FINAL PERFORMANCE REPORT
South Carolina State Wildlife Grant [T-31-R]
Assessing introgressive hybridization within and habitat requirements of native South Carolina redeye bass

October 1, 2007 – September 30, 2012

GRANT OBJECTIVES

1. Assess the degree of hybridization and introgression of Alabama spotted bass alleles throughout the middle and upper Savannah River reservoirs and headwater tributaries/streams, and identify genetically 'pure' populations in these systems.
2. Develop and implement simple, rapid and cost-effective genetic assays for the short and long-term monitoring of the hybridization between redeye bass and other black bass species throughout the Savannah River system.
3. Use Geographic Information System (GIS) technology and population genetic data to identify specific aquatic and shoreline habitat that is associated with high-frequencies of native, non-native and hybrid bass species.
4. Genetically assay redeye bass, and four other co-distributed species from Santee drainage. Assess variation with respect to Savannah drainage populations as an examination of native or introduced status of redeye bass. Assess presence and degree of hybridization in Santee redeye bass populations.

ACTIVITY OVERVIEW:

1. Assess the degree of hybridization and introgression of Alabama spotted bass alleles throughout the middle and upper Savannah River reservoirs and headwater tributaries/streams, and identify genetically 'pure' populations in these systems.

Activity: Genetic analysis was completed for N=669 black bass collected in 2004, and N=632 black bass collected in 2010 from reservoir sites on Lakes Russell, Hartwell, Keowee and Jocassee. Species composition was compared, and shows a precipitous decline in redeye bass collected from 2004 to 2010. Our 2004 survey indicated redeye bass had been virtually eliminated from Lakes Keowee and Russell, where they comprised 0% and 2% of black bass collected, respectively. Collections in 2010 show little change in redeye bass proportions from these two lakes, but a decline is evident in Lakes Hartwell and Jocassee. For Lake Hartwell redeye bass comprised 26% of fish collected in 2004 and 8% in 2010. There is a corresponding increase in hybrids between redeye and Alabama spotted bass, from 26% to 43%. For Lake Jocassee genetically pure redeye bass comprised 39% percent of black bass collected in 2004, and only 14% in 2010. Hybrids between redeye and Alabama spotted bass increased, from 29% in 2004 to 54% in 2010. Also of interest on Lake Jocasee is new collections of hybrids between redeye bass and smallmouth bass, which comprised 5% of fish analyzed from the lake in 2010 (Table 1).

In examining the proportions of redeye bass, Alabama spotted bass and their hybrids alone, an increase in pure Alabama spotted bass is evident for Lakes Hartwell and Russell. The proportion of fish in this classification from Lake Hartwell increased from 1% in 2004 to 10% in 2010. From Lake Russell proportions increased from 30% to 50% (Table 2).

Table 1. Percent composition of black bass species classifications collected from Lakes Jocassee, Keowee, Hartwell and Russell in 2004 and 2010; redeye bass (REB), Alabama spotted bass (ASB), largemouth bass (LMB), smallmouth bass (SMB). Percent values are reported as proportions for each lake, and are average proportions over all sites sampled.

	Jocassee		Keowee		Hartwell		Russell	
Species	2004 N=127	2010 N=140	2004 N=161	2010 N=137	2004 N=171	2010 N=183	2004 N=144	2010 N=172
REB	.39	.14	-	<.01	.26	.08	.02	-
ASB	-	.01	.25	.26	<.01	.05	.17	.27
REBxASB	.29	.54	.38	.42	.26	.43	.37	.27
LMB	.20	.15	.37	.31	.47	.42	.44	.46
SMB	.12	.11	-	-	-	-	-	-
Other hybrids	-	.05	-	<.01	-	.01	-	<.01

Table 2. Average proportions of redeye bass (REB), Alabama spotted bass (ASB) and hybrids between the two species collected from Lakes Jocassee, Keowee, Hartwell and Russell in 2004 and 2010. Proportions reported for each lake are average proportions over all sites sampled.

