytiscidae and similarities between bridge subsites and downstream subsites supporting Simulidae.

Fishes

Fishes are listed in Appendix A, Table 16. Data for all fish subsite collections are in Appendix A, Tables 17a,b through 22a,b. A Friedman's test on fish species assemblages for all subsites was significant (T^2 [F] = 5.5242, (1763 df), Critical t = 1.9613, and $P < 0.0001$), and significant subsite pair-wise multiple comparison results are in Appendix A, Tables 23a,b,c. Significant results were found in 20.3% of the 1722 possible comparisons. Percent of results by stream order are listed in Table 3.2 and graphed in Figure 3.3. Percent values remained less than or equal to 50% in Figure 3.3 with increasing differences between lower order streams and higher order streams, and a first order stream value at 15% was lower than other values. These could be supported by higher similarity between lower order streams.

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, and $P = 0.0038$), 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, $P = 0.3609$). A graph of mean number of species in fish assemblages by stream order (Appendix B, Figure 3.4) was generated, and appeared to display the highest values at bridge subsites, next highest at downstream subsites, and lowest at upstream subsites.

Fish species numbers organized into guilds based on habitat use (Appendix A, Table 24) 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 and $P < 0.0001$), and the Scheffé multiple comparisons test supported the use of guilds identifying differences between guilds (Critical Value = 4.93954, $P < 0.0001$) (Appendix A, Table 25). Figure 3.5 shows that the greatest species diversity in habitat guilds occurred at bridge subsites, then at downstream subsites, and lowest at upstream subsites. This pattern may be related to the greater diversity of habitats found in bridge subsite runs and side pools. The debris guild at each subsite was substantially less diverse than other guilds, which were more similar in mean numbers and pattern. Results were possibly due to anthropogenic clearing of obstructive debris from riparian systems. Fish assemblage data for each subsite was run in Primer 6 (Clarke & Gorley 2006) generating PCAs identifying species that show the highest levels of variation across subsites (Appendix B, Figures 9 through 11). PCA of upstream subsite fish assemblages (Appendix B, Figure 9) identified *Labidesthes sicculus* and *Gambusia holbrooki* as species with the most variability. These results could have been generated by the drought with disruption of lotic upstream subsites generating variations in *L. sicculus* populations and supporting a broad distribution of the highly adaptive *G. holbrooki*. PCA of bridge subsites species assemblages (Appendix B, Figure 10) identified *Micropterus salmoides* and *Centrarchus macropterus* as species with the most variability. These results could be a product of the lentic nature of the bridge subsites' runs and side pools. PCA of downstream subsite species assemblages (Appendix B, Figure 11) identified *L. sicculus* and *G. holbrooki* as species with the most variability. These results, like those for upstream subsites, could have been generated by the drought with disruption of lotic upstream subsites generating variations in *L. sicculus* populations and supporting a broad distribution of the highly adaptive *G. holbrooki*. These similar results might support drought generated similarities between upstream and downstream subsites.

Preparation of Data for Regression Analyses

All data sets were tested for normality using a Shapiro-Wilk test (StatSoft Inc., 2012) and sets that failed the normality test were transformed using log natural (ln x), log to the 10th (log10 x), squared (x^2), and square-root ($\sqrt{x}$) values; and were then retested for normality. Normalizing and standardizing of data sets prior to statistical analyses beyond Friedman's is strongly recommended (Snodgrass et al., 1996). The transformations that generated normality and had the greater P value (≥ 0.05) were used to replace the original data sets. Appendix A, Tables 26a through 26e, contain normalized and transformed data sets. Fish and macroinvertebrate species numbers for bridge subsites were standardized by hectometers prior to being included in Appendix A, Table 26a.

Macroinvertebrate and fish data sets from Appendix A, Table 26a were run in Primer 6 (Clarke & Gorley, 2006) generating a Curtis-Bray similarity analysis used to develop a cluster diagram that allowed a comparison of species similarity between the bridge sites in Appendix B, Figure 12 for macroinvertebrates and Figure 13 for fishes. Macroinvertebrate cluster analysis (Appendix B, Figure 12) did not produce a discernible pattern in that first through fourth order streams often formed branches or sister clades that did not involve the same or closely related stream orders. An exception to this is the upper most clade involving 4CB and 4AB, which both possess the same stream order, but the node separating them is weak with a similarity value of approximately 45%.

Fish cluster analysis (Appendix B, Figure 13) also did not provide a strong discernible pattern of stream order relationships, but was stronger than the macroinvertebrate data. In the fish similarity data, the upper most branch of the cluster diagram includes only second and third order streams. The central or middle branch includes one fourth order stream and all the remaining streams are first through third order. The lower most branches consist of all fourth order streams with the exception of one second order stream. Except for the fourth order stream in the upper most branches and the second order stream in the lower most branches, sister clades in general involve streams of the same order or the next order up or down. Similarity values are generally weak to moderately strong for the nodes.

Data sets from Appendix A, Tables 26a through 26e were run through Principal Component Analysis (PCA) in Primer 6 and Discriminant Function Analysis (DFA) in Statistica to identify potentially significant independent variables for multiple and stepwise regression analyses involving macroinvertebrate and fish assemblages as dependent variables. Two DFAs were run, one with macroinvertebrate species assemblages as the dependent variable and one with fish species assemblages as the dependent variable (Appendix A, Table 27).

PCA and DFA results for macroinvertebrates were run against macroinvertebrate species assemblage diversity data in forward and backward stepwise regressions, and multi-linear regression analyses. The resulting regression analyses of variance and selected variables are in Table 3.3 multi-linear, Table 3.4 forward stepwise and Table 3.5 backward stepwise. Compiling these results and considering the related *P* values, it could be accurate, due to reoccurring selection and significant *P* values, to consider bridge run perimeter as an influencing variable for macroinvertebrate species assemblage diversity at bridge subsites for first through fourth order streams.

The PCA for bridge subsites and DFA for fishes were run against the fish species assemblage diversity data in forward stepwise regression, backward stepwise regression, and multi-linear regression analyses are in Tables 3.3 multi-linear, Table 3.4 forward stepwise, and Table 3.5 backward stepwise. Compiling these results and considering the related *P* values, it could be accurate, due to reoccurring selection and significant *P* values, to consider pH in summer and Total Surface Area as variables influencing fish species diversity at bridge subsites for first through fourth order streams.

Table 3.1. Percentage of significant multiple subsite pair-wise comparisons. Results for macroinvertebrate assemblages converted into percentage. Stream order is identified by the numerical values of 1 through 4. Subsites are identified by U = upstream, B = bridge, and D = downstream. Tabular results were converted in to a graph using stream order comparisons in Figure 3.1.

	1U	**1B**	**1D**	**2U**	**2B**	**2D**	**3U**	**3B**	**3D**	**4U**	**4B**	**4D**
1U	17	22	0	22	11	22	11	22	22	20	27	60
1B		17	0	33	0	11	22	33	22	13	13	20
1D				0	0	0	0	11	11	0	20	20
2U					22	22	0	0	33	33	47	87
2B							0	22	11	0	7	13
2D							22	22	11	0	7	13
3U									33	27	33	73
3B									33	13	33	67
3D									33	20	27	27
4U										10	20	40
4B											10	16
4D												0

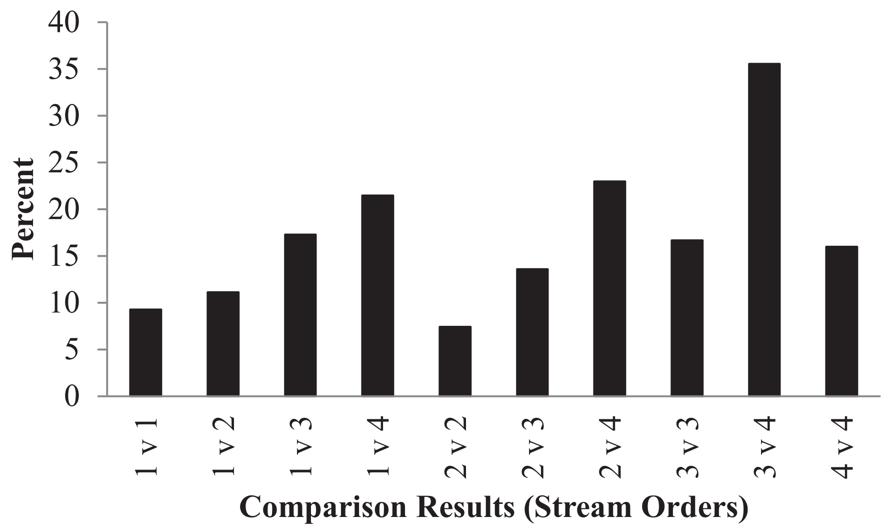

Figure 3.1. Comparison results using Percent. Significant subsite pair-wise multiple comparison results for macroinvertebrate assemblages were converted into percent.

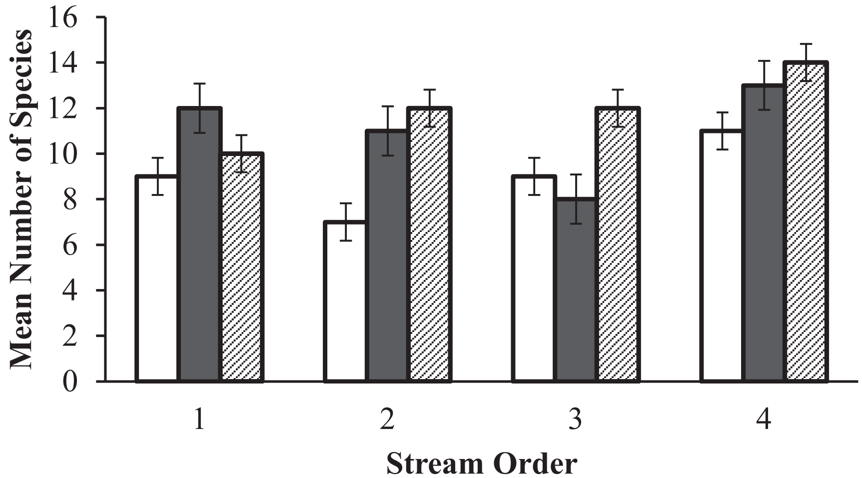

Figure 3.2. Mean number of macroinvertebrate species in assemblages for each subsite by stream order. Open bars are upstream subsite, solid bars are bridge subsite, and slash bars are downstream subsite.

Table 3.2. Significant subsite pair-wise multiple comparison results for fish assemblages converted into percent. Stream order is identified by the numerical values of 1 through 4. Subsites are identified by U = upstream, B = bridge, and D = downstream. Tabular results were converted in to a graph using stream order comparisons in Figure 3.3.

	1U	**1B**	**1D**	**2U**	**2B**	**2D**	**3U**	**3B**	**3D**	**4U**	**4B**	**4D**
1U	0	56	0	22	67	33	33	33	44	33	80	73
1B		0	33	44	67	0	22	33	33	20	40	13
1D			0	11	67	33	22	33	33	33	73	53
2U				17	67	22	33	33	44	27	67	60
2B					33	56	67	56	56	60	47	60
2D						17	22	56	33	47	47	13
3U							17	33	33	33	53	40
3B								33	44	60	53	40
3D									33	40	47	33
4U										25	56	36
4B											20	28
4D												10

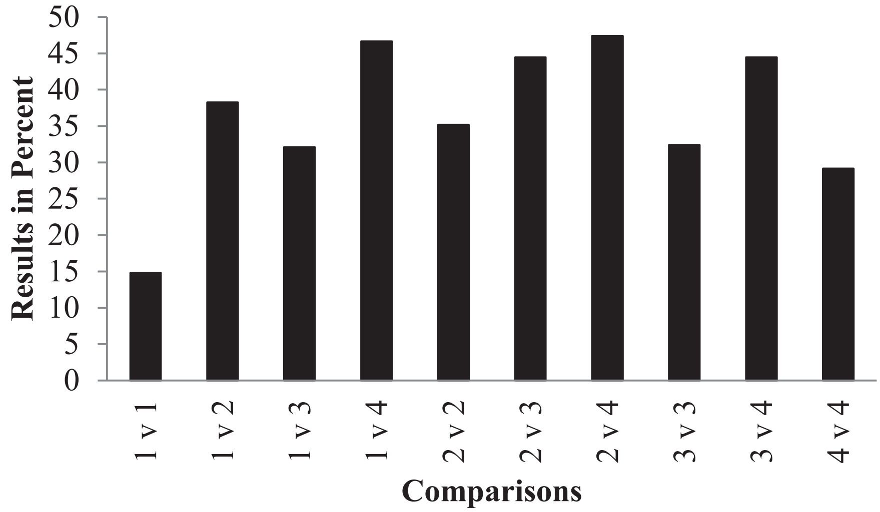

Figure 3.3. Results by compared stream order. Significant subsite pair-wise multiple comparison results for fish assemblages converted into percent.

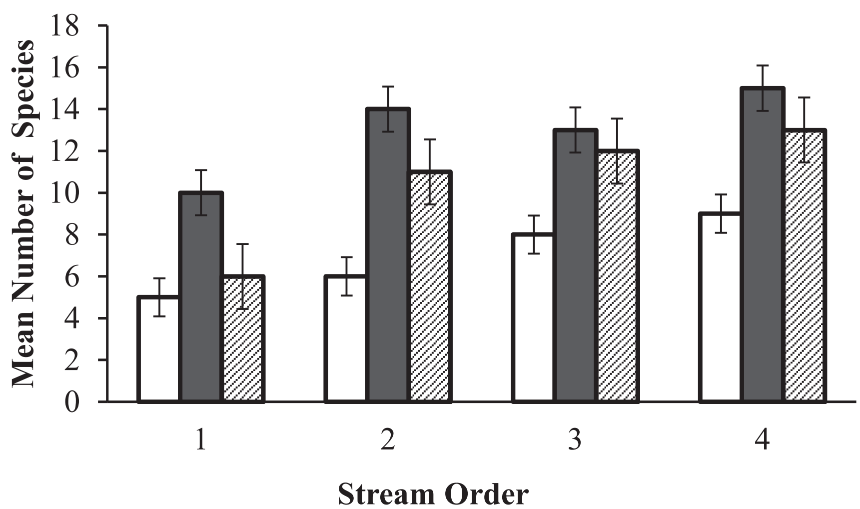

Figure 3.4. Mean number of fish species in assemblages for each subsite by stream order. Open bars are upstream subsite, solid bars are bridge subsite, and slash bars are downstream subsite.

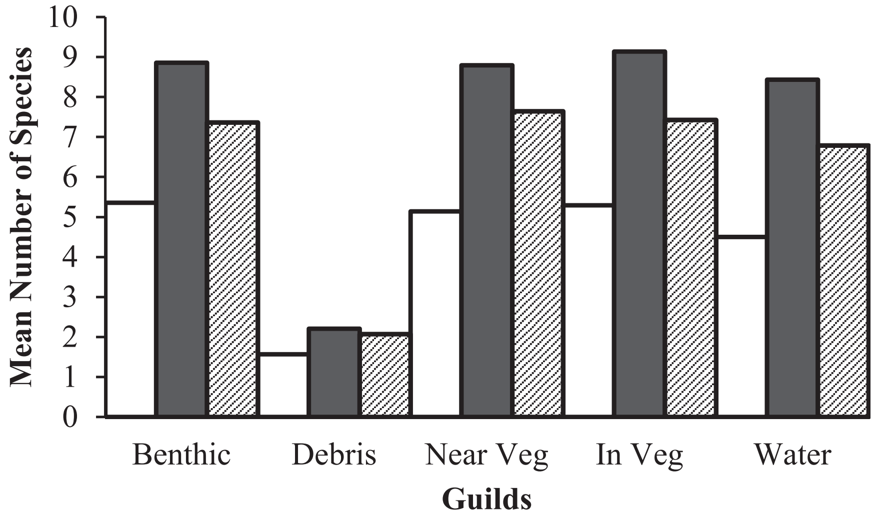

Figure 3.5. Mean number of fish species in guild assemblages by subsites. Open bars are upstream subsite, solid bars are bridge subsite, and slash bars are downstream subsite. Veg represents vegetation.

