FRESHWATER FISHERIES STATEWIDE RESEARCH

ANNUAL PROGRESS REPORT

F-63

January 1, 2005 – December 31, 2005

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Study Title: **STATEWIDE RESEARCH**

Job Title: **Annulus Validation And Age Structure Of Two Catostomids In
                         The Broad River, SC**

Period Covered   January 1, 2005 – December 31, 2005

Results and Discussion

Annulus Validation

On 5 October 2001 we collected 91 "brassy" jumprock *Scartomyzon spp* (mean TL 321 mm;

range 190 – 445 mm TL) and 33 notchlip redhorse *Moxostoma collapsum* (mean TL 399 mm; range

217 – 475 mm TL) from the lower Broad River near Columbia, SC. Each of the fish collected

received an intraperitoneal or intramuscular injection of 0.5 cc of Liquamycin LA-200® (OTC

injection) to produce a chemical mark on its otoliths that could be used to document single annulus

formation per year. The fish were transported to the Cheraw State Fish Hatchery and placed in grow-

out ponds. On 5 November 2002, 12 "brassy" jumprock and 14 notchlip redhorse were harvested

from the grow-out ponds. Survival of "brassy" jumprock (13%) in grow-out ponds was poor while

that of notchlip redhorse was much better (43%).

The otoliths (lapilli) from each fish were removed to estimate age and examined for chemical

marks. Estimated ages of "brassy" jumprock ranged from 3 to 13 and estimated ages of notchlip

redhorse ranged from 4 to 13. Marking efficacy was 100%, with each of the 26 otoliths examined

containing an OTC mark positioned on or very near the last fully formed annulus. Based on

distances between successive annuli, we estimate that there was a year's growth between the last

fully formed annulus and the margin. It appears that "brassy" jumprock and notchlip redhorse

otoliths form one annulus each year; therefore otoliths are an appropriate structure to use for age estimation.

The position of the OTC mark on the last fully formed annulus was unexpected. One interpretation of this result is that annulus formation occurs during fall, which would be inconsistent with the annulus formation of other Catostomids studied in different locales. An alternative explanation is that the stress associated with handling, marking and transporting caused an anomalous check on the otolith that was incorrectly interpreted as an annulus. However, the spacing of annuli on the otoliths was consistent with yearly growth and two fish had otoliths with an incomplete annulus forming on the margin; therefore we believe that annulus formation in these species occurs once per year during the fall. A separate yearlong study involving monthly collections of "brassy" jumprock and notchlip redhorse otoliths would be needed to resolve the question of when annulus formation occurs in these species.

Age Structure

One hundred twenty "brassy" jumprock (mean TL 311 mm; range 103 – 424 mm TL) and 200 notchlip redhorse (mean TL 367 mm; range 157 – 485 mm TL) were collected from 10 sites along the Broad River during fall 2001. To estimate age, otoliths (lapilli) were removed from 75 "brassy" jumprock and 121 notchlip redhorse and read independently by two experienced readers. Reader agreement was 77% and 70% for "brassy" jumprock and notchlip redhorse respectively. For "brassy" jumprock reader disagreement ranged from 1 to 3 years with the majority of disagreements (94%) being 1 year. For notchlip redhorse reader disagreement ranged from 1 to 4 years with the majority of disagreements (86%) being 1 year. Final estimated age was determined by concurrence between the two original readers and a third experienced reader when disputed otoliths were reread simultaneously and discussed. When reader-estimated age was plotted against final estimated age

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some trends were apparent reader 1 tended to overestimate fish age while reader 2 tended to underestimate fish age (Figure 1). Most disagreements in estimated age were due to differences identifying the first annulus.
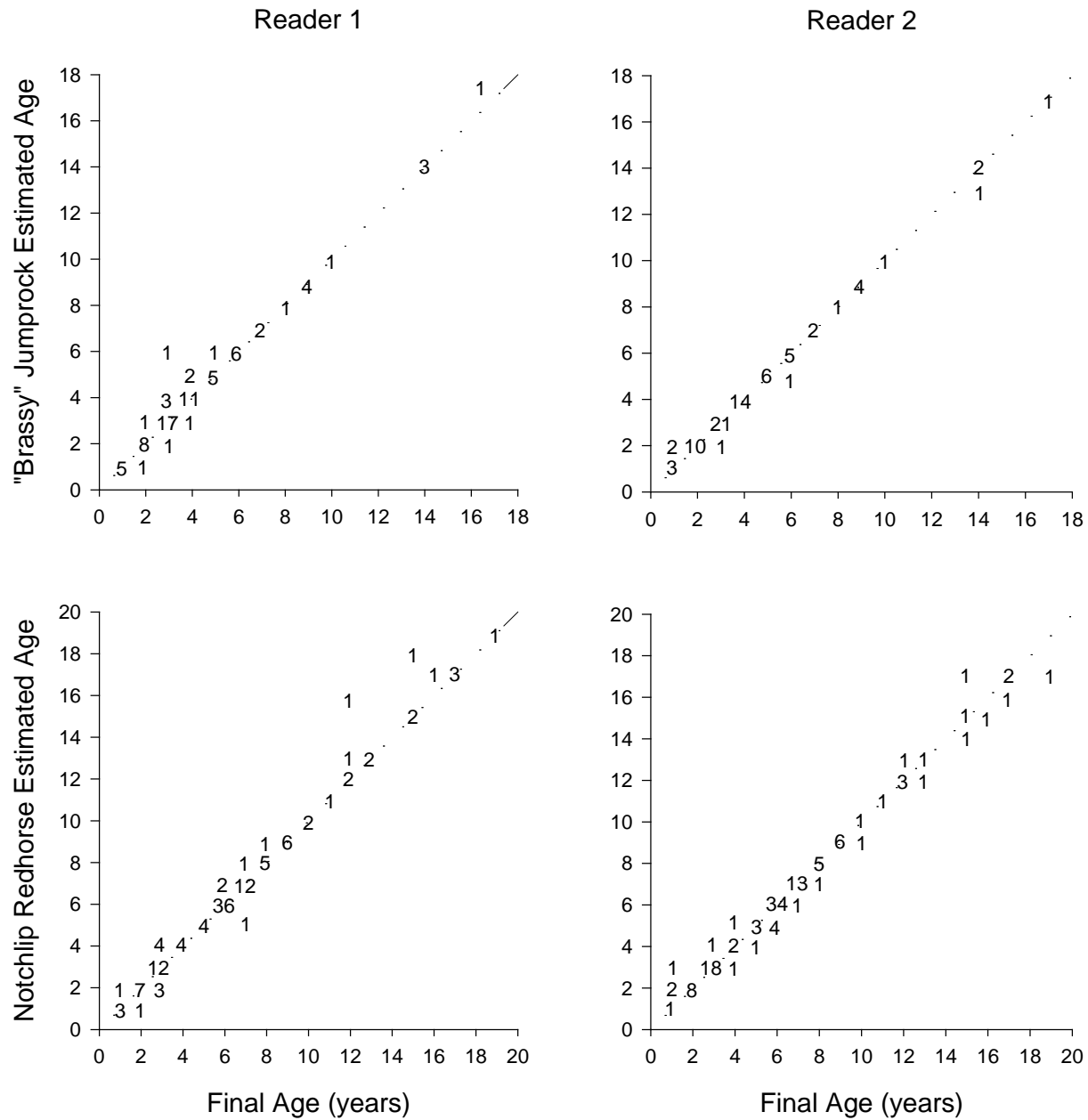
Figure 1.   Age-bias graphs for two readers of "brassy" jumprock and notchlip redhorse otoliths. Numbers indicate sample size. Dashed line represents agreement

between reader-estimated age and final estimated age as determined by a concert read.

"Brassy" jumprock and notchlip redhorse are relatively long-lived species in the Broad River, SC. Estimated ages for "brassy" jumprock ranged from 1 to 17 and estimated ages of notchlip redhorse ranged from 1 to 19 (Table 1). Plots of age-class frequency distribution (Figure 2) indicated that both species recruit to boat electrofishing gear in their 3rd year of growth. The dominant age class for "brassy" jumprock was 3 year-olds, accounting for 29% of the fish collected. The dominant age class for notchlip redhorse was 6 year-olds, accounting for 32% of the fish collected. Age-4 and age-5 notchlip redhorse were grossly underrepresented in our samples, potentially indicating poor recruitment of those year classes.

Table1.   Mean total length at estimated age for "brassy" jumprock and notchlip redhorse collected from the Broad River during fall 2001. Number of aged fish in parentheses.

Age	Mean TL (mm)	
	"Brassy" jumprock	Notchlip redhorse
1	155 (5)	240 (4)
2	201 (10)	220 (8)
3	278 (22)	300 (19)
4	326 (14)	338 (4)
5	331 (6)	375 (4)
6	381 (6)	376 (38)
7	383 (2)	426 (14)
8	390 (1)	397 (6)
9	381 (4)	385 (6)
10	363 (1)	440 (2)
11		450 (1)
12		399 (4)
13		399 (2)
14	409 (3)	
15		439 (3)
16		435 (1)
17	386 (1)	437 (3)
19		419 (1)
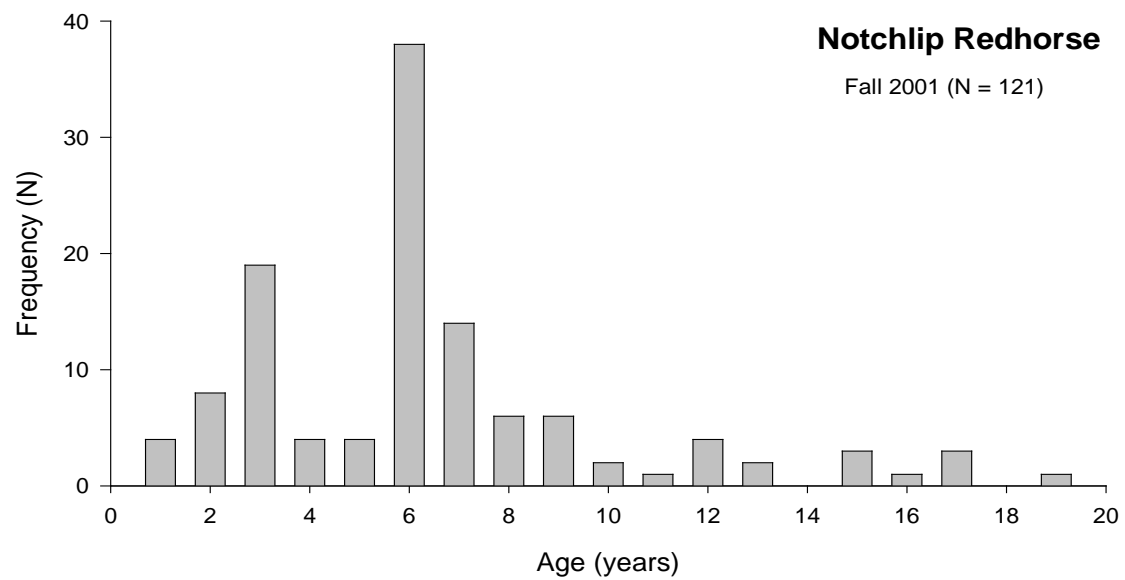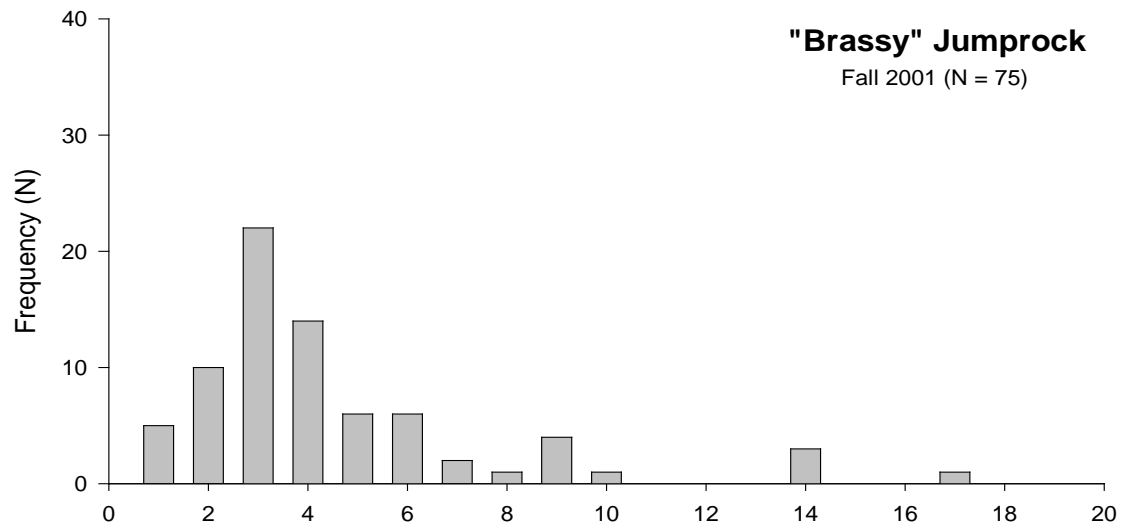
Figure 2.   Age class frequency distributions of "brassy" jumprock and notchlip redhorse
collected from the Broad River, SC, during fall 2001.

Recommendations

- Investigate growth differences in both species among sites sampled or river sections.

- Resolve question of when annulus formation occurs in both species.

- Produce technical report by 1 April 2006

Prepared By: <u>James Bulak</u>   Title: <u>Research Regional Coordinator</u>

Job Title: **Water quality and productivity gradients in Lake Murray**

Period Covered October 1, 2004 – December 31, 2005

Results and Discussion

A general limnological assessment of Lake Murray was performed in May and June 2005. The objective of conducting this survey was to characterize productivity and water quality characteristics at various potential stocking locations for striped bass juveniles. The working hypothesis was that areas of relatively high productivity might be superior stocking sites.

Lake Murray was divided into an upper and lower lake zone of approximately equal area. During three two-day sampling periods in May and June 2005, temperature, dissolved oxygen, conductivity, incident light extinction, and chlorophyll a profiles were taken at 2 m depth intervals at multiple upper and lower zone sampling sites (Figure 1). From light extinction data, we determined the depth of the photic zone (i.e. the depth that receives 1% of the incident light). Mean temperature, dissolved oxygen and conductivity values were determined using measurements taken at the surface, 2, 4, 6, and 8 m. Mean chlorophyll concentration was calculated by using measurements that were wholly or partly within the photic zone (i.e. if the photic zone extended to 7 m and chlorophyll measurements were taken at 6 and 8 m, both values would be included in the calculation). The significance of differences between the mean values of temperature, dissolved oxygen, depth of photic zone, and chlorophyll concentration were evaluated using the two sample T-test for unequal variances at alpha = 0.05. To estimate the mass of chlorophyll in the photic zone per unit area of lake surface (mg/m^2), mean chlorophyll concentration (mg/m^3) was multiplied by the depth of the photic zone (m) for each site.
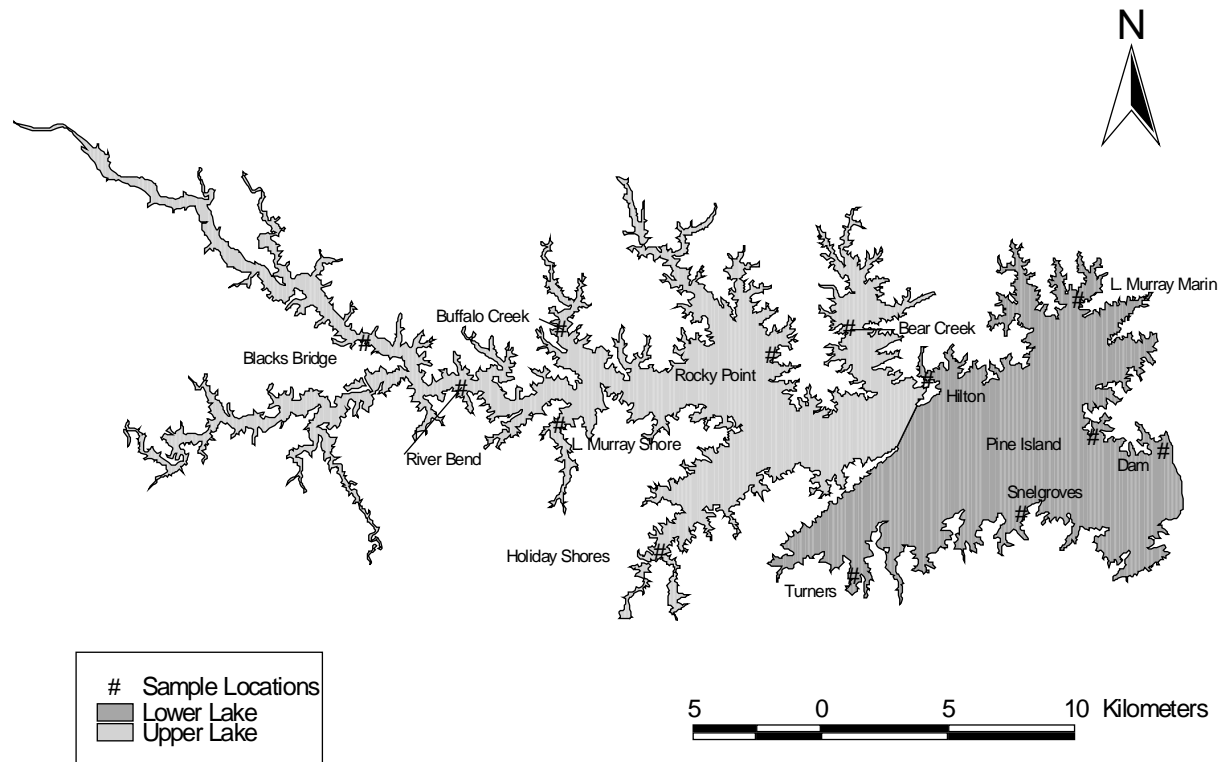
Figure 1.　　　Limnological sampling sites for Lake Murray in 2005.

Within their respective photic zones, the upper zone of the lake had substantially different water quality and was more productive than the lower basin (Table 1). While mean temperature was slightly warmer in the upper zone, the difference was not significant. Dissolved oxygen was significantly lower in the upper zone. This statistical difference was generally due to shallower, more nutrient rich conditions in the upper zone, which caused hypoxic condition s in the hypolimnion during this time period. Mean chlorophyll concentrations within the photic zone were significantly higher in the upper zone, probably associated with higher nutrient concentrations

generally observed in the upper end of reservoirs. The depth of the photic zone was significantly less

in the upper lake, probably due to a combination of higher primary productivity and increased

turbidity in the upper lake. The mean mass of chlorophyll in the photic zone per unit area of lake

surface was 52.8 mg/m^3 and 35.1 mg/m^3 in the upper and lower lake zones, respectively. If survival

of striped bass juveniles were directly correlated with productivity, this would suggest stocking 60%

of the stocked fish in the upper lake and 40% in the lower lake. This suggestion assumes that

predation potential is equal in each zone.

Table 1.   Mean water quality and primary productivity measurements obtained from
multiple sites within the upper and lower half of Lake Murray. Sampling was
conducted on during three separate sampling periods – mid-May, early June,
and mid-June, 2005. Calculation methods are provided in text.

Zone	N	Temperature (C)	Dissolved Oxygen (mg/L)	Depth of photic zone (meters)	Chlorophyll (mg/m3)	Chlorophyll (mg/m2)
Lower	15	22.2	7.3	7.0	5.1	35.1
Upper	14	22.6	5.4	4.0	13.5	52.8

While the upper lake was substantially more productive, there was variation within each

zone. Overall, the most productive sites were located at a zone located between the Buffalo

Creek/Lake Murray Shores area in the upper lake to the upper boundary of the lower zone (i.e. Hilton

and Turners) (Table 2). Information suggests the Hollow Creek, Beaverdam Creek, and Crystal Lake

embayments are more productive than the Bear Creek and Lake Murray Marina embayment.

Additional sampling is needed in 2006 to better define productivity gradients within the main stem

and major embayments of Lake Murray. Once the productivity gradient is defined, stocking

decisions (number and location) could be directly related to primary production expected at or near a stocking site.

Table 2.   Mean mass of chlorophyll within the photic zone at Lake Murray sites
sampled in 2005. Sites are listed from the most upstream to the most
downstream locations. The highest five chlorophyll measurements are
bolded.

Site	N	Chlorophyll (mg/m2)
Black's Bridge	3	42.8
River Bend	3	39.5
Lake Murray Shores	3	**67.0**
Buffalo Creek	2	**67.4**
Rocky Point	2	**51.7**
Holiday Shores	1	**46.4**
Bear Creek	1	18.6
Hilton	3	40.6
Turners	1	**56.7**
Pine Island	3	36.8
Snelgroves	1	17.2
Lake Murray Marina	3	29.6
Dam	3	37.8

Recommendations

- Use 2005 data to help plan a stocking strategy in 2006.

- Perform additional limnological sampling in 2006 that would more clearly define the main lake and major embayment productivity gradient. Evaluate correlation of existing SCDHEC/ EPA/SCE&G monitoring data with observed trends in primary productivity.

- If resources allow, compare the growth rate of stocked striped bass juveniles in a high and low productivity area to further evaluate the hypothesis that faster growth will occur in high productivity areas.

Prepared By: <u>James Bulak</u>   Title: <u>Research Regional Coordinator</u>

Job Title: **Assessing Hybridization Among Native and Introduced Black Bass Species in the Savannah River Drainage**

Period Covered   January 1, 2005 – December 31, 2005

Results and Discussion

There are two primary objectives of this study. One is to describe the geographic structure of redeye bass *Micropterus coosae* throughout its range. We reported last year that fish were collected from nine streams in the Savannah. In the past year redeye bass were collected from two additional steam locations in the Savannah drainage. We collected N=12 redeye from Eastatoee Creek and N=8 from Little Coldwater Creek. This brings our total stream and river collections on the Savannah to N=169 fish from 11 populations. Samples have also been secured from the Saluda River, the only location in the Santee drainage where redeye bass are found, and from the Chatahoochee and Mobile Bay drainages, which represent the Gulf Slope portion of the fishes range (Figure 1.).

The phylogenetic relationships among the drainages represented have been examined. Results indicate that populations of the Atlantic slope drainages are distinct from those of the Gulf Slope (Figure 2.). The genetic distance observed between Saluda River and Savannah River populations are small in comparison, and are even small compared to some distances observed within the Savannah drainage (Figure 3.). This indicates that the Saluda River population is the result of one or more translocations from Savannah drainage streams, and not part of the fish's original range.

The other objective of this study is to assess hybridization among redeye bass, and other *Micropterus spp*. of the Savannah drainage. These include the native largemouth bass *M. salmoides*, and the introduced smallmouth bass *M. dolomieu* and spotted bass *M. punctulatus*. Work in 2005

toward this objective has focused on the development of 5 genetic markers, the mtDNA Cytchrome *b*

locus, and the nuclear DNA loci Actin Intron, ITS Intron2, S7 Intron1, and LDHA Intron 6. We
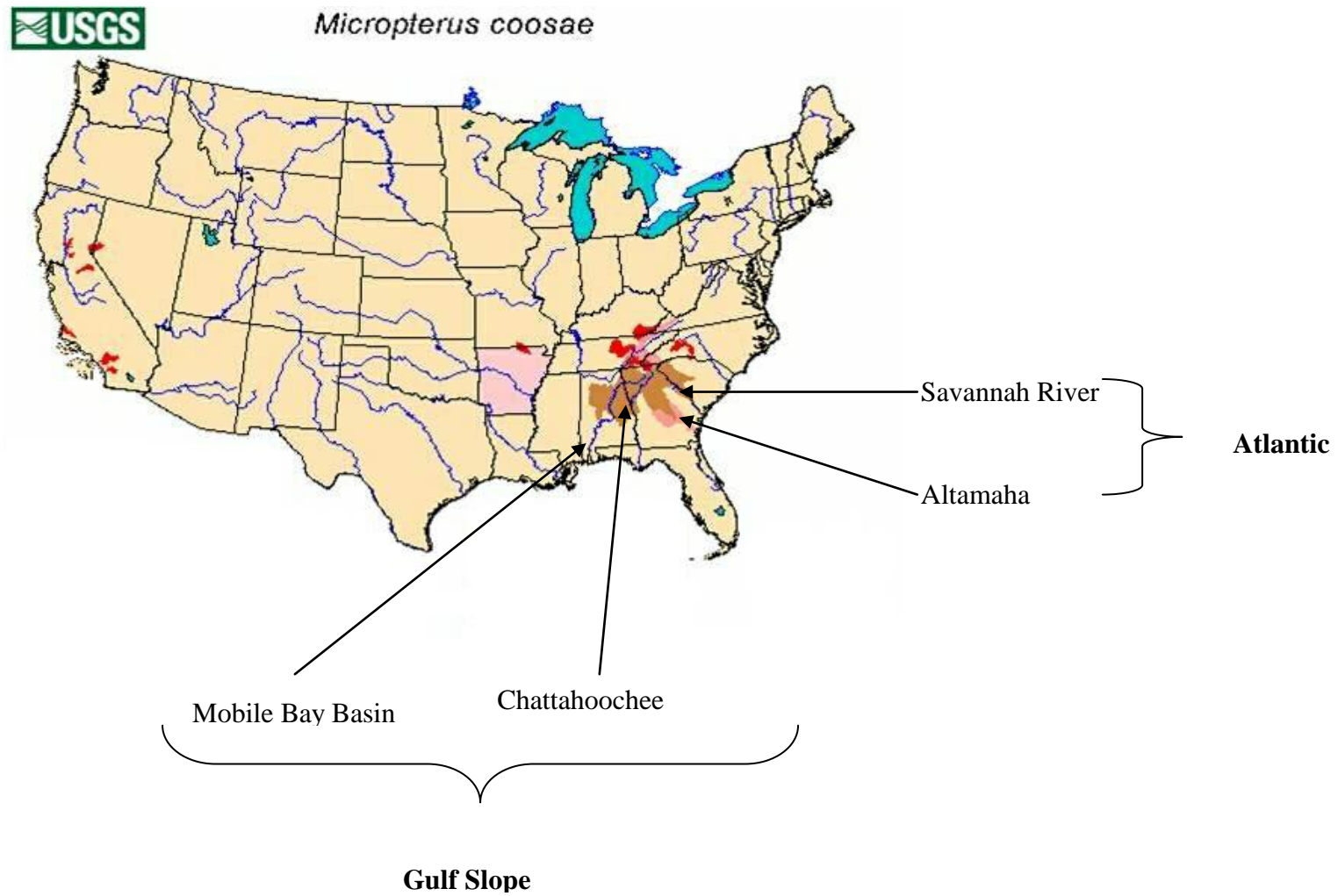
Figure 1. Map showing the drainages of the redeye bass *Micropterus coosae* native range.