	Jocassee		Keowee		Hartwell		Russell	
Species	2004 N=86	2010 N=97	2004 N=101	2010 N=90	2004 N=89	2010 N=102	2004 N=81	2010 N=93
REB	.57	.20	-	.01	.49	.14	.04	-
ASB	-	.02	.39	.37	.01	.10	.30	.50
REBxASB	.43	.78	.61	.62	.49	.77	.66	.50

Genotype scores were generated for individual redeye bass, Alabama spotted bass, and hybrids between these two species, by counting the number of Alabama spotted bass alleles at each of the three nDNA loci examined. Scores ranged from 0 for ‘pure’ redeye bass to 6 for ‘pure’ Alabama spotted bass. Results, similarly to species proportions, reflect little change for Lake Keowee. All other reservoir populations show a decrease in ‘pure’ redeye bass (score of 0) and a corresponding increase in ‘pure’ Alabama spotted bass (score of 6). Over all scores increased over time for each lake. This is most notable for Lake Hartwell which scored 1.3 in 2004 and 3.2 in 2010. Increased genotype scoring reflects the increase in fish classified as pure Alabama spotted bass, as well as an increase in the segment of Alabama spotted bass alleles among hybrid individuals From each population in 2010, a portion of the individuals scoring as pure Alabama spotted bass possessed redeye bass haplotypes at the mitochondrial locus, indicating a portion of fish classified as pure Alabama spotted bass are actually the result of high order backcrossing (Figure 1, Bangs 2011).

This study has documented the decline of redeye bass in Savannah basin reservoirs over time, and certainly indicates that the species is on a path to elimination from these habitats, by hybridization. The decline in collections of pure redeye bass from Jocassee and Hartwell indicate these populations will eventually reach species composition levels already seen in Lake Keowee and Lake Russell, where Alabama bass were initially introduced to the Savannah basin.