Table 3.3. Multiple Linear Regression results. $Y_{Macroinvertebrates}$ and Y_{Fish} identify the dependent variables in each the analysis. The analysis of variance precedes the selected variables and their individual "P" values, and "None" signifies that no variables were selected by the regression. PCA and DFA are the methods used to select the data prior to performing the regression analyses.

Method	**Analysis of Variance and Selected Variables**							
PCA	$Y_{Macroinverts}$:							
	Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
	Regression	12	2.333	0.194	0.354	0.881	0.809	0.000
	Residual	1	0.549	0.549				
	None							
DFA	$Y_{Macroinverts}$:							
	Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
	Regression	11	2.257	0.205	0.657	0.739	0.783	0.000
	Residual	2	0.625	0.312				
	None							
PCA	Y_{Fish}:							
	Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
	Regression	12	322.919	26.910	9.629	0.247	0.991	0.888
	Residual	1	2.795	2.795				
	None							
DFA	Y_{Fish}:							
	Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
	Regression	10	303.175	30.317	4.035	0.139	0.931	0.700
	Residual	3	22.539	7.513				
	pH Summer(P=0.024), Total Surface Area(P=0.035)							

Table 3.4. Forward Stepwise Regression results. $Y_{Macroinvertebrates}$ and Y_{Fish} identifythe dependent variables in each the analysis. The analysis of variance precedes the selected variables and their individual "P" values, and "None" signifies that no variables were selected by the regression. PCA and DFA are the methods used to select the data prior to performing the regression analyses.

Method — **Analysis of Variance and Selected Variables**

PCA — $Y_{Macroinverts}$:

Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
Regression	1	1.390	1.390	11.180	0.006	0.482	0.439
Residual	12	1.492	0.124				

Bridge Run Perimeter(P=0.006)

DFA — $Y_{Macroinverts}$:

Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
Regression	1	1.390	1.390	11.180	0.006	0.482	0.439
Residual	12	1.492	0.124				

Bridge Run Perimeter(P=0.006)

PCA — Y_{Fish}:

Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
Regression	2	186.391	93.196	7.358	0.009	0.572	0.494
Residual	11	139.323	12.666				

pHSummer(P=0.041), Total Surface Area(P=0.004)

DFA — Y_{Fish}:

Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
Regression	3	228.674	76.225	7.855	0.006	0.702	0.613
Residual	10	97.040	9.704				

pH Summer(P=0.009), Bridge Width(0.063), and
Total Surface Area(P=0.010)

Table 3.5. Backward Stepwise Regression results. $Y_{Macroinvertebrates}$ and Y_{Fish} identify the dependent variables in each the analysis. The analysis of variance precedes the selected variables and their individual "P" values, and "None" signifies that no variables were selected by the regression. PCA and DFA are the methods used to select the data prior to performing the regression analyses.

Method — **Analysis of Variance and Selected Variables**

PCA — $Y_{Macroinverts}$:

Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
Regression	1	1.390	1.390	11.180	0.006	0.482	0.439
Residual	12	1.492	0.124				

Bridge Run Perimeter(0.006)

DFA — $Y_{Macroinverts}$:

Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
Regression	5	2.068	0.414	4.065	0.039	0.718	0.541
Residual	8	0.814	0.102				

Conductivity Winter(0.011), Temperature Winter(0.003), Current(0.053), Sand(0.021), and Silt(0.069)

PCA — Y_{Fish}:

Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
Regression	6	308.161	51.360	20.482	<0.001	0.946	0.900
Residual	7	17.553	2.508				

pH Summer(P=0.002), Biomass(0.004), Sand(<0.001), Elevation(0.009), Bridge Run Perimeter(P=0.016), and Total Surface Area(<0.001)

DFA — Y_{Fish}:

Group	**DF**	**SS**	**MS**	**F**	**P**	**R_{sqr}**	**Adj R_{sqr}**
Regression	7	300.695	42.956	10.301	0.006	0.923	0.834
Residual	6	25.020	4.170				

pH Summer(0.001), Clay(0.062), Depth(0.041), Since Built(0.011), Elevation(0.044), Bridge Run Perimeter(0.006), and Total Surface Area(<0.001)

Chapter IV

DISCUSSION

Drought in 2011 affected many riparian systems in the southeastern United States. The Suwannee River basin was not immune to these affects with the United States Geologic Survey annual water data reports from the stations on the upper Alapaha River and Withlacoochee River recorded low flow means between 0.02 and 0.00 cfs. These means persisted July through December of 2011. The drought provided an opportunity to assess the impact of bridges on low order streams during drought events as possible sites of refugia.

Most research supports the concept that bridge construction generates negative perturbations that disturb normal stream conditions and benthic community structure (Cline et al. 1982; Larsen, 1993). These perturbations are generated by pillars, dredging, and embankments that are involved in bridge construction producing deleterious impacts on aquatic habitats such as channelization and deep run formation (Cline et al., 1982; Resh et al., 1988; Larsen, 1993). Channelization and deep run formation constitute an ecological disturbance for existing fish and macroinvertebrate assemblages, which can result in extended periods of altered sediment grain size and current velocity at sites of anthropogenic disturbance (Resh et al., 1988; Death, 1996; Blettler & Marchese, 2005). Benthic invertebrates that indicate stream health are affected by changes in sediment grain size and current velocity to an extent that they are also beneficial as indicators of anthropogenic disturbance (Death, 1996). Research supports sites upstream from the bridges with silt-clayed sediments demonstrate higher macroinvertebrate and fish species richness and higher levels of benthic biomass than bridge and downstream sites (Blettler & Marchese, 2005). Recent research has demonstrated that r-strategist species assemblages related to sandy unstable sediments can colonize the habitats successfully in less than one year after disturbances (Blettler & Marchese, 2005; Death, 1996). It must be kept in mind that the potential for positive effects of bridges on riparian ecosystems does not occur initially following construction but should be considered following a period of naturalization, and a "naturalized" bridge site may have a return of the riffle and run habitat, as well as sensitive species (Death, 1996; Lau et al., 2006). Negative effects of bridge construction on riparian ecosystems have been well documented on fifth and higher order streams for macroinvertebrates, game fish, and vegetation (Blettler & Marchese, 2005).

A few studies have considered macroinvertebrate assemblages on fourth and lower order streams following a period of naturalization, but no studies have considered the effects on fish assemblages at fourth and lower order stream bridge sites (Joy & Death, 2000; Blettler & Marchese, 2005). Since existing fauna could have resulted from geology, amount of human habitation, distance from species source populations, or many other factors, I considered a broader range of past research studies than those just occurring at bridge sites (Joy & Death, 2000). There are many studies that have identified the macroinvertebrate and fish species composition of stream orders in several regions of the United States, but very few have been done in the area of the Suwannee River basin of southern Georgia. While many research studies have been performed at bridge sites to study stream health using macroinvertebrates, some have been performed to assess the effects of urbanization on riparian systems, and a few have assessed the presence or absence of game fish along riparian systems. More research assessing the macroinvertebrate and fish species assemblages together at bridge sites needed to be performed. My work supports the positive effect bridges have on fish species assemblage's diversity, and provides support for the bridge sites as having some positive effects downstream from the bridge subsite. Macroinvertebrates share a similar pattern with the fish that is altered towards the greatest level of positive effects being generated downstream from the bridge subsites. Both the results on the macroinvertebrates and the fish assemblages support the concept that first through fourth order steams can serve as refuges for both taxa if properly engineered.

Differences and similarities in macroinvertebrate species assemblages between the upstream, bridge, and downstream were supported by analyses of the data set. Each subsite had species that were most often found between the same subsites at different sites, but also upstream and downstream subsites shared more species in common, than upstream and bridge subsites. Worthy of note were the greater number of similarities than differences in macroinvertebrate species assemblages between bridge and downstream subsites. Additionally, macroinvertebrate assemblage data and stream health numbers both supported bridge and downstream subsites as each individually providing greater species diversity than upstream subsites. Considering that upstream subsites could serve as the control in that they were less impacted by bridge site construction; it is of interest that they had low levels of macroinvertebrate species diversity while higher levels of macroinvertebrate species diversity were extant at bridge and downstream subsites which were more heavily impacted by bridge site construction. The potential for beneficial affects originating at bridge subsites, and being conferred to downstream subsites was supported. Overall, at bridge and downstream subsites macroinvertebrate species diversity was the greatest at downstream subsites. However, an exception to this overall pattern was bridge subsites at third order streams that broke from the trend of all other bridge subsites. When considering the geomorphological and substrate volume differences that existed at these two sites, it is possible that these factors could have generated the variances that were seen in macroinvertebrate species assemblages at these two third order bridge subsites. 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.

Fish species were collected during base flow or lower to provide the maximum possible accuracy for assessing the diversity in fish species assemblages (Lau et al., 2006; Chadwick et al., 2006). Differences and similarities in fish species assemblages between upstream, bridge, and downstream subsites were supported by analyses of the data set, but also upstream and downstream subsites shared more species in common, than upstream and bridge subsites, or downstream and bridge subsites. Worthy of note were the greater species diversity levels of both bridge and downstream subsites, when each were compared to upstream subsites. There were differences in the species assemblages of the bridge subsites and downstream subsites, but the bridge subsites had higher levels of species diversity for all stream orders. Considering upstream subsites could serve as the control that was minimally affected by bridge site construction. It is critical to note that these subsites had low levels of fish species diversity, while higher levels of fish species diversity existed at bridge and downstream subsites that were affected by bridge site construction. If increased fish species diversity is seen as beneficial, then beneficial affects originating at bridge subsites, and being conferred to downstream subsites was supported. 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 fishes. It also suggests that around fourth to fifth order streams, the impact of bridge site construction shifts from positive to negative. It is possible that the factor (bridge run surface area) that was important in increased fish species diversity at bridge sites might decrease as a factor as the flow and width of riparian systems increase, leading to a mean threshold point for most systems occurring above fourth order streams.

Fish species were organized into guilds by habitat use to provide an ecological measure for the affects generated by bridge construction. The guild data matched the species assemblage data in all cases despite the guild data foci being habitat use as opposed to species diversity. Thus, fish habitat use data matched fish species assemblage diversity data in all the aforementioned trends. These results support a conceptualization of the bridge sites as not just generating species diversity, but also generating habitat diversity. Thus, converting small portions (bridge subsites) of a riparian system from a moderately productive low order stream state to a maximally productive medium order stream state, with elevated levels of habitat use diversity.

Macroinvertebrate species assemblage data used as the dependent variable in regression analyses resulted in bridge run perimeter being selected as the variable that had the most influence on the bridge site species assemblage diversity data with $r^2 = 0.482$ and $P = 0.006$. The variable bridge run perimeter emphasizes the importance of the littoral zone for the diversity of macroinvertebrate species assemblages. At bridge subsites, vegetation (macrophytes, algae, and submerged terrestrial) was most often located along the littoral zone of the bridge subsites, generating higher levels of habitat diversity along the bridge run perimeter. Bridge run perimeter can proxy for littoral habitat diversity at the bridge subsite supporting greater macroinvertebrate species diversity if the bridge run perimeter is maximized during bridge site construction and throughout subsequent bridge site renovation events.

Fish species assemblage data used as the dependent variable in regression analyses resulted in pH summer and total surface area being selected as the variables that had the most influence on the fish assemblage diversity data. Lower pH could indicate elevated levels of DOC generated by concentrated levels of fulvic and humic acids in quiescent 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. Due to the similarities in the measurements of total surface area and bridge run perimeter, each can function as proxies for the other, in that they both are related to habitat diversity and through that, to vegetation, pH, and oxygen. The pH during summer and total surface area of the water at the bridge subsite 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.

The purpose of this thesis is to appraise the impact of naturalized bridge sites, along fourth and lower order streams in the Suwannee River basin of south Georgia, as it relates to macroinvertebrate and fish assemblages. Bridges create environments that often differ from undisturbed stream environments with respect to many physiochemical and biological properties. Variations in physiochemical and biological factors were assessed for their effects on the assemblage structures so as to assess 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 if naturalized 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 the fish species found at bridge sites along first through fourth order streams. For both macroinvertebrates and fish, it is also the first such work done in south Georgia as an area predominated by flatwoods habitat. 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 in light of 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).

Looking at the river continuum concept we find that first through third order streams belong to the headwater stream set, while fourth through sixth order streams belong to the medium stream 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 all alter the bridge subsite and bring it closer to the physical and species state of the fourth through sixth order medium 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 would not be remiss to consider that bridges provide a constructive effect of elevating the river continuum measure of the first through third order streams.

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 subsites used as controls. Also, the distance of the effect could help in the maximizing of 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 could broaden the applicability of the research.

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Appendix A:

Tables 1 - 27

Table 1. Bridge sites sampled including: site labels, descriptions, locations, and date sampled 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

Table 2. Variable data sets prior to normalization organized into construction, physical, chemical and biological.

Rows	Construction	Physical
1	Depth (D)	Stream Order (SO)
2	Bridge Run Length (BRL)	Current (Cu)
3	Bridge Run Width (BRW)	Gravel (G)
4	Total Length (TL)	Sand (Sa)
5	Total Length hectometers (TLH)	Silt (Si)
6	Since Built (SB)	Clay (Cla)
7	Year Built (YB)	Elevation (E)
8	Side Pools (SP)	
9	Bridge Length (BL)	**Chemical**
10	Bridge Width (BW)	Oxygen Summer (O_2S)
11	Side Pool Length 1 (SPL1)	pH S (pHS)
12	Side Pool Width 1 (SPW1)	Conductivity Summer (CS)
13	Side Pool Length 2 (SPL2)	Temperature Summer (TS)
14	Side Pool Width 2 (SPW2)	Oxygen Winter (O_2W)
15	Side Pool Length 3 (SPL3)	pH W (pHW)
16	Side Pool Width 3 (SPW3)	Conductivity Winter (CW)
17	Bridge Run Perimeter (BRP)	Temperature Winter (TW)
18	Bridge Run Surface Area (BRSA)	
19	Side Pool Perimeter 1 (SPP1)	**Biological**
20	Side Pool Surface Area 1 (SPSA1)	Bridge Vegetation Width (BVW)
21	Side Pool Perimeter 2 (SPP2)	Side Pool 1 Vegetation Width (P1VW)
22	Side Pool Surface Area 2 (SPSA2)	Side Pool 2 Vegetation Width (P2VW)
23	Side Pool Perimeter 3 (SPP3)	Side Pool 3 Vegetation Width (P3VW)
24	Side Pool Surface Area 3 (SPSA3)	Total Vegetation Width (TVW)
25	Total Surface Area (TSA)	Algae (A)
26	Total Perimeter (TP)	Macroinvertebrate Sp. (ISp)
27		Macroinvertebrate Sp. hectometers (ISH)
28		Stream Health (SH)
29		Organic Matter (OM)
30		Biomass (BM)

Table 3a. Construction generated independent variables. Column labels D – BW correspond with column 1, rows 1-10 in Table 2.