Figure 2. Phylogentic tree showing drainage level relationships among populations of redeye bass *Micropterus coosae* surveyed.
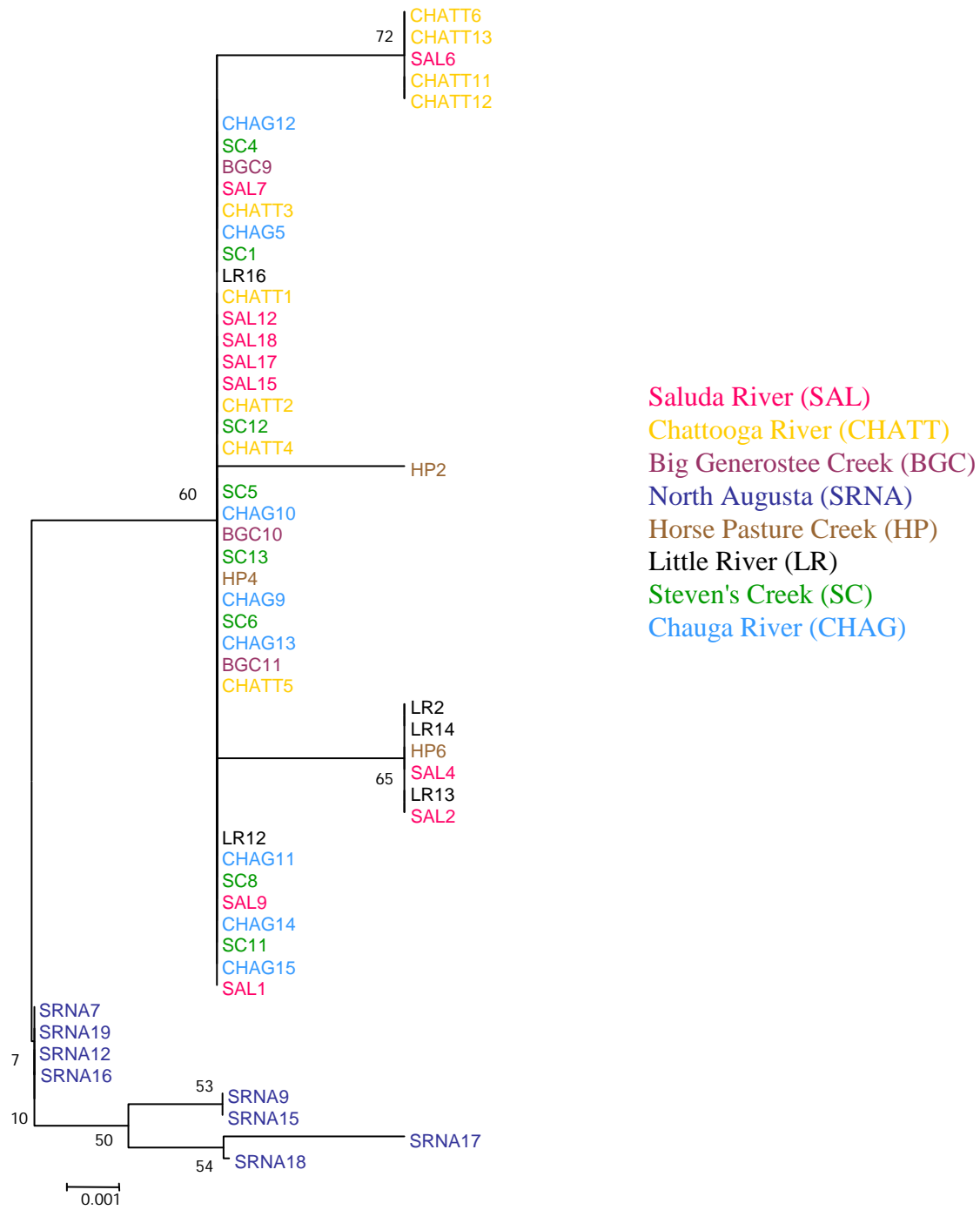
Figure 3. Phylogenetic tree showing relationships among individuals of the Savannah drainage and the Saluda River.

reported last year that N=1175 bass were collected from Lakes Jocassee, Keowee, Hartwell, and Richard B. Russell. Genetic sequencing of these fish at the markers developed will be underway soon.

Recommendations

This genetic survey is funded through August of 2006. In 2006, complete genetic sequencing for all stream samples collected. Efforts are underway to collect individuals from the Altamaha drainage. Once collections and sequencing are complete, include this drainage in analysis to better define the genetic structure of the redeye bass, and to better assess the uniqueness of the Savannah drainage populations. Complete marker development and genetic sequencing of all reservoir samples. Quantitatively assess hybridization in the reservoirs and assess the purity of redeye bass populations in the associated streams. Final reporting will evaluate the present and potential genetic impact of introduced *Micropterus sp*. on the redeye bass of the Savannah drainage.

In addition to tissues for genetic analysis, length and weight data, and otoliths are available for most reservoir samples. Upon completion of genetic evaluations, individual specific data can be combined with available age and growth information to compare hybrids and pure species. An evaluation of the impact of hybridization on the black bass fisheries of the Savannah Lakes may be possible.

Prepared By: Jean Leitner   Title: Fisheries Biologist

Job Title: **Zoogeography of Centrarchidae of the South Atlantic Slope**

Period Covered   January 1, 2005 – December 31, 2005

Results and Discussion

The objective of this study is to evaluate levels of within and among population diversity for

six species from the family Centrarchidae; redbreast sunfish *Lepomis auritus* (RBS), redear sunfish

L. microlophus (RES), warmouth *L. gulosus* (WAR), dollar sunfish *L. marginatus* (DSF), spotted

sunfish *L. punctatus* (SOS), and mud sunfish *Acantharchus pomotis* (MDS). We have sampled

populations from the Savannah, Edisto, Santee, and Pee Dee drainages. We reported previously that

our initial data set did not show sufficient variation to assess population structure in two of the

species studied, mud sunfish and dollar sunfish. In this study period work has focused on increasing

our data set through further genetic sequencing, and on final data compilation and analysis.

Significant progress has been made toward that end. We will report here on available results, and

expect a comprehensive final report to be completed by March 15 of this year.

Our data set consists of DNA sequences of approximately 610 nucleotides of the 5' and 3'

ends of the mitochondrial ND2 locus. Table 1 shows the number of sequences successfully obtained

by species/drainage. The low number of sequences from mud sunfish are due to difficulty

encountered in resolving sequences for that species.

Table 1.   Number of sequences included in analysis. Data is listed by species/drainage.

No. Seq. / Drainage	Species					
	DSF	**RBS**	**RES**	**SOS**	**WAR**	**MDS**
Savannah	7	5	5	10	8	0
Edisto	10	8	10	10	10	5
Santee	9	10	8	10	10	2
Pee Dee	10	6	7	8	9	7

Sequence data shows variation within all six species of sunfish surveyed, but that variation differs markedly in magnitude. Nucleon diversities calculated for each species varied from 0.02 – 0.65, with mud sunfish having the lowest observed diversity and redbreast the highest (Table 2). With the exception of the dollar and mud sunfishes, all other species were appreciably diverse.

Table 2.   Nucleon diversity, by species, for six species of sunfish surveyed.

Species	Nucleon Diversity
RBS	0.65
RES	0.53
WAR	0.51
SOS	0.43
DSF	0.11
MDS	0.02

This hierarchy of nucleon diversity is mirrored in computed Φ_{st} values, which partition total variance into within and among drainage components (Table 3.). Those species with small amounts of haplotype diversity (DSF, MDS) likewise had the lowest components of between drainage variation. Similarly, those species with relatively high haplotype diversities had, at least in two cases (WAR, RES), fairly large components of between population variance.

Table 3.   Partitioning of nucleon diversity to among and within drainage sources for six sunfish species.

Species	Proportion of Nucleon Diversity	
	Among Drainages	Within Drainages
WAR	0.35	0.65
RBS	0.20	0.80
RES	0.03	0.97
SOS	0.02	0.98
DSF	0.01	0.99
MDS	0.01	0.99

We hypothesized that somewhat deep evolutionary divergences among populations would be observed. The present data does support the existence of management units in warmouth and redbreast sunfish, but not in the other four species surveyed. This pattern is contrary to the large components of among drainage variation observed in other freshwater species of the Atlantic Slope. In previous surveys madtoms *Noturus sp*., pygmy sunfishes *Elassoma sp*., and shortnose sturgeons *Acipenser brevirostrum* all were shown to exhibit divergences at least consistent with the existence of individual management units (Bennetts *et al*. 1999, Quattro *et al*. 2001, Quattro *et al*. 2002).

There may be a correlation between the published range of each species, our observed level of haplotype diversity and the degree of genetic differentiation among drainages. For example, both warmouth and redbreast sunfish have very large ranges from essentially Canada along the Atlantic coast of the US and through the Gulf of Mexico. The four other species are relatively confined from as far north of New York south along the Atlantic coast into the Gulf (Rhode *et al*. 1994). Perhaps these species represent remnants of species adversely affected by glaciation during the past 100,000 years.

This and other possible explanations for the pattern shown in our data set will be further explored in the coming months. Between now and March 15 work will focus on some limited remaining lab work, additional data truthing and analysis, and production of a final report. Lab work will include filling in missing 5' or 3' sequences for select individuals. DNA will also be re-extracted from some mud sunfish in an effort to increase sample sizes from certain drainages. Further analyses will include the incorporation of computed genetic distances among the haplotypes observed.

Recommendations

Complete lab work and data analyses over next three months. Produce final report by March 15, 2006.

Literature Cited

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Prepared By: Jean Leitner   Title: Fisheries Biologist

Job Title: **Statewide Stream Classification Based on Fish Assemblage Structure**

Period Covered January 1, 2005 - December 31, 2005

Results and Discussion

Fish Assemblage Structure Across South Carolina

I tested the utility of various aquatic classifications in organizing the predominant patterns of variation in fish species composition and abundance among 116 stream sites sampled in 2003 and 2004. The 50 sites sampled in 2003, located in three major drainages and seven ecoregions, were selected based on the best judgment of biologists to represent best quality ("reference") streams in their respective districts. The 64 sites sampled in 2004 were randomly selected from the Santee (Enoree, Upper Broad), Pee Dee (Waccamaw, Black), and ACE (Combahee) river basins without regard to ecoregion. One site sampled in 2004 produced no fish and was omitted from the analysis. Sixteen sites were outliers compared to remaining sites due to low richness (<4 species) and were also omitted, resulting in a total of 98 sites. Fishes were sampled following the procedures described in Thomason *et al.* (2002); the total number of fishes collected from all electrofishing passes was used in the analyses presented below.

The sites were categorized based on their physiographic, ecoregional, and drainage basin characteristics (Table 1, Figure 1, Figure 2). A series of strata were defined based on the intersection of drainage and physiographic classes of varying area. The finest stratum (STRAT1) was created by combining unique combinations of minor river drainage by Level IV ecoregion (25 levels). The second stratum was created by combining major river drainage by Level IV ecoregion (STRAT2, 19

Table 1.   Major river drainages and ecoregions sampled in the 2003-2004 Stream Survey. Codes for each drainage and ecoregion are used in subsequent tables and figures.

Major River Drainage (HUC6)	Code
Savannah	030601
ACE	030502
Santee	030501
Pee Dee	030402
Minor River Drainage (DRAIN2)	**Code**
Savannah	1
ACE	2
Saluda	3
Broad	4
Lower Santee	6
Black	7
Waccamaw	10
Level IV Ecoregion (ECOREG4)	**Code**
Crystalline Blue Ridge	661
Inner Piedmont	451
Outer Piedmont	452
Carolina Slate Belt	453
Kings Mountain	454
Carolina Flatwoods	631
Mid-Atlantic Floodplains & Low Terraces	632
Sand Hills	651
Atlantic Southern Loam Plains	652
Southeastern Floodplains and Low Terraces	653
Level III Ecoregion (ECOREG3)	**Code**
Crystalline Blue Ridge	66
Piedmont	45
Southeastern Plains	65
Middle Atlantic Coastal Plain	63
Physiographic Region	**Code**
Crystalline Blue Ridge	66
Piedmont	45
Coastal Plain	64

Level III Ecoregions of the Conterminous United States

Map Source: USEPA, 2003
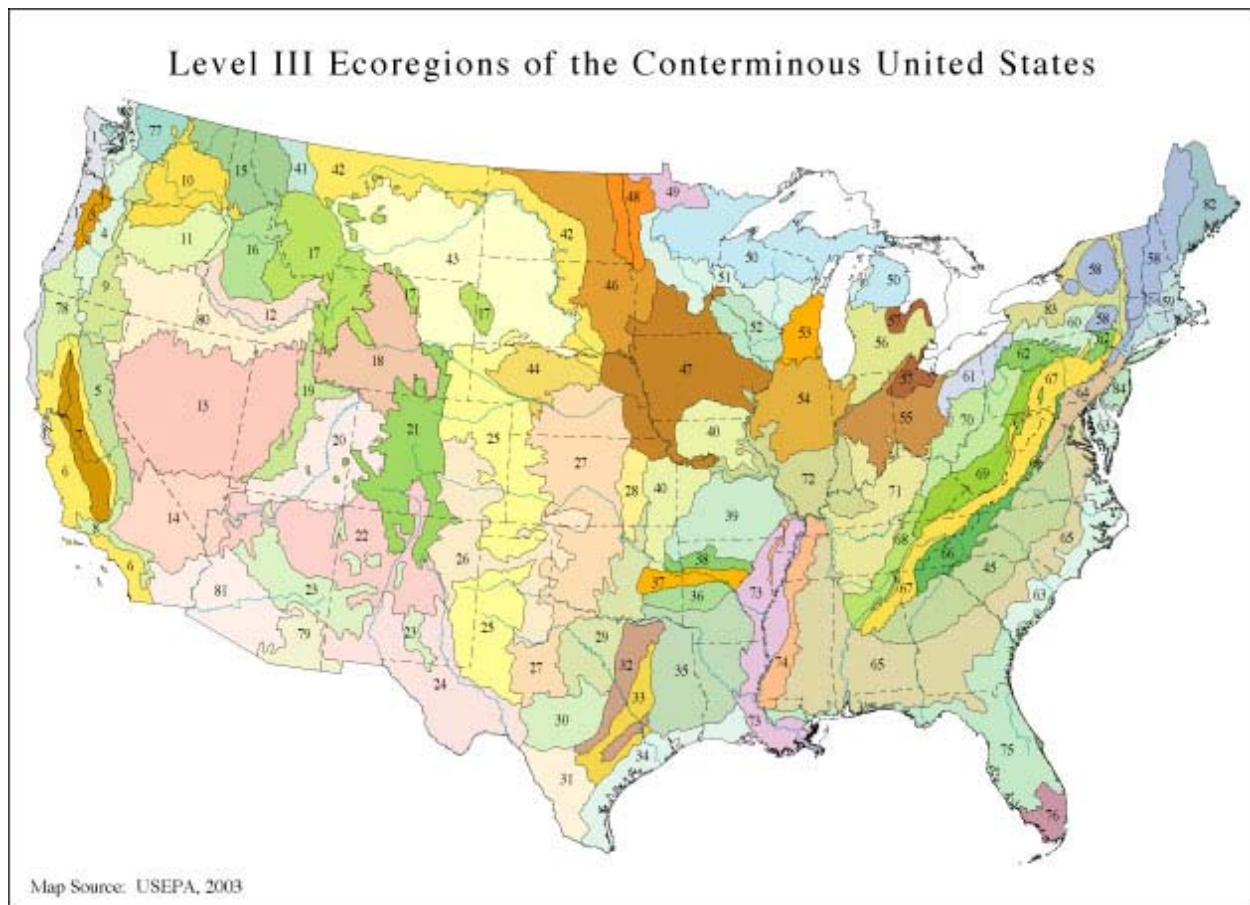
Figure 1.   Map showing Level III ecoregions of the U.S. Under Level IV, these areas are subdivided into additional ecoregion classes (see Table 1).
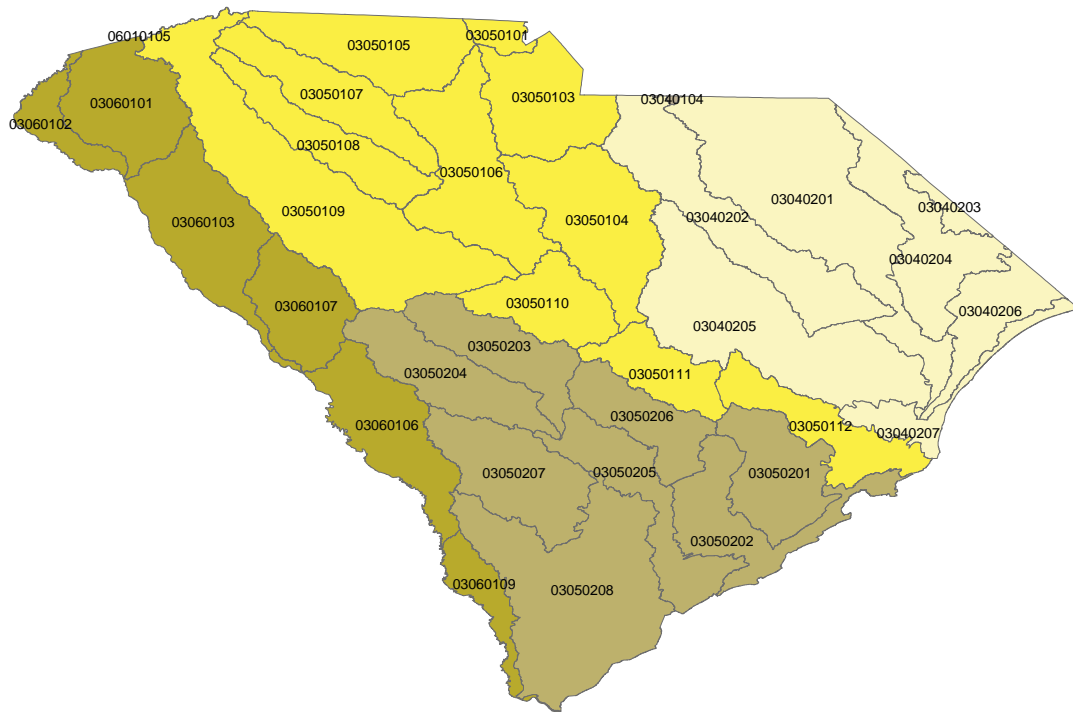
Figure 2.   Map showing major river drainages of South Carolina. Six digit Hydrologic
Unit Codes are unique to Savannah, ACE, Santee, and Pee Dee river systems
(see Table 1).

levels). The third stratum was formed by joining Level III ecoregion with minor river drainage (STRAT3, 14 levels). The fourth stratum was Level III ecoregion by major river drainage (STRAT4, 11 levels). The fifth stratum was physiographic region by minor river drainage (STRAT5, 10 levels), and the last stratum was physiographic region by major river drainage (STRAT6, 7 levels).

Predominant patterns in species composition and abundance among collections were extracted by an indirect-gradient ordination method, non-metric multidimensional scaling (NMS), implemented with PCOrd software (McCune and Mefford 1997). NMS requires no assumptions about distributions or forms of relationships in biological data (Minchin 1987). Only the rank order of dissimilarities (ecological distance) among samples is used, thus NMS estimates nonlinear monotonic relationships in the data. Rare species (single individual collected) were omitted prior to analysis. Abundances of 76 species at the 98 sites were fourth-root transformed prior to analysis to reduce the influence of very abundant species (Clarke 1993). Bray-Curtis dissimilarity values were calculated for each pair of collections as a measure of ecological distance (Faith *et al*. 1987). Two dimensions accounted for 87% of the variance in ecological distance among sites (final stress=15.3), significantly greater than would be expected in random data (Monte Carlo test, p<0.05). The second dimension (Axis 2) accounted for the most variance (R^2=0.74).

A plot of site scores on the two NMS axes illustrates faunal similarity among sites; sites located near each other in the two-dimensional space have similar fish assemblages. When sites are coded according to their drainage and/or region, the clustering or separation of groups apparent in the plot serves as an illustration of the degree that various classifications are useful for partitioning variation in assemblage structure (Figure 3). A classification based on major river drainage, for example, shows sites in the Santee drainage scattered amongst other drainage sites and occurring at extreme ends of both axes, indicating that Santee sites are highly variable (Figure 3). By
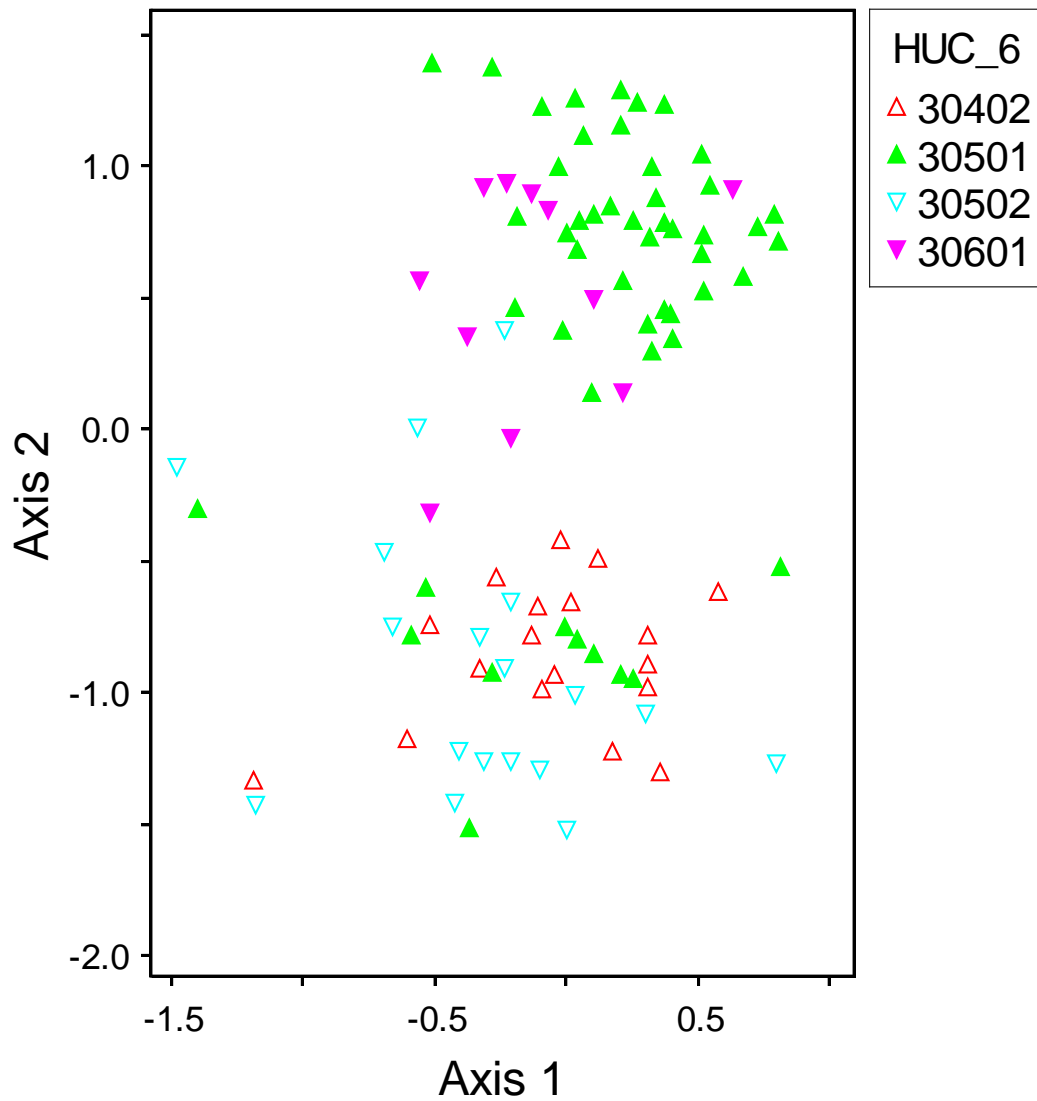
Figure 3. Plot of site scores on two ordination axes produced by nonmetric multidimensional scaling. Sites located in a similar region of the graph are characterized by similar fish species composition and abundance. Sites are coded by major river drainage, so the degree to which drainages blend together, cluster or separate from one another in the 2-dimensional space illustrates similarity or dissimilarity in species composition among drainages (see Table 1 for legend code definitions).

contrast, sites in the ACE and Pee Dee drainages score mostly on the negative end of Axis 2, and Savannah sites score mostly on the positive end of Axis 2, suggesting some differences in assemblage structure. Overall, however, the interspersion of sites in different drainages suggests that fish assemblages among the four drainages are not highly distinct from each other. Sites coded by minor river drainage show similar interspersion as that found under the major drainage classification, with Saluda, Broad, and Savannah sites clustered together on the positive end of Axis 2 and ACE, Lower Santee, Black, and Waccamaw drainage sites clustered together on the negative end (Figure 4). This indicates that a finer classification by minor drainage is not an overall improvement over major drainage for partitioning variation in assemblage structure.

Sites coded by physiographic province produced a clear faunal gradient (Figure 5). The lone Blue Ridge site is located on the extreme upper-right edge of the Piedmont site cluster, which is nearly completely separated from the grouping of Coastal Plain sites, suggesting that physiography is an important way to classify fish assemblages. Sites coded by Level III ecoregion, which simply breaks out Southeastern Plains (or inner Coastal Plain) from Middle Atlantic Coastal Plain (or outer Coastal Plain), showed that this distinction was not reflected in fish assemblages (Figure 6). Sites coded by Level IV ecoregion appeared to reflect an elevational gradient as sites were ordered from upper right toward lower left according to ecoregion: Blue Ridge, Inner Piedmont, Kings Mt., Outer Piedmont, Slate Belt, Sand Hills (Figure 7). The other ecoregional divisions within the Coastal Plain were interspersed, indicating more uniform fish assemblages.