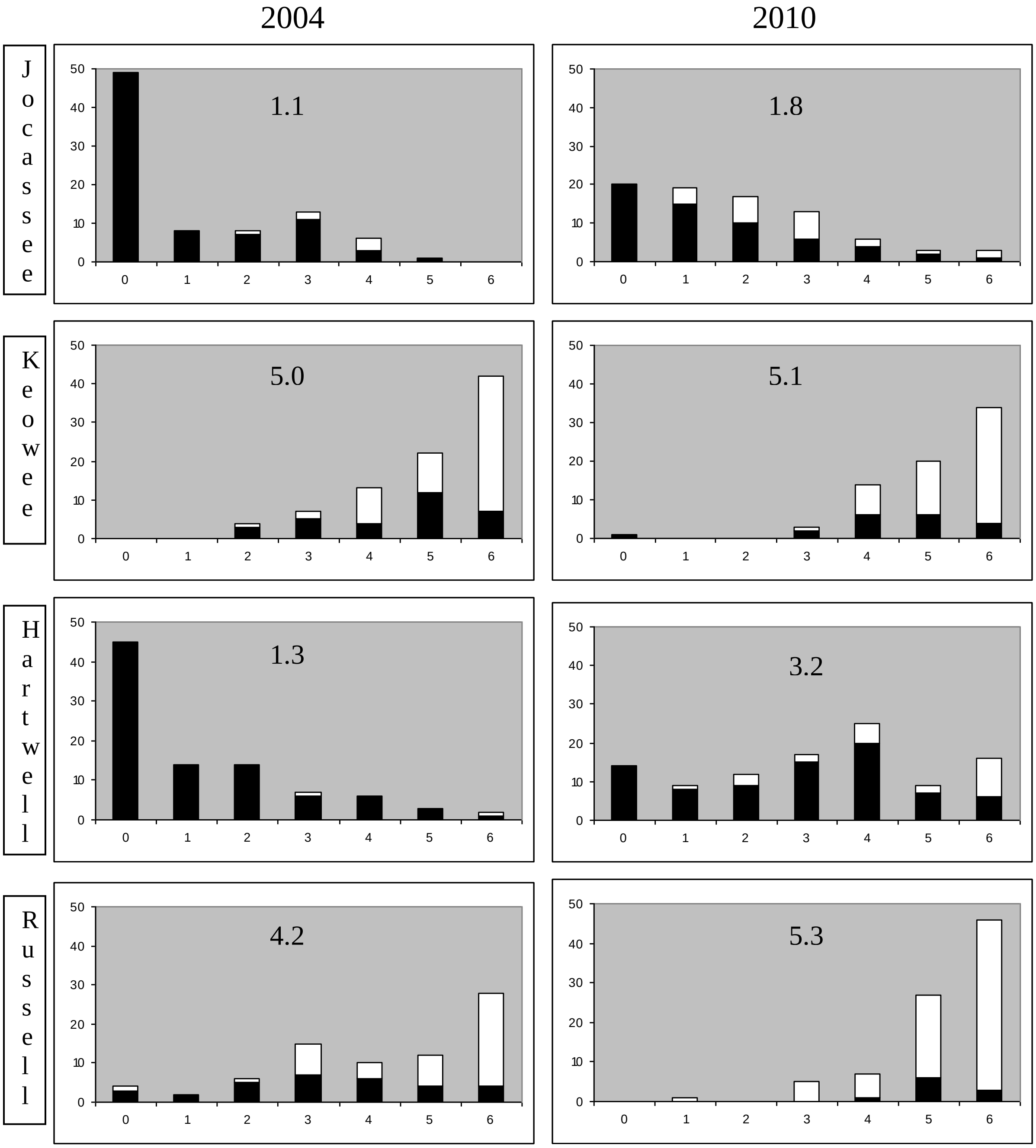


Figure 1*: Genotype scoring across three nDNA loci. Numbers on the x-axis represent the total number of ASB alleles across three nDNA loci, so 0 represents "pure REB" and 6 represents "pure ASB". White bars represent ASB mtDNA and black bars represent REB mtDNA. Y-axis is number of individuals. The number at the top of each graph is the average genotype score for that lake at that time period.

*From Bangs, Max. 2011. Decline of the Savannah River redeye bass (*Micropterus coosae*) due to introgressive hybridization with invasive Alabama spotted bass (*M. punctulatus henshalli*). Masters Thesis, University of South Carolina.

Genetic anlaysis of black bass collected from Savannah basin tributaries in 2009 and 2010 confirmed non-natives and/or hybrids from 5 of 9 collection sites (Table 3.). Hybrids were collected from at least one tributary associated with each of three reservoirs (we did not sample any tributaries associated with Lake Jocassee). Three of these collections, Chauga River lower, Big Generostee Creek and Little Coldwater Creek represent new documentation of Alabama spotted bass hybrids, as we collected only native black bass from each of these sites in 2004. Some hybrids collected from Chauga River lower carried a rare mtDNA haplotype previously only seen in Lake Hartwell. The potential for the spread of Alabama spotted bass and their hybrids from the reservoirs to additional tributary populations is indicated.

A new incidence of the non-native smallmouth bass was also documented by our survey of tributary populations (Table 3.). In 2004 we sampled the main stem of the Savannah River at Augusta Shoals, a site well below the impoundments included in this work. That collection yielded only genetically pure redeye bass and largemouth bass. In 2010 however we collected smallmouth bass also, as well as their hybrids with redeye bass, confirming angler reports that smallmouth bass were present in this area of the Savannah River. This shoal area of the Savannah River is near the lower extent of the range of redeye bass in the basin. It represents a portion of the range that as yet has not been colonized by the Alabama spotted bass that are expanding in the upper part of the basin. The presence of smallmouth and especially their hybrids with redeye there is disappointing.