Subsite	D	BRL	BRW	TL	TLH	SB	YB	SP	B L	B W
1AB	0.83	40.80	10.85	62.69	0.63	21	1991	2	80.00	12.64
1BB	0.95	55.81	10.48	55.81	0.56	25	1987	0	70.00	25.00
1CB	1.01	95.40	15.28	142.45	1.42	31	1981	2	362.00	13.80
2AB	0.96	98.80	11.04	98.80	0.99	64	1948	0	126.00	30.15
2BB	1.47	54.86	12.95	101.36	1.01	26	1986	1	80.00	14.63
2CB	1.16	31.60	18.24	31.60	0.32	7	2005	0	60.00	12.76
3AB	0.41	34.50	10.30	34.50	0.35	43	1969	0	150.00	12.35
3BB	0.52	65.50	10.64	65.50	0.66	6	2006	0	124.00	12.15
3CB	0.17	69.30	8.03	99.00	0.99	5	2007	2	415.00	12.40
4AB	0.71	78.64	15.00	100.54	1.01	7	2005	3	102.00	14.40
4BB	1.25	53.04	13.66	106.80	1.07	42	1970	2	72.00	10.57
4CB	0.38	76.00	9.78	169.60	1.70	6	2006	1	262.00	12.54
4DB	1.08	85.29	18.51	85.29	0.86	6	2006	0	214.00	14.43
4EB	0.42	57.70	10.55	57.70	0.58	3	2009	0	216.00	13.00

Table 3b. Construction generated independent variables. Column labels SPL1 – BRSA correspond with column 1, rows 11-18 in Table 2.

Subsite	SPL1	SPW1	SPL2	SPW2	SPL3	SPW3	BRP	BRSA
1AB	17.65	5.10	4.24	8.00	0.00	0.00	133.00	614.00
1BB	0.00	0.00	0.00	0.00	0.00	0.00	183.00	760.00
1CB	19.05	3.13	28.00	7.80	0.00	0.00	308.00	1777.00
2AB	0.00	0.00	0.00	0.00	0.00	0.00	179.00	785.00
2BB	46.50	8.90	0.00	0.00	0.00	0.00	266.00	1276.00
2CB	0.00	0.00	0.00	0.00	0.00	0.00	137.00	1082.00
3AB	0.00	0.00	0.00	0.00	0.00	0.00	130.00	790.00
3BB	0.00	0.00	0.00	0.00	0.00	0.00	212.00	1561.00
3CB	8.25	24.30	21.45	6.75	0.00	0.00	251.00	1185.00
4AB	6.30	8.10	7.80	15.80	7.80	7.60	236.00	2310.00
4BB	22.66	13.72	31.10	12.19	0.00	0.00	197.00	1807.00
4CB	93.60	14.74	0.00	0.00	0.00	0.00	268.00	1214.00
4DB	0.00	0.00	0.00	0.00	0.00	0.00	236.00	1811.00
4EB	0.00	0.00	0.00	0.00	0.00	0.00	220.00	1640.00

Table 3c. Construction generated independent variables. Column labels SPP1 – TP correspond with column 1, rows 19-26 in Table 2.

Subsite	**SPP1**	**SPSA1**	**SPP2**	**SPSA2**	**SPP3**	**SPSA3**	**TSA**	**TP**
1AB	43.00	102.00	25.00	45.00	0.00	0.00	761.00	201.00
1BB	0.00	0.00	0.00	0.00	0.00	0.00	760.00	183.00
1CB	191.00	729.00	91.00	215.00	0.00	0.00	2721.00	590.00
2AB	0.00	0.00	0.00	0.00	0.00	0.00	785.00	179.00
2BB	82.00	319.00	0.00	0.00	0.00	0.00	1595.00	348.00
2CB	0.00	0.00	0.00	0.00	0.00	0.00	1082.00	137.00
3AB	0.00	0.00	0.00	0.00	0.00	0.00	790.00	130.00
3BB	0.00	0.00	0.00	0.00	0.00	0.00	1561.00	212.00
3CB	70.00	275.00	62.00	159.00	0.00	0.00	1619.00	383.00
4AB	74.00	231.00	44.00	116.00	42.00	117.00	2774.00	396.00
4BB	177.00	1281.00	65.00	231.00	0.00	0.00	3319.00	439.00
4CB	270.00	2508.00	0.00	0.00	0.00	0.00	3722.00	538.00
4DB	0.00	0.00	0.00	0.00	0.00	0.00	1811.00	236.00
4EB	0.00	0.00	0.00	0.00	0.00	0.00	1640.00	220.00

Table 4a. Physical independent variables are non-construction generated natural environmental variables. Column labels SO – E correspond with column 2, rows 1-7 in Table 2.

Subsite	SO	Cu	G	Sa	Si	Cla	E
1AU	1	0.222	0	47.5	2.5	0	58
1BU	1	0.133	0	5	5	40	48
1CU	1	0.2	0	50	0	0	32
2AU	2	0	0	15	5	30	51
2BU	2	0	0.5	48.5	1	0	39
2CU	2	0	0	37.5	2.5	10	42
3AU	3	0.25	0	50	0	0	32
3BU	3	0.1	0	48.5	1	0.5	24
3CU	3	0.333	2.5	47.5	0	0	43
4AU	4	0.071	0	49.5	0.5	0	79
4BU	4	0	0	15	1	34	73
4CU	4	0	0	15	10	25	50
4DU	4	0.026	0	48.5	1	0.5	43
4EU	4	0.091	1	46	2.5	0.5	39
1AB	1	0.043	0.5	43.5	10	5	58
1BB	1	0.048	5	10	25	32.5	48
1CB	1	0.2	1	46	2.5	0	32
2AB	2	0.021	0	35	5	10	51
2BB	2	0	0.5	42	5	2.5	39
2CB	2	0	0	34	1	15	42
3AB	3	0.125	1	48	1	0	32

Table 4b. Physical independent variables are non-construction generated natural environmental variables. Column labels SO – E correspond with column 2, rows 1-7 in Table 2.

Subsite	**SO**	**Cu**	**G**	**Sa**	**Si**	**Cla**	**E**
3BB	3	0.167	5	44	1	0	24
3CB	3	0.25	2.5	47.5	0	0	43
4AB	4	0.05	0.5	44.5	2.5	2.5	79
4BB	4	0	0	10	10	30	73
4CB	4	0	10	20	10	10	50
4DB	4	0	1	45.5	2.5	1	43
4EB	4	0.071	0.5	47.5	1	1	39
1AD	1	0.125	0.5	46	1	2.5	58
1BD	1	0.25	2.5	32.5	5	1	48
1CD	1	0.2	0	49	1	0	32
2AD	2	0	2.5	37.5	10	0	51
2BD	2	0.167	2.5	46	1	0	39
2CD	2	0.033	0	39	1	10	42
3AD	3	0.2	10	30	5	5	32
3BD	3	0.167	0	48.5	1	0.5	24
3CD	3	0.111	2.5	47.5	0	0	43
4AD	4	0.071	0.5	46	2.5	1	79
4BD	4	0	15	29	1	5	73
4CD	4	0	0	46.5	1	2.5	50
4DD	4	0.1	5	44.5	0.5	0	43
4ED	4	0.043	0	30	10	10	39

Table 5a. Chemical independent variables for all subsites U, B, and D including both summer (S) and winter (W) measurements. Column labels O_2S – TW correspond with column 2, rows 10-17 in Table 2.

Subsites	**O_2S**	**pHS**	**CS**	**TS**	**O_2W**	**pHW**	**CW**	**TW**
1AU	4	3.46	112.5	22.5	3.25	3.67	128.4	14.5
1BU	7	5.7	181.4	23.7	3.6	5.93	128.6	18.1
1CU	4.43	3.41	201	26.9	5	5	108.2	10.8
2AU	1	4.3	121.2	22.3	3.82	3.79	105.8	14.7
2BU	2.4	5.82	69.3	24.7	6.25	5.02	98	11.8
2CU	2.1	7.58	1168	25.2	6.4	7.91	723	13.4
3AU	3	7.19	335	25.3	4.03	6.96	295	14.7
3BU	2.5	7.4	389	24.9	5.24	7.04	273	14.6
3CU	6.8	6.82	145	32	15.45	7.78	293	12.32
4AU	6	7.29	660	25.6	12.2	7.88	905	6.6
4BU	1.53	7.05	602	23.9	12.91	7.9	871	7.3
4CU	1.2	6.8	170	21.8	11.87	7.82	406	8.7
4DU	3.24	6.71	337	26.1	12.75	7.74	375	8.9
4EU	4.16	6.54	216	26.4	3.32	6.38	173.2	17.4
1AB	4	3.45	107.4	23.3	3.35	3.72	131.1	14.1
1BB	6.8	5.4	176.6	23.6	3.1	5.87	116.5	18.3
1CB	4.5	3.42	198	27	8.6	7.6	192.3	9.8
2AB	1	4.46	106.2	23.2	3.07	3.85	109.3	14.7
2BB	1.91	5.8	69	24.7	6.98	4.94	97.8	11.6
2CB	2	7.55	1096	25.1	5.4	8.9	707	13.2
3AB	2	7.2	344	25.3	4.08	6.83	293	14.6

Table 5b. Chemical independent variables for all subsites U, B, and D including both summer (S) and winter (W) measurements. Column labels O_2S – TW correspond with column 2, rows 10-17 in Table 2.

Subsites	**O_2S**	**pHS**	**CS**	**TS**	**O_2W**	**pHW**	**CW**	**TW**
3BB	2.5	7.37	389	25	4.74	7.05	276	14.6
3CB	7	6.91	131	32	15.48	7.76	292	12.6
4AB	6	7.3	615	25.8	11.29	7.54	905	6.4
4BB	4.01	7.38	423	28.5	12.01	7.87	872	7
4CB	2	7.25	152	25	12.04	7.55	405	8.6
4DB	2.99	6.67	335	26.4	12.22	7.92	376	8.9
4EB	4.28	6.53	216	26.4	4.96	6.75	172.5	17.3
1AD	4.4	3.54	106.4	22.7	3.27	3.79	129.2	14
1BD	6.6	5.3	178	23.7	3.16	6.04	138.3	18
1CD	4.43	3.62	195.5	26.9	4.41	5.57	109.5	9
2AD	1	4.56	107.2	23.2	3.22	3.9	114.6	14.7
2BD	3.51	5.84	67.7	25.1	6.8	5.04	96.8	11.3
2CD	3	7.45	1030	25.6	4.7	9	702	13.1
3AD	2.5	7.21	348	25.3	3.5	6.77	285	14.7
3BD	4	7.36	363	24.9	3.2	7.12	277	14.9
3CD	6.1	7.32	127	32.2	14.05	7.51	290	12.8
4AD	4.8	7.1	660	25	11.37	7.55	902	6.9
4BD	1.14	7.15	396	25.5	12.01	7.76	872	6.8
4CD	0.5	6.95	148.1	24.4	12.07	7.57	405	7.8
4DD	2.94	6.25	346	26.6	12.77	7.65	373	8.6
4ED	4.2	6.85	212	26.5	4.74	6.8	171.7	17.4

Table 6. Biological independent variables at bridge subsites. Column labels BVW – A correspond with column 2, rows 20-25 in Table 2.

Subsite	BVW	P1VW	P2VW	P3VW	TVW	A
1AB	1.01	0	0	0	5.05	1
1BB	3.02	0	0	0	15.08	0
1CB	0.70	0	0	0	4.87	1
2AB	2.76	0	0	0	24.8	0
2BB	0	3.33	0	0	13.3	1
2CB	0.55	0	0	0	2.2	0
3AB	0	0	0	0	0	0
3BB	0	0	0	0	0	0
3CB	0.43	0	1.2	0	4.55	1
4AB	6.18	0	0	0	30.88	0
4BB	0	0	0	0	0	1
4CB	0	0	0	0	0	1
4DB	0	0	0	0	0	0
4EB	0	0	0	0	0	0

Table 7a. Biological independent variables collected from all subsites. Column labels ISp – BM correspond with column 2, rows 26-30 in Table 2.

Subsite	**ISp**	**ISH**	**SH**	**OM**	**BM**
1AU	11.00	275.00	18.00	1.61	10.24
1BU	6.00	211.00	9.00	1.30	8.28
1CU	9.00	34.00	14.00	0.01	0.05
2AU	6.00	188.00	8.00	1.18	7.50
2BU	7.00	190.00	8.00	0.11	0.72
2CU	7.00	126.00	11.00	0.58	3.70
3AU	8.00	60.00	12.00	0.03	0.16
3BU	7.00	280.00	10.00	2.66	16.91
3CU	11.00	52.00	18.00	0.04	0.22
4AU	15.00	116.00	24.00	2.32	14.74
4BU	9.00	74.00	13.00	0.93	5.89
4CU	13.00	200.00	20.00	0.34	2.15
4DU	11.00	134.00	16.00	0.07	0.44
4EU	8.00	120.00	12.00	0.03	0.18
1AB	14.00	283.00	23.00	0.76	4.86
1BB	9.00	320.00	14.00	0.88	5.63
1CB	12.00	224.00	19.00	0.06	0.36
2AB	11.00	231.00	18.00	0.70	4.42
2BB	12.00	486.00	19.00	0.54	3.44
2CB	11.00	381.00	16.00	0.97	6.17
3AB	11.00	195.00	20.00	0.02	0.15

Table 7b. Biological independent variables collected from all subsites. Column labels ISp – BM correspond with column 2, rows 26-30 in Table 2.

Subsite	ISp	ISH	SH	OM	BM
3BB	5.00	130.00	7.00	0.27	1.72
3CB	8.00	324.00	13.00	0.01	0.08
4AB	16.00	92.00	25.00	0.05	0.34
4BB	10.00	184.00	18.00	0.62	3.95
4CB	17.00	244.00	29.00	0.40	2.53
4DB	12.00	233.00	18.00	0.09	0.59
4EB	12.00	238.00	21.00	0.10	0.64
1AD	10.00	599.00	17.00	2.24	14.26
1BD	9.00	328.00	14.00	2.87	18.29
1CD	11.00	922.00	17.00	0.35	2.21
2AD	9.00	294.00	13.00	0.88	5.60
2BD	12.00	302.00	19.00	0.14	0.86
2CD	14.00	173.00	22.00	0.56	3.59
3AD	11.00	177.00	17.00	0.32	2.06
3BD	10.00	136.00	15.00	5.39	34.30
3CD	16.00	211.00	28.00	0.08	0.49
4AD	13.00	226.00	20.00	1.53	9.72
4BD	14.00	408.00	19.00	0.34	2.13
4CD	15.00	291.00	23.00	0.08	0.54
4DD	18.00	261.00	29.00	0.16	0.99
4ED	11.00	283.00	18.00	0.42	2.67

Table 8. Macroinvertebrates collected form all sites taxonomically identified to the family level of taxonomic organization, and some to genus or species.

Phylum, Class, & Order	Family	Genus & Species
Nematodes		
Annelida Hirudinea		
Insecta Tricoptera		
	Dipseudopsidae	
	Hydropsychidae	
Insecta Coleoptera		
	Dytiscidae	
	Gyrinidae	
Insecta Odonata Anisoptera		
	Cordulegastridae	
	Gomphidae	
	Libellulidae	
Insecta Diptera		
	Chironomidae	
	Corydalidae	
	Simulidae	
	Tabanidae	
	Tipulidae	
Insecta Hemiptera		
	Belostomatidae	
	Corixidae	
	Gerridae	
	Nepidae	
Crustacea Amphipoda		
	Gammaridae	*Synurella sp.*
Crustacea Isopoda		
	Armadillidiae	*Armadillidium vulgar*
Crustacea Decapoda		
	Procambridae	*Procambarus spiculifer*
	Procambridae	*Procambarus clarkia*
	Palaemonidae	*Palaemontes sp.*
Mollusca Gastropoda		
	Physidae	*Physella gyrina*
	Planorbidae	*Helisoma anceps*
	Viviparidae	*Viviparus georgianus*
Mollusca Bivalvia		
	Corbiculidae	*Corbicula fluminea*
	Unionidae	*Elliptio buckleyi*

Table 9. Macroinvertebrate counts at upstream subsites for first through third order streams.