When sites were classified by unique drainage by ecoregion strata, similar patterns were apparent indicating that physiography is an important means of classifying fish assemblages. Starting with the coarsest stratification, physiographic province by major river drainage showed that sites
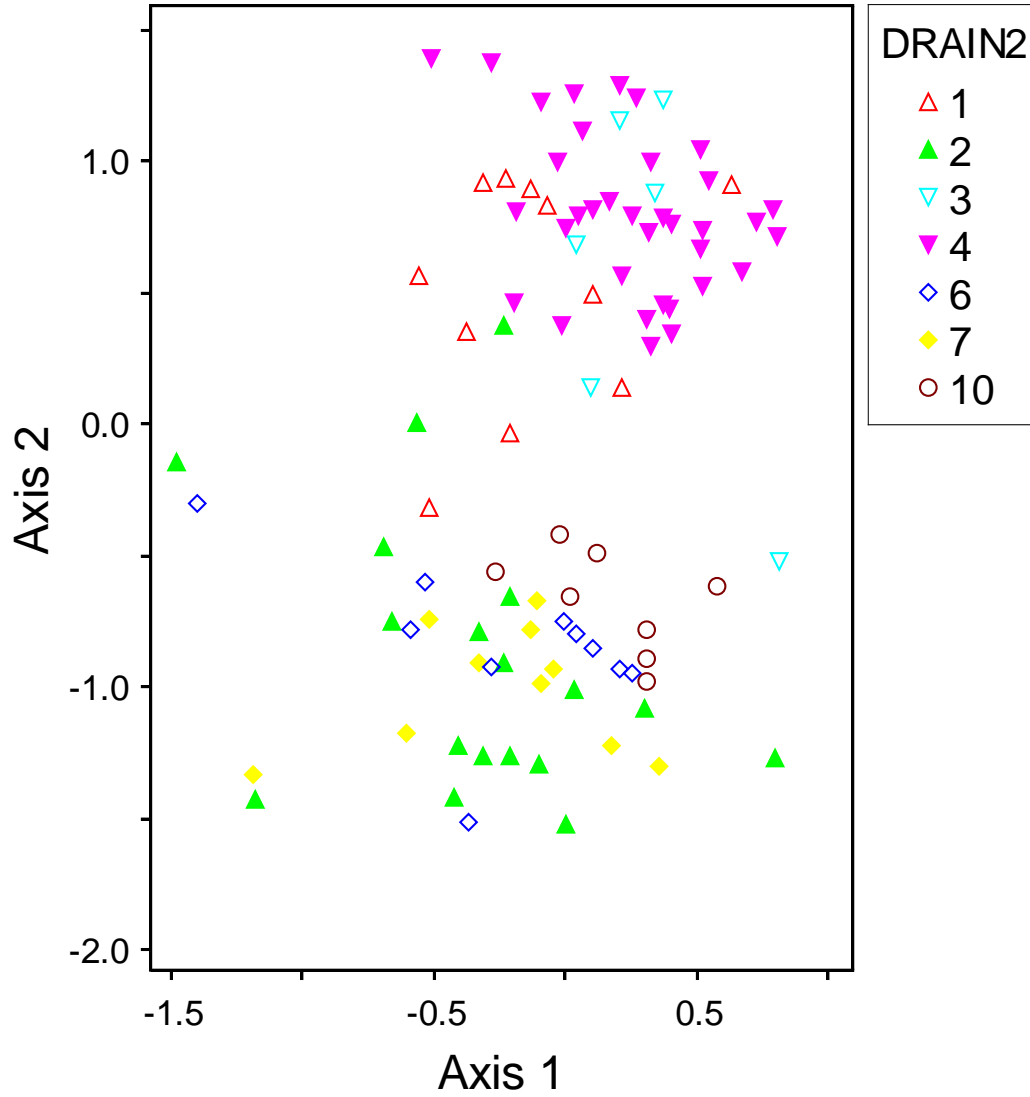
Figure 4. Plot of site scores on the two NMS ordination axes, grouped by minor river drainage (see Table 1 for legend code definitions).
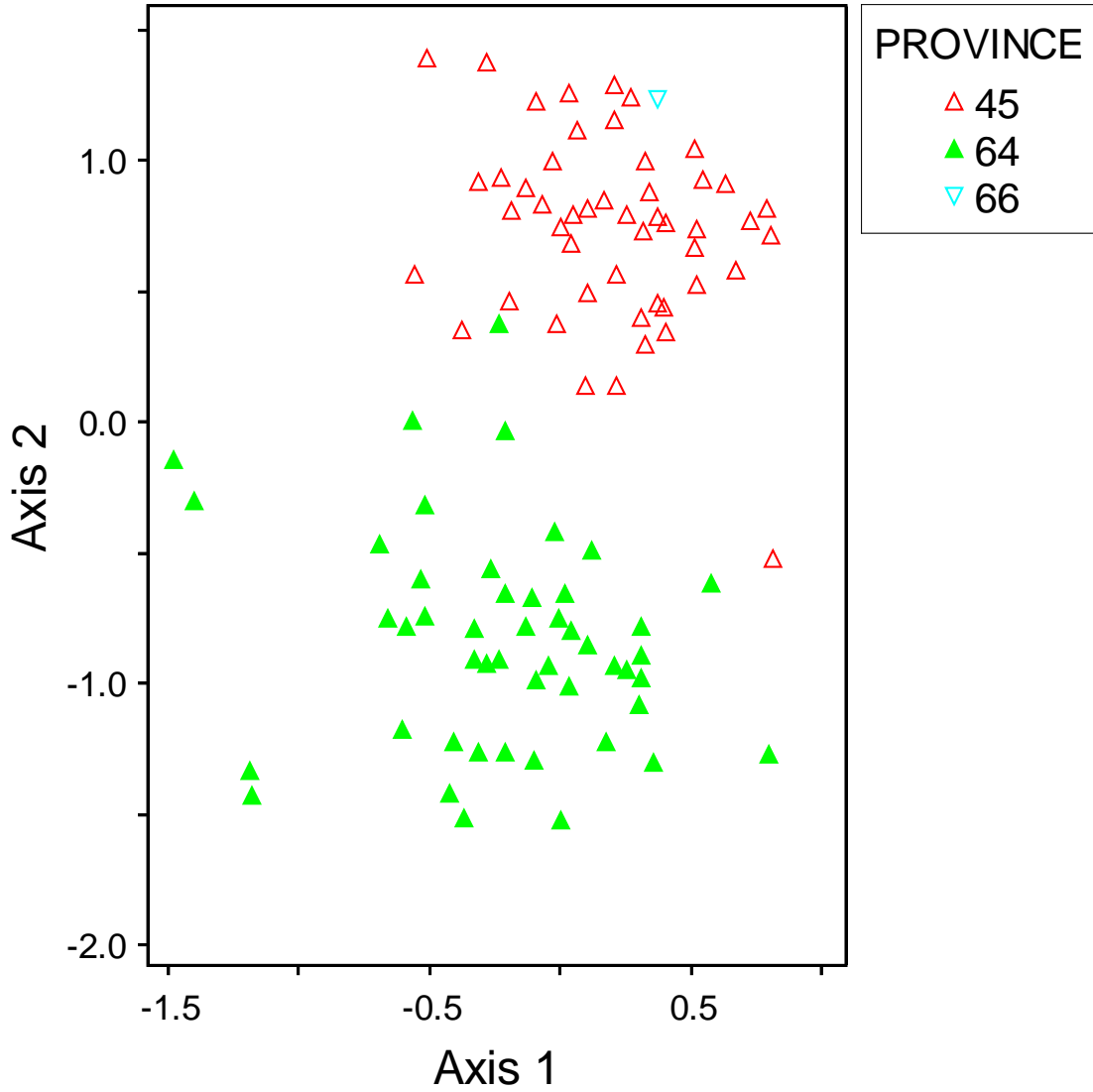
Figure 5. Plot of site scores on the two NMS ordination axes, grouped by physiographic province (see Table 1 for legend code definitions).
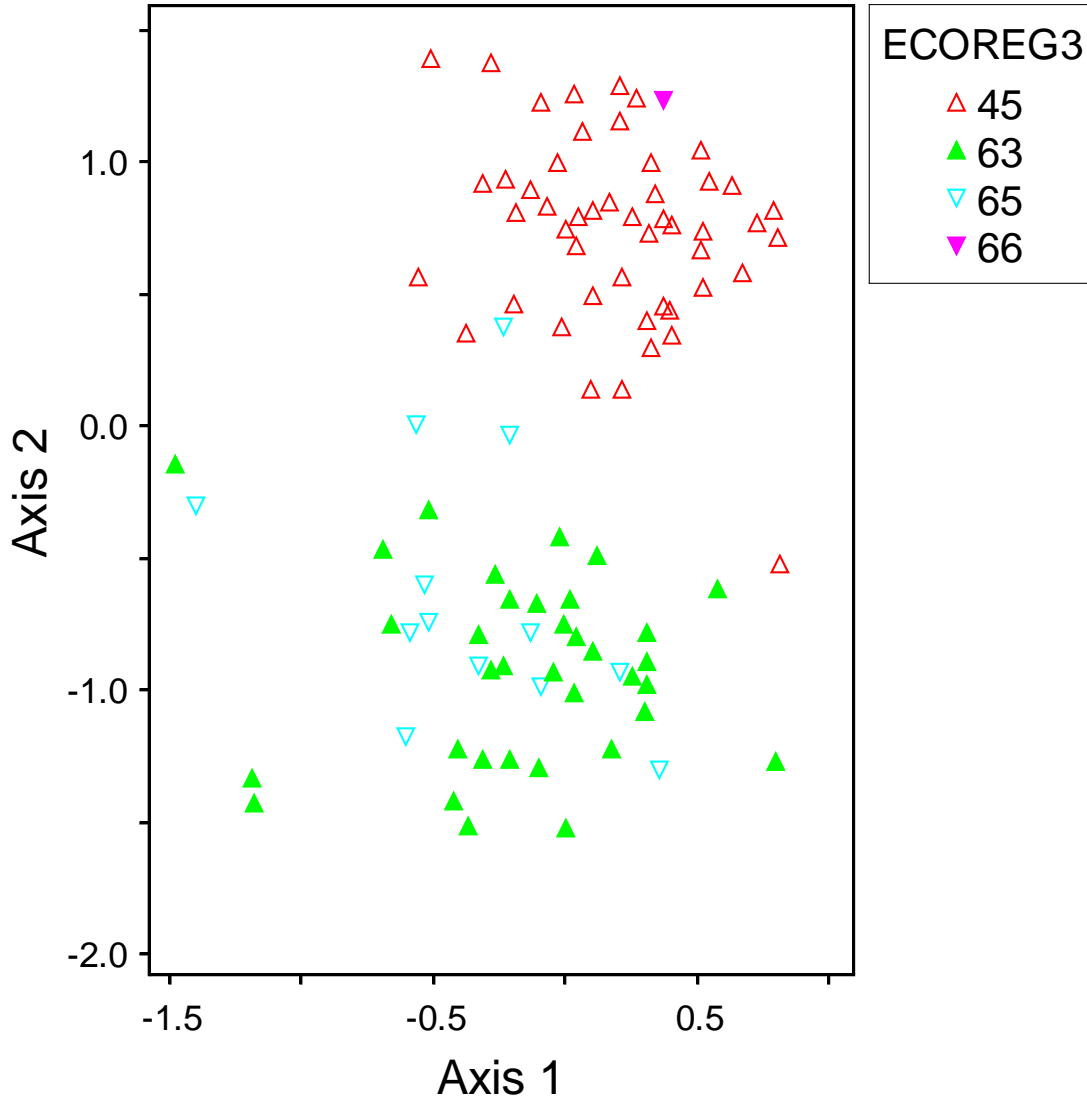
Figure 6. Plot of site scores on the two NMS ordination axes, grouped by Level III
ecoregion (see Table 1 for legend code definitions).
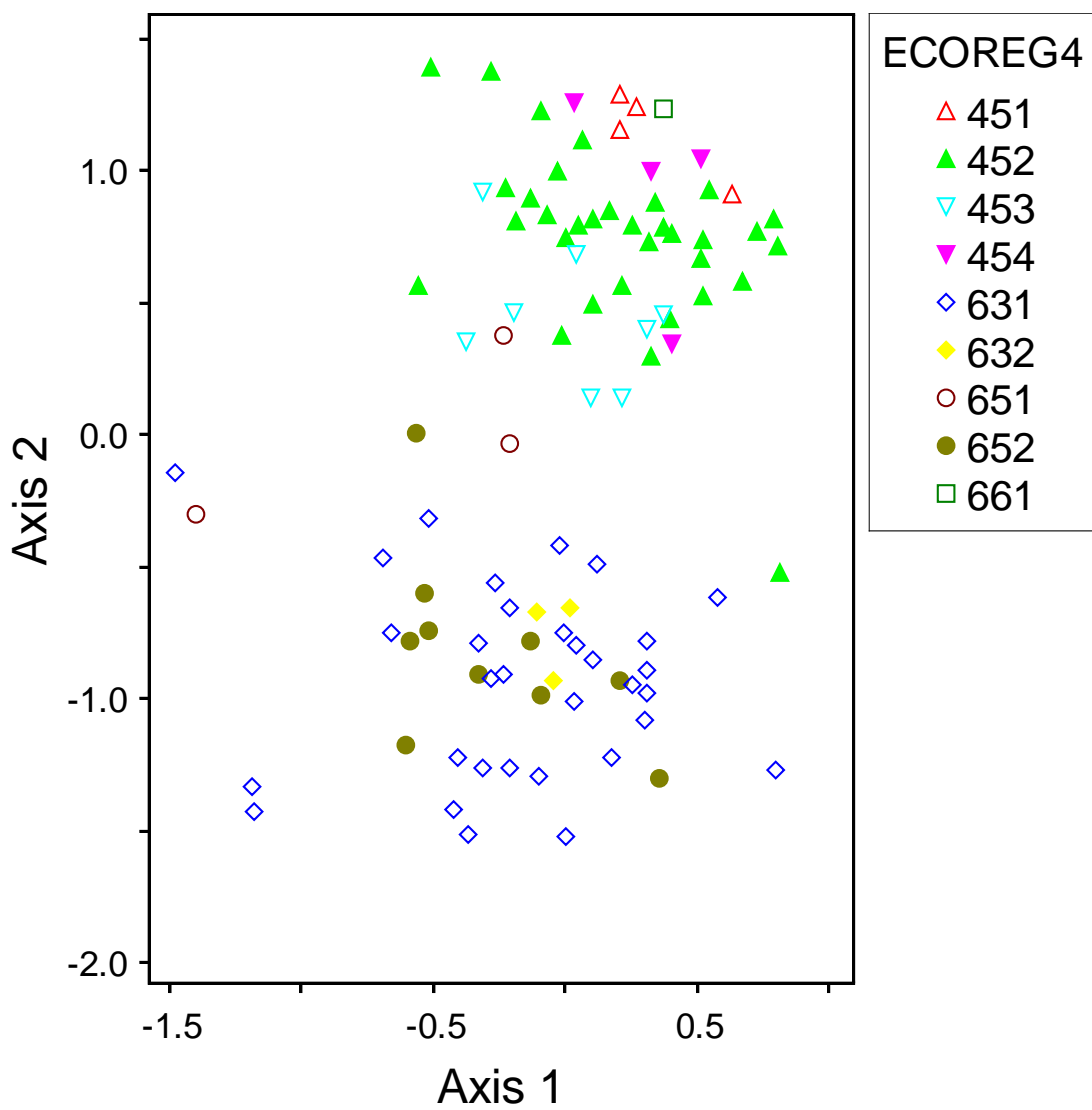
Figure 7. Plot of site scores on the two NMS ordination axes, grouped by Level IV ecoregion (see Table 1 for legend code definitions).

tended to cluster within province and were more interspersed with respect to drainage (Figure 8). The classification of physiography by minor river drainage again shows little faunal distinction among Piedmont Savannah, Saluda, or Broad River sites, nor among Coastal Plain Savannah, ACE, Lower Santee, Black, or Waccamaw River sites (Figure 9). Level III ecoregion by major river drainage again shows little variation according to drainage, although the location of Inner Coastal Plain ACE and Savannah sites appears to be grading from coastal plain into Piedmont assemblages (Figure 10). Further classification of Level III ecoregion into minor river drainages does not partition variation more effectively because drainages within the Piedmont and within the Coastal Plain are interspersed (Figure 11). Plots of Level IV ecoregion by river drainage could not be printed due to software limitations on the number of different classes that can be produced.

The plots are useful for visual examination of various aquatic classifications, but are decidedly subjective. A more objective approach is to use a randomization procedure such as multi-response permutation procedures (MRPP). MRPP is a non-parametric procedure for testing the hypothesis of no difference between two or more groups of entities. Discriminant analysis is a parametric procedure that can be used on the same general class of questions. However, MRPP has the advantage of not requiring assumptions (such as multivariate normality and homogeneity of variances) that are seldom met with ecological community data (McCune and Grace). In this case, average ecological distance among sites within strata were statistically compared to average distance between strata (Table 2). Only those strata that contained more than three sites were tested, which omitted Blue Ridge, Sand Hills, Mid-Atlantic Floodplains & Low Terraces, and Southeastern Floodplains and Low Terraces from all comparisons. For the MRPP tests of remaining strata, the cutoff for statistical significance was designated as $p<0.01$ due to multiple comparisons being made.
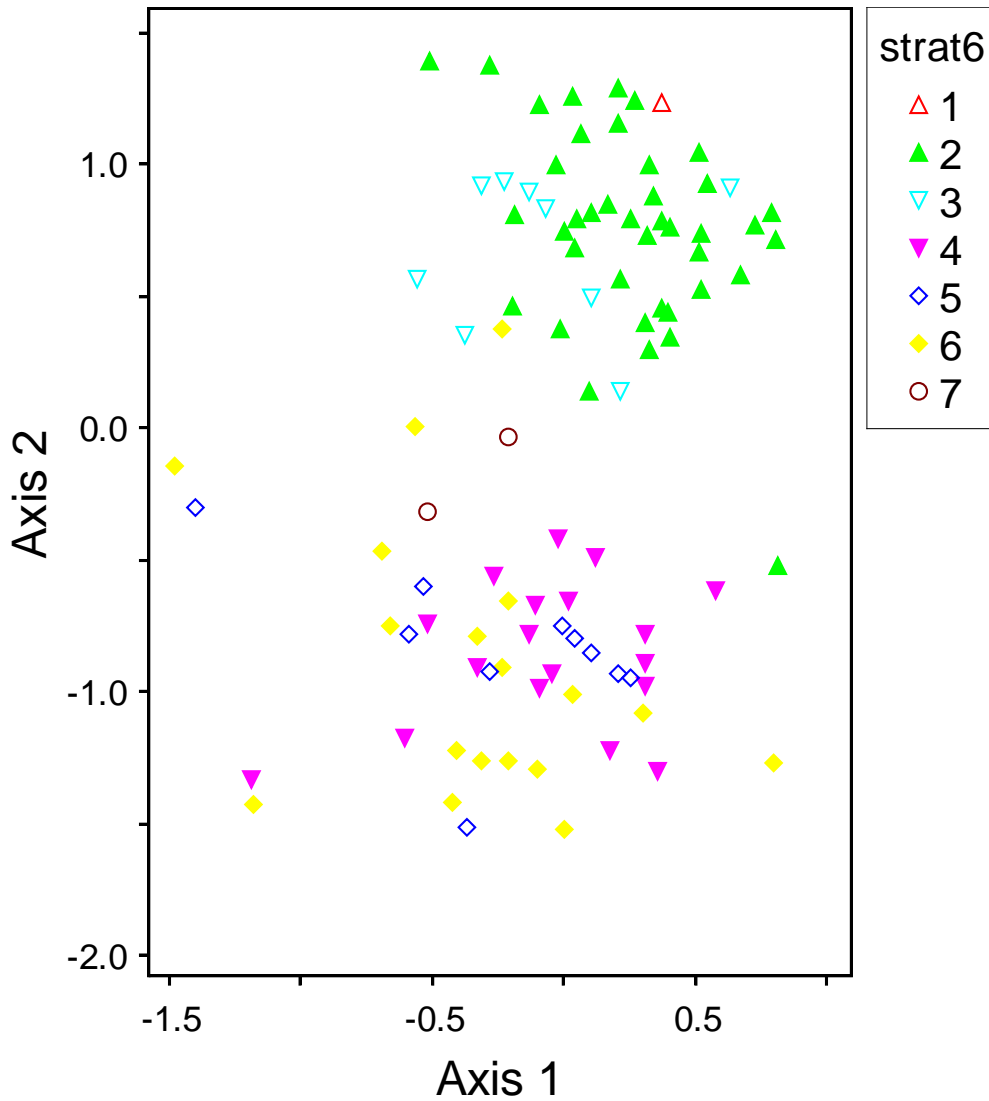
Figure 8. Plot of site scores on the two NMS axes with sites grouped according to
unique strata defined by intersections of physiography and major river drainage
(1 – Blue Ridge Santee, 2- Piedmont Santee, 3 - Piedmont Savannah, 4 -
Coastal Plain PeeDee, 5 - Coastal Plain Santee, 6 - Coastal Plain ACE, 7 -
Coastal Plain Savannah).
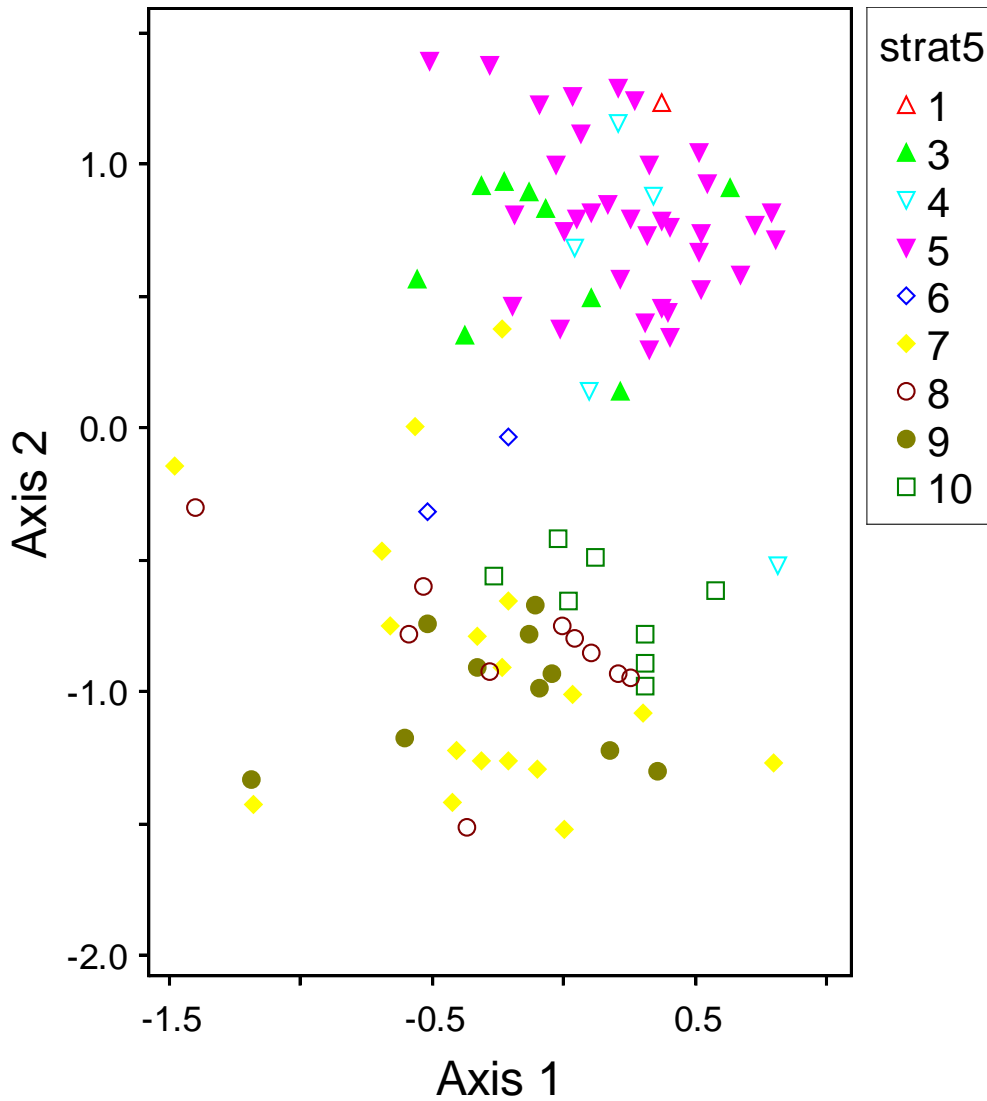
Figure 9. Plot of site scores on the two NMS axes with sites grouped according to unique strata defined by intersections of physiography and minor river drainage (1 – Blue Ridge Saluda, 3 - Piedmont Savannah, 4 - Piedmont Saluda, 5 - Piedmont Broad, 6 - Coastal Plain Savannah, 7 - Coastal Plain ACE, 8 - Coastal Plain Lower Santee, 9 - Coastal Plain Black, 10 - Coastal Plain Waccamaw).
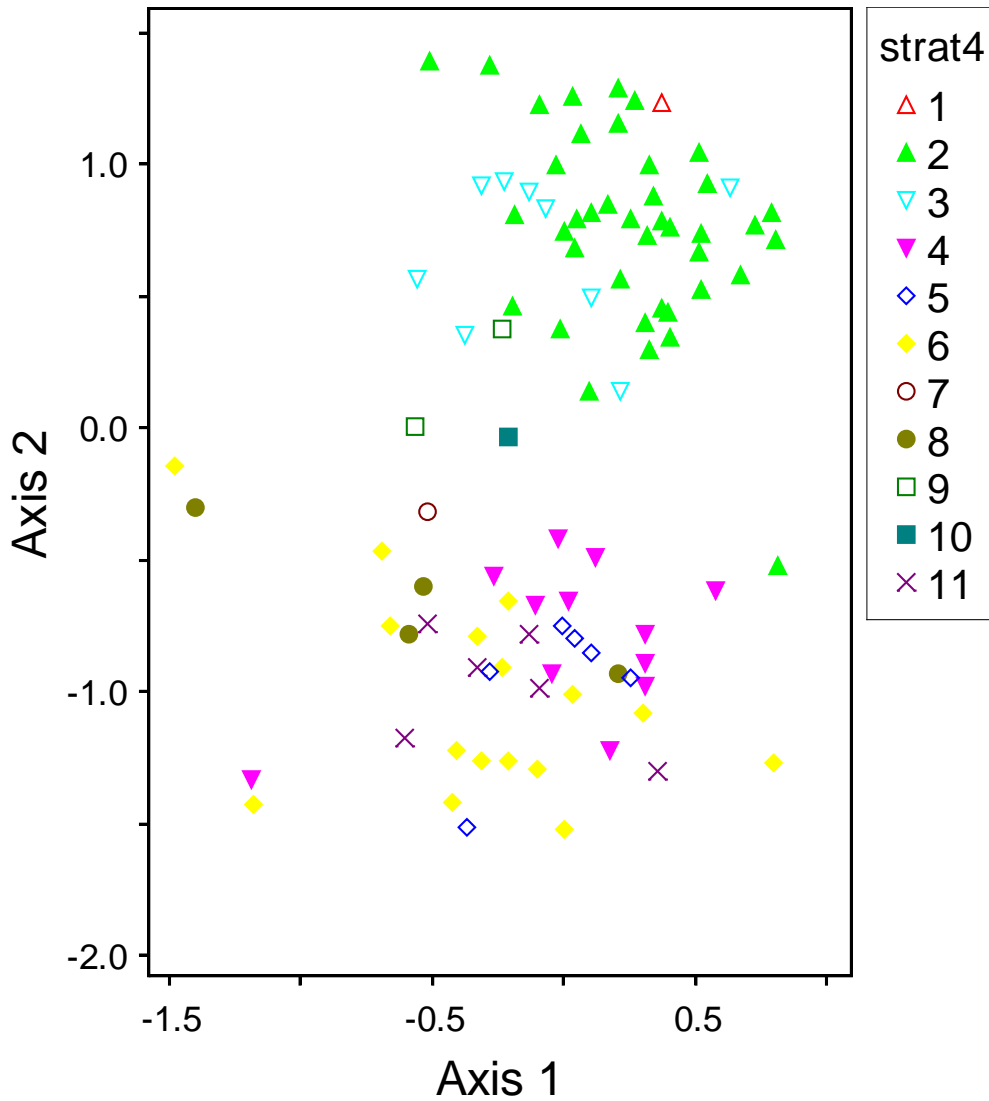
Figure 10. Plot of site scores on the two NMS axes with sites grouped according to unique strata defined by intersections of Level III ecoregion and major river drainage (1 – Blue Ridge Santee, 2- Piedmont Santee, 3 - Piedmont Savannah, 4 – Middle Atlantic (Outer) Coastal Plain PeeDee, 5 - Middle Atlantic (Outer) Coastal Plain Santee, 6 - Middle Atlantic (Outer) Coastal Plain ACE, 7 - Middle Atlantic (Outer) Coastal Plain Savannah, 8 – Southeastern (Inner) Plains Santee, 9 - Southeastern (Inner) Plains ACE, 10 - Southeastern (Inner) Plains Savannah, 11 - Southeastern (Inner) Plains PeeDee.
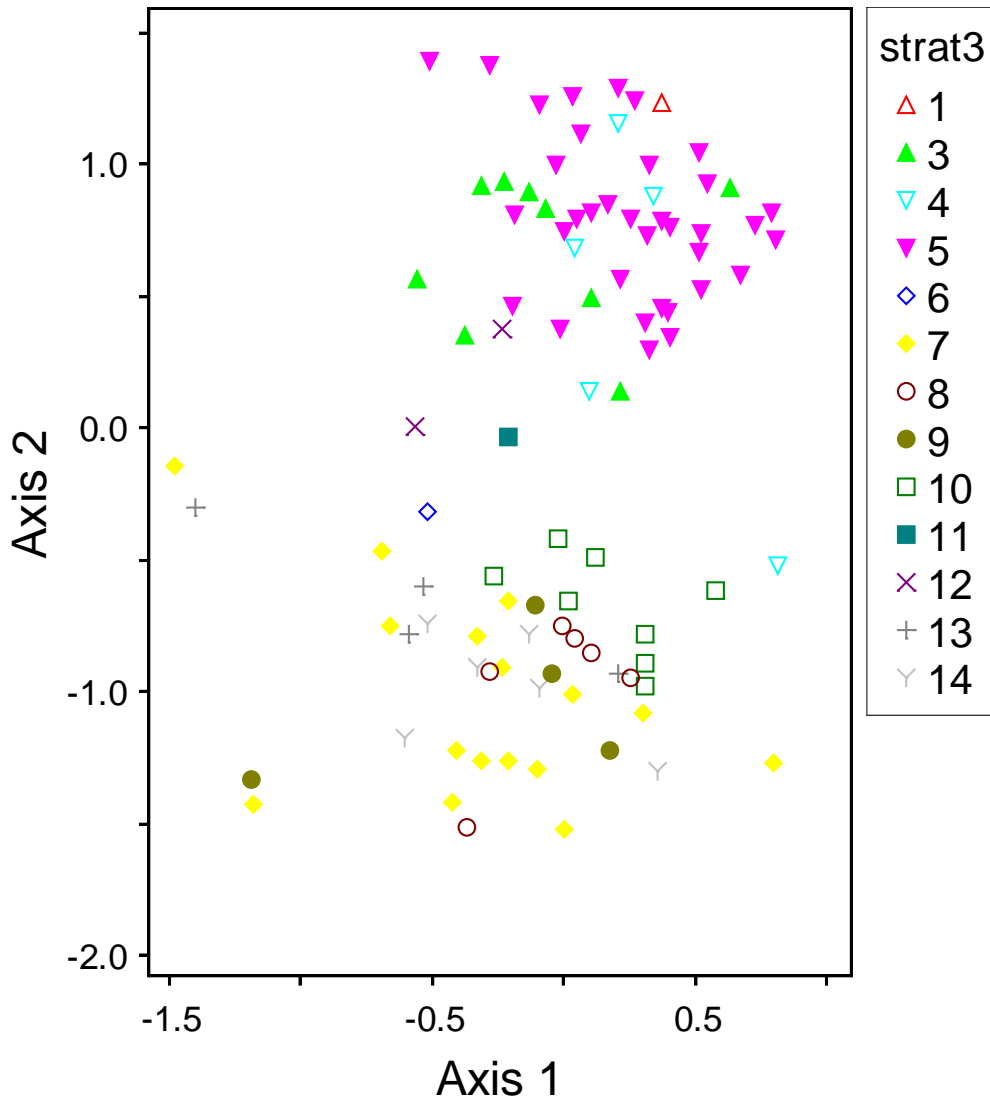
Figure 11. Plot of site scores on the two NMS axes with sites grouped according to unique strata defined by intersections of Level III ecoregion and minor river drainage (1 – Blue Ridge Saluda, Santee, 3 - Piedmont Savannah, 4 – Piedmont Saluda, 5 - Piedmont Broad, 6 - Middle Atlantic (Outer) Coastal Plain Savannah, 7 - Middle Atlantic (Outer) Coastal Plain ACE, 8 – Middle Atlantic (Outer) Coastal Plain Santee, 9 - Middle Atlantic (Outer) Coastal Plain Black, 10 - Middle Atlantic (Outer) Coastal Plain Waccamaw, 11 - Southeastern (Inner) Plains Savannah, 12 - Southeastern (Inner) Plains ACE, 13 - Southeastern (Inner) Plains Santee, 14 - Southeastern (Inner) Plains Black.