Table 3. Genetic identifications of black bass collected from Savannah basin streams in 2009 and 2010; redeye bass (REB), largemouth bass (LMB), Alabama spotted bass (ASB), smallmouth bass (SMB), hybrid (HYB). Streams are grouped by the reservoir they are associated with, or as a direct tributary to the Savannah River.

			Species (N)					
Tributary to	Stream	Date	REB	LMB	ASB	ASB x REB	SMB	SMB x REB
L. Keowee	Eastatoee Ck.	9/24/09	8	0	0	0	0	0
	Little River	9/30/09	2	0	0	2	0	0
L. Hartwell	Chauga River lower	9/14/09, 9/29/09	9	2	0	8	0	0
	Chauga River - upper	9/29/09	15	0	0	0	0	0
	Chatooga River	8/4/10	18	0	0	0	0	0
L. Russell	Big Generostee Ck.	7/30/09	14	0	0	1	0	0
	Little Coldwater Ck.	9/1/10	18	3	0	3	0	0
Savannah R.	Steven’s Ck.	7/29/09	15	6	0	0	0	0
	Savannah River	9/16/10	15	4	0	0	6	2

Significant Deviations: None.

2. **Develop and implement simple, rapid and cost-effective genetic assays for the short and long-term monitoring of the hybridization between redeye bass and other black bass species throughout the Savannah River system.**

Activity: Assays for the mtDNA locus ND2 were successfully developed, using the program Web-based Allele-Specific Primers (WASP) by Pongsakorn et al. (2007). Primers were designed for haplotypes specific for the five species of black bass present or having genetic influence in South Carolina; largemouth bass, Florida bass, Alabama bass, redeye bass, and smallmouth bass (Figure 2).

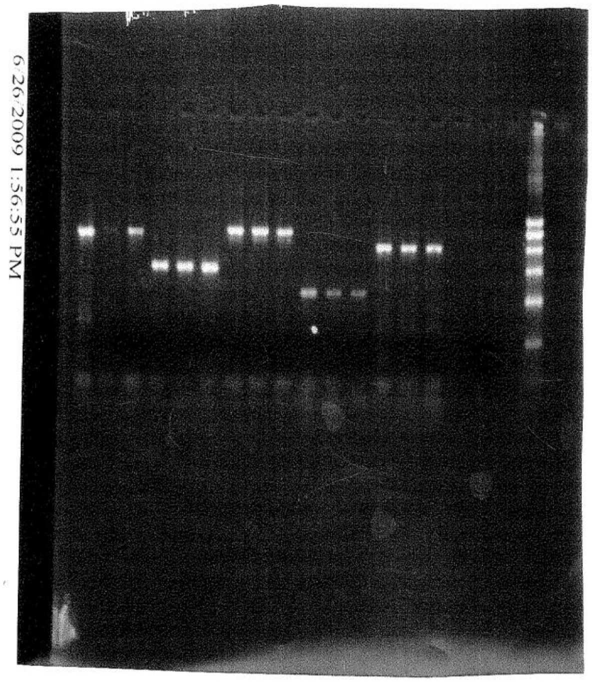


Figure 2:. Agarose gel run to confirm diagnostic differences in fragment lengths, in base pairs (bps) for ND2 assays for five black bass species. In lanes 1-3 beginning on the right are Alabama bass (744 bps), 4-6 are smallmouth bass (432 bps), 7-9 are largemouth bass (800 bps), 10-12 are Florida bass (284 bps), and 13-15 are redeye bass (647 bps). A ladder of fragments of known bps is in the last lane

Development of new assays for nuclear loci was not successful, however. Loci either did not show enough polymorphism for the design of assays that would quantify the contribution of genes from all five species in question, or allelic diversity within and among species gave complicated and confusing banding patterns on agarose gels. After considerable effort, it was determined the best course of action was to abandon the development of new nuclear DNA genetic assays and move forward with DNA sequencing.