Macroinvert.	**1AU**	**1BU**	**1CU**	**2AU**	**2BU**	**2CU**	**3AU**	**3BU**	**3CU**
Nematodes	144	120	24	84	120	60	24	120	24
Hirudea	0	0	0	0	0	0	0	0	0
Dipseudopsidae	1	0	0	0	0	0	0	0	0
Hydropsychidae	0	1	0	0	0	0	0	1	0
Dytiscidae	1	0	1	0	1	0	0	0	1
Gyrinidae	0	0	1	0	0	0	0	0	0
Cordulegastridae	0	0	2	0	0	0	0	0	1
Gomphidae	0	0	1	0	0	0	0	0	0
Libellulidae	0	0	2	0	0	0	0	0	0
Chironomidae	67	72	0	82	28	60	18	120	0
Corydalidae	0	0	0	0	0	0	0	0	0
Simulidae	25	0	0	0	20	0	0	27	1
Tabanidae	0	0	0	0	0	0	0	0	0
Tipulidae	1	0	0	0	0	0	0	0	0
Belostomatidae	0	0	0	0	0	0	0	0	1
Corixidae	0	0	1	0	4	0	0	0	1
Gerridae	0	0	0	0	0	0	0	0	0
Nepidae	1	0	1	8	5	0	1	0	0
Synurella sp.	26	14	0	1	12	1	1	4	0
A. vulgar	3	0	0	0	0	0	0	0	0
P. spiculifer	0	0	0	0	0	0	1	7	0
P. clarkii	4	3	0	12	0	2	1	0	3
Palaemontes sp.	2	1	1	1	0	1	2	1	1
P. gyrina	0	0	0	0	0	0	0	0	0
H. anceps	0	0	0	0	0	0	0	0	0
V. georgianus	0	0	0	0	0	0	0	0	4
C. fluminea	0	0	0	0	0	1	12	0	14
E. buckleyi	0	0	0	0	0	1	0	0	1

Table 10. Macroinvertebrate counts at upstream subsites for fourth orders streams.

Macroinvert.	**4AU**	**4BU**	**4CU**	**4DU**	**4EU**
Nematodes	36	36	12	60	36
Hirudea	0	0	0	0	0
Dipseudopsidae	0	0	0	0	0
Hydropsychidae	0	0	0	0	0
Dytiscidae	2	1	4	0	0
Gyrinidae	4	0	1	0	0
Cordulegastridae	1	0	1	3	0
Gomphidae	1	0	0	0	1
Libellulidae	0	0	0	0	0
Chironomidae	3	18	0	48	60
Corydalidae	0	0	0	0	0
Simulidae	0	4	0	0	0
Tabanidae	0	0	0	0	0
Tipulidae	0	0	0	0	0
Belostomatidae	1	0	4	0	0
Corixidae	8	2	7	4	12
Gerridae	0	0	0	6	0
Nepidae	3	0	12	1	0
Synurella sp.	0	6	2	1	1
A. vulgar	0	0	0	0	0
P. spiculifer	0	0	0	0	0
P. clarkii	3	0	5	3	2
Palaemontes sp.	1	1	1	1	6
P. gyrina	1	0	0	0	0
H. anceps	20	0	0	0	0
V. georgianus	0	0	146	0	0
C. fluminea	31	5	3	6	2
E. buckleyi	1	1	2	1	0

Table 11. Macroinvertebrate counts at bridge subsites for first through third order streams.

Macroinvert.	**1AB**	**1BB**	**1CB**	**2AB**	**2BB**	**2CB**	**3AB**	**3BB**	**3CB**
Nematodes	144	240	96	144	200	240	128	122	240
Hirudea	0	0	0	0	0	1	0	0	0
Dipseudopsidae	0	0	0	0	0	0	0	0	0
Hydropsychidae	1	1	2	0	1	0		0	0
Dytiscidae	8	0	24	4	1	1	1	0	0
Gyrinidae	5	0	3	0	1	0	1	0	0
Cordulegastridae	0	0	0	0	0	1	1	0	1
Gomphidae	2	1	2	2	1	1	2	0	0
Libellulidae	2	0	1	2	1	1	0	0	0
Chironomidae	84	60	84	60	96	120	52	0	72
Corydalidae	0	0	0	0	0	0	1	0	0
Simulidae	0	0	0	0	180	0	0	4	0
Tabanidae	0	0	0	0	0	0	0	0	0
Tipulidae	1	0	0	0	0	0	0	0	0
Belostomatidae	0	0	0	1	0	0	0	0	0
Corixidae	1	0	3	0	0	0	0	0	0
Gerridae	0	0	0	0	0	0	0	0	1
Nepidae	1	5	1	1	1	7	0	0	0
Synurella sp.	22	1	6	9	1	7	0	0	0
A. vulgar	3	1	0	0	0	0	0	1	0
P. spiculifer	0	0	0	0	0	0	2	2	0
P. clarkii	8	10	1	6	2	0	1	0	0
Palaemontes sp.	1	1	1	1	1	1	1	0	1
P. gyrina	0	0	0	0	0	0	0	0	0
H. anceps	0	0	0	0	0	0	0	0	0
V. georgianus	0	0	0	1	0	0	0	0	6
C. fluminea	0	0	0	0	0	1	5	1	2
E. buckleyi	0	0	0	0	0	0	0	0	1

Table 12. Macroinvertebrate counts at bridge subsites for fourth order streams.

Macroinvert.	**4AB**	**4BB**	**4CB**	**4DB**	**4EB**
Nematodes	12	120	72	144	122
Hirudea	0	0	0	0	0
Dipseudopsidae	1	0	0	0	0
Hydropsychidae	0	0	0	0	0
Dytiscidae	4	1	2	1	1
Gyrinidae	2	0	1	0	0
Cordulegastridae	1	1	0	1	1
Gomphidae	0	0	1	1	0
Libellulidae	1	0	0	0	0
Chironomidae	13	36	12	48	96
Corydalidae	0	0	0	0	0
Simulidae	0	0	0	24	0
Tabanidae	0	0	0	0	1
Tipulidae	0	0	0	0	0
Belostomatidae	1	0	6	0	0
Corixidae	0	0	40	0	1
Gerridae	0	0	0	1	1
Nepidae	0	0	2	0	0
Synurella sp.	4	4	6	1	5
A. vulgar	0	0	2	0	0
P. spiculifer	0	0	0	0	1
P. clarkii	1	0	2	2	1
Palaemontes sp.	18	11	24	1	2
P. gyrina	6	1	32	7	0
H. anceps	17	6	9	0	0
V. georgianus	8	0	20	0	0
C. fluminea	2	3	5	2	6
E. buckleyi	1	1	8	0	0

Table 13. Macroinvertebrate counts at downstream subsites for first through third order streams.

Macroinvert.	**1AD**	**1BD**	**1CD**	**2AD**	**2BD**	**2CD**	**3AD**	**3BD**	**3CD**
Nematodes	432	136	852	180	144	84	122	72	96
Hirudea	0	0	0	0	0	0	0	0	0
Dipseudopsidae	0	1	0	0	0	0	0	0	0
Hydropsychidae	1	1	1	1	0	0	0	1	0
Dytiscidae	1	0	1	21	1	0	41	0	2
Gyrinidae	1	0	1	0	0	0	0	0	4
Cordulegastridae	0	0	0	0	0	1	1	1	1
Gomphidae	0	0	2	0	1	1	1	0	1
Libellulidae	0	0	0	0	1	2	1	0	2
Chironomidae	108	108	36	72	84	24	1	34	60
Corydalidae	0	0	0	0	0	0	0	0	0
Simulidae	0	0	0	0	0	38	0	0	0
Tabanidae	0	0	0	0	0	0	0	0	0
Tipulidae	1	0	0	0	0	1	0	0	0
Belostomatidae	0	0	1	0	0	0	0	0	1
Corixidae	0	0	2	10	0	0	0	0	1
Gerridae	0	1	0	0	1	3	2	1	1
Nepidae	0	2	0	1	1	1	4	3	0
Synurella sp.	36	72	24	1	60	6	1	5	12
A. vulgar	1	0	1	0	1	0	0	0	0
P. spiculifer	0	0	0	0	2	1	1	5	0
P. clarkii	17	6	0	7	5	1	0	0	0
Palaemontes sp.	1	1	1	1	1	1	0	2	2
P. gyrina	0	0	0	0	0	0	0	0	0
H. anceps	0	0	0	0	0	0	0	0	1
V. georgianus	0	0	0	0	0	0	0	0	9
C. fluminea	0	0	0	0	0	9	2	12	12
E. buckleyi	0	0	0	0	0	0	0	0	6

Table 14. Macroinvertebrate counts at downstream subsites for fourth order streams.

Macroinvert.	**4AD**	**4BD**	**4CD**	**4DD**	**4ED**
Nematodes	144	246	199	120	132
Hirudea	0	0	2	0	0
Dipseudopsidae	0	0	0	1	0
Hydropsychidae	0	0	0	1	0
Dytiscidae	2	3	1	2	1
Gyrinidae	0	0	0	3	0
Cordulegastridae	1	0	0	0	3
Gomphidae	0	2	1	2	2
Libellulidae	1	0	0	1	0
Chironomidae	42	64	24	60	120
Corydalidae	0	0	0	0	0
Simulidae	0	34	0	36	0
Tabanidae	0	0	0	0	0
Tipulidae	0	0	0	0	0
Belostomatidae	0	0	1	0	0
Corixidae	0	1	5	12	0
Gerridae	1	2	0	1	0
Nepidae	8	8	5	2	1
Synurella sp.	1	25	1	2	10
A. vulgar	0	0	0	1	0
P. spiculifer	0	0	0	0	0
P. clarkii	5	0	2	1	3
Palaemontes sp.	6	3	4	1	9
P. gyrina	0	12	14	3	0
H. anceps	5	3	1	0	0
V. georgianus	0	0	15	0	0
C. fluminea	8	1	16	12	1
E. buckleyi	2	4	0	0	1

Table 15a. Pair-wise multiple comparison results of macroinvertebrate data for variance between subsites. The first letters in the subsite labels are combined with the stream order number to generate the site labels. U = upstream subsite, B = bridge subsite, and D = downstream subsite are in the title and at to the end of individual subsites to generate labels.

Macroinvertebrates U, B, D				
1AU vs. 1BU	2AU vs. 4DD	3AU vs. 4CD	4EU vs. 3CD	3BB vs. 4CD
1AU vs. 2CU	2BU vs. 4AU	3AU vs. 4DD	4EU vs. 4AD	3BB vs. 4DD
1AU vs. 3AU	2BU vs. 1AB	3AU vs. 4ED	4EU vs. 4BD	3BB vs. 4ED
1AU vs. 3BB	2BU vs. 4AB	3BU vs. 4AU	4EU vs. 4CD	3CB vs. 4CB
1BU vs. 4AU	2BU vs. 4CB	3BU vs. 4CU	4EU vs. 4DD	3CB vs. 3CD
1BU vs. 4CU	2BU vs. 3CD	3BU vs. 1AB	1AB vs. 1BB	3CB vs. 4BD
1BU vs. 1AB	2BU vs. 4AD	3BU vs. 4AB	1AB vs. 3BB	3CB vs. 4CD
1BU vs. 2AB	2BU vs. 4BD	3BU vs. 4CB	1AB vs. 3CB	3CB vs. 4DD
1BU vs. 4AB	2BU vs. 4CD	3BU vs. 3CD	1AB vs. 2AD	4BB vs. 4CB
1BU vs. 4CB	2BU vs. 4DD	3BU vs. 4AD	1AB vs. 3BD	4BB vs. 3CD
1BU vs. 2BD	2CU vs. 4AU	3BU vs. 4BD	1BB vs. 4CB	4BB vs. 4BD
1BU vs. 2CD	2CU vs. 4CU	3BU vs. 4CD	1BB vs. 3CD	4BB vs. 4DD
1BU vs. 3CD	2CU vs. 1AB	3BU vs. 4DD	1BB vs. 4BD	4CB vs. 4DB
1BU vs. 4AD	2CU vs. 2AB	3CU vs. 4CB	1BB vs. 4CD	4CB vs. 1AD
1BU vs. 4BD	2CU vs. 2CB	3CU vs. 3CD	1BB vs. 4DD	4CB vs. 1BD
1BU vs. 4CD	2CU vs. 4AB	3CU vs. 4BD	1CB vs. 3BB	4CB vs. 1CD
1BU vs. 4DD	2CU vs. 4CB	3CU vs. 4DD	1CB vs. 4CB	4CB vs. 2AD
1BU vs. 4ED	2CU vs. 4EB	4AU vs. 4BU	2AB vs. 3BB	4CB vs. 3AD

Table 15b. Pair-wise multiple comparison results of macroinvertebrate data for variance between subsites. The first letters in the subsite labels are combined with the stream order number to generate the site labels. U = upstream subsite, B = bridge subsite, and D = downstream subsite are in the title and at to the end of individual subsites to generate labels.

Macroinvertebrates U, B, D				
1CU vs. 4AU	2CU vs. 2BD	4AU vs. 4EU	2BB vs. 4CB	4CB vs. 3BD
1CU vs. 1AB	2CU vs. 2CD	4AU vs. 3BB	2BB vs. 3CD	4DB vs. 3CD
1CU vs. 4AB	2CU vs. 3CD	4BU vs. 1AB	2BB vs. 4BD	4DB vs. 4BD
1CU vs. 4CB	2CU vs. 4AD	4BU vs. 4AB	2BB vs. 4DD	4DB vs. 4DD
1CU vs. 3CD	2CU vs. 4BD	4BU vs. 4CB	2CB vs. 3BB	1BD vs. 3CD
1CU vs. 4AD	2CU vs. 4CD	4BU vs. 3CD	3AB vs. 4CB	1BD vs. 4BD
1CU vs. 4BD	2CU vs. 4DD	4BU vs. 4AD	3AB vs. 3CD	1BD vs. 4DD
1CU vs. 4CD	2CU vs. 4ED	4BU vs. 4BD	3AB vs. 4BD	1CD vs. 4BD
1CU vs. 4DD	3AU vs. 4AU	4BU vs. 4CD	3AB vs. 4DD	2AD vs. 3CD
2AU vs. 4AU	3AU vs. 4CU	4BU vs. 4DD	3BB vs. 4AB	2AD vs. 4BD
2AU vs. 4CU	3AU vs. 1AB	4CU vs. 3BB	3BB vs. 4CB	2AD vs. 4DD
2AU vs. 1AB	3AU vs. 4AB	4DU vs. 4CB	3BB vs. 4EB	3AD vs. 3CD
2AU vs. 4AB	3AU vs. 4CB	4DU vs. 3CD	3BB vs. 1AD	3AD vs. 4BD
2AU vs. 4CB	3AU vs. 2BD	4DU vs. 4BD	3BB vs. 2BD	3AD vs. 4DD
2AU vs. 3CD	3AU vs. 2CD	4DU vs. 4DD	3BB vs. 2CD	3BD vs. 3CD
2AU vs. 4AD	3AU vs. 3CD	4EU vs. 1AB	3BB vs. 3CD	3BD vs. 4BD
2AU vs. 4BD	3AU vs. 4AD	4EU vs. 4AB	3BB vs. 4AD	3BD vs. 4DD
2AU vs. 4CD	3AU vs. 4BD	4EU vs. 4CB	3BB vs. 4BD	

Table 16. Fish species that were collected during the research were identified down to the genus and species.