Table 2.   Results of multi-response permutation procedures testing the hypothesis that
             sites within strata have more similar fish faunas than sites between strata.
             Strata that are significantly different may be useful for biological
             classification of freshwater streams, whereas those that are not different may
             be pooled.

Significantly Different (p<0.01)	Not Significantly Different
Physiographic Region	
Piedmont vs. Coastal Plain	
Major River Drainage	
Santee vs. ACE, Savannah, Pee Dee	ACE vs. Pee Dee
Savannah vs. ACE, Pee Dee	
Minor River Drainage	
Savannah vs. Broad, Lower Santee, Black, Waccamaw	Savannah vs. Saluda
Saluda vs. ACE, Lower Santee, Black, Waccamaw	Saluda vs. Broad
Broad vs. Lower Santee, ACE, Black, Waccamaw	ACE vs. Lower Santee, Black
Lower Santee vs. Waccamaw	Lower Santee vs. Black
Black vs. Waccamaw	
Level III Ecoregion	
Piedmont vs. Southeastern Plains	
Piedmont vs. Middle Atlantic Coastal Plain	
SEastern Plains vs. Mid Atlantic Coastal Plain	
Level IV Ecoregion	
Inner Piedmont vs. Outer Piedmont and all lower State ecoregions	Inner Piedmont vs. Kings Mt.
Significantly Different (p<0.01)	Not Significantly Different
Outer Piedmont vs. Atlantic Southern Loam Plains, Carolina Flatwoods,	Outer Piedmont vs. Kings Mt.
Kings Mt. vs. Atlantic Southern Loam Plains, Carolina Flatwoods	Outer Piedmont vs. Slate Belt
Slate Belt vs. Atlantic Southern Loam Plains, Carolina Flatwoods	Kings Mt. vs. Slate Belt
	Carolina Flatwoods vs. Atlantic Southern Loam Plains
STRAT6 – Physiographic Region by Major River Drainage	
* All results mirror those found above in physiographic and major drainage classes	
STRAT5 - Physiographic Region by Minor River Drainage	
* All results mirror those found above in major and minor drainage classes	
STRAT4 – Level III Ecoregion by Major River Drainage	
*Coastal Savannah, Inner Coastal ACE had too few sites to evaluate	

Table 1. Continued.

Significantly Different (p<0.01)	Not Significantly Different
Piedmont Savannah vs. all other classes	SEastern Plains vs. Mid Atlantic Coastal Plain of all drainages
Piedmont Santee vs. all other classes	
STRAT3– Level III Ecoregion by Minor River Drainage	
Piedmont Savannah vs. Piedmont Broad	Piedmont Savannah vs. Piedmont Saluda
All Piedmont drainages vs. Coastal Plain drainages	Piedmont Saluda vs. Piedmont Broad
Waccamaw vs. all other classes except Mid Atlantic (Outer) Coastal Black	SEastern Plains vs. Mid Atlantic Coastal Plain of all drainages except Waccamaw Mid Atlantic Coastal Plain Waccamaw vs. Mid Atlantic Coastal Plain Black
STRAT2– Level IV Ecoregion by Major River Drainage	
Results mirror STRAT4 and STRAT1	
STRAT1– Level IV Ecoregion by Minor River Drainage	
Only those classes containing at least 4 sites are reported	
Outer Piedmont Savannah vs. other classes except Slate Belt Savannah	Outer Piedmont Savannah vs. Slate Belt Savannah
Significantly Different (p<0.01)	Not Significantly Different
Slate Belt Savannah vs. other drainages	Outer Piedmont Broad vs. Kings Mt. Broad
Outer Piedmont Broad vs. Coastal drainages	SEastern Plains Black, Carolina Flatwoods ACE, Santee, and Black
Atlantic Southern Loam Plains Black vs. Carolina Flatwoods Waccamaw	

Clearly the strongest pattern among classes to emerge from these results was the physiographic shift between upper State and lower State assemblages. Although the details of the testing are too numerous to go into exhaustively, the major patterns are 1) some degree of uniqueness among Savannah drainage sites, 2) considerable uniqueness of Waccamaw drainage sites, and 3) the similarity of remaining Coastal Plain drainages. The similarity of ACE basin and Black River faunas is remarkable given the distance between them. Kings Mt. does not seem to warrant a separate class for the purposes of aquatic classification. Major river drainage was just as efficient as minor drainage in capturing faunal variation, with the exception of the distinctive Waccamaw drainage.

Overall, ecoregional and drainage level classification appears to be helpful for partitioning variation in fish assemblage structure in South Carolina. The data analyzed here were insufficient to evaluate all strata comprehensively, therefore a conservative approach would be to retain a fine-scale stratification for future work to assess stream habitat.

Recommendations

Landscape Classification using Fish Assemblages

An understanding of the principal environmental influences, both man-made and natural, on aquatic communities is necessary for effective management of freshwater resources. This analysis details the different levels of landscape variation and documents variation in fish assemblage structure associated with these natural influences. Currently, I recommend stratifying freshwater streams of the State according to "ecobasins", defined as unique combinations of Level IV ecoregion by major river drainage, with the modifications that Kings Mt. be absorbed into the Outer Piedmont ecoregion and that the Waccamaw basin be separate from the greater Pee Dee system.

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Thomason, C, J Bettinger, D Rankin, D Crochet, L Rose and H Beard. 2002. The South Carolina Standard Operating Procedures for Sampling Fish in Wadeable Streams. South Carolina Department of Natural Resources, Freshwater Fisheries Section, Columbia, SC.

Prepared By: <u>Mark Scott</u>   Title: <u>Research Fisheries Biologist</u>

Job Title: **Recovery of the Main Stem Reedy River Fish Community from a Major Oil Spill.**

Period Covered   January 1, 2005 – December 31, 2005

Results and Discussion

In response to the June 26, 1996 diesel oil pipeline spill of 22,800 barrels (957,600 gallons) that killed an estimated 35,000 fish along 37 km of the Reedy River south of Greenville, South Carolina (Rankin *et al*. 1996), a longitudinal sampling framework was implemented to monitor the recovery of the affected river section. Five fixed sites—an undisturbed reference site approximately 5 km upstream of the oil spill origin and four sites ranging from 2-30 km downstream within the disturbed section—were each sampled once in August 1996 (1.5 months post-disturbance), October 1996 (4 months post-disturbance), October 1997 (16 months post-disturbance), October 1998 (28 months post-disturbance), October 2000 (52 months post-disturbance), and September-October 2005 (112 months post-disturbance) (Table 1). Site A was not sampled in August 1996; therefore, 29 samples were conducted altogether.

Table 1.   Sites employed in the Reedy River main stem recovery monitoring study and their positions relative to the oil spill origin.

Site	Type	Position Relative to Spill Site (river km)
Reference	Reference	5.4 km upstream
A	Disturbed	1.8 km downstream
B	Disturbed	14.2 km downstream
C	Disturbed	20.6 km downstream
D	Disturbed	29.5 km downstream

Fish sampling consisted of three-pass depletion electrofishing by 12-15-person crews using tandem backpack and barge-mounted electrofishing gear. The entire wetted channel was sampled

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over a reach length of 150 m, with the same reach sampled year to year for each site. All fish were

collected and subsequently identified, sorted, measured (TL mm), and weighed (g) after each pass,

then released upon completion of all passes. Questionable specimens were preserved on site and

identified at a later time.

A total of 8,454 fish representing 33 species from eight families was collected (Table 2).

Total fish abundance by sample ranged from 14 (site B, August 1996) to 768 (reference site, 2005),

and species richness was characterized by a low of four (site B, August 1996) and a high of 22 (site

D, 2000 and 2005; Table 3). Expectedly, the lowest abundance and richness occurred at a disturbed

site during the earliest sample period following the oil spill.

From a species standpoint, mosquitofish *Gambusia holbrooki* was the most abundant species

overall (1,755), whereas brown bullhead *Ameiurus nebulosus* and rosyface chub *Hybopsis rubrifrons*

were represented by one individual each. Frequency of occurrence ranged from one sample (brown

bullhead, common carp *Cyprinus carpio*, rosyface chub, and seagreen darter *Etheostoma*

thalassinum) to 29 (bluegill *Lepomis macrochirus*).

Recovery Analysis

Recovery was defined and assessed as a function of relative fish community structure among

sites within and between years, under the assumption that the disturbed sites would exhibit early

dissimilarity in community structure to the undisturbed reference site but subsequently become

increasingly similar to the reference site over time (i.e., with recovery). Furthermore, recovery rates

among disturbed sites were expected to vary in proportion to their spatial relation to potential

Table 2.   Species collected in the 29 Reedy River main stem samples, August 1996 –
           October 2005, and their associated three-letter codes.

Family	Common Name	Scientific Name	SCDNR Species Code
Cyprinidae	Greenfin shiner	*Cyprinella chloristia*	GFS
	Whitefin shiner	*Cyprinella nivea*	WFS
	Common carp	*Cyprinus carpio*	CRP
	Rosyface chub	*Hybopsis rubrifrons*	RFC
	Bluehead chub	*Nocomis leptocephalus*	BHC
	Golden shiner	*Notemigonus crysoleucas*	GLS
	Spottail shiner	*Notropis hudsonius*	STS
	Yellowfin shiner	*Notropis lutipinnis*	YFS
	Sandbar shiner	*Notropis scepticus*	SBS
Centrarchidae	Flier	*Centrarchus macropterus*	FLR
	Redbreast sunfish	*Lepomis auritus*	RBS
	Green sunfish	*Lepomis cyanellus*	GSF
	Pumpkinseed	*Lepomis gibbosus*	PPS
	Warmouth	*Lepomis gulosus*	WAR
	Bluegill	*Lepomis macrochirus*	BLG
	Redear sunfish	*Lepomis microlophus*	RES
	Largemouth bass	*Micropterus salmoides*	LMB
	Black crappie	*Pomoxis nigromaculatus*	BLC
Ictaluridae	Snail bullhead	*Ameiurus brunneus*	SBH
	White catfish	*Ameiurus catus*	WCF
	Yellow bullhead	*Ameiurus natalis*	YBH
	Brown bullhead	*Ameiurus nebulosus*	BBH
	Flat bullhead	*Ameiurus platycephalus*	FBH
	Channel catfish	*Ictalurus punctatus*	CCF
	Margined madtom	*Noturus insignis*	MGM
Catostomidae	White sucker	*Catostomus commersoni*	WHS
	Creek chubsucker	*Erimyzon oblongus*	CCS
	Northern hogsucker	*Hypentelium nigricans*	NHS
	Striped jumprock	*Scartomyzon rupiscartes*	STJ
Poeciliidae	Eastern mosquitofish	*Gambusia holbrooki*	MSQ
Percidae	Seagreen darter	*Etheostoma thalassinum*	SGD
Clupeidae	Gizzard shad	*Dorosoma cepedianum*	GZS
Esocidae	Redfin pickerel	*Esox americanus*	RFP

Table 3.   Fish abundance and diversity attributes for the five Reedy River main stem
            sites by sample period, August 1996 – October 2005.

Sample Period	Months Post-Disturbance	Site	Total Fish Abundance	Species Richness	Shannon-Weiner Diversity
August 1996	1.5	Reference	268	15	1.93
		B	14	4	1.12
		C	31	9	1.58
		D	118	10	0.73
October 1996	4	Reference	266	12	2.06
		A	126	15	2.10
		B	35	9	1.50
		C	49	10	1.70
		D	125	15	1.42
October 1997	16	Reference	485	14	1.88
		A	431	13	1.20
		B	576	21	1.50
		C	153	15	2.00
		D	309	16	1.99
October 1998	28	Reference	616	14	1.76
		A	211	13	2.01
		B	192	19	2.02
		C	113	17	2.09
		D	211	14	1.63
October 2000	52	Reference	276	12	1.69
		A	205	10	1.80
		B	232	19	2.29
		C	163	15	2.20
		D	439	22	2.41
October 2005	112	Reference	768	15	2.19
		A	600	17	1.99
		B	494	20	2.19
		C	391	21	2.53
		D	561	22	2.39

recolonization sources such as the undisturbed upstream section as well as tributaries. The magnitude of the disturbance and resulting fish kill was presumed to be distributed relatively evenly along the affected section based on samples conducted immediately after the incident; thus, for the purposes of this analysis it was assumed that all the disturbed sites began the recovery process from the same initial level of disturbance.

Fish community similarity was measured through non-metric multidimensional scaling (NMS) ordination using PC-ORD Version 4.33 software (McCune and Mefford 1999). NMS extracts and relativizes units (i.e., samples) along indirect gradients of ecological similarity derived from ecological distance comparisons among all pairs of samples. Sorensen/Bray-Curtis dissimilarity was employed as the distance measure herein. Samples are plotted within one or more dimensions according to unitless scores representing relative similarity; thus, points (i.e., sites or species) closer together in the NMS output are more similar to one another.

The species-abundance matrix was adjusted prior to analysis. Species representing $\leq 0.1\%$ of the grand total abundance of all species combined were excluded to avoid effects of extremely low abundance on community structure. Ten species fell below the minimum abundance threshold, resulting in 23 remaining species. Additionally, mosquitofish were removed due to their extreme variability in population size within seasons, small adult size, and habitat preference for shallow side channels and backwaters, all of which carry inherent sampling inconsistencies across long sample intervals. Finally, abundances of the remaining 22 species were relativized within samples to reduce the influence of absolute abundance variation due to variable site area.

Ordination Results

NMS ordination extracted significant structure in the data (Monte Carlo test, $p < 0.02$), with two dimensions explaining 93% of the variation in Sorensen distance, or fish community similarity, among samples. Axes 1 and 2 (Figure 1) accounted for 33% and 60% of the variation, respectively.

The disturbance-recovery sequence was temporally and spatially evident in the NMS output by samples (Figure 1). Early dissimilarity in community structure between the reference and disturbed sites was apparent in the opposing positions of the 1996 reference and disturbed samples in ordination space. Such dissimilarity was expected given the reduction in abundance and diversity at the disturbed sites as a result of the fish kill. Of note, however, is the initial ordination position of site A (October 1996), which exhibited greater early similarity to the reference site than the other disturbed sites, suggesting an increased rate of recolonization due to its proximity to the upstream undisturbed section and a major tributary (Table 1).

Indicative of recovery, the disturbed sites collectively increased in community similarity to the reference site over time, which was reflected by a net positive shift of disturbed sites along Axes 1 and 2 towards the reference site from 1996-2005 (Figure 1). The reference site expectedly remained stable relative to the disturbed sites, essentially cycling in a localized portion of ordination space. Site A became very similar to the reference site by 1997 and subsequently cycled concurrently to the reference site, further supporting a proximity influence on community dynamics.

A relationship between time and community stability emerged when the study duration was divided into two relatively equal periods, August 1996-October 2000 (52 months) and October 2000-October 2005 (60 months). The net change in community structure (i.e., net change in ordination position) of each respective disturbed site was greater from 1996-2000 than 2000-2005. That is, the

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All Samples August 1996 - October 2005
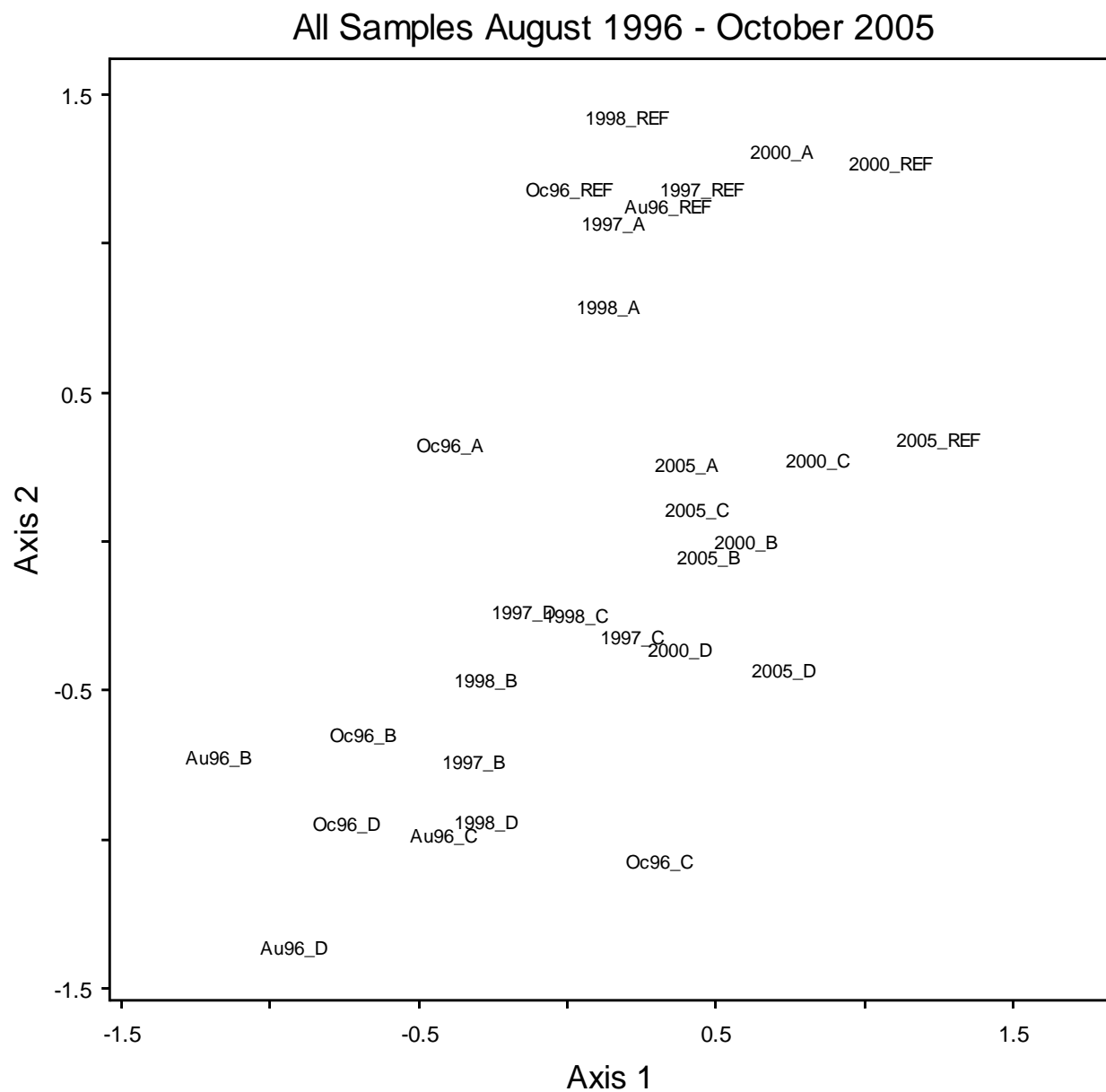
Figure 1. NMS ordination plot of all Reedy River main stem samples, August 1996 –
October 2005. Each sample/site is denoted by the sample period followed by
site code (e.g., Au96_REF = August 1996, reference site). Samples closer
together are more similar to one another.

disturbed sites exhibited greater stability over the last 60 months of monitoring than in the first 52 months, suggesting recovery towards pre-disturbance levels of variability. However, as no samples were conducted between 2000-2005, this observation is limited to net change alone for that time period; the degree of interannual variability remains unknown. The exception to this pattern was the reference site and, to some extent, site A, which displayed a greater net shift from 2000-2005. However, these changes were likely the product of an increase in the abundance of a single species rather than an overall shift in community structure and will be addressed hereafter. An additional consideration with significant temporal implications is the influence of hydrology over the study duration. The study coincided with a regional drought and subsequent reversal to above-average rainfall, which undoubtedly affected community structure during the recovery process.

Species characterizing the disturbance-recovery dynamics were apparent (Figure 2). Species whose relative abundance was most associated with the disturbed communities included bluegill and pumpkinseed *Lepomis gibbosus*, whereas flat bullhead *Ameiurus platycephalus*, spottail shiner *Notropis hudsonius*, and northern hogsucker *Hypentelium nigricans*, among others, occurred most abundantly in reference and later samples (Figure 2). Such associations generally agree with a gradient from resilient, opportunistic or generalist species to sensitive, K-selected or specialized species from the disturbance (lower left) to reference/recovery (upper/middle) regions of the plot, respectively. The increase in relative abundance of spottail shiners at the reference site and site A from 2000-2005 is believed to account for the shifts observed at those sites over this sample interval.

Altogether, recovery of the affected section was evident in the distinct shift from disturbed towards reference conditions among the downstream sites over time. By 2005, all sites occurred within the same general area on the ordination plot, indicating convergence in community structure, although the specific level of recovery remains unknown due to the inherent inability to define an
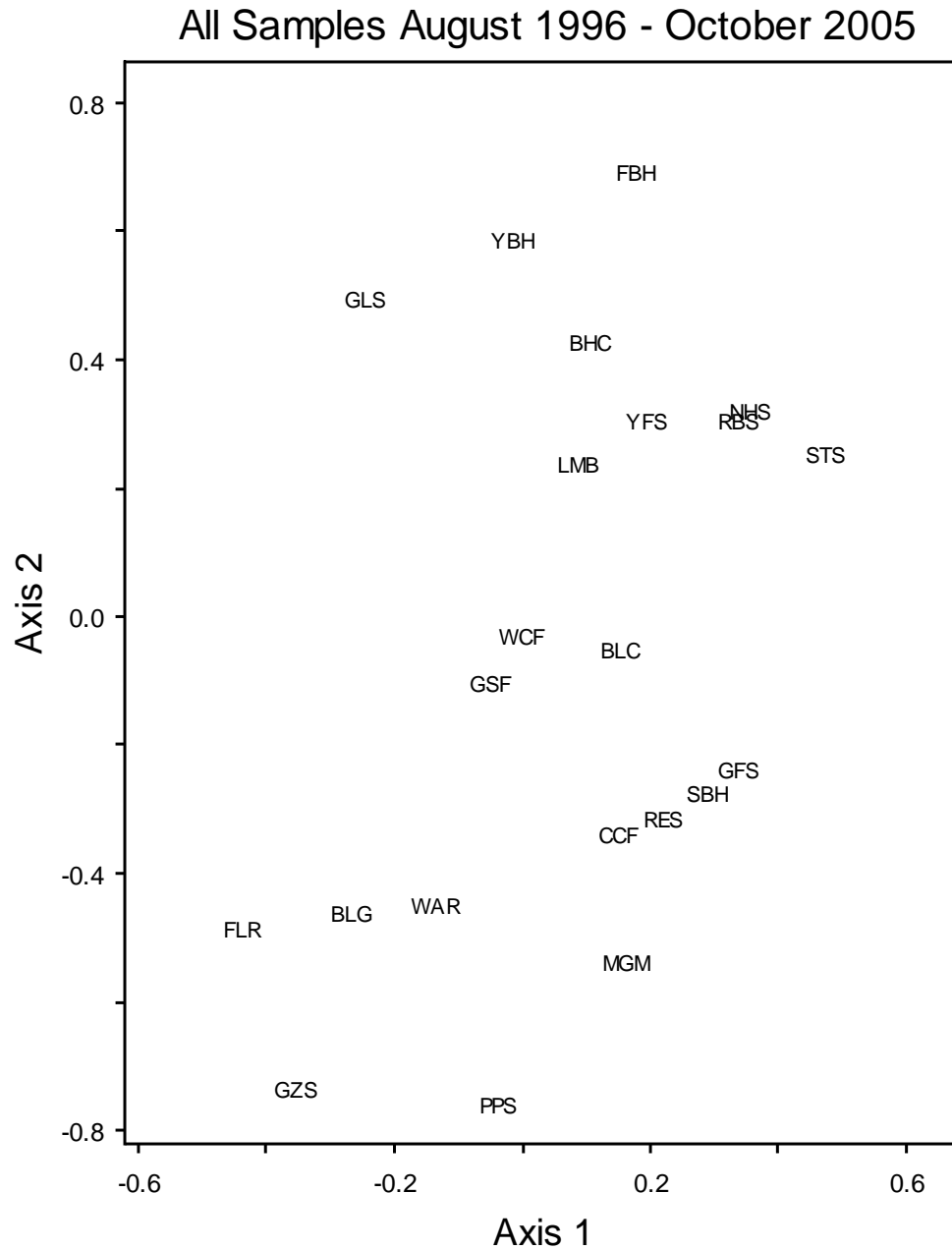
Figure 2. NMS ordination plot of species from all Reedy River main stem samples, August 1996 – October 2005. See Table 2 for species codes.

absolute recovery endpoint in the absence of pre-disturbance data. Furthermore, development increased substantially within the Reedy River watershed over the monitoring period, rendering the attainment of "pre-oil spill" fish community structure unlikely; therefore, stabilization may be a better relative indication of community recovery. The remaining differences among sites may in fact be the reflection of a natural longitudinal (i.e., upstream-downstream) gradient in community structure, in which case the fish community of the Reedy River may be more fully recovered than is apparent in the present analysis alone. A study is in progress on a similar but less disturbed river to examine longitudinal patterns in fish community structure, thus elucidating the recovery progress of the Reedy River (see following job report).