Family: Genus species	**Family: Genus species**
Lepisosteidae	**Atherinopsidae**
• *Lepisosteus osseus*	• *Labidesthes sicculus*
• *Lepisosteus platyrhincus*	**Centrarchidae**
Amiidae	• *Micropterus notius*
• *Amia calva*	• *Micropterus salmoides*
Aphrododeridae	• *Centrarchus macropterus*
• *Aphredoderus sayanus*	• *Lepomis auritus*
Umbridae	• *Lepomis gulosus*
• *Umbra pygmaea*	• *Lepomis macrochirus*
Esocidae	• *Lepomis marginatus*
• *Esox americanus*	• *Lepomis punctatus*
• *Esox niger*	• *Pomoxis nigromaculatus*
Cyprinidae	• *Enneacanthus gloriosus*
• *Notemigonus crysoleucas*	• *Enneacanthus obesus*
• *Opsopoeodus emiliae*	• *Acantharchus pomotis*
• *Notropis petersoni*	**Elassomatidae**
• *Notropis texanus*	• *Elassoma evergladei*
• *Cyprinella venusta*	• *Elassoma okefenokee*
• *Pteronotropis hypselopterus*	• *Elassoma zonatum*
Catostomidae	**Percidae**
• *Minytrema melanops*	• *Percina nigrofasciata*
• *Erimyzon sucetta*	• *Etheostoma edwini*
Ictaluridae	• *Etheostoma fusiforme*
• *Ameiurus brunneus*	
• *Ameiurus nebulosus*	
• *Noturus gyrinus*	
• *Noturus leptacanthus*	
Fundulidae	
• *Fundulus chrysotus*	
• *Fundulus lineolatus*	
• *Leptolucania ommata*	
Poeciliidae	
• *Gambusia holbrooki*	
• *Heterandria formosa*	

Table 17a. Fish species counts at upstream subsites for first through third order streams.

Fish Sp.	1AU	1BU	1CU	2AU	2BU	2CU	3AU	3BU	3CU
L. osseus	0	0	0	0	0	0	0	0	0
L. platyrhincus	0	0	0	0	0	0	0	0	0
A. calva	0	0	0	0	0	0	0	0	0
A. sayanus	1	2	0	0	4	0	1	1	3
U. pygmaea	0	0	0	0	0	0	0	0	0
E. americanus	2	1	0	19	2	0	0	0	0
E. niger	1	0	0	0	0	0	0	0	0
N. crysoleucas	0	0	0	0	0	0	0	0	0
O. emiliae	0	0	0	0	0	0	0	0	0
N. petersoni	0	0	0	0	0	0	3	1	15
N. texanus	0	0	0	0	1	0	41	0	0
C. venusta	0	0	0	0	0	0	0	1	5
P. hypselopterus	0	0	0	0	0	0	0	0	0
M. melanops	0	0	0	0	0	0	0	0	0
E. sucetta	0	0	0	0	0	0	0	0	0
A. brunneus	0	0	0	0	0	0	0	0	0
A. nebulosus	0	0	0	0	0	0	0	0	0
N. gyrinus	0	0	0	0	1	0	0	0	5
N. leptacanthus	0	0	0	0	0	0	0	0	2
F. chrysotus	0	0	0	0	0	0	0	0	0
F. lineolatus	0	0	0	0	2	0	0	0	0
L. omatta	0	0	0	0	0	0	0	0	0

Table 17b. Fish species counts at upstream subsites for first through third order streams.

Fish Sp.	**1AU**	**1BU**	**1CU**	**2AU**	**2BU**	**2CU**	**3AU**	**3BU**	**3CU**
G. holbrooki	0	6	0	0	6	0	3	1	98
H. formosa	0	0	0	0	0	0	0	0	22
L. sicculus	0	1	0	0	7	5	14	0	0
M. notius	0	0	0	0	0	0	0	0	0
M. salmoides	0	0	0	0	1	1	2	0	3
C. macropterus	49	1	8	18	0	0	0	0	1
L. auritus	0	0	0	0	0	0	2	0	0
L. gulosus	6	0	0	19	0	0	0	0	0
L. macrochirus	3	0	2	0	10	0	7	0	60
L. marginatus	0	0	0	0	0	0	0	0	0
L. punctatus	0	0	0	0	0	0	0	1	0
P. nigromaculatus	0	0	0	0	1	0	0	0	1
E. gloriosus	0	0	0	0	0	0	0	0	0
E. obesus	0	0	1	0	0	0	0	0	0
E. evergladei	0	0	0	0	0	0	0	0	0
E. okefonokee	0	0	0	0	1	0	0	0	0
E. zonatum	0	0	0	0	0	0	0	0	0
P. nigrofasciata	0	0	0	0	4	3	17	0	10
E. edwini	0	0	0	0	0	0	0	0	0
E. fusiforme	0	0	0	0	0	0	0	0	0
A. pomotis	0	0	0	0	0	0	0	0	0

Table 18a. Fish species counts at upstream subsites for fourth order streams.

Fish Sp.	**4AU**	**4BU**	**4CU**	**4DU**	**4EU**
L. osseus	0	0	0	0	0
L. platyrhincus	0	0	0	0	0
A. calva	0	0	0	0	0
A. sayanus	8	0	14	3	2
U. pygmaea	0	0	0	0	0
E. americanus	1	0	12	0	0
E. niger	0	0	4	3	0
N. crysoleucas	0	0	0	0	0
O. emiliae	0	0	0	0	9
N. petersoni	5	0	0	0	0
N. texanus	0	0	0	0	0
C. venusta	0	0	0	0	2
P. hypselopterus	0	0	0	0	0
M. melanops	0	0	1	0	0
E. sucetta	0	0	0	0	0
A. brunneus	0	0	0	0	0
A. nebulosus	0	0	0	0	0
N. gyrinus	0	0	0	0	1
N. leptacanthus	0	0	2	0	0
F. chrysotus	0	0	0	0	0
F. lineolatus	0	0	0	0	0
L. omatta	0	0	0	0	0

Table 18b. Fish species counts at upstream subsites for fourth order streams.

Fish Sp.	4AU	4BU	4CU	4DU	4EU
G. holbrooki	12	0	7	4	0
H. formosa	0	0	0	3	0
L. sicculus	15	0	5	234	435
M. notius	0	0	0	0	0
M. salmoides	3	0	0	3	1
C. macropterus	14	0	1	0	0
L. auritus	6	0	2	2	0
L. gulosus	0	0	0	0	0
L. macrochirus	3	0	16	15	8
L. marginatus	0	0	4	0	0
L. punctatus	10	0	6	1	0
P. nigromaculatus	0	0	1	0	0
E. gloriosus	0	0	0	0	0
E. obesus	0	0	0	0	0
E. evergladei	0	0	0	0	0
E.okefonokee	0	0	0	0	0
E. zonatum	0	1	2	0	0
P. nigrofasciata	0	0	2	0	10
E. edwini	0	0	0	5	0
E. fusiforme	1	0	0	0	0
A. pomotis	0	0	1	0	0

Table 19a. Fish species counts at bridge subsites for first through third order streams.

Fish Sp.	1AB	1BB	1CB	2AB	2BB	2CB	3AB	3BB	3CB
L. osseus	0	0	0	0	0	0	0	0	0
L. platyrhincus	0	3	0	3	0	0	0	0	0
A. calva	0	0	0	1	0	0	0	0	0
A. sayanus	0	9	0	43	7	5	0	2	5
U. pygmaea	0	1	0	1	0	0	0	0	0
E. americanus	4	0	0	6	2	0	0	0	0
E. niger	2	1	2	7	4	0	0	0	1
N. crysoleucas	0	1	0	0	0	0	0	5	2
O. emiliae	0	0	0	0	0	0	0	0	0
N. petersoni	0	0	0	0	0	20	6	7	18
N. texanus	0	0	0	0	0	0	8	1	27
C. venusta	0	0	0	0	0	0	2	53	6
P. hypselopterus	0	0	0	0	0	0	0	0	0
M. melanops	0	0	0	0	15	0	0	0	0
E. sucetta	0	0	0	0	1	0	0	0	0
A. brunneus	0	0	0	0	0	0	0	0	0
A. nebulosus	0	0	0	1	0	0	0	0	0
N. gyrinus	0	0	0	1	2	0	0	0	2
N. leptacanthus	0	1	0	0	3	0	2	0	1
F. chrysotus	0	0	0	0	0	0	0	0	0
F. lineolatus	0	0	0	2	14	0	0	0	2
L. omatta	1	0	13	5	0	0	0	0	0

Table 19b. Fish species counts at bridge subsites for first through third order streams.

Fish Sp.	1AB	1BB	1CB	2AB	2BB	2CB	3AB	3BB	3CB
G. holbrooki	61	21	134	25	88	0	4	3	181
H. formosa	0	1	0	0	0	0	0	0	4
L. sicculus	0	1	0	1	119	7	1	3	3
M. notius	0	1	0	1	0	0	0	0	0
M. salmoides	0	0	0	0	14	1	0	0	8
C. macropterus	19	0	42	117	1	1	0	0	1
L. auritus	0	1	0	0	0	0	1	1	14
L. gulosus	4	1	1	4	0	0	0	0	2
L. macrochirus	0	4	30	11	80	0	0	0	103
L. marginatus	0	0	0	0	0	0	0	0	0
L. punctatus	0	0	0	0	0	0	0	2	2
P. nigromaculatus	0	0	0	0	1	0	0	0	3
E. gloriosus	1	0	1	1	1	0	0	0	0
E. obesus	1	0	3	0	0	0	0	0	0
E. evergladei	0	0	0	0	0	0	0	0	0
E. okefonokee	0	0	0	7	6	0	0	0	0
E. zonatum	0	0	1	0	3	0	0	0	0
P. nigrofasciata	0	0	0	0	3	0	21	0	12
E. edwini	0	0	0	0	0	0	0	0	0
E. fusiforme	0	1	0	0	4	0	0	1	2
A. pomotis	0	0	0	1	0	0	0	0	0

Table 20a. Fish species counts at bridge subsites for fourth order streams.

Fish Sp.	4AB	4BB	4CB	4DB	4EB
L. osseus	0	0	1	0	0
L. platyrhincus	3	8	0	0	0
A. calva	0	0	0	0	0
A. sayanus	10	4	24	3	3
U. pygmaea	0	0	0	0	0
E. americanus	0	0	2	0	0
E. niger	0	1	19	2	0
N. crysoleucas	0	1	0	0	0
O. emiliae	0	0	0	0	25
N. petersoni	0	1	0	1	0
N. texanus	0	0	0	1	11
C. venusta	3	0	0	1	4
P. hypselopterus	0	0	0	0	0
M. melanops	0	2	1	0	0
E. sucetta	0	0	0	0	0
A. brunneus	0	0	0	0	0
A. nebulosus	0	0	0	0	0
N. gyrinus	0	0	0	0	0
N. leptacanthus	0	0	0	0	0
F. chrysotus	1	0	6	0	0
F. lineolatus	1	0	2	0	0
L. omatta	0	0	0	0	0

Table 20b. Fish species counts at bridge subsites for fourth order streams.

Fish Sp.	**4AB**	**4BB**	**4CB**	**4DB**	**4EB**
G. holbrooki	113	35	41	19	1
H. formosa	0	0	3	0	0
L. sicculus	78	15	154	280	109
M. notius	0	1	0	0	0
M. salmoides	22	12	17	132	0
C. macropterus	28	8	0	0	1
L. auritus	21	1	0	3	0
L. gulosus	5	0	0	0	0
L. macrochirus	26	44	23	22	10
L. marginatus	0	0	0	0	0
L. punctatus	2	3	4	1	0
P. nigromaculatus	0	1	4	1	0
E. gloriosus	0	0	0	0	0
E. obesus	0	0	0	0	0
E. evergladei	0	0	1	0	0
E. okefonokee	0	0	0	0	0
E. zonatum	6	6	18	0	0
P. nigrofasciata	2	0	1	1	20
E. edwini	0	0	0	2	0
E. fusiforme	3	1	75	2	0
A. pomotis	0	0	0	0	0

Table 21a. Fish species counts at downstream subsites for first through third order streams.

Fish Sp.	**1AD**	**1BD**	**1CD**	**2AD**	**2BD**	**2CD**	**3AD**	**3BD**	**3CD**
L. osseus	0	0	0	0	0	0	0	0	0
L. platyrhincus	0	0	0	0	0	0	0	0	0
A. calva	0	0	0	0	0	0	0	0	0
A. sayanus	17	8	2	95	8	3	2	1	5
U. pygmaea	0	0	0	0	0	0	0	0	0
E. americanus	1	0	0	12	1	0	1	0	0
E. niger	0	0	0	2	0	0	0	0	1
N. crysoleucas	0	0	0	0	0	0	0	0	0
O. emiliae	0	0	0	0	3	0	0	0	0
N. petersoni	0	0	0	0	0	9	6	3	57
N. texanus	0	0	0	0	7	0	43	37	3
C. venusta	0	0	0	0	0	0	11	29	4
P. hypselopterus	0	13	0	0	0	0	0	2	0
M. melanops	0	0	0	0	20	0	0	0	0
E. sucetta	0	0	0	0	0	0	0	0	2
A. brunneus	0	0	0	1	0	0	0	0	0
A. nebulosus	0	0	0	0	0	0	0	0	0
N. gyrinus	0	0	0	0	1	0	0	0	0
N. leptacanthus	0	0	0	0	0	0	0	0	0
F. chrysotus	0	0	0	0	0	0	0	0	0
F. lineolatus	0	0	0	0	2	0	0	0	0
L. omatta	0	0	0	0	0	0	0	0	0

Table 21b. Fish species counts at downstream subsites for first through third order streams.

Fish Sp.	**1AD**	**1BD**	**1CD**	**2AD**	**2BD**	**2CD**	**3AD**	**3BD**	**3CD**
G. holbrooki	0	18	0	0	4	6	5	3	52
H. formosa	0	0	0	0	0	0	0	0	5
L. sicculus	0	1	0	0	31	34	30	5	199
M. notius	0	0	0	0	0	0	1	0	0
M. salmoides	0	0	0	0	2	3	0	0	8
C. macropterus	7	2	9	1	1	2	0	0	0
L. auritus	0	1	0	0	1	1	0	0	0
L. gulosus	9	1	0	8	0	0	0	0	2
L. macrochirus	3	2	16	0	9	19	4	5	80
L. marginatus	0	0	0	0	0	0	0	0	0
L. punctatus	0	2	0	0	0	0	0	0	2
P. nigromaculatus	0	0	0	0	2	0	0	0	3
E. gloriosus	0	0	0	0	0	0	0	0	0
E. obesus	0	0	0	0	0	0	0	0	0
E. evergladei	0	0	0	0	0	0	0	0	0
E. okefonokee	0	0	0	0	0	0	0	0	0
E. zonatum	0	0	0	0	0	0	0	0	1
P. nigrofasciata	0	1	0	0	23	9	9	3	31
E. edwini	0	0	0	0	0	0	0	0	0
E. fusiforme	0	0	0	0	3	1	0	0	3
A. pomotis	0	0	0	0	0	0	0	0	0

Table 22a. Fish species counts at downstream subsites for fourth order streams.

Fish Sp.	**4AD**	**4BD**	**4CD**	**4DD**	**4ED**
L. osseus	0	0	0	0	1
L. platyrhincus	0	0	0	0	0
A. calva	0	0	0	0	0
A. sayanus	6	2	15	1	0
U. pygmaea	0	0	0	0	0
E. americanus	1	2	1	1	0
E. niger	0	0	9	3	0
N. crysoleucas	0	0	0	0	0
O. emiliae	0	0	30	0	14
N. petersoni	11	1	2	249	0
N. texanus	0	0	0	0	3
C. venusta	0	0	0	0	1
P. hypselopterus	0	0	0	0	0
M. melanops	0	0	0	0	0
E. sucetta	0	0	0	0	0
A. brunneus	0	0	0	0	0
A. nebulosus	0	0	0	0	0
N. gyrinus	0	0	0	0	0
N. leptacanthus	1	0	0	0	0
F. chrysotus	0	0	0	0	0
F. lineolatus	0	0	0	0	0
L. omatta	0	0	0	0	0

Table 22b. Fish species counts at downstream subsites for fourth order streams.

Fish Sp.	**4AD**	**4BD**	**4CD**	**4DD**	**4ED**
G. holbrooki	10	15	27	41	5
H. formosa	0	0	2	1	0
L. sicculus	7	78	253	323	47
M. notius	0	0	0	0	0
M. salmoides	2	0	29	70	1
C. macropterus	17	0	7	0	0
L. auritus	2	1	0	0	0
L. gulosus	1	1	0	0	1
L. macrochirus	3	22	43	54	29
L. marginatus	0	1	1	0	0
L. punctatus	7	8	1	0	0
P. nigromaculatus	0	1	1	0	0
E. gloriosus	0	0	0	0	0
E. obesus	0	0	0	0	0
E. evergladei	0	0	0	0	0
E. okefonokee	0	0	0	0	0
E. zonatum	0	3	8	0	0
P. nigrofasciata	1	0	0	9	4
E. edwini	0	0	0	3	0
E. fusiforme	0	0	8	1	1
A. pomotis	0	0	0	0	0

Table 23a. Pair-wise multiple comparison results of fish data for variance between subsites. The first letters in the subsite labels are combined with the stream order number to generate the site labels. U = upstream subsite, B = bridge subsite, and D = downstream subsite are in the title and at to the end of individual subsites to generate labels.

Fish U, B, D				
1BUvs.1BB	1BBvs.1CD	1BUvs.4BD	1CUvs.2BB	2ADvs.3CD
1ABvs.2AB	1BBvs.1CU	1BUvs.4BD	1CUvs.2BD	2ADvs.4AB
1ABvs.2BB	1BBvs.2AU	1BUvs.4BU	1CUvs.2BU	2ADvs.4BB
1ABvs.2CU	1BBvs.2BB	1BUvs.4DB	1CUvs.3AD	2ADvs.4BB
1ABvs.3CB	1BBvs.2CB	1BUvs.4DD	1CUvs.3CB	2ADvs.4BD
1ABvs.3CD	1BBvs.2CU	1CBvs.1CD	1CUvs.3CD	2ADvs.4BU
1ABvs.4AB	1BBvs.3BU	1CBvs.2AB	1CUvs.3CU	2ADvs.4BU
1ABvs.4BB	1BBvs.3CB	1CBvs.2BB	1CUvs.4AB	2AUvs.2AB
1ABvs.4BD	1BBvs.3CD	1CBvs.2CU	1CUvs.4AD	2AUvs.2BB
1ABvs.4BU	1BBvs.4BB	1CBvs.3BU	1CUvs.4AU	2AUvs.2BD
1ADvs.2AB	1BBvs.4BU	1CBvs.3CB	1CUvs.4BB	2AUvs.3CB
1ADvs.2BB	1BDvs.2AB	1CBvs.3CD	1CUvs.4BB	2AUvs.3CD
1ADvs.2BD	1BDvs.2BB	1CBvs.4AB	1CUvs.4BD	2AUvs.3CU
1ADvs.3CB	1BDvs.2BD	1CBvs.4BB	1CUvs.4BD	2AUvs.4AB
1ADvs.3CD	1BDvs.3CB	1CBvs.4BD	1CUvs.4BU	2AUvs.4AD
1ADvs.3CU	1BDvs.3CD	1CBvs.4BU	1CUvs.4DB	2AUvs.4BB
1ADvs.4AB	1BDvs.4AB	1CDvs.2AB	1CUvs.4DD	2AUvs.4BB
1ADvs.4BB	1BDvs.4BB	1CDvs.2BB	1CUvs.4ED	2AUvs.4BD
1ADvs.4BB	1BDvs.4BB	1CDvs.2BD	2ABvs.2AD	2AUvs.4BU
1ADvs.4BD	1BDvs.4BD	1CDvs.2BU	2ABvs.2CB	2AUvs.4DB
1ADvs.4BU	1BDvs.4BU	1CDvs.3CB	2ABvs.2CD	2AUvs.4DD
1ADvs.4DB	1BDvs.4BU	1CDvs.3CD	2ABvs.2CU	2BBvs.2CB
1ADvs.4DD	1BUvs.1BB	1CDvs.3CU	2ABvs.3AB	2BBvs.2CD
1AUvs.2AB	1BUvs.1CB	1CDvs.4AB	2ABvs.3AD	2BBvs.2CU
1AUvs.2BB	1BUvs.2AB	1CDvs.4AD	2ABvs.3AU	2BBvs.3AB
1AUvs.2BD	1BUvs.2BB	1CDvs.4AU	2ABvs.3BB	2BBvs.3AD

Table 23b. Pair-wise multiple comparison results of fish data for variance between subsites. The first letters in the subsite labels are combined with the stream order number to generate the site labels. U = upstream subsite, B = bridge subsite, and D = downstream subsite are in the title and at to the end of individual subsites to generate labels.

Fish U, B, D				
1AUvs.3CB	1BUvs.2BD	1CDvs.4BB	2ABvs.3BD	2BBvs.3AU
1AUvs.3CD	1BUvs.2BU	1CDvs.4BB	2ABvs.3BU	2BBvs.3BB
1AUvs.3CU	1BUvs.3CB	1CDvs.4BD	2ABvs.4BU	2BBvs.3BD
1AUvs.4AB	1BUvs.3CD	1CDvs.4BD	2ABvs.4DU	2BBvs.3BU
1AUvs.4BB	1BUvs.3CU	1CDvs.4BU	2ABvs.4EB	2BBvs.3CU
1AUvs.4BB	1BUvs.4AB	1CDvs.4DB	2ABvs.4ED	2BBvs.4AD
1AUvs.4BD	1BUvs.4AD	1CDvs.4DD	2ABvs.4EU	2BBvs.4AU
1AUvs.4BU	1BUvs.4AU	1CDvs.4ED	2ADvs.2BB	2BBvs.4BD
1AUvs.4DB	1BUvs.4BB	1CUvs.1CB	2ADvs.2BD	2BBvs.4BU
1AUvs.4DD	1BUvs.4BB	1CUvs.2AB	2ADvs.3CB	2BBvs.4DB
2BBvs.4DD	2CDvs.4BU	3BBvs.4AB	3CDvs.4BU	4BUvs.4DU
2BBvs.4DU	2CDvs.4BU	3BBvs.4BB	3CDvs.4DU	4BUvs.4EB
2BBvs.4EB	2CUvs.3AD	3BBvs.4BD	3CDvs.4EB	4BUvs.4EB
2BBvs.4ED	2CUvs.3CB	3BBvs.4BU	3CDvs.4ED	4BUvs.4ED
2BBvs.4EU	2CUvs.3CD	3BBvs.4BU	3CDvs.4EU	4BUvs.4EU
2BDvs.2CB	2CUvs.3CU	3BDvs.3CB	3CUvs.3CB	4DDvs.4EU
2BDvs.2CU	2CUvs.4AB	3BDvs.3CD	3CUvs.3CD	
2BDvs.3AB	2CUvs.4AD	3BDvs.4AB	3CUvs.4BB	
2BDvs.3AU	2CUvs.4AU	3BDvs.4BB	3CUvs.4BU	
2BDvs.3BB	2CUvs.4BB	3BDvs.4BB	4ABvs.4BD	
2BDvs.3BD	2CUvs.4BB	3BDvs.4BD	4ABvs.4BU	
2BDvs.3BU	2CUvs.4BD	3BDvs.4BU	4ABvs.4DU	
2BDvs.3CB	2CUvs.4BD	3BUvs.3CB	4ABvs.4EB	
2BDvs.4BU	2CUvs.4BU	3BUvs.3CD	4ABvs.4ED	
2BDvs.4DU	2CUvs.4DB	3BUvs.3CU	4ABvs.4EU	
2BDvs.4EB	2CUvs.4DD	3BUvs.4AB	4ADvs.4BB	

Table 23c. Pair-wise multiple comparison results of fish data for variance between subsites. The first letters in the subsite labels are combined with the stream order number to generate the site labels. U = upstream subsite, B = bridge subsite, and D = downstream subsite are in the title and at to the end of individual subsites to generate labels.

Fish U, B, D			
2BDvs.4EU	2CUvs.4ED	3BUvs.4AD	4ADvs.4BU
2BUvs.2BB	3ABvs.3CB	3BUvs.4AU	4AUvs.4BB
2BUvs.2CU	3ABvs.3CD	3BUvs.4BB	4AUvs.4BD
2BUvs.3BU	3ABvs.4AB	3BUvs.4BB	4AUvs.4BU
2BUvs.3CB	3ABvs.4BB	3BUvs.4BD	4BBvs.4DB
2BUvs.3CD	3ABvs.4BB	3BUvs.4BD	4BBvs.4DU
2BUvs.4BB	3ABvs.4BD	3BUvs.4BU	4BBvs.4DU
2BUvs.4BD	3ABvs.4BU	3BUvs.4DB	4BBvs.4EB
2BUvs.4BU	3ABvs.4BU	3BUvs.4DD	4BBvs.4EB
2CBvs.3CB	3ADvs.3CB	3BUvs.4ED	4BBvs.4ED
2CBvs.3CD	3ADvs.3CD	3CBvs.4AD	4BBvs.4EU
2CBvs.3CU	3ADvs.4AB	3CBvs.4AU	4BBvs.4EU
2CBvs.4AB	3ADvs.4BB	3CBvs.4BB	4BDvs.4BB
2CBvs.4AD	3ADvs.4BD	3CBvs.4BD	4BDvs.4BD
2CBvs.4BB	3ADvs.4BU	3CBvs.4BU	4BDvs.4DU
2CBvs.4BB	3AUvs.3CB	3CBvs.4BU	4BDvs.4EB
2CBvs.4BD	3AUvs.3CD	3CBvs.4DB	4BDvs.4ED
2CBvs.4BU	3AUvs.4AB	3CBvs.4DD	4BDvs.4EU
2CBvs.4DB	3AUvs.4BB	3CBvs.4DU	4BUvs.4BB
2CBvs.4DD	3AUvs.4BB	3CBvs.4EB	4BUvs.4BB
2CDvs.3CB	3AUvs.4BD	3CBvs.4ED	4BUvs.4BD
2CDvs.3CD	3AUvs.4BU	3CBvs.4EU	4BUvs.4BU
2CDvs.4AB	3AUvs.4BU	3CDvs.4AD	4BUvs.4DB
2CDvs.4BB	3BBvs.3CB	3CDvs.4AU	4BUvs.4DD
2CDvs.4BD	3BBvs.3CD	3CDvs.4BD	4BUvs.4DU

Table 24. Fish species organized into guilds by habitat use for all life stages and actions. Some species are abbreviated: americanus = a-canus, hypselopterus = h-opterus, leptacanthus = l-anthus, macrochirus = m-chirus, macropterus = m-terus, marginatus = m-atus, nigrofasciata = n-ciata, nigromaculatus = n-culatus, platyrhincus = p-incus.

Open Water	**Near Vegetation**	**In Vegetation**	**Debris**	**Benthic**
L. osseus	*L. osseus*	*A. calva*	*A. calva*	*A. calva*
L. p-incus	*L. p-incus*	*A. sayanus*	*A. sayanus*	*A. sayanus*
E. niger	*A. calva*	*U. pygmaea*	*U. pygmaea*	*U. pygmaea*
N. petersoni	*A. sayanus*	*E. a-canus*	*E. a-canus*	*E. a-canus*
N. texanus	*E. a-canus*	*E. niger*	*E. niger*	*E. niger*
C. venusta	*E. niger*	*N. crysoleucas*	*P. h-opterus*	*O. emiliae*
M. melanops	*N. crysoleucas*	*O. emiliae*	*E. sucetta*	*N. petersoni*
E. sucetta	*O. emiliae*	*P. h-opterus*	*N. gyrinus*	*M. melanops*
A. brunneus	*N. texanus*	*F. chrysotus*		*E. sucetta*
A. nebulosus	*P. h-opterus*	*F. lineolatus*		*A. brunneus*
F. chrysotus	*N. l-anthus*	*L. ommata*		*A. nebulosus*
F. lineolatus	*F. chrysotus*	*G. holbrooki*		*N. gyrinus*
L. ommata	*F. lineolatus*	*H. formosa*		*N. l-anthus*
G. holbrooki	*L. ommata*	*M. salmoides*		*M. notius*
L. sicculus	*G. holbrooki*	*C. m-terus*		*M. salmoides*
M. notius	*M. salmoides*	*L. auritus*		*C. m-terus*
M. salmoides	*C. m-terus*	*L. gulosus*		*L. auritus*
C. m-terus	*L. auritus*	*L. m-chirus*		*L. gulosus*
L. auritus	*L. gulosus*	*L. punctatus*		*L. m-atus*
L. gulosus	*L. m-chirus*	*P. n-culatus*		*L. punctatus*
L. m-chirus	*L. m-atus*	*E. gloriosus*		*P. n-culatus*
L. m-atus	*P. n-culatus*	*E. obesus*		*E. gloriosus*
P. n-culatus	*A. pomotis*	*A. pomotis*		*E. obesus*
	E. edwini	*E. evergladei*		*A. pomotis*
	E. fusiforme	*E. okefenokee*		*E. evergladei*
		E. zonatum		*E. okefenokee*
		E. edwini		*E. zonatum*
		E. fusiforme		*P. n-ciata*
				E. edwini
				E. fusiforme

Table 25. Scheffé multiple comparisons test of fish guilds data for variance between subsites. U = upstream subsite, B = bridge subsite, and D = downstream subsite are in the title and at to the end of individual subsites to generate labels.

Fish Guilds U, B, D	
Open Water B - Debris D	Debris B - Open Water B
Near Vegetation B - Debris D	Debris B - Near Vegetation B
In Vegetation B - Debris D	Debris B - In Vegetation B
Debris U - Open Water B	Debris B - Near Vegetation D
Debris U - Near Vegetation B	Debris D - Near Vegetation D
Debris U - In Vegetation B	Debris D - In Vegetation D
Debris U - Benthic B	Benthic B - Debris B
Debris U - Open Water D	Benthic B - Debris D
Debris U - Near Vegetation D	
Debris U - In Vegetation D	
Debris U - Benthic D	

Table 26a. Normalized data sets to be used in PCA and DFA for fish and macroinvertebrate assemblages. Column labels ISH – TVW correspond with Table 2. To conserve space "fish species by length in hectometers" = FSH.