Recommendations

The Reedy River oil spill represents a valuable empirical context from which to address disturbance in aquatic community ecology. Ensuing efforts will be aimed at further analyzing and interpreting the data and producing a completion report as well as manuscript for publication in an applicable scientific journal. Additional samples are scheduled for 2008 and 2012.

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Rankin, D. M., W. R. Geddings, and W. E. Hayes. 1996. Reedy River Fish Kill Report. Appendix III *In* Geddings, W. R., and D. M. Rankin. Fisheries Investigations in Lakes and Streams. District I. Annual Progress Report F-63-1-1. South Carolina Department of Natural Resources, Freshwater Fisheries Section, Columbia, SC.

Prepared By: Kevin Kubach   Title: Fisheries Biologist

Job Title: **Main Stem Reedy River – Main Stem North Tyger River Longitudinal Comparison**

Period Covered January 1, 2005 – December 31, 2005

Results and Discussion

The June 1996 Reedy River oil spill and subsequent fish community recovery study occurred over a large spatial scale, with five sites spanning approximately 35 river km. Although overall fish community recovery was observed from 1996-2005, differences in community structure among sites remained (previous job report, Figure 1). Given the large spatial and temporal scales, it is likely that the remaining differences among sites are due in part to a natural longitudinal (i.e., upstream-downstream) riverine gradient in community structure rather than persisting effects of the disturbance alone.

To assess the potential influence of a natural longitudinal gradient on the Reedy River study sites, a comparative sampling design was implemented on a similar but undisturbed river in the context of the oil spill. The North Tyger River was selected as the optimal reference river because it was the most physically and faunally comparable river within the upper Santee River basin on which a free flowing reach of sufficient length was present. In addition, with less overall development than the Reedy River watershed, the North Tyger River permitted the potential comparison of fish communities under different levels of land use. However, such an analysis would only be valid following the determination that the Reedy River has largely stabilized in community structure. Stabilization is a more appropriate term than recovery to "pre-oil spill" conditions, since the Reedy River watershed experienced a simultaneous increase in land use over the monitoring period and will not likely attain "pre-oil spill" conditions. Observation of a longitudinal fish community gradient on the North Tyger River similar to that of the Reedy River, however, in conjunction with the observed

recovery in community structure (see previous report), would suggest that the Reedy River has stabilized substantially relative to its condition following the oil spill.

Four sites spanning 25.1 river km and altogether dropping 50 m in elevation (0.199% slope) were selected on the North Tyger River, a very similar physical gradient to the Reedy River study section at 54 m over 34.9 river km (0.155% slope; Table 1). Sites were sampled in identical fashion to the Reedy River, with three-pass depletion electrofishing of 150 m. Sampling will be completed in November 2005 and is scheduled again for fall 2010.

Table 1.   Elevations and inter-site distances of the Reedy River and North Tyger River study sections.

Reedy River			North Tyger River		
Site	Elevation (m)	Distance Between Sites (river km)	Site	Elevation (m)	Distance Between Sites (river km)
1	225				
		7.2	1	233	
2	208				12.6
		12.4	2	203	
3	192				5.1
		6.4	3	196	
4	183				7.4
		8.9	4	183	
5	171				

Recommendations

Potential analyses for the presence and significance of a longitudinal fish community gradient include correlations (or lack thereof) among measures of fish community structure with elevation as well as inter-site distance. Additionally, sites could be tested for differences in community structure. For example, are differences in community structure significant among all sites, or perhaps only

between the upper- and lowermost sites? Ordination could be employed to visually compare longitudinal patterns in fish community structure between the Reedy and North Tyger Rivers.

If gradients are present and similar, analysis could expand to examine differences in community structure based on land use, with the North Tyger River serving as a less-developed reference river. Of interest would be potential differences in fish community integrity, such as diversity index scores or presence and relative abundance of species of conservation concern. Hypotheses could be formulated around the assumption that the fish community of the North Tyger River will be more even, diverse, and support greater variety and numbers of sensitive species than that of the Reedy River.

Prepared By: <u>Kevin Kubach</u>   Title: <u>Fisheries Biologist</u>

Job Title: **Fish Assemblage Integrity Among Reedy River Tributaries**

Period Covered   January 1, 2005 – December 31, 2005

Results and Discussion

The rate and spatial distribution of development within the Reedy River watershed make it conducive to an assessment of fish community integrity among tributaries exhibiting a steep gradient in land use level and type. Objectives include the examination of potential correlations among land use (type, level, and distribution), stream physical (i.e., habitat) properties, and fish community structure, as well as the identification of thresholds in land use at which fish community integrity exhibits significant decline.

Stream size and location criteria were established to objectively identify the set of potential study sites. Field verification determined that watersheds between 10-40 km^2 corresponded to stream sites appropriate for sampling and large enough to support fish communities sufficient for comparison. Additionally, sites were required to be >1 km from the Reedy River main stem, out of the immediate influence of reservoirs, and not nested within the same watershed.

Fifteen sites within the Reedy River watershed met the above criteria and access requirements (Table 1). Recent digital elevation model (DEM) land cover data were used to quantify the land use distribution within each watershed. Sites ranged along a gradient of approximately 19-87% in non-agricultural development level. To increase representation of low development watersheds, five sites from the adjacent Saluda River system and within the latitudes and elevation range of the Reedy River tributaries (152-300 m) were selected, bringing the total to 20 watersheds ranging from 15-87% developed, or 12-79% forested (Table 1).

Table 1. The 15 Reedy River and five Saluda River tributaries sampled June – October 2005, by development level within river system. Note: development values are preliminary.

Stream	Latitude (°N)	Longitude (°W)	Elevation (m)	Approximate Development Level (%)*
Reedy River System				
Brushy Creek	34.79914	82.39190	262	87
Richland Creek	34.85457	82.38395	269	81
Huff Creek	34.71488	82.35223	270	69
Rocky Creek	34.70389	82.29763	246	64
Laurel Creek	34.77899	82.34481	243	61
Langston Creek	34.88538	82.42379	293	57
Baldwin Creek	34.72433	82.30769	248	56
Reedy River headwater	34.94153	82.46429	300	40
Tributary to Baker Creek	34.66114	82.34817	217	29
Little Creek	34.62658	82.31021	215	25
Harrison Creek	34.66914	82.29473	228	24
Walnut Creek	34.40212	82.17350	152	23
Horse Creek	34.52373	82.26418	183	20
Beaverdam Creek	34.49901	82.23488	183	20
Martin Creek	34.58704	82.24868	203	19
Saluda River System				
Carpenter Creek	34.96404	82.57513	298	17
Gibson Creek	34.38450	82.30067	179	16
Shoal Creek	34.94024	82.57446	294	15
Tributary to Mountain Creek	34.53848	82.34586	205	15
Broad Mouth Creek	34.49065	82.42612	222	N/A

*Approximate percent combined low-, medium-, and high-density non-agriculturally developed land cover

Fish were sampled by depletion electrofishing according to the SCDNR Standard Operating Procedures (Thomason *et al*. 2002). Comprehensive habitat measurements (Fitzpatrick 1998) were conducted within the same reach at which fish were sampled. Channel morphology and riparian characterization are scheduled. Data loggers were installed at each site to continuously monitor water level and temperature. Sites were sampled once each between June – October 2005.

2005 Results Summary

Sites yielded from six to 17 of 37 total species representing seven families (Table 2; Table 3).
Total fish density ranged from 1,979-12,353 fish/ha, and communities corresponded to Shannon-Weiner Diversity values of 1.15-2.06 (Table 2). The Reedy River tributaries were dominated by cyprinids and centrarchids, followed by ictalurids, catostomids, and percids. The most abundant species on average were bluehead chub *Nocomis leptocephalus*, yellowfin shiner *Notropis lutipinnis,* bluegill *Lepomis macrochirus*, redbreast sunfish *Lepomis auritus*, and northern hogsucker *Hypentelium nigricans* (Table 3). Bluehead chub and redbreast sunfish were present at all 20 sites, while 13 species occurred at only one site each.

Table 2.   Fish abundance and diversity attributes of the Reedy and Saluda River tributaries sampled June – October 2005, by total fish density.

Stream	Mean Wetted Width (m)	Sample Area (ha)	Total Fish Abundance	Total Fish Density (fish/ha)[1]	Species Richness	Shannon-Weiner Diversity
Beaverdam Creek	3.4	0.034	420	12,353	16	2.06
Walnut Creek	4.7	0.047	568	12,085	17	1.59
Gibson Creek[2]	3.1	0.031	318	10,258	9	1.40
Martin Creek	3.7	0.037	290	7,838	17	2.01
Baldwin Creek	3.9	0.039	246	6,308	12	1.74
Broad Mouth Creek[2]	6.9	0.083	486	5,855	15	1.74
Little Creek	5.1	0.052	299	5,750	16	1.93
Tributary to Baker Creek	3.8	0.038	215	5,658	10	1.69
Tributary to Mountain Creek[2]	4.1	0.041	222	5,415	6	1.15
Carpenter Creek[2]	5.6	0.063	330	5,238	14	1.59
Huff Creek	7.2	0.131	669	5,107	9	1.44
Reedy River headwater	5.6	0.063	260	4,127	13	1.85
Langston Creek	4.5	0.050	198	3,960	10	1.70
Laurel Creek	5.1	0.052	187	3,596	11	1.64
Richland Creek	6.1	0.073	243	3,329	8	1.21
Brushy Creek	5.9	0.070	230	3,286	13	1.82
Harrison Creek	2.8	0.028	92	3,286	10	1.31
Horse Creek	5.4	0.059	146	2,475	15	1.87
Rocky Creek	7.7	0.123	303	2,463	12	1.59
Shoal Creek[2]	4.7	0.047	93	1,979	10	1.97

[1]calculated from three-pass electrofishing catch without projection for actual abundance
[2]Saluda River system

Table 3.   Relative abundance and frequency of occurrence of the 37 fish species present among Reedy and Saluda River tributaries, June – October 2005.

Family	Common Name	Scientific Name	Mean Relative Abundance	Frequency of Occurrence
Cyprinidae	Central stoneroller*	*Campostoma anomalum*	<0.001	1
	Rosyside dace	*Clinostomus funduloides*	0.001	2
	Fieryblack shiner*	*Cyprinella pyrrhomelas*	<0.001	1
	Eastern silvery minnow	*Hybognathus regius*	<0.001	1
	Rosyface chub*	*Hybopsis rubrifrons*	0.006	1
	Bluehead chub	*Nocomis leptocephalus*	0.314	20
	Golden shiner	*Notemigonus crysoleucas*	<0.001	2
	Spottail shiner	*Notropis hudsonius*	0.032	7
	Yellowfin shiner	*Notropis lutipinnis*	0.275	19
	Sandbar shiner	*Notropis scepticus*	0.002	3
	Fathead minnow	*Pimephales promelas*	0.004	1
	Creek chub	*Semotilus atromaculatus*	0.035	17
Centrarchidae	Flier	*Centrarchus macropterus*	0.002	1
	Redbreast sunfish	*Lepomis auritus*	0.061	20
	Green sunfish	*Lepomis cyanellus*	0.023	13
	Pumpkinseed	*Lepomis gibbosus*	0.002	6
	Warmouth	*Lepomis gulosus*	0.012	9
	Bluegill	*Lepomis macrochirus*	0.091	19
	Redear sunfish	*Lepomis microlophus*	0.001	1
	Redeye bass*	*Micropterus coosae*	<0.001	1
	Largemouth bass	*Micropterus salmoides*	0.010	17
	Black crappie	*Pomoxis nigromaculatus*	<0.001	2
Ictaluridae	Yellow bullhead	*Ameiurus natalis*	0.019	12
	Brown bullhead	*Ameiurus nebulosus*	0.001	2
	Flat bullhead	*Ameiurus platycephalus*	0.008	12
	Channel catfish	*Ictalurus punctatus*	<0.001	1
	Margined madtom	*Noturus insignis*	0.004	7
Catostomidae	White sucker	*Catostomus commersoni*	0.001	3
	Creek chubsucker	*Erimyzon oblongus*	<0.001	1
	Northern hogsucker	*Hypentelium nigricans*	0.044	15
	Striped jumprock	*Scartomyzon rupiscartes*	0.011	8
Percidae	Swamp darter	*Etheostoma fusiforme*	0.001	2
	Tessellated darter	*Etheostoma olmstedi*	0.001	1
	Seagreen darter	*Etheostoma thalassinum*	0.010	6
	Yellow perch	*Perca flavescens*	<0.001	1
Poeciliidae	Eastern mosquitofish	*Gambusia holbrooki*	0.027	7
Esocidae	Redfin pickerel	*Esox americanus*	0.001	1

*Species present only in Saluda River system samples

Recommendations

Such variability in abundance and diversity suggests a wide range in fish community structure among the tributaries sampled. Assemblage integrity, however, cannot be fully addressed until community structure is interpreted according to appropriate functional measures. Integrity by definition refers to fish community condition relative to that of historic or reference communities in the context of a changing independent variable over time or along an independent variable gradient at a given time, respectively. Therefore, ensuing efforts should strive to identify and interpret the underlying relationships observed between fish community integrity and land use within the Reedy River watershed.

Sites will be re-sampled summer-fall 2006 to provide additional information on temporal variability in fish community structure. Findings will ultimately be used to identify watersheds at greatest risk of biotic degradation for conservation and restoration candidacy. The potential also exists to develop an index of biotic integrity with regional applicability.

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Thomason, C., J. Bettinger, D. Rankin, D. Crochet, L. Rose, and H. Beard. 2002. The South Carolina Standard Operating Procedures for Sampling Fish in Wadeable Streams. South Carolina Department of Natural Resources, Freshwater Fisheries Section, Columbia, SC.

Prepared By: Kevin Kubach   Title: Fisheries Biologist

Job Title: **Development Of A Dynamic Water Quality Model For Lake
Greenwood, SC**

Period Covered   January 1, 2005-December 31, 2005

Results and Discussion

Lake Greenwood is the first major impoundment on the Saluda River, located approximately 100 km downstream from the Saluda headwaters in the SC Blue Ridge Mountains. With a total surface area of 11,400 A, the reservoir has a productive fishery although excess nutrient loading and eutrophication may threaten water quality and biotic habitat. The primary goal of this two-year study is to develop a dynamic simulation model of water quality in Lake Greenwood. The model will help quantify interactions among lake hydrology, nutrient loading and water quality in the lake. Furthermore, the model will help predict implications of alternate management plans for water quality protection and will help formulate long-term plans for water quality enhancement and aquatic habitat protection. Once developed for Lake Greenwood, this model could be expanded to examine related issues of water and habitat quality for the series of river/reservoir segments along the Saluda River and other drainage basins.

The basic conceptual scope of this modeling effort (Figure 1) is to link information on inputs from the larger watershed (point-source dischargers and nonpoint source runoff) to ecological/water quality patterns and interactions within the lake. We plan to use a state-of-the-art, reservoir-modeling platform (CE-QUAL-W2) to simulate in-lake processes as they respond to input hydrology and nutrient loading. The primary objective of the initial phase of study (2004-05) has been to develop a detailed, comprehensive data set on key parameters needed for model development and calibration (phosphorus distributions; algal productivity, biomass, and taxonomic composition; and rates of oxygen depletion).

**MODELING NUTRIENT LOADS
AND LAKE EUTROPHICATION**
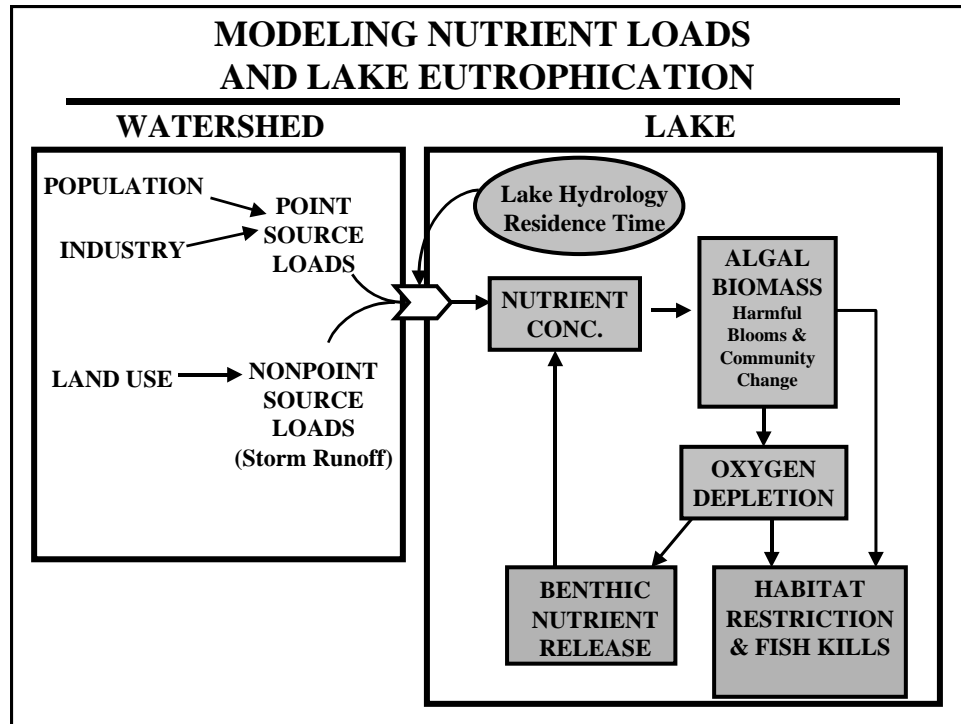
| WATERSHED | LAKE |

Figure 1.   Conceptual diagram of watershed/water quality interactions.

Field Sampling

A total of 12 sampling sites were established (Figure 2) to quantify the spatial detail in the reservoir from the input tributaries (Saluda and Reedy River Arms) to the downstream forebay. Most of the sampling sites were located along the main axis of the lake, with one site in a mid-lake embayment (Mid-Cane Creek Embayment, Figure 2).   Table 1 indicates the study components at each of the stations.   In addition, ongoing work by Clemson University and the Saluda-Reedy Watershed Consortium will be available to quantify trends in land use, water quality and nutrient loading in the major catchments flowing into Lake Greenwood.

LAKE GREENWOOD

Drainage Area : 1,170 mi^2
Surface Area : 11,394 Acres
Mean Depth : 7.0 m
Volume : 320.7 x 10^6 m^3
Avg. Res. Time: 3.45 Months

Sampling Stations
(2004-05)
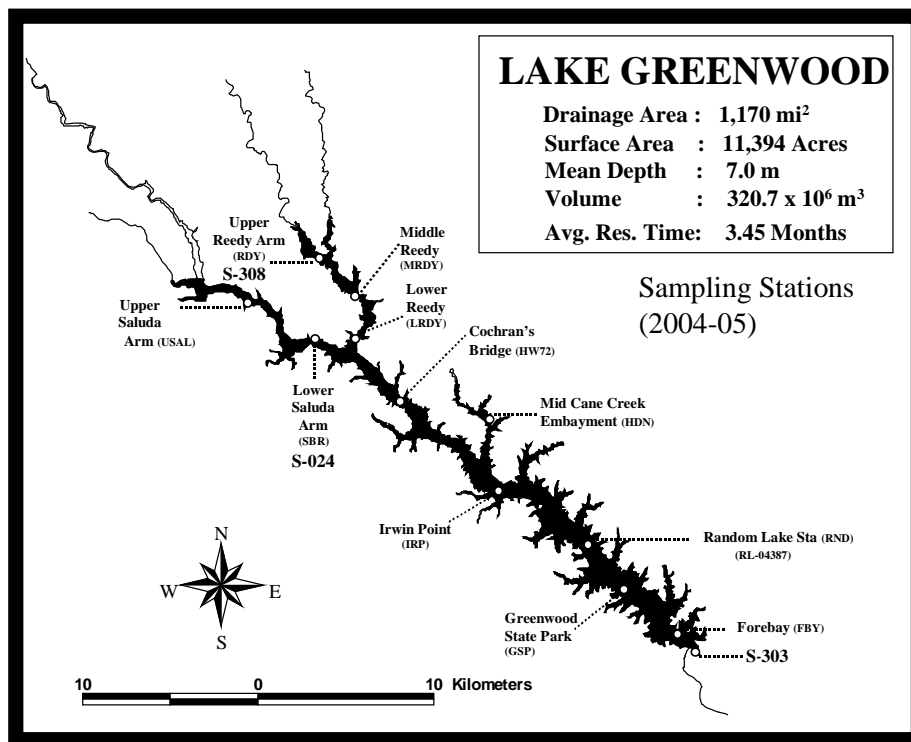
Figure 2. Sampling stations. SCDNR monitoring sites are shown as named stations (with 3-4 letter data codes). Additional data will be available from SCDHEC monitoring sites (S-306, S-024, S-303, and RL-04387)

Sampling Schedule and Analysis

Starting in January 2004, the original sampling protocol included monthly sampling for all study components (Table 1) throughout the annual cycle. While this sampling protocol was adequate to capture broad-scale seasonal patterns, it was not designed to quantify key, short- term events such as short-lived algal blooms and storm events. With additional support from the Saluda-Reedy Watershed Consortium, we increased sampling frequency to twice monthly through the active growing season (May-Oct 2004) with additional sampling during major storm events. This additional

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Table 1.   Site locations and study components.

Site Locations	Temperature/ Oxygen Profiles	Phosphorus/ Chlorophyll Concentrations	Plankton Productivity
Upper Saluda Arm	*	*	
Saluda Bridge	*	*	
Upper Reedy Arm	*	*	*
Middle Reedy Arm	*		
Lower Reedy Arm	*		
Highway 72 Bridge	*	*	*
Irvin's Point	*		
Cane Creek Embayment	*	*	
Irwin's Point	*		
Random Lake Station	*	*	*
Greenwood State Park	*		
Forebay	*	*	*

support also provided funds for quantifying algal community structure (pigment analyses and microscopic examinations) to complement our own studies of algal biomass and production. During 2004, we sampled the lake for distributions of oxygen, phosphorus, algae distributions and productivity on 35 days, including 9 days of sampling before and after 3 major storm events (Tropical storm Bonnie and Hurricane Frances, and Hurricane Jeanne). During 2005, we resumed sampling in March and continued monthly sampling through the extended growing season (Apr-Nov) to provide a second annual cycle of data for model validation. Additional effort during 2005 was focused on quantifying detailed vertical profiles of organic/inorganic forms of particulate and dissolved phosphorus during the period of lake stratification (Aug-Nov). These data will help

quantify phosphorus cycling dynamics in the lake, especially with respect to the potential release of phosphorus from anaerobic benthic sediments.

Field Measurements and Laboratory Analyses

Temperature/Oxygen Profiles.

At all 12 sampling sites (Figure 2), a detailed vertical profile of water temperature and dissolved oxygen (YSI-58 DO Meter) was examined at 1-m depth intervals from the surface to the bottom. The oxygen sensor was air-calibrated and checked daily; the YSI-58 thermistor was calibrated against a certified, NIST-traceable thermometer (FisherBrand, SN:1295). To insure a high level of quality control in field data collection, our laboratory secured SC certification for field measures of temperature and oxygen profiles (Lab.ID 40570, 21 May 2004).

Phosphorus and Chlorophyll Concentrations.

At 7 of the sampling sites, we collected -water samples for analysis of algal biomass (chlorophyll-a) and phosphorus, a critical limiting nutrient for algal production and eutrophication. Surface water samples for chlorophyll-a were placed in opaque HDPE bottles, labeled and placed immediately on ice. Phosphorus samples were collected from both surface and bottom waters and were partitioned into 3 HDPE bottles designated for analysis of total phosphorus, total soluble phosphorus, and soluble ortho-phosphate. Samples for total phosphorus were preserved with 1 ml H_2SO_4. Only surface water samples were collected at the upper tributary arms (Upper Saluda and Upper Reedy) which were < 5m total depth. All sample bottles were immediately labeled, placed on ice and transported to a certified analytical laboratory within 24 hrs of sample collection. Shealy Environmental Services (Lab. ID32010) performed the phosphorus analyses, using acid-persulfate digestion and ascorbic acid reduction (EPA Method 365.2). Two sets of samples for chlorophyll-a

analyses were collected and placed on ice in amber or foil-covered HDPE bottles. The first set was analyzed by SEAUS, Inc (Cert. Lab ID 36001) using acetone extraction and fluorometric analysis (APHA 1998, Standard Method 10200H). The SCDNR Freshwater Fisheries Research Lab analyzed the second set using acetone extraction and a modified, non-acidification fluorometric analysis (Welschmeyer 1994, Arar and Collins 1997, APHA 1998,); the DNR lab was subsequently certified (Cert. ID 4057) for continued studies in 2005.

Algal Productivity.

During 2004, the vertical distribution of algal productivity was quantified monthly, based on oxygen changes in a vertical array of light and dark bottles incubated in situ at 4 of the sampling sites (Table 1, Figure 3). The 4 stations were selected to provide a wide range of nutrient and light conditions for robust estimates of production coefficients in the model. At each station and time, a 15-L sample of surface water was collected, stirred vigorously to insure homogenous conditions, and then used to fill 14 light bottles (300 ml BOD bottles) and 4 dark bottles. After the initial oxygen concentration was determined in 2 of the bottles (using a aYSI-58 DO meter and 5905 bottle probe) the remaining light bottles were suspended at 0.1, 0.3, 0.6, 1.1, 1.6, 2.1, 2.6, 3.1, 4.1, 5.1. and 6.1 m depths, with duplicate bottles at the 0.1m level. The depth range from 0 to 6.1 m was usually in the photic zone at these stations. Dark bottles were suspended in duplicate near the surface (0.1m) and just below the lower light bottles. After a 4 to 6-hr in situ incubation, the bottles were retrieved and the change in oxygen concentration determined. Net productivity (Pn, mg L-1 h-1) for each depth in the vertical array was calculated as (L-I)/t, where L was the final oxygen concentration in each light bottle, I was the initial oxygen concentration, and t was the time of incubation (h). Respiration (R, mg L-1 h-1) was calculated as (I-D)/t where D was the final oxygen concentration in the dark bottles. Gross productivity (Pg, mg L-1 h-1) was then calculated for each depth in the vertical array as Pn +
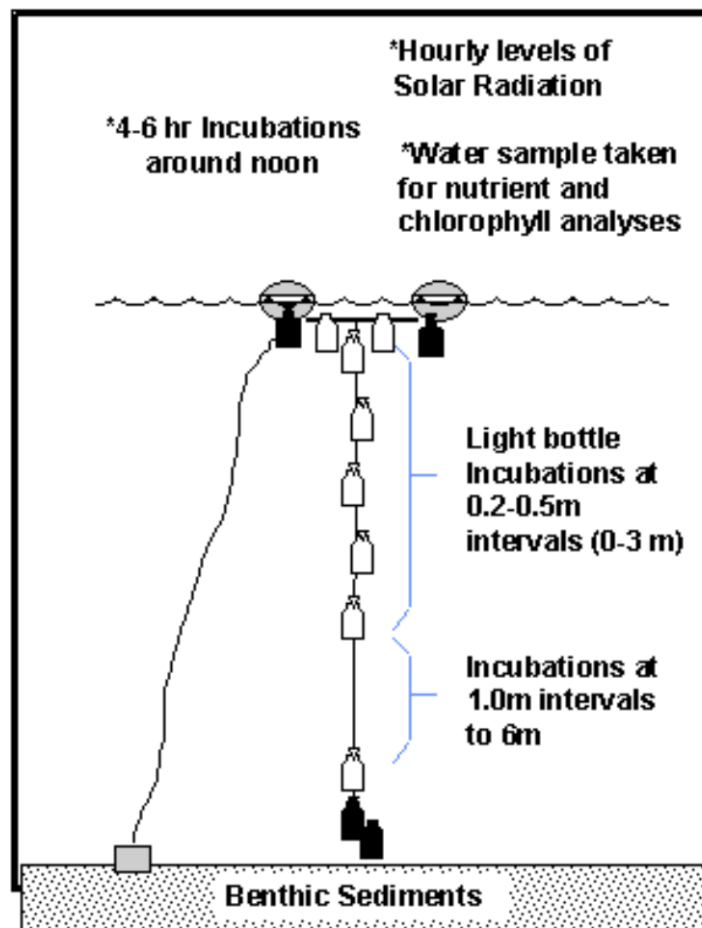
Figure 3. Schematic view of the vertical array of light and dark bottles for evaluating algal production and water column respiration. Stations at the Forebay, Lower-Lake, and Mid-Lake were > 6m deep, so the "Benthic Sediments" were well below the vertical array. (The station in the upper Reedy River Arm was < 3 m deep so the vertical array extended only to the 2.1 m level).

R. During winter and fall, R was evenly distributed between the upper and lower levels of the vertical array and the mean R from all 4 dark bottles was used to calculate Pg for each depth in the vertical array. However, during periods of thermal stratification in the top 6 m (May-August), the deeper dark bottles were often cooler (2-7°C) than surface dark bottles and exhibited correspondingly

lower rates of respiration. During these periods, R for each level in the vertical array was computed as an exponential function of the observed temperature profile as follows:

$$R = (k1) \, e \, (k2)T$$

Where k1 and k2 were determined from an exponential regression (EXCEL) of R vs T for the shallow and deeper dark bottles. Hourly levels of gross productivity were extrapolated to daily rates (mg L-1 d-1) by the following calculation:

$$Pg(mg \, L^{-1} \, d^{-1}) = Pg(mg \, L^{-1} \, h^{-1})(t)(Ld)/Li$$

where t = the duration of incubation (h), Ld = total solar radiation for the day (μmol m-2 da-1), and Li = total solar radiation during the incubation (μmol m-2). Ld and Li were derived from continuous recordings of photosynthetically active radiation (PAR) using a LiCor Li-190SA quantum sensor and Campbell CR21X data logger, deployed on a dock at a mid-lake location near Station HW72 (Fig. 2). Additional information on light distribution through the water column was gained by vertical profiles of PAR at 0.5 to 1.0m intervals throughout the photic zone at each station (LiCor 250A underwater quantum meter). Secchi disk observations were also recorded as an additional indication of water clarity at each station and time.

Phytoplankton Taxonomy

To determine the succession of algal dominants during this study and to assess the potential for harmful algal blooms, additional samples were collected for taxonomic analysis by the SC Algal Ecology Lab in Charleston SC (Hollings Marine Lab and SCDNR Marine Resources Research Inst). This analysis included microscopic screening of preserved water samples and High Performance Liquid Chromatography (HPLC) analysis of extracted pigments of known taxonomic importance to algal identification. The details of methodology and results are provided in Appendix A.

While most of the data for 2005 is still being analyzed, the following charts provide an overview of the 2004 data set and the kinds of information and preliminary interpretations gained thus far.

Basic Hydrology

Water quality in reservoirs typically responds directly or indirectly to changes in basic hydrology such as tributary inflows, outflows and resultant residence time and water level. During 2004, water level in Lake Greenwood was maintained according to an operating "rule curve" approved by FERC (Figure 4). The rule curve calls for water level reductions through the late fall and winter to a late January minimum of 434.5 ft (MSL). Beginning in February, the water level increased gradually to 439 ft by mid-April. This level was maintained through summer and early fall with a gradual drawdown beginning again in November. Some fluctuations in water level (+/- 1 ft) occurred in response to several major storm events in September and December.

The Saluda and Reedy Rivers represent the major sources of inflow to Lake Greenwood. Long-term mean daily discharge (since 1939) in the Saluda (976 cfs, http://nwis.waterdata.usgs.gov) is about 2.8 times that in the Reedy (352 cfs). This relative relationship was roughly the same during 2004, except for a few short-term runoff events in February and July, when flow in the Reedy was approximately equivalent to those in the Saluda (Figure 4). The major hydrologic events of the year were a series of tropical storms in September (Frances, Ivan, and Jeanne). The peak discharge in the Saluda following Hurricane Frances (11,225 cfs) was more than 10 times the long-term annual mean and was about 70% of the highest daily flow on record (16,100 cfs, Aug 27, 1995). The mean flow for September 2004 (2,837 cfs) was almost 5 times higher than the average flow for this month (594 cfs) and about 52% higher than the long-term maximum flows for September (1,862 cfs). Similar statistics for the Reedy were not available because of stream gauge damage during these storms.
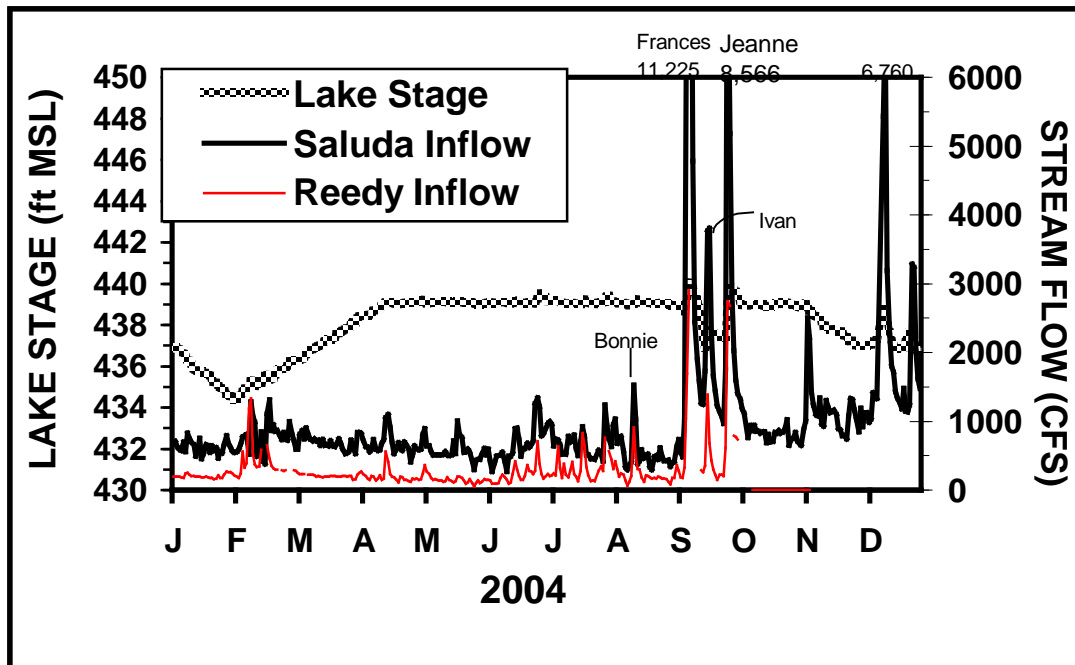
Figure 4.   Daily water level (lake stage) in Lake Greenwood and the major inflows from
the Saluda and Reedy Rivers for 2004.   Data from
http://nwis.waterdata.usgs.gov.

Phosphorus Distributions

Phosphorus concentrations in the upper reaches of the lake (both the Saluda and Reedy River

Arms, > 30 km upstream from the dam) were typically elevated above the SC water quality standard

of 0.06 mg/L (Figure 5).   This observation was consistent with SCDHEC placement of Lake

Greenwood on the State list of impaired waters due to high phosphorus concentrations.   The overall

mean concentration of total phosphorus in the Upper Saluda Arm (0.13 ± 0.03 mg $L^{-1;}$ Mean ±

Std.Err) was very similar to the Upper Reedy (0.11± 0.02 mg L^{-1}), both with >70 % exceedence of

the 0.06 mg L^{-1} standard.   The highest concentrations (0.40-0.48 mg L^{-1}) were observed in both arms

during discharge peaks related to Hurricanes Frances (Sep 9) and Jeanne (Sep 28, Figure 4).   These

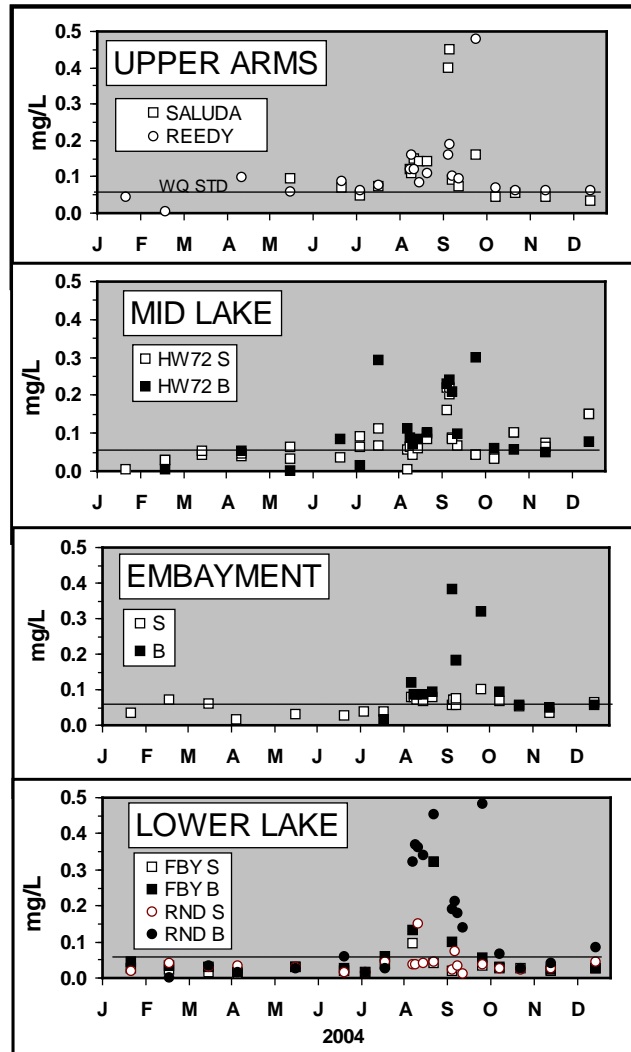results suggest the importance of nonpoint sources of phosphorus in both tributaries during storm events.

Figure 5. Distributions of total phosphorus in surface (S) and bottom waters (B) of Lake Greenwood. The dashed line at 0.06 mg/L represents the SC water quality standard for total phosphorus.

Further downstream, surface water concentrations typically declined substantially (Figure 5), probably due to particulate matter sedimentation and algal uptake. However, bottom water

concentrations at the downstream, deeper stations were much higher than in the surface waters during the late summer. At the Lower Lake stations (15-20 m deep), average bottom water concentrations in Aug and Sep (0.15-0.30 mg L^{-1}) were 3-6 times higher than in the surface waters (0.05 mg L^{-1}). This increase of bottom water phosphorus was perhaps due to the accumulation of settling particulate matter and the potential of phosphorus release from anaerobic benthic sediments (see section on Oxygen Depletion, p. 15). For extended sampling during 2005, we sampled detailed profiles of phosphorus fractions (total, soluble, and ortho-phosphate) at mid-lake (HW72) and lower-lake stations (RND and Forebay) throughout the peak of late-season stratification (Aug-Oct). These data will help quantify the relative importance of the internal phosphorus loading from anaerobic in comparison with the external loading from the Reedy and Saluda rivers.

Algal Distributions

Algal biomass (chlorophyll-a) also exhibited higher concentrations in the upper reaches of the lake (Figure 6), extending downstream to the middle sections (20 km upstream) including the mid-lake tributary embayment (Cane Creek). Algal biomass reached moderate levels (10-20 µg L-1 chlorophyll a) throughout the lake during summer, with clear domination by cyanobacteria (blue-green algae) during August and September (Appendix A). Cyanobacteria are nitrogen-fixing species that commonly bloom in nutrient rich conditions with limited hydrodynamic flushing, particularly during the warmer months. Some genera of known toxin-producing species of Cyanophytes were identified (Microcystis, Anabaena, Nitzchia, Aphanizamenon, and Anabaeneopsis) although none of these genera was found in high concentrations (Appendix A). On the other hand, total algal biomass (as indicated by chlorophyll a concentrations) occasionally exceeded state standards (40 µg L-1) in the upper and mid-lake sections. The pronounced bloom at the Mid-Lake station in late fall (60-80 µg L-1, Figure 6) was confirmed by both laboratories and was dominated by alloxanthin pigments

(Cryptophytes, Appendix A). Cryptophytes are motile, protozoan-like algae that are not typically associated with harmful, toxin-producing algae. The highest persistent chlorophyll a concentrations occurred in the mid-lake tributary embayment, suggesting more pronounced blooms in protected embayments with low flushing. Cryptophytes also dominated the algal community in the embayment during these fall blooms (Appendix A).
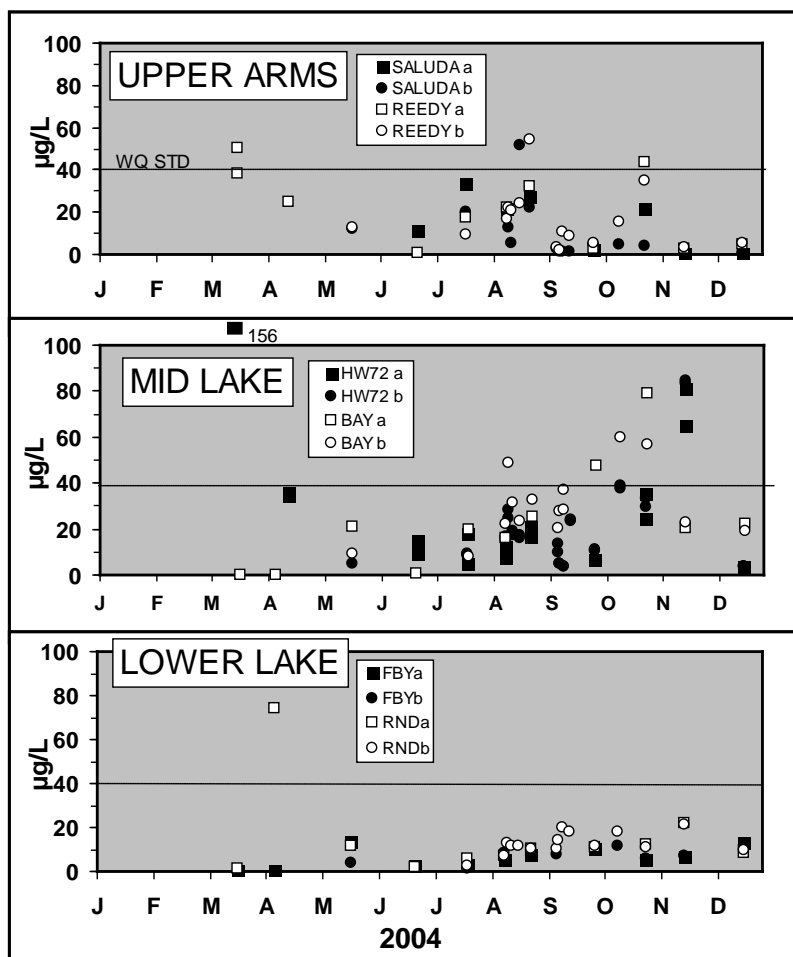

Figure 6. Chlorophyll-a distribution in Lake Greenwood. The black and white symbols represent separate stations in each lake zone. The square and circle symbols (a and b) represent results from 2 laboratories, SEAUS, Inc (squares) and SCDNR, Freshwater Fisheries Lab (circles). The dashed lines indicate the water quality standard of 40 μg L-1. (The spikes observed during March and April were part of the initial start-up period and are currently being considered as outliers.)

Algal Productivity

Vertical patterns and the spatial variability of algal productivity typically exhibited some general correlations with phosphorus concentration, algal biomass, and turbidity. For example, during mid-summer conditions (Figure 7, Table 2), the Upper Reedy Arm exhibited the highest levels of surface productivity (Pg(max), perhaps in response to higher phosphorus concentration and algal biomass. However, productivity in the Upper Reedy attenuated rapidly with depth due to more turbid conditions in the upper lake. The Mid-Lake station had similar phosphorus levels, although this station displayed somewhat less surface production, due in part to less algal biomass. However, at this station, water clarity was higher, light attenuation was lower and productivity extended through deeper levels of the water column, yielding the highest level of total water column production (Pg(int), Table 2). At the downstream regions of the lake (Lower Lake and Forebay), phosphorus and chlorophyll levels were much reduced, corresponding to considerably less algal production at the surface and through the water column. Continued analysis of these kinds of correlations will help quantify model parameters related to light, nutrient and biomass limitations of algal productivity in Lake Greenwood.

The maximum productivity in the surface waters (Pg(max)) exhibited a distinct seasonal patterns with a rapid increase at all stations in early spring (Figure 8, upper panel). Throughout the rest of the growing season (May-Oct), Pg(max) was typically higher in the Upper Reedy and decreased from upper lake to lower lake stations (Figure 8, Table 3), similar to patterns for total phosphorus and chlorophyll. The major deviation from this growing season pattern was in late September, when runoff from Hurricane Jeanne (Figure 4) produced extremely high turbidity in the upper and mid-lake stations (secchi disk observations $\cong 0.1$ m). This high turbidity greatly inhibited algal productivity at the upper and mid-lake stations. During this same time, surface water turbidity

in the lower lake remained relatively low (Secchi disk values > 1 m) and productivity exhibited a
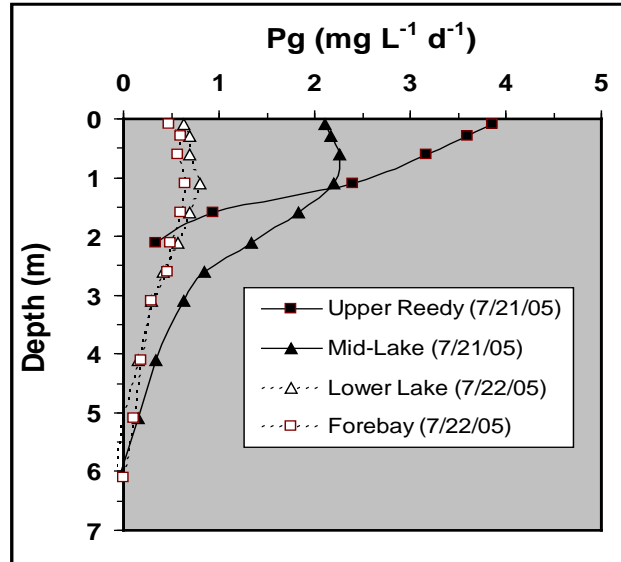
moderate fall peak.

Figure 7. Example of typical vertical patterns of gross oxygen production at 4 stations in
Lake Greenwood (7/21-22/04).

Table. 2.   Daily oxygen production, phosphorus concentration, chlorophyll-*a*, and light
attenuation in Lake Greenwood (7/21-22/04). Pg(max) is the maximum
volumetric production rate in the water column, Pg(int) is the vertically
integrated, area-based production through the water column, Kext is the light
extinction coefficient.

Station	Pg(max) $(mg\ L^{-1}\ d^{-1})$	Pg (int) $(g\ m^{-2}\ d^{-1})$	Chlorophyll $(\mu g\ L^{-1})$	Tot. P $(mg\ L^{-1})$	Secchi (m)	Kext (m^{-1})
Upper Reedy	3.86	4.31	17.7	0.077	0.5	3.05
Mid-Lake	2.27	5.71	5.2	0.076	1.3	1.12
Lower Lake	0.80	2.07	6.1	0.042	2.7	0.78
Forebay	0.64	2.04	2.9	0.038	>3.0	0.82

The daily integrated area-based productivity (Pg(int)), displayed a similar seasonal pattern (Figure 8, lower panel). However, the spatial pattern of Pg(int) during growing season was more variable (Figure 8, Table 3), reflecting combined influences of nutrients and turbidity. While phosphorus concentrations (and algal biomass) was typically higher in the upper and mid-lake stations, the lower turbidity in the lower lake stations allowed productivity to extend to deeper levels often resulting in higher integrated productivity. This was particularly evident in early spring and fall, when the vertically integrated productivity at both the Lower-Lake and Forebay stations was higher than in the Upper and Mid-Lake stations (Figure 8). Respiration rates (R(int)) indicated higher rates of oxygen demand and general heterotrophic conditions (P:R ratio < 1) in the photic zone of the Upper to Mid-Lake areas (Table 3). In contrast, the lower lake stations indicated a more autotrophic photic zone (P:R ratio > 1) with a net production of organic matter through the growing season.

Oxygen Depletion

Lake Greenwood has a history of hypolimnetic oxygen depletion that affects habitat availability (Snoots 1993). During the 2004 sampling season, the distribution of dissolved oxygen in Lake Greenwood exhibited a rapid depletion in the bottom waters from well-mixed conditions in March and early April to highly stratified conditions from May through October Figure 9 illustrates the changes in temperature and dissolved oxygen in the lower end of Lake Greenwood. A linear regression of the mean oxygen concentration below 10 m over time indicates a relatively consistent rate of hypolimnetic oxygen depletion (0.14 mg L^{-1} d^{-1}; 4.2 mg L^{-1} mo^{-1}, $r^2 > 0.94$) throughout the lower end of the lake. A pronounced thermocline extended throughout most of the lake from mid-May through the fall.
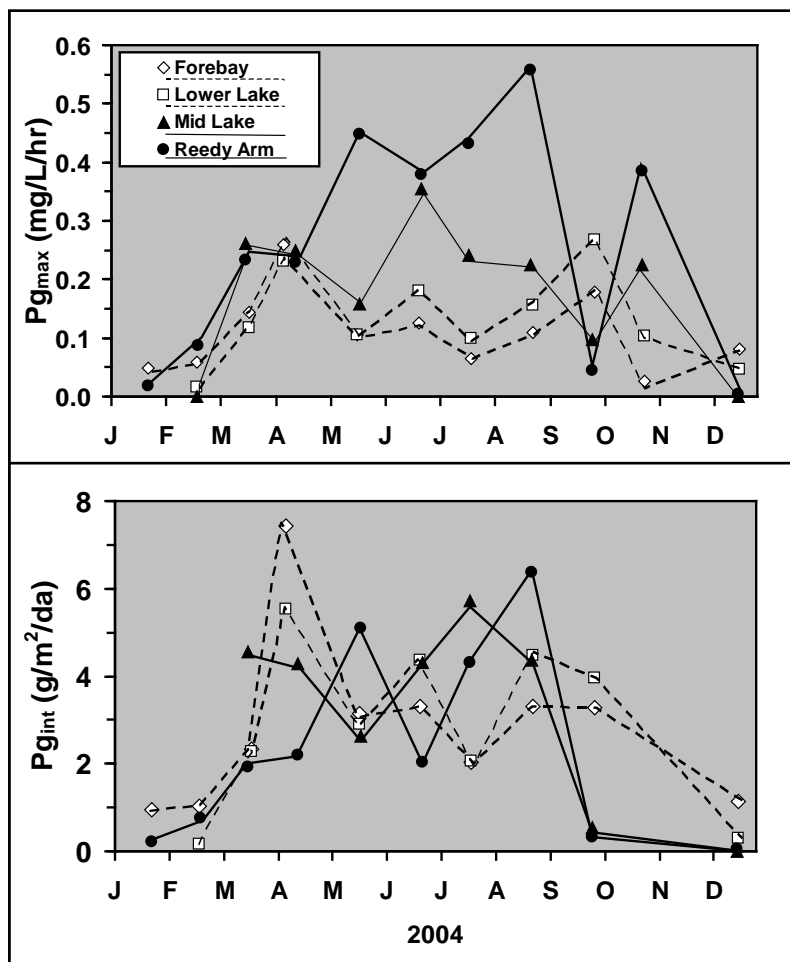
Figure 8.   Seasonal patterns of maximum surface productivity (Pg(max)) and vertically integrated productivity (Pg(int)) in Lake Greenwood, 2004.

Table 3.   Growing season means (± standard error) for Pg(max), Pg(int), R(int), and P:R ratios; May-Oct, 2004

Stations	Pg(max) (mg L^{-1} h^{-1})	Pg(int) (g m^{-2} d^{-1})	R(int) (g m^{-2} d^{-1})	P:R Ratio
Upper Reedy	0.375 ± 0.071	3.63 ± 1.09	3.80 ± 0.67	0.96
Mid-Lake	0.217 ± 0.035	3.51 ± 0.89	6.96 ± 2.20	0.50
Lower Lake	0.152 ± 0.027	3.55 ± 0.47	2.65 ± 0.84	1.34
Forebay	0.101 ± 0.021	3.01 ± 0.25	2.36 ± 1.09	1.27

Figure 9 also illustrates oxygen and temperature thresholds that indicate the volume or thickness of high quality habitat in the lake (blue shading). The left-hand vertical line at 4 mg/L DO indicates the lower limit of dissolved oxygen preferred by most fish species in the reservoir. The right-hand vertical line at 25°C indicates the upper temperature preferred by some important predatory fish like striped bass. Lower quality habitat, indicated by the red shading in Figure 9, develops whenever a DO drop below 4 mg/L or temperature exceeds 25oC. By the end of May in Lake Greenwood forebay, high quality habitat was restricted to a depth zone between 2 and 6 m. From June through September, high quality habitat was very limited at the lower end of the lake. Figure 10 illustrates the spatial patterns of habitat quality throughout the lake during the spring of 2004. By the end of June, the volume of high quality (as well as marginal quality) habitat became severely limited, a condition which persisted through August. By September, the inflow of cooler water restored some higher quality habitat in the upper levels of the water column, especially in the upstream end of the lake (deeper parts of the lake still contained oxygen depleted water).