Subsites	FSH	ln ISH	OM	BM	TVW
1AB	12	3.135494216	0.764	4.863	5.05
1BB	25	2.833213344	0.884	5.627	15.08
1CB	6	2.197224577	0.056	0.356	4.87
2AB	19	2.48490665	0.695	4.424	24.8
2BB	18	2.48490665	0.54	3.437	13.3
2CB	15	3.555348061	0.97	6.174	2.2
3AB	23	3.465735903	0.024	0.153	0
3BB	15	2.079441542	0.27	1.719	0
3CB	21	2.197224577	0.013	0.083	4.55
4AB	15	2.772588722	0.053	0.337	30.88
4BB	15	2.302585093	0.621	3.953	0
4CB	10	2.397895273	0.397	2.527	0
4DB	17	2.63905733	0.092	0.586	0
4EB	15	3.044522438	0.1	0.637	0

Table 26b. Normalized data sets to be used in PCA and DFA for fish and macroinvertebrate assemblages. Column labels O_2S – pHW correspond with Table 2.

Subsites	O_2S	pHS	ln CS	ln TS	O_2W	pHW
1AB	4	3.45	4.6858281	3.19047635	3.35	3.72
1BB	6.8	5.4	5.1795338	3.20274644	3.1	5.87
1CB	4.5	3.42	5.2933048	3.33220451	8.6	7.6
2AB	1	4.46	4.6746962	3.18635263	3.07	3.85
2BB	1.91	5.8	4.2484952	3.24649099	6.98	4.94
2CB	2	7.55	7.0003345	3.26193531	5.4	8.9
3AB	2	7.2	5.8435444	3.26956894	4.08	6.83
3BB	2.5	7.37	5.9661467	3.25809654	4.74	7.05
3CB	7	6.91	4.8828019	3.49650756	15.48	7.76
4AB	6	7.3	6.423247	3.28840189	11.29	7.54
4BB	4.01	7.38	6.0497335	3.38439026	12.01	7.87
4CB	2	7.25	5.0304379	3.25809654	12.04	7.55
4DB	2.99	6.67	5.8171112	3.31054301	12.22	7.92
4EB	4.28	6.53	5.3798974	3.31054301	4.96	6.75

Table 26c. Normalized data sets to be used in PCA and DFA for fish and macroinvertebrate assemblages. Column labels CW – Cu correspond with Table 2.

Subsites	ln CW	TW	*SQRT x SO*	SQRT x Cu
1AB	4.883559212	14.1	1	0.20736441
1BB	4.766438334	18.3	1	0.21908902
1CB	5.264243386	9.8	1	0.4472136
2AB	4.703203926	14.7	1.414213562	0.14491377
2BB	4.593097605	11.6	1.414213562	0
2CB	6.562444094	13.2	1.414213562	0
3AB	5.683579767	14.6	1.732050808	0.35355339
3BB	5.624017506	14.6	1.732050808	0.40865633
3CB	5.680172609	12.6	1.732050808	0.5
4AB	6.809039306	6.4	2	0.2236068
4BB	6.771935556	7	2	0
4CB	6.00635316	8.6	2	0
4DB	5.932245187	8.9	2	0
4EB	5.156177599	17.3	2	0.26645825

Table 26d. Normalized data sets to be used in PCA and DFA for fish and macroinvertebrate assemblages. Column labels G – D correspond with Table 2.

Subsites	SQRT x G	Sa	SQRT x Si	SQRT x Cla	D
1AB	0.1	0.87	0.447213595	0.316227766	0.83
1BB	0.3162278	0.2	0.707106781	0.806225775	0.95
1CB	0.1414214	0.92	0.223606798	0	1.01
2AB	0	0.7	0.316227766	0.447213595	0.96
2BB	0.1	0.84	0.316227766	0.223606798	1.47
2CB	0	0.68	0.141421356	0.547722558	1.16
3AB	0.1414214	0.96	0.141421356	0	0.41
3BB	0.3162278	0.88	0.141421356	0	0.52
3CB	0.2236068	0.95	0	0	0.17
4AB	0.1	0.89	0.223606798	0.223606798	0.71
4BB	0	0.2	0.447213595	0.774596669	1.25
4CB	0.4472136	0.4	0.447213595	0.447213595	0.38
4DB	0.1414214	0.91	0.223606798	0.141421356	1.08
4EB	0.1	0.95	0.141421356	0.141421356	0.42

Table 26e. Normalized data sets to be used in PCA and DFA for fish and macroinvertebrate assemblages. Column labels TLH – E correspond with Table 2.

Subsites	**TLH**	**ln SB**	**YB**	**BW**	**ln BL**	**BRP**	**TSA**	**E**
1AB	0.627	3.09	1991	13	4.39445	133	761	58
1BB	0.5581	3.26	1987	25	4.26268	183	760	48
1CB	1.4245	3.47	1981	14	5.8944	308	2721	32
2AB	0.988	4.17	1948	31	4.84419	179	785	51
2BB	1.0136	3.3	1986	15	4.39445	266	1595	39
2CB	0.316	2.08	2005	13	4.11087	137	1082	42
3AB	0.345	3.78	1969	12	5.01728	130	790	32
3BB	0.655	1.95	2006	12	4.82831	212	1561	24
3CB	0.99	1.79	2007	12	6.03069	251	1619	43
4AB	1.0054	2.08	2005	14	4.63473	236	2774	79
4BB	1.068	3.76	1970	12	4.29046	197	3319	73
4CB	1.696	1.95	2006	13	5.57215	268	3722	50
4DB	0.859	1.95	2006	14	5.37064	236	1811	43
4EB	0.577	1.39	2009	13	5.3799	220	1640	39

Table 27. Variable data sets after normalization and selection by Principle Component Analysis or Discriminant Function Analysis identified by data set in preparation for regression analyses. Column labels correspond with Table 2.

PCA	**DFA Fish**	**DFA Macroinvertebrates**
Biological	Biological	Biological
PC #1	ISH	BM
TVW	Chemical	Chemical
PC #2	pHS	O_2S
BM	Physical	CW
OM	$\sqrt{Cu}$	ln CS
Chemical	$\sqrt{Cla}$	Physical
PC #1	Construction	ln TS
pHW	D	TW
O_2W	TLH	$\sqrt{Cu}$
O_2S	SB	$\sqrt{Cla}$
PC #2	BW	$\sqrt{G}$
pHS	E	Sa
O_2S	TSA	Si
pHW	BRP	Construction
Physical		TLH
PC #1		BRP
E		
PC #2		
$\sqrt{SO}$		
$\sqrt{Cla}$		
$\sqrt{Sa}$		
Construction		
PC #1		
TSA		
PC #2		
BRP		

Appendix B:

Figures 1 - 13

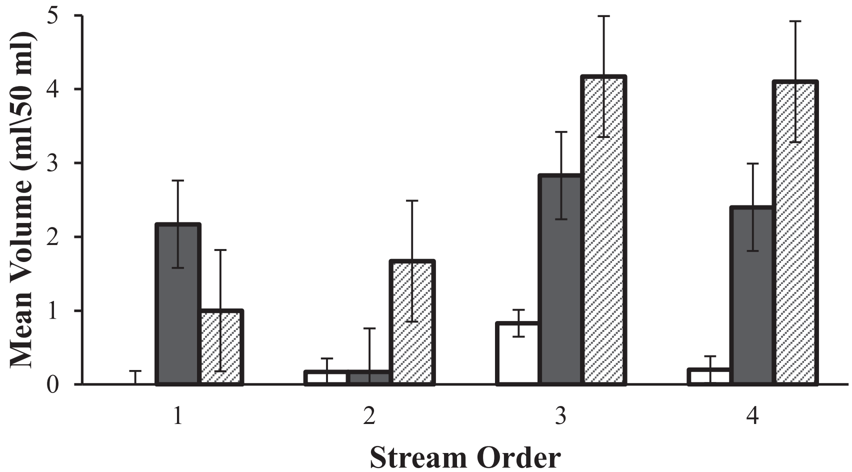

Figure 1. Mean gravel volume for each subsite by stream order. Open bars are upstream subsite, solid bars are bridge subsite, and slash bars are downstream subsite.

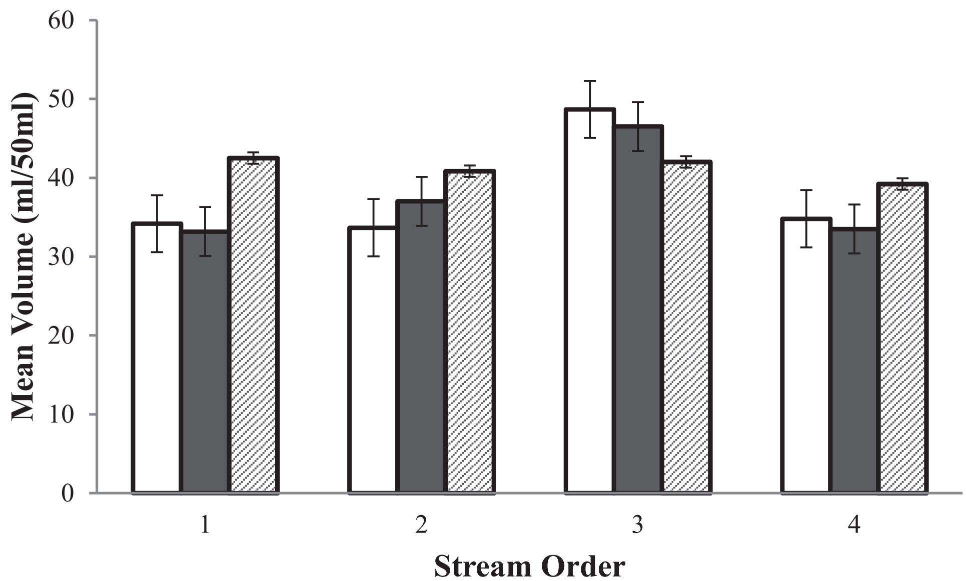

Figure 2. Mean sand volume for each subsite by stream order. Open bars are upstream subsite, solid bars are bridge subsite, and slash bars are downstream subsite.

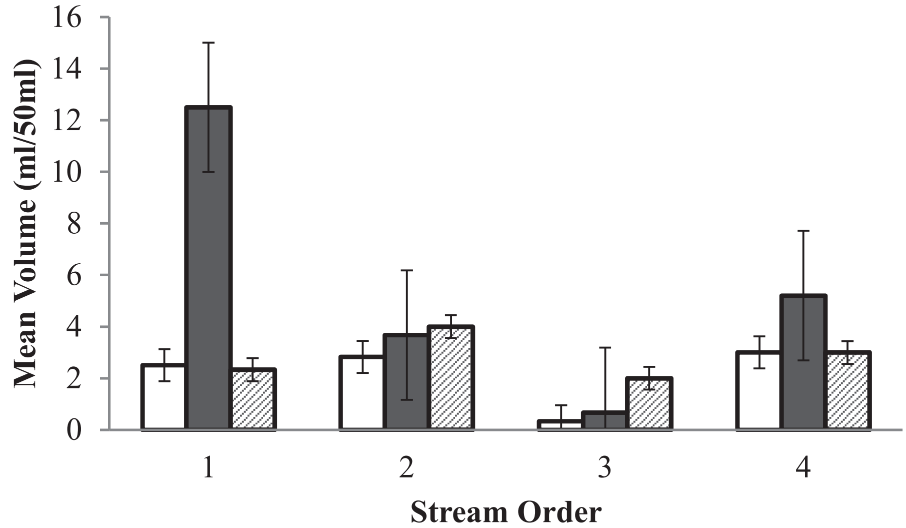

Figure 3. Mean silt volume for each subsite by stream order. Open bars are upstream subsite, solid bars are bridge subsite, and slash bars are downstream subsite.

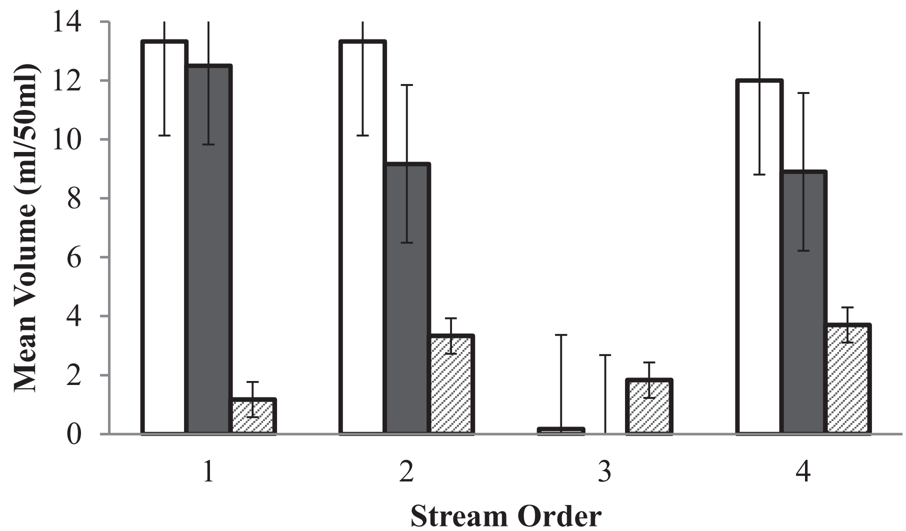

Figure 4. Mean clay volume for each subsite by stream order. Open bars are upstream subsite, solid bars are bridge subsite, and slash bars are downstream subsite.

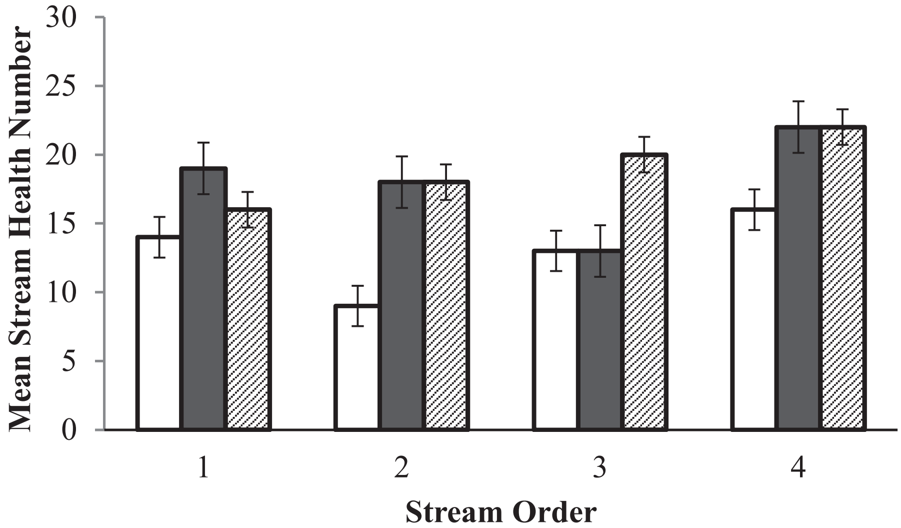

Figure 5. Mean stream health number for each subsite by stream order. Open bars are upstream subsite, solid bars are bridge subsite, and slash bars are downstream subsite.