Although oxygen decline in the hypolimnion is a natural process, the intensity, duration, and spatial extent of hypoxic conditions in Lake Greenwood are related in part to the high rates of nutrient loading and eutrophic conditions in the upper regions of the lake. Since the pattern of oxygen distribution represents a key component of water quality (which responds directly to levels of nutrient loading and algal production), a major goal of the developing model will be to predict spatial and temporal distributions of oxygen as functions of hydrology and management alternatives. Understanding the patterns of temperature and oxygen distributions will contribute to a dynamic assessment of extent and variability of habitat quality in Lake Greenwood.
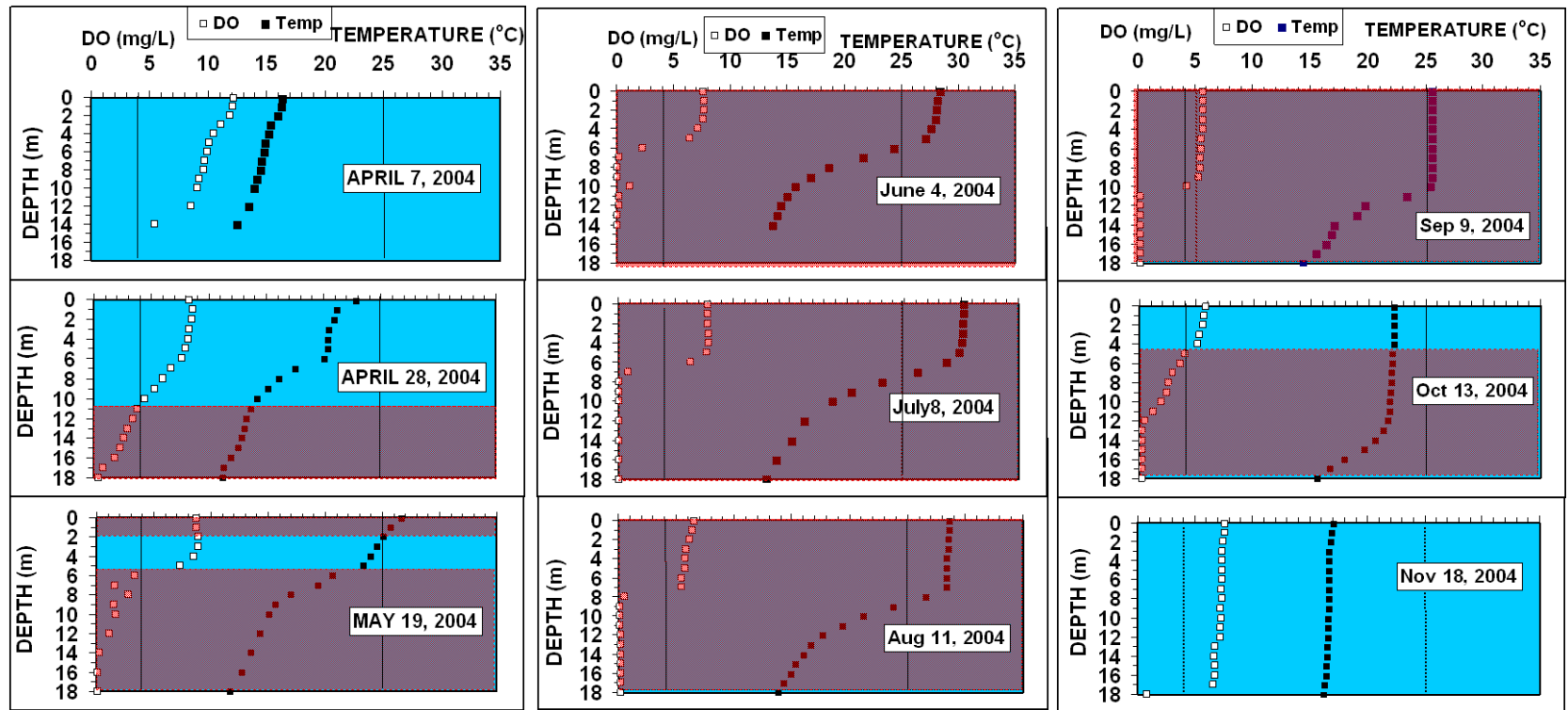
Figure 9.   Vertical patterns of dissolved oxygen (DO) and water temperature in the forebay of Lake Greenwood; April-Nov, 2004. The blue shading indicates zones of high quality habitat, where DO > 4 mg/L and temperature is < 25oC. The red shading indicates zones of poorer quality habitat as defined by these thresholds.
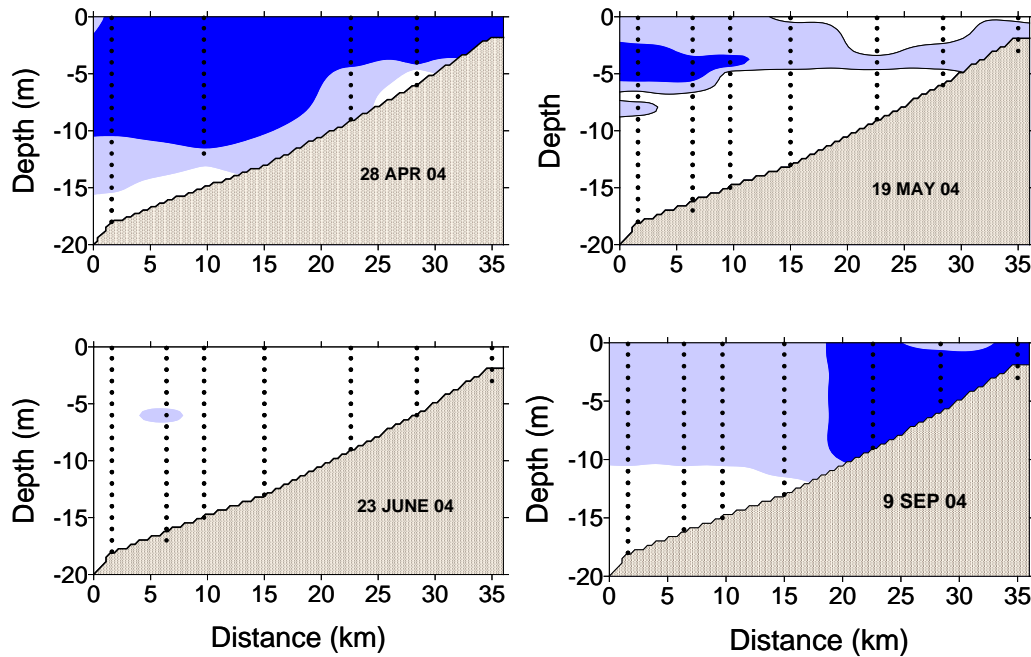
Figure 10. Spatial patterns of habitat quality through the spring 2004 in Lake
    Greenwood. Dark blue indicates "High Quality" habitat where DO>4 mg/L
    and Temperature is < 25ºC. Light blue indicates "Marginal" habitat where DO
    is between 2 and 4 mg/L and temperature is between 25 and 27ºC. White areas
    indicate "Poor" quality habitat where DO < 2 mg/L and temperature is > 27ºC.

<u>Future Plans and Recommendations</u>

The database collected for 2004-05 represents a sound foundation for model development,

calibration, and validation. We plan to complete data analysis and initiate model development by the

1st quarter of 2006. A thorough calibration and testing of the model should be completed by the 2nd

quarter of 2006, with final applications and assessment of potential watershed management options

by the end of 2006.

A specific question related to watershed management options for phosphorus loading is "how

much of the total phosphorus load to Lake Greenwood derives from external loading (from the

Saluda-Reedy watersheds) as compared to internal loading from bottom sediments?" The relative

contributions of these two sources of phosphorus loading has direct implication to the response of

lake water quality to watershed management options such an implementation of a TMDL for external

loads. As data analysis is completed and model development proceeds, we recommend a focused

analysis of the issue of internal vs external loading and implications to water and habitat quality.

Analysis of the 2004 data set (this report) clearly identified the potential effects of phosphorus

release from benthic sediments into the bottom waters (see Figure 5). However, to accurately

quantify the effect of benthic phosphorus releases, we needed information from more detailed

vertical profiles of phosphorus through the water column. Our extended sampling through 2005,

focused specifically on phosphorus profile analysis through peak period of stratification (July-Nov);

this effort provided data to accurately incorporate related functions of sediment phosphorus release

into the overall water quality model. Once the model is thoroughly calibrated and tested, we

recommend a comprehensive sensitivity analysis of key factors (external loads and internal lake

processes) with respect to their effects on water quality and habitat quality in Lake Greenwood. One

approach to evaluating habitat quality involves delineation of zones of optimal, marginal, and poor

quality based on threshold levels of dissolved oxygen and temperature (see Fig. 10- as an example of

habitat quality zonation based on 2004 field data). CE-QUAL-W2 specifically incorporates

functions to chart predicted zones of habitat quality based on external influences (i.e. rainfall, runoff,

phosphorus loads) and internal processes (i.e. algal production, sediment phosphorus release, etc). In

the near future we expect continued population growth and land-use change in the watershed to

affect patterns of stormwater runoff, nonpoint source loading and wastewater discharges to the

Saluda-Reedy watershed. Furthermore, the ongoing "Total Maximum Daily Load" (TMDL) analysis

by SCDHEC and ENSR Inc is expected to lead to future reuctions in wastewater discharges as well

as nonpoint source runoff. We recommend use of the model to evaluate predicted changes in habitat quality in Lake Greenwood (in terms of depth distributions, areal coverage, and duration) in response to these expected changes in phosphorus loading.

The scope of the current modeling effort will include a dynamic simulation of in-lake interactions and water quality patterns in Lake Greenwood in response to the total nutrient loading from the contributing watersheds. While this model will represent a powerful tool in developing management plans for Lake Greenwood, long-term management plans for aquatic resources in the entire basin will require more comprehensive **modeling of lake/watershed interactions**. A modeling effort of this scope would seek to integrate information on land-use changes and resultant nonpoint sources of runoff, with additional information on projected population growth and related changes in wastewater processing and point source discharges**. We recommend continued model development that would couple the developing lake model (CE-QUAL-W2) with a state-of-the-art watershed simulation model.** Such a model would combine information on land use, soils, and meteorology to simulate runoff and nonpoint source loads from the network of catchments in the Saluda-Reedy Watershed. The model would further combine results with information on point source discharges and reservoir release rates to route water through the basin and to simulate water quality dynamics in the receiving streams and lakes. The final product would help evaluate the impacts of loading patterns to Lake Greenwood as functions of land-use changes and point source discharge regulations in the basin. This scope of watershed modeling would address issues of water quality and aquatic resources throughout the entire Saluda-Reedy watershed, facilitating coordinated basin management. The objective will be facilitated by collaboration with ongoing analysis of temporal trends in water quality throughout the watershed (Hargett, *et al*), trends in land use and storm water runoff (Jeffrey Allen and Steve Klaine, Clemson University) , wet-weather patterns of

point source discharges (Anderson, Furman University), and laboratory evaluations of benthic sediment phosphorus release (Deanhart, Lander University).

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Prepared By: Hank McKellar   Title: Fisheries Biologist

APPENDIX A

Phytoplankton Taxonomic Composition

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HPLC Analysis. Phytoplankton community composition was estimated by High Performance Liquid Chromatographic (HPLC) pigment analysis (Kempton, *et al*. 2002a, Lewitus, *et al*. 2005). A volume of between 60 and 120 mL of whole water from each sample was filtered onto 25 mm GF/F filters and stored frozen until analyses. Filters were extracted in 2-3 mL of acetone, and filtered into an amber vial for HPLC analyses. Extracts were analyzed according to the method of Van Heukelem and Thomas (2001) on an Agilent Technologies (Palo Alto, CA) 1100 series HPLC. Briefly, 150 µL of extract was injected onto a C8, reverse-phase column (Eclipse™; Agilent Technologies). Methanol was the mobile phase, with tetrabutyl ammonium acetate added as an ion-pairing agent. Separate pigments were quantified by absorbance under visible light (450-665 nm), via diode array detector (Agilent Technologies), and identified by comparison with pure standards.

This method determines the concentration of 18 pigments of known chemotaxonomic importance to algal identification, Table 1 (Van Heukelem and Thomas, 2001). Data is presented on nine of these that are relatively less ambiguous with respect to taxonomic relevance. The marker pigments included fucoxanthin, peridinin, alloxanthin, 19'-hexanoyloxyfucoxanthin (19'-hex), chlorophyll c_3, chlorophyll b, zeaxanthin, canthaxanthin, and prasinoxanthin. Fucoxanthin is widely used as a marker for diatoms, a group in which it is universally present in high relative amounts. However, it is also found in some species of chrysophytes and prymnesiophytes (aka haptophytes), as

well as a subset of dinoflagellates. Therefore, caution is warranted in extrapolating fucoxanthin

values

Table 1.   Photopigments used for phytoplankton community analyses and their corresponding taxa.

Photopigment	Associated Taxa
Chlorophyll *a*	All algae
Chlorophyll *b*	Chlorophytes, euglenophytes, prasinophytes
Chlorophyll c_1	some chrysophytes, diatoms, dinoflagellates, haptophytes
Chlorophyll c_2	Chrysophytes, cryptophytes, diatoms, dinoflagellates, haptophytes
Chlorophyll c_3	Some dinoflagellates, some haptophytes
Fucoxanthin	Chrysophytes, diatoms, some dinoflagellates, haptophytes
Prasinoxanthin	some prasinophytes
Violaxanthin	Chlorophytes, prasinophytes
Zeaxanthin	Chlorophytes, cyanobacteria, euglenophytes, some prasinophytes
Neoxanthin	Chlorophytes, euglenophytes
Diatoxanthin	Chrysophytes, diatoms, dinoflagellates, euglenophytes, haptophytes prymnesiophytes
Diadinoxanthin	Chrysophytes, diatoms, dinoflagellates, euglenophytes, haptophytes
Alloxanthin	Cryptophytes
Peridinin	some dinoflagellates
19'-butanoyloxyfucoxanthin	some chrysophytes, some dinoflagellates, some haptophytes prymnesiophytes, some
19'-hexanoyloxyfucoxanthin	some dinoflagellates, some haptophytes
Lutein	Chlorophytes, euglenophytes, some prasinophytes
Canthaxanthin	Some cyanobacteria
Carotenes	Most photosynthetic algae

to diatom biomass. Peridinin is found in some species of dinoflagellates but no other phytoplankton. Therefore it is a specific marker for a subset of dinoflagellates. Alloxanthin is a specific marker for cryptophytes. 19'-hex is generally used as an indicator of some haptophytes, but also can occur in some dinoflagellates. Chlorophyll c_3 is a normally uncommon pigment that is associated with some dinoflagellates and haptophytes. Chlorophyll *b* is generally associated with green algae. Zeaxanthin has been used as a marker pigment for cyanobacteria (blue-green algae) but is also found in

chlorophytes, prasinophytes, raphidophytes, and euglenophytes. Canthaxanthin can be found in some cyanobacteria and prasinoxanthin in some prasinophytes.

A formula was used to estimate the relative contribution of cyanobacteria vs. green algae to the zeaxanthin signal in HPLC profiles. While analyzing the pigment ratios of several species used in calibrating a pigment modeling program, CHEMTAX, Lewitus et al. (2005) found that the ratio of $\mu g \, L^{-1}$ chlorophyll b to $\mu g \, L^{-1}$ zeaxanthin in *Chlorella* sp.(a coccoid chlorophyte) and *Ankistrodesmus* sp. (a benthic conjugatophyte) was 23 (mean of all treatments). Based on this limited data set, the contribution of green algae to zeaxanthin concentrations should be chlorophyll b concentration divided by 23. Subtracting this number from zeaxanthin concentration should give a qualitative index of the relative contribution of cyanobacteria to zeaxanthin concentration:

$$\mu g \, L^{-1} \, Zeaxanthin - (\mu g \, L^{-1} \, chlorophyll \, b/23)$$

By this reasoning, the contribution of cyanobacteria and green algae to zeaxanthin should be roughly equivalent when this index is 1. Values above 1 would correspond to greater relative contributions of cyanobacteria.

Microscopic Screening. Samples were received in 100 mL volumes fixed in 3% Lugol's solution. The samples were agitated gently to re-suspend settled cells, and 2 mL was placed in a Labtek ® chambered cover glass. Each sample was settled for at least 10 min and examined on a Nikon TE-2000 inverted microscope at 10X, 20X, and 40X objective magnification with both bright field and Differential Interference Contrast. Each sample was examined in a raster pattern in order to adequately cover the entire sample area. Algal species were identified to the highest taxonomic level possible given the limitations of Lugol's fixation. Therefore, it should be noted that the number of genera identified is certainly an underestimate the total number present.

Results

Pigment Analysis. Figures A1-A7 show three aspects of the HPLC pigment analyses for each sampling site. The top panel in each graph provides the marker pigment concentrations normalized to chlorophyll *a* concentration and therefore represents the relative contribution of marker pigment biomass to overall community biomass. The middle panel provides the absolute concentration (in μg L^{-1}) of each marker pigment, and the bottom panel, presents the zeaxanthin index, that shows qualitative estimates of the relative contribution of cyanobacteria (above an index value of 1) and green algae (below 1).

At most sample sites, cyanobacteria dominated the July 2004 phytoplankton communities, and a peak in cyanobacteria biomass occurred on 12 August 2004. A relatively high contribution of cyanobacteria over this period was indicated by a high proportion of zeaxanthin among accessory pigments, and a zeaxanthin index > 1 in all cases but at site FBY in July 2004. Cyanobacteria did not appear to be important contributors to overall phytoplankton community biomass for the rest of the experimental period, which instead was dominated by taxa containing fucoxanthin (e.g. diatoms and other chromophytes) and alloxanthin (cryptophytes). Cryptophytes were an important contributor to phytoplankton biomass on 28 October 2004, and made up > 50% of accessory pigment biomass in the Lower Saluda Arm (SBR), Mid-Lake (Hwy 72), Lower Lake (RND), and the Forebay (FBY). Peridinin, an indicator of a subset of dinoflagellates, was rare except for relatively high contributions to community composition in the Forebay (FBY) on 9 July and 22 July 2004.

Microscopic Screening. In addition to cyanobacteria, the following algal groups were identified: xanthophytes (rare), haptophytes (rare), euglenophytes, dinoflagellates, diatoms, cryptophytes, chlorophytes, and raphidophytes. Chlorophytes and cryptophytes were the most diverse
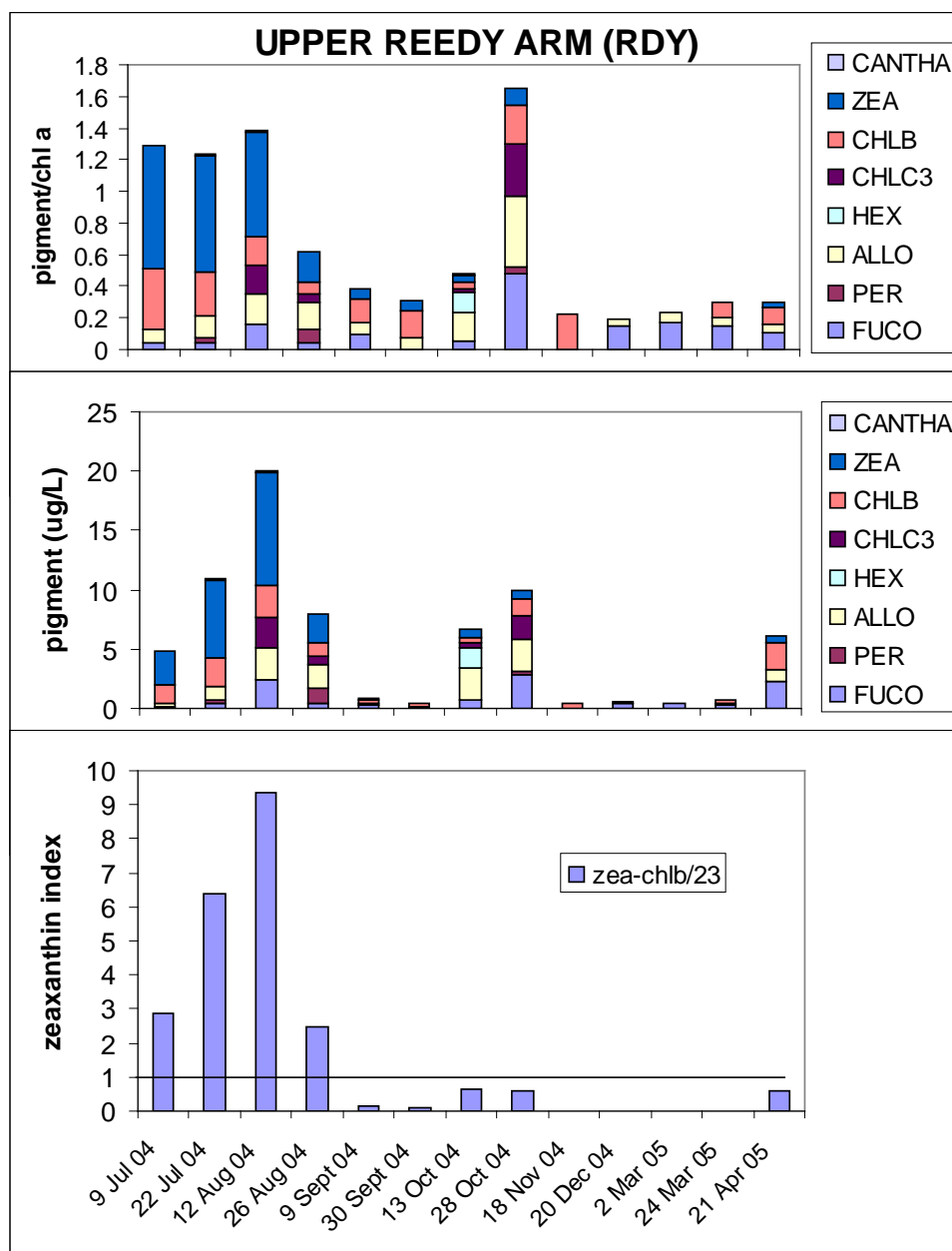
Figure. A1. HPLC pigment ratios to chlorophyll a (upper panel), pigment concentrations (middle panel) and zeaxanthin index (lower panel) for the Upper Reedy Arm (July 2004-Apr 2005). The index shows qualitative estimates of the relative contribution of cyanobacteria (values > 1) and green algae (values < 1).
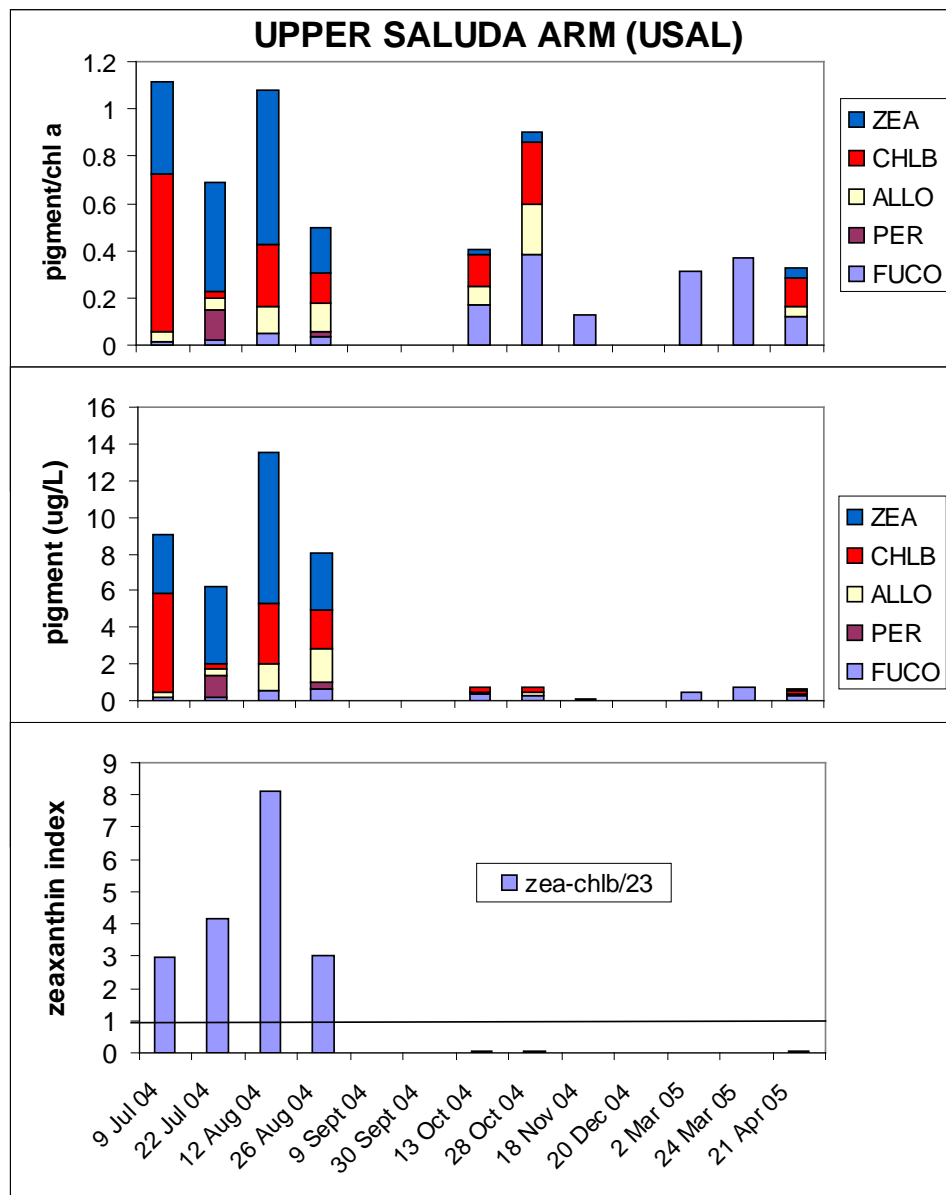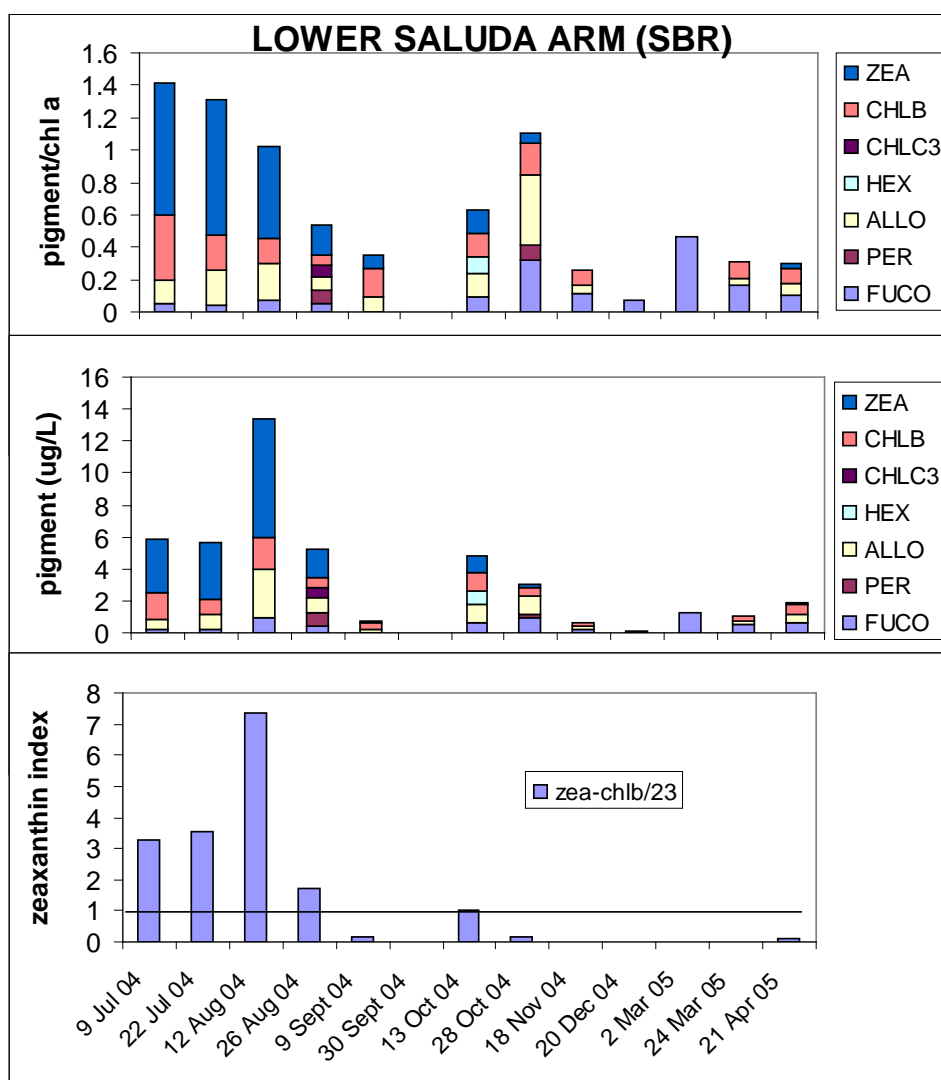
Figure A2. HPLC pigment ratios to chlorophyll a (upper panel), pigment concentrations (middle panel) and zeaxanthin index (lower panel) for the Upper Saluda Arm (USAL); (July 2004-Apr 2005). The index shows qualitative estimates of the relative contribution of cyanobacteria (values > 1) and green algae (values < 1).