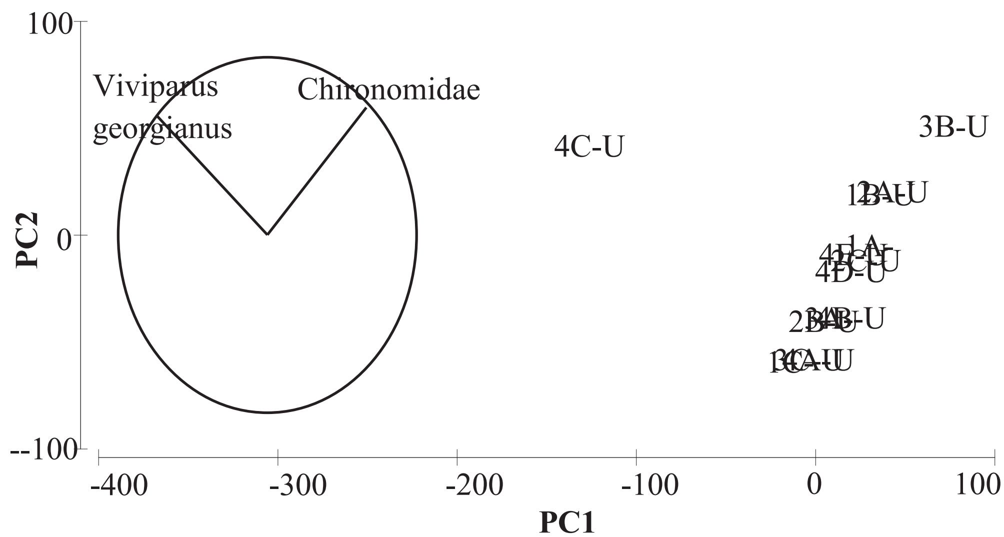

Figure 6. Macroinvertebrate species data for upstream subsites. Graphed eigenvectors provide greater than 90% of all variation.

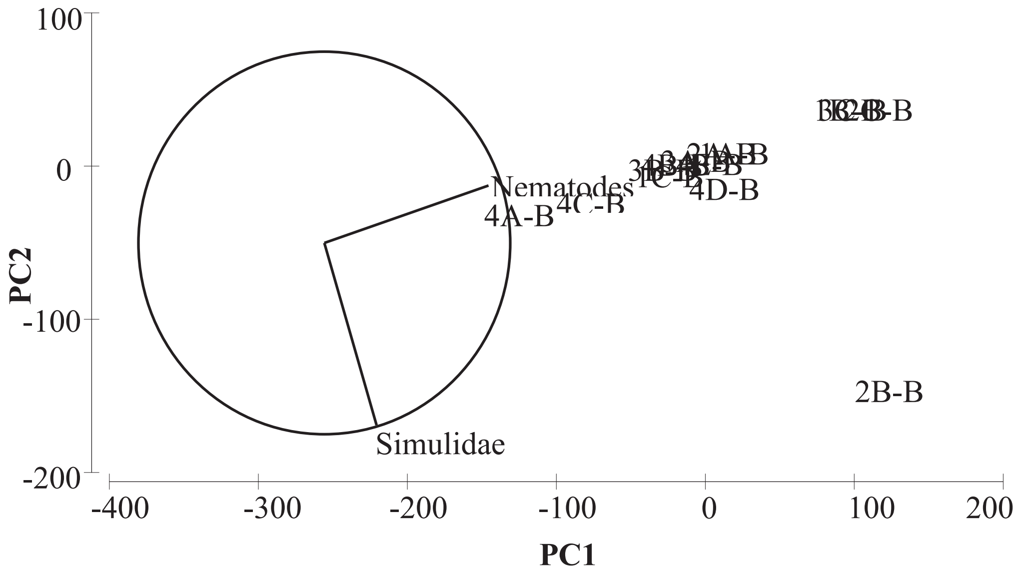

Figure 7. Macroinvertebrate species data for bridge subsites. Graphed eigenvectors provide greater than 90% of all variation.

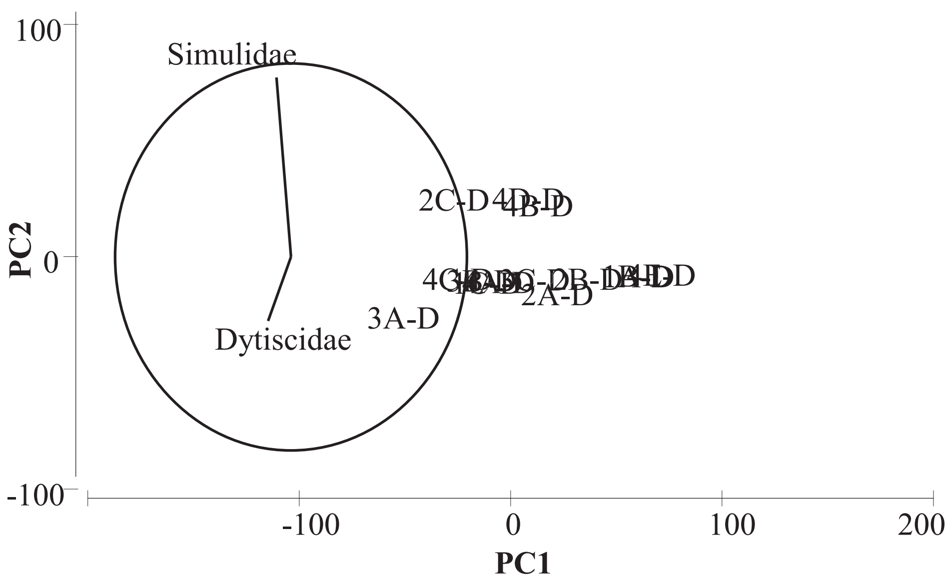

Figure 8. Macroinvertebrate species data for downstream subsites. Graphed eigenvectors provide greater than 90% of all variation.

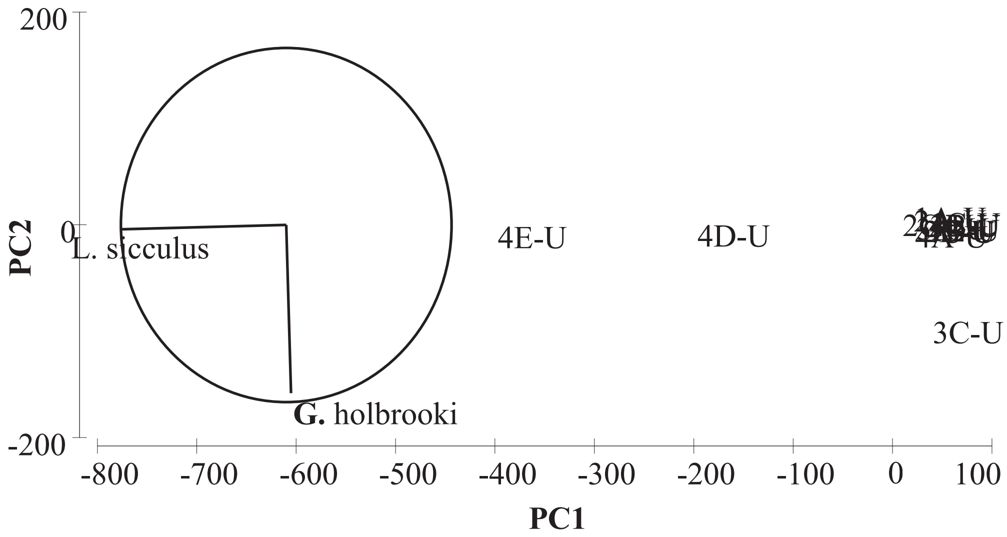

Figure 9. Fish species data for upstream subsites. Graphed eigenvectors provide greater than 90% of all variation.

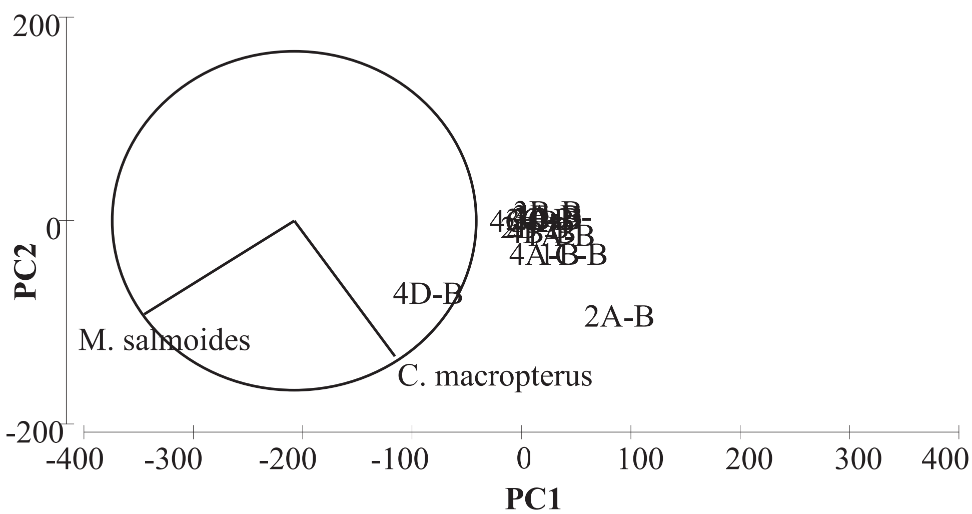

Figure 10. Fish species data for bridge subsites. Graphed eigenvectors provide greater than 90% of all variation.

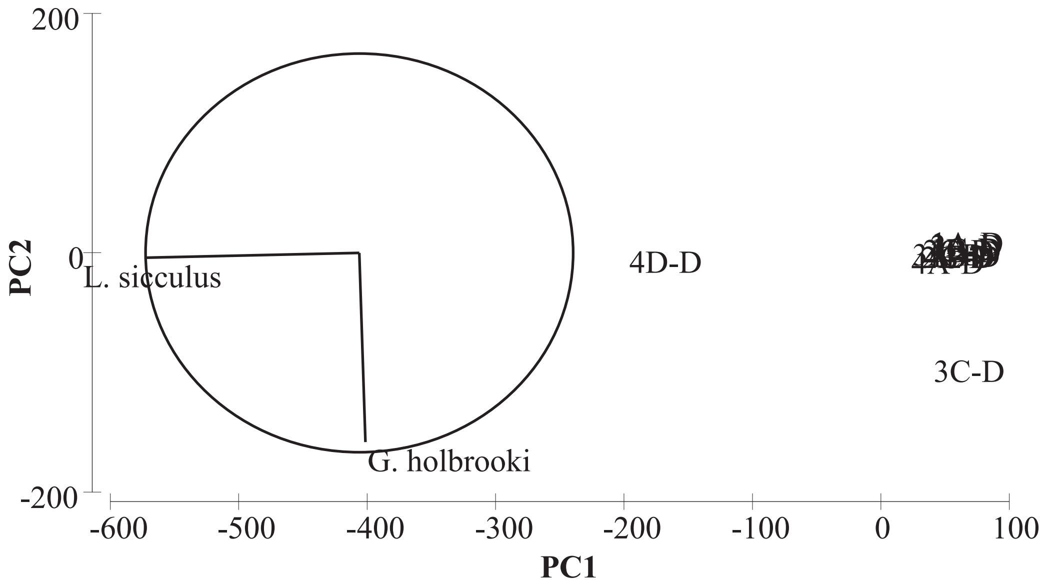

Figure 11. Fish species data for downstream subsites. Graphed eigenvectors provide greater than 90% of all variation.

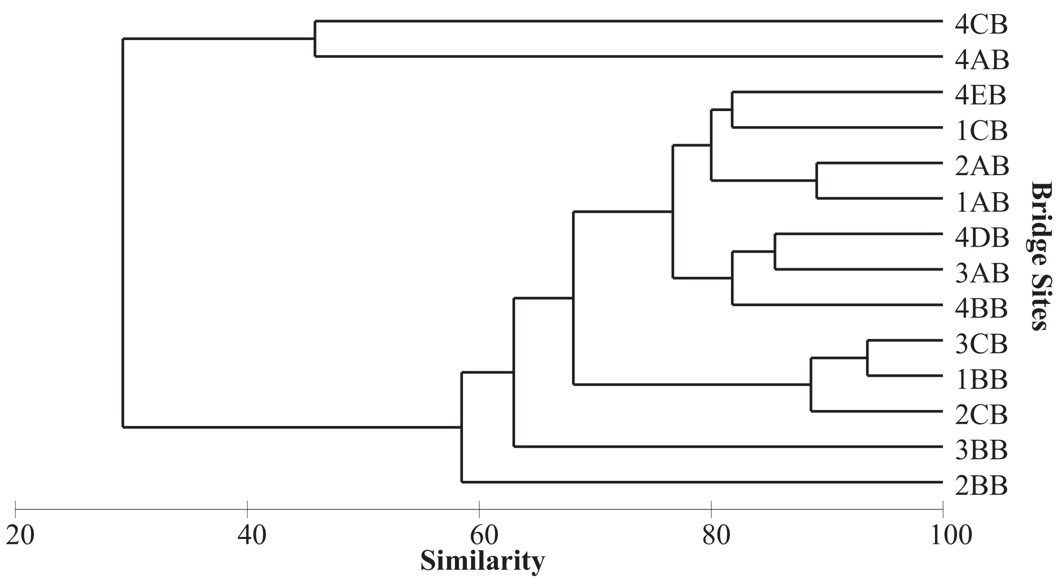

Figure 12. Cluster Analysis of macroinvertebrate species assemblages at bridge subsites.

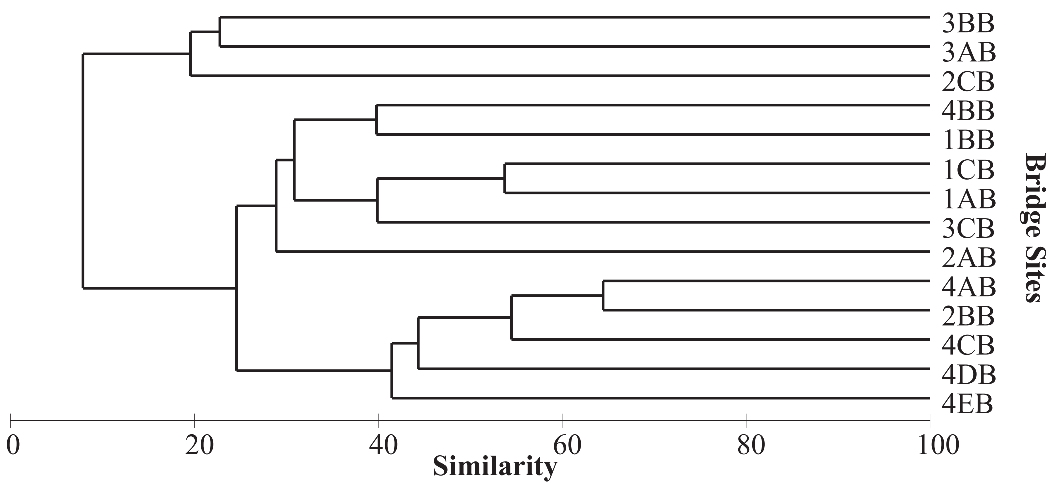

Figure 13. Cluster Analysis of fish species assemblages at bridge subsites.

Appendix C:

Animal Use Approval

VALDOSTA STATE UNIVERSITY
Building for Our Next Century

October 24, 2011

Dr. David Bechler
Department of Biology
Valdosta State University

RE: AUP-00031-2010
Directed Study (BIOL 4950 & 6950)
Survey of fish fauna in barrow pits

Dear Dr. Bechler:

The continuation of your Animal Use Protocol referenced above has been approved by the Institutional animal Care and Use Committee under Animal Welfare Assurance Number A4578-01. This continuation approval is through August 12, 2012 at which time you will be required to submit an annual report and request for continuation through the protocol expiration date of August 12, 2013.

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

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

Sincerely,

Barbara Gray

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

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

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