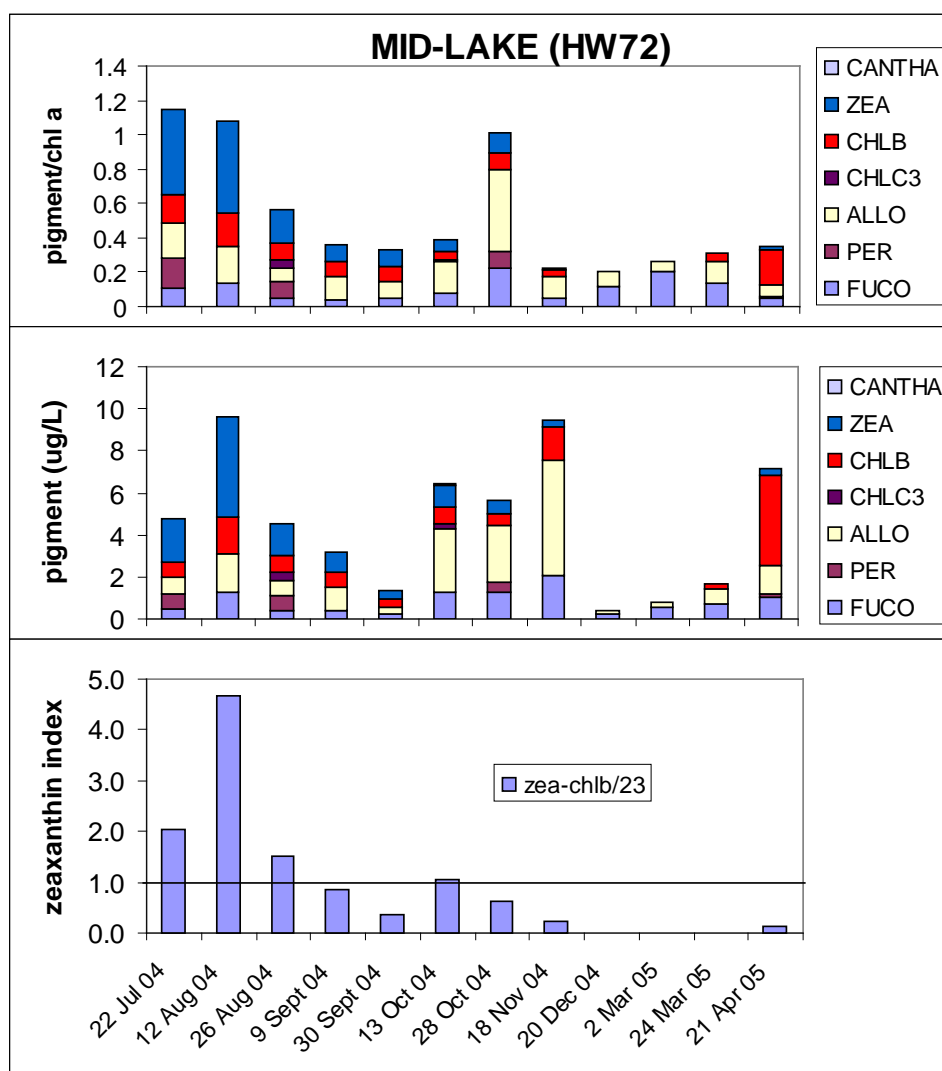Figure A3. HPLC pigment ratios to chlorophyll a (upper panel), pigment concentrations (middle panel) and zeaxanthin index (lower panel) for the Lower Saluda Arm (SBR)(July 2004-Apr 2005). The index shows qualitative estimates of the relative contribution of cyanobacteria (values > 1) and green algae (values < 1).
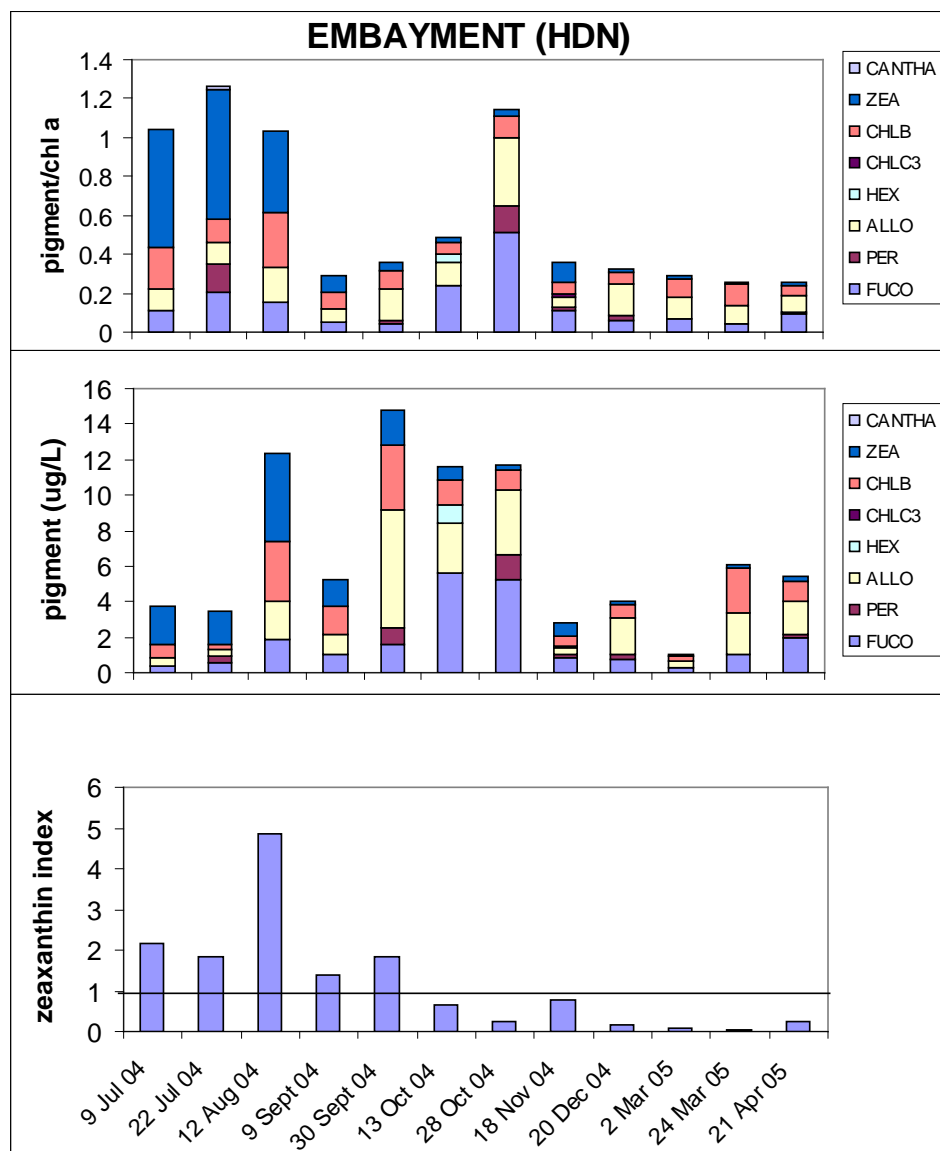
Figure A4. HPLC pigment ratios to chlorophyll a (upper panel), pigment concentrations (middle panel) and zeaxanthin index (lower panel) for the Mid-Lake station (HW72) (July 2004-Apr 2005). The index shows qualitative estimates of the relative contribution of cyanobacteria (values > 1) and green algae (values < 1).

Figure A5. HPLC pigment ratios to chlorophyll a (upper panel), pigment concentrations (middle panel) and zeaxanthin index (lower panel) for the Mid-Lake Embayment station on Cane Creek (HDN) (July 2004-Apr 2005). The index shows qualitative estimates of the relative contribution of cyanobacteria (values > 1) and green algae (values < 1).
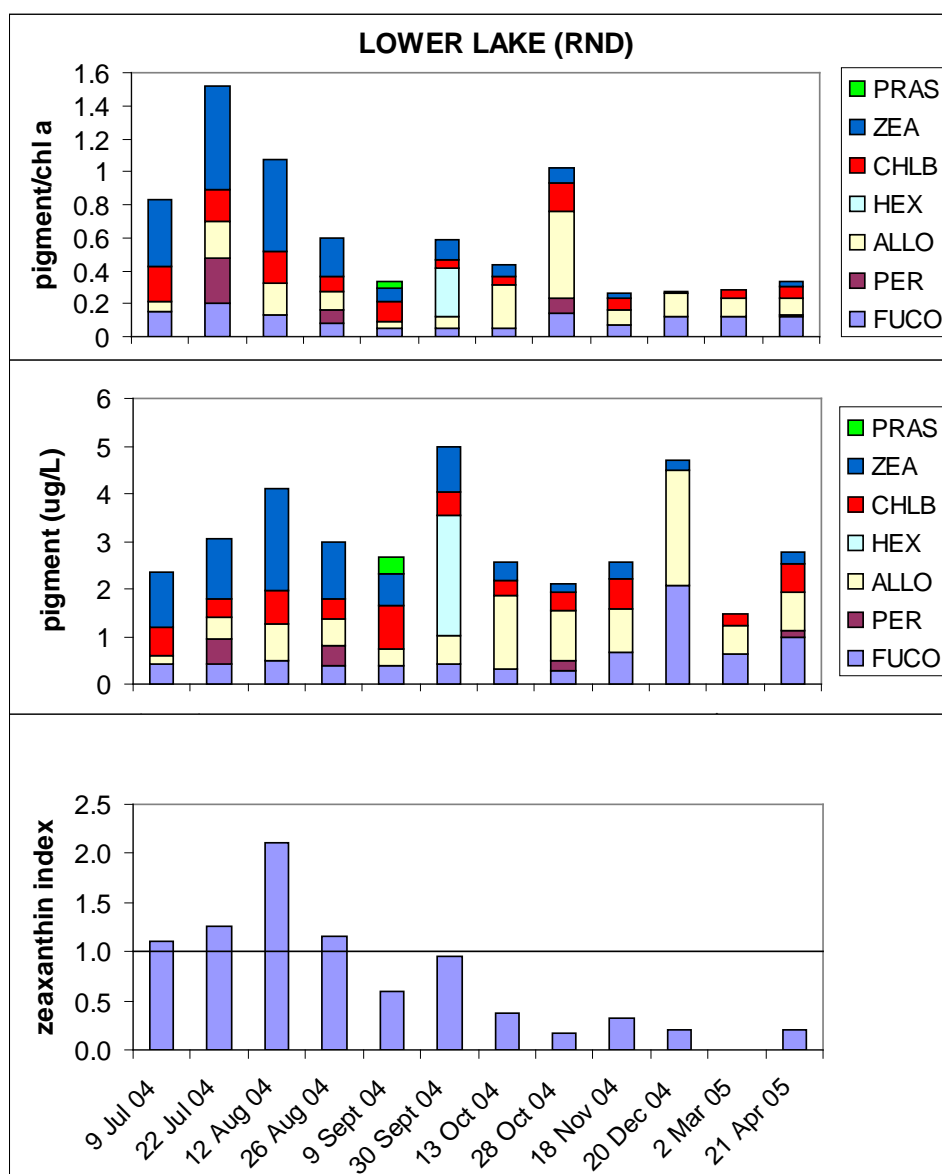
Figure A6. HPLC pigment ratios to chlorophyll a (upper panel), pigment concentrations (middle panel) and zeaxanthin index (lower panel) for the Lower Lake station (RND) (July 2004-Apr 2005). The index shows qualitative estimates of the relative contribution of cyanobacteria (values > 1) and green algae (values < 1).
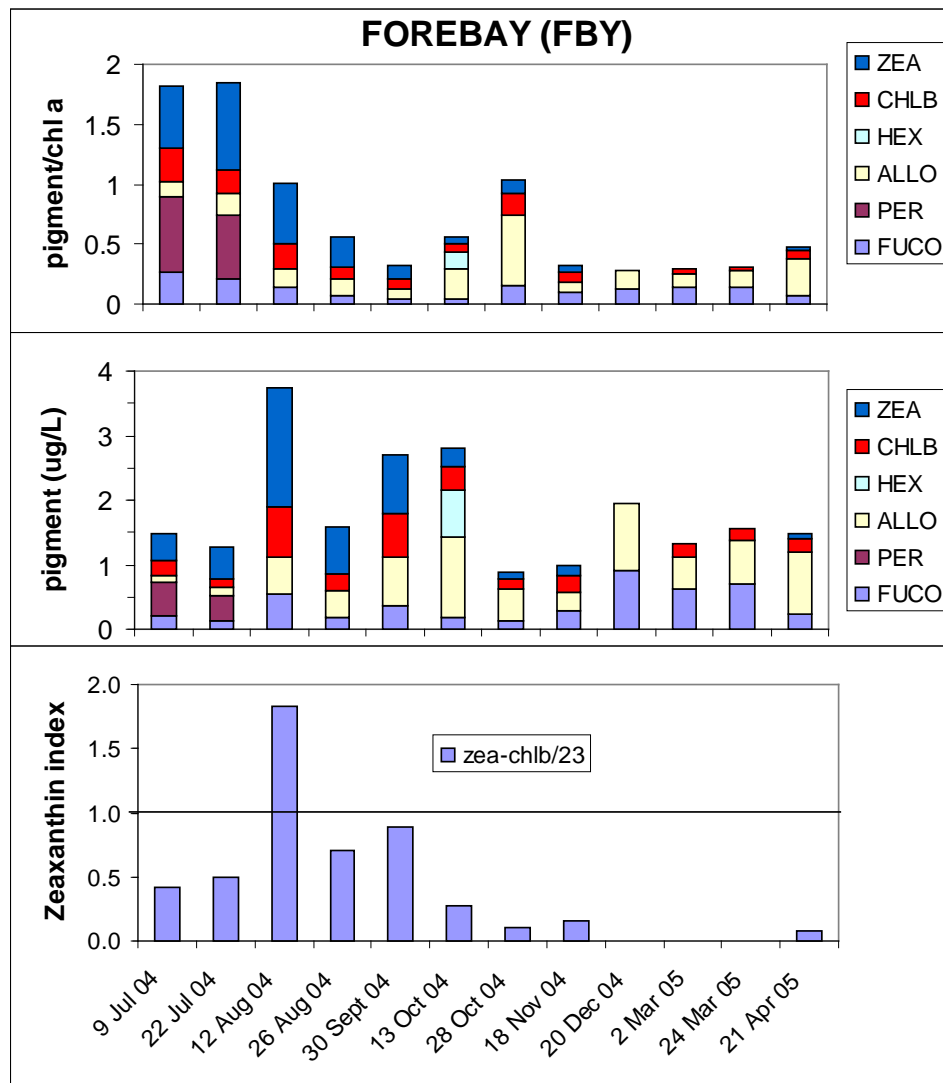
Figure A7. HPLC pigment ratios to chlorophyll a (upper panel), pigment concentrations (middle panel) and zeaxanthin index (lower panel) for the Forebay (FBY) (July 2004-Apr 2005). The index shows qualitative estimates of the relative contribution of cyanobacteria (values > 1) and green algae (values < 1).

groups, with respect to numbers of genera. The dominant taxa in all sites were cryptophytes, including nearly monogeneric blooms of *Cryptomonas* in November of 2004, and March and April of 2005. Other dominant classes were chlorophytes, notably the genera of *Chloroella*, *Scendesmus* and *Staurastrum*. Some genera of known toxin-producing species were identified, including *Microcystis*, *Anabaena*, *Nitzchia*, *Aphanizamenon*, and *Anabaeneopsis*. However, all these genera were sparsely represented in samples.

The relative distribution of taxonomic classes is shown as a percentage of total genera at each station in Figures A8-A10. In the upper tributary arms of Lake Greenwood, the Upper Reedy showed a total of 126 genera over the 7 sampling dates (Figure A8, upper panel). This site showed dynamic changes in diversity, with the summer dominance of chlorophytes dropping sharply in September 2004 and March 2005. Chrysophytes, represented by the genera *Chromulina*, *Dinobryon*, *Epipyxis*, and *Mallomonas* were present only in October 2004 and April 2005.

In the Upper Saluda Arm (Figure A8, middle panel), a total of 57 genera were identified, giving this site the least-diverse algal assemblage. Diatoms, though not always dominant, were better-represented throughout the year. December of 2004 was completely dominated by a single diatom genus, *Synedra*. The April 2005 sampling date showed that diversity among groups was returning, with a near-equal representation of Chlorophytes, diatoms, cryptophytes and haptophytes. In the Lower Saluda Arm (Figure A8, lower panel) a total of 91 genera were identified. A notable decrease in assemblage diversity occurred from July-September 2004 (chlorophytes, cyanobacteria, diatoms, and cryptophytes, in descending order of dominance) to one in which Chlorophytes made up >60% of genera in December 2004. Euglenophytes emerged as a dominant group in March and April of 2005.

Further downstream, a total of 129 genera were identified at the Mid-Lake station (HW72, Figure A9). Chlorophytes were the dominant genera, except for December 2004, when Diatoms and
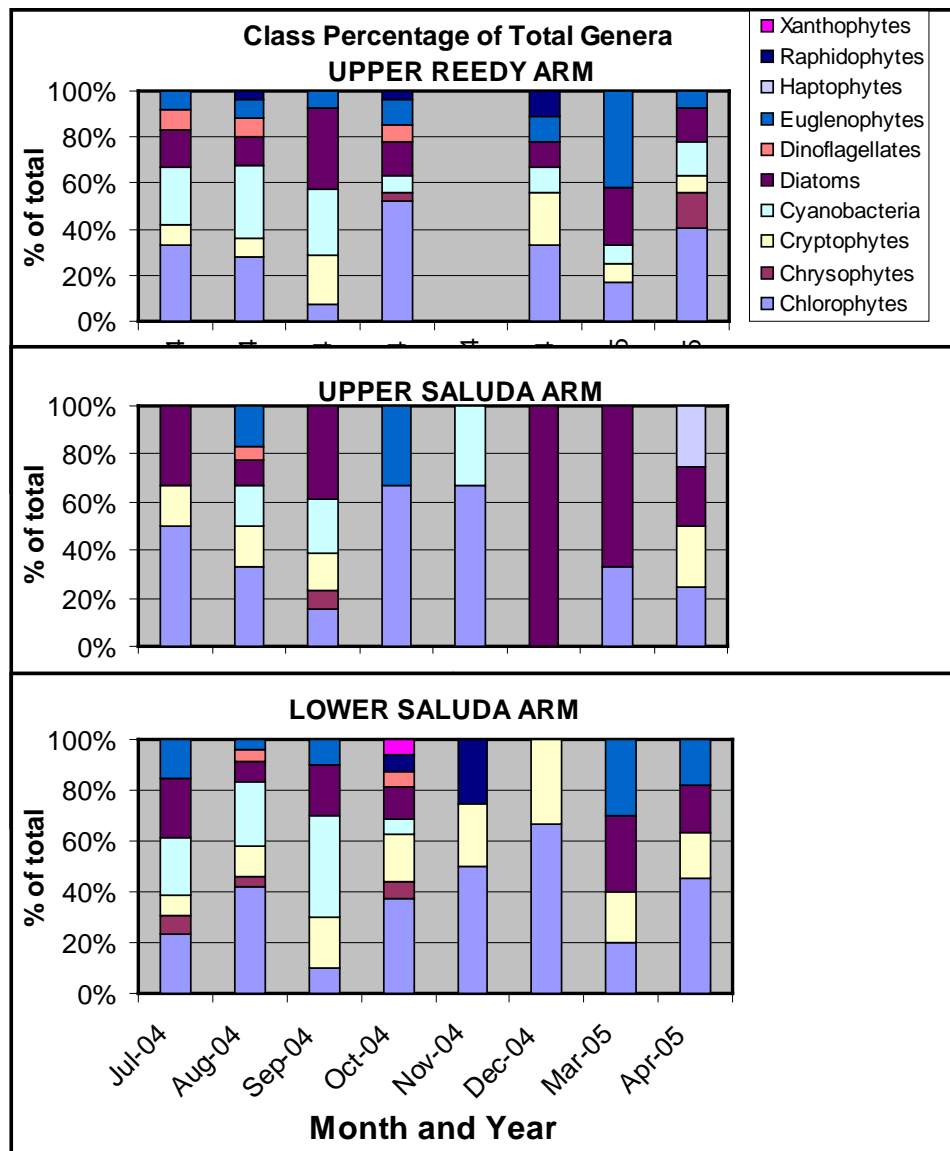
Figure A8. Percentage of total genera represented by each taxonomic group in the upper
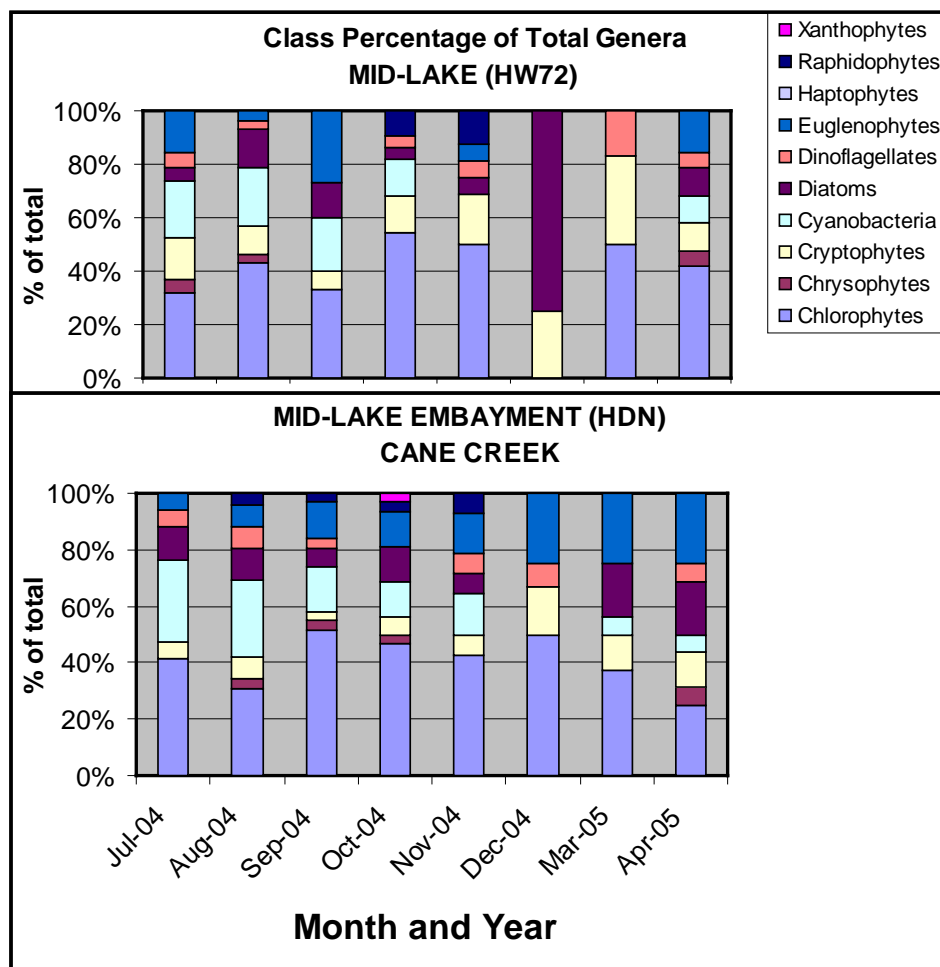   arms of Lake Greenwood (July 2004-Apr 2005)

Figure A9. Percentage of total genera represented by each taxonomic group at the Mid-Lake station (HW72) and the mid-lake embayment (HDN) of Lake Greenwood (July 2004-Apr 2005)
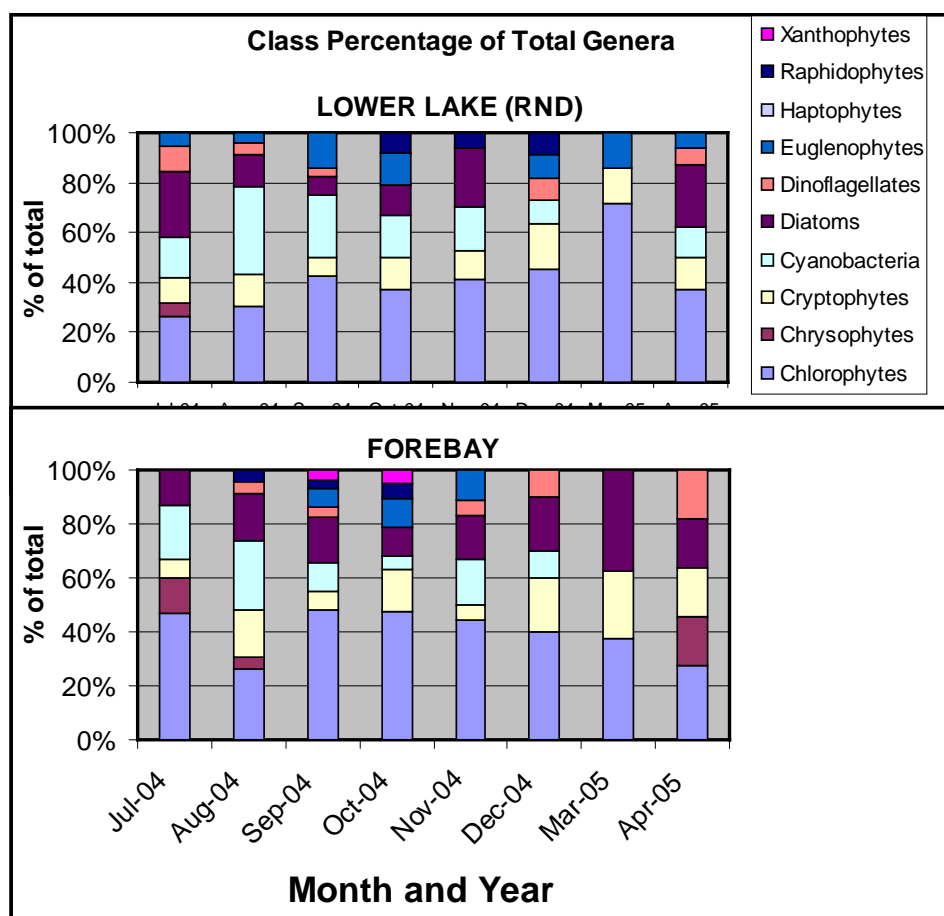
Figure A10. Percentage of total genera represented by each taxonomic group at the Lower Lake station (RND) and the Forebay (FBY) of Lake Greenwood (July 2004-Apr 2005)

Cryptophytes made up 75% and 25% of genera, respectively. Raphidophytes were noted in October and November of 2004, yet absent in other months. In the mid-lake embayment (HDN), a total of 164 genera were identified, making this site the most diverse assemblage (Figure A9, lower panel). Chlorophytes and cryptophytes dominated in nearly every month, except for April 2005. The percent of euglenophyte genera increased from July 2004 to April 2005. Cyanobacteria were co-dominants during late summer but were reduced in the fall and were entirely absent by December 2004.

In the most downstream sections of the lake, a total of 145 genera were identified at the Lower Lake station (Figure A10 upper panel), with chlorophytes clearly dominating. Cyanobacterial genera were well-represented in this site, with the greatest diversity found in August 2004 (8 genera, including *Anabaena* and *Microcystis*). The raphidophytes *Gonyostomum* and *Vacuolaria* were present from October to December 2004. In the Forebay (Figure A10, lower panel), a total of 133 genera were identified. Chlorophytes were the most diverse group of genera in nearly all months, followed by cryptophytes, diatoms and cyanobacteria. The most diverse assemblage of genera was noted in September, 2004, and the fewest number of genera were found in March 2005. Two rare xanthophyte genera, *Pseudostaurastrum* and *Goniochloris* were found in September and October of 2004.

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