While our mtDNA assay development will certainly prove valuable to future studies, we chose not to implement their use in this survey. Results from earlier sequenced individuals showed the presence of a rare redeye bass haplotype in Lake Hartwell, and also in hybrid individuals in a Hartwell tributary. We chose to sequence remaining individuals for the ND2 locus to preserve the potential to assess the origin of certain hybrid fish collected from the tributaries.

Significant Deviations: None.

3. **Use Geographic Information System (GIS) technology and population genetic data to identify specific aquatic and shoreline habitat that is associated with high-frequencies of native, non-native and hybrid bass species.**

Activity: A GIS database was developed that includes all Savannah and Santee basin black bass collections associated with this study (2004 – present), all Savannah and Santee basin South Carolina DNR stream team collections made within the range of redeye bass (2008 to present), and all historic South Carolina stream database collections that include record of redeye bass (1962 – 2002; Figure 3). When mapped together historic stream database collections and current collections imply that redeye bass are absent now from areas of the lower Piedmont Savannah basin where they were historically collected. This may be due to factors associated with sampling protocols however. The stream team samples are from randomly chosen sites, and include a prescribed length of stream based in part on its width at the sampling site. The total area of this stretch is sampled and any habitat included in it. Sites are not selected to include particular habitat or substrate types, so habitat specialist that may be present within a stream, could still be absent from the sampled reach. Redeye bass in Savannah and Santee basin streams are found almost exclusively in association with shoal habitats, or with hard rocky structure in pools. Recent stream team collections from tributaries to the Enoree River did not include any redeye bass, while collections targeting redeye bass for this study found them to be common in larger shoal habitats of the Enoree River main stem.

Over the course of this study, our target species redeye bass, Alabama bass and their hybrids were not encountered over sandy or silty habitats in tributaries, or over soft bottoms in the reservoirs with the exception of a single individual collected from a cove area of Lake Russell that is fed by a stream known to support a redeye population. The sampling effort for this study was extensive, and the need to maximize collections of redeye bass, Alabama bass and their hybrids to accomplish Objective 1 (N = 783) ultimately required targeting of shoal habitats, and shorelines with constructed rip rap or natural hard rock outcrops. The need to target specific habitat types impacts our ability to further evaluate those species associations with a range habitat types. Still, we have gained valuable insight through this study into what habitat types are most likely to hold redeye bass and their hybrids with Alabama bass. High quality shoal habitats and hard structure typical of our Piedmont tributaries and rivers are clearly of great importance to redeye bass in the Savannah basin, and necessary for streams to support robust redeye populations.

The spatial distribution of tributary collections that produced hybrids between Alabama bass and redeye bass provides important information with respect to the spread of non-native black bass alleles in the Savannah Basin (Figure 4). Tributary populations where hybrids have been collected were those in closest geographic proximity to the reservoirs, within which non-native alleles are already widespread. Our results indicate that spread of Alabama bass alleles into the sampled tributary populations is the result of upstream movement from the reservoirs. This is supported by the presence of the rare mtDNA haplotype in hybrids collected from the Lower Chauga River site (Objective 1), as in previous studies this haplotype was only sampled from within the Lake Hartwell population.

Results as noted earlier also indicate an increased spread into tributaries over time. Non-native alleles were sampled from four of six tributaries directly associated with a reservoir. Three of these collections represent new occurrences of Alabama bass alleles since the earlier study in 2004. Another new incidence of non-native alleles involves the collection of smallmouth bass and their hybrids with redeye bass from Augusta Shoals on the Savannah River main stem. In total the work supported by this grant has greatly furthered our knowledge of where and how non-native alleles are impacting native redeye bass populations of the Savannah basin. Our increased understanding has allowed resource managers to narrow their focus within the Savannah basin to specific tributaries that may provide refuge areas for pure populations of redeye bass. Work is underway to identify and delineate those refuge areas, and to quantify the habitats associated with them. An evaluation of in place barriers to upstream movement, and their potential to create and maintain genetically pure refuge areas is also included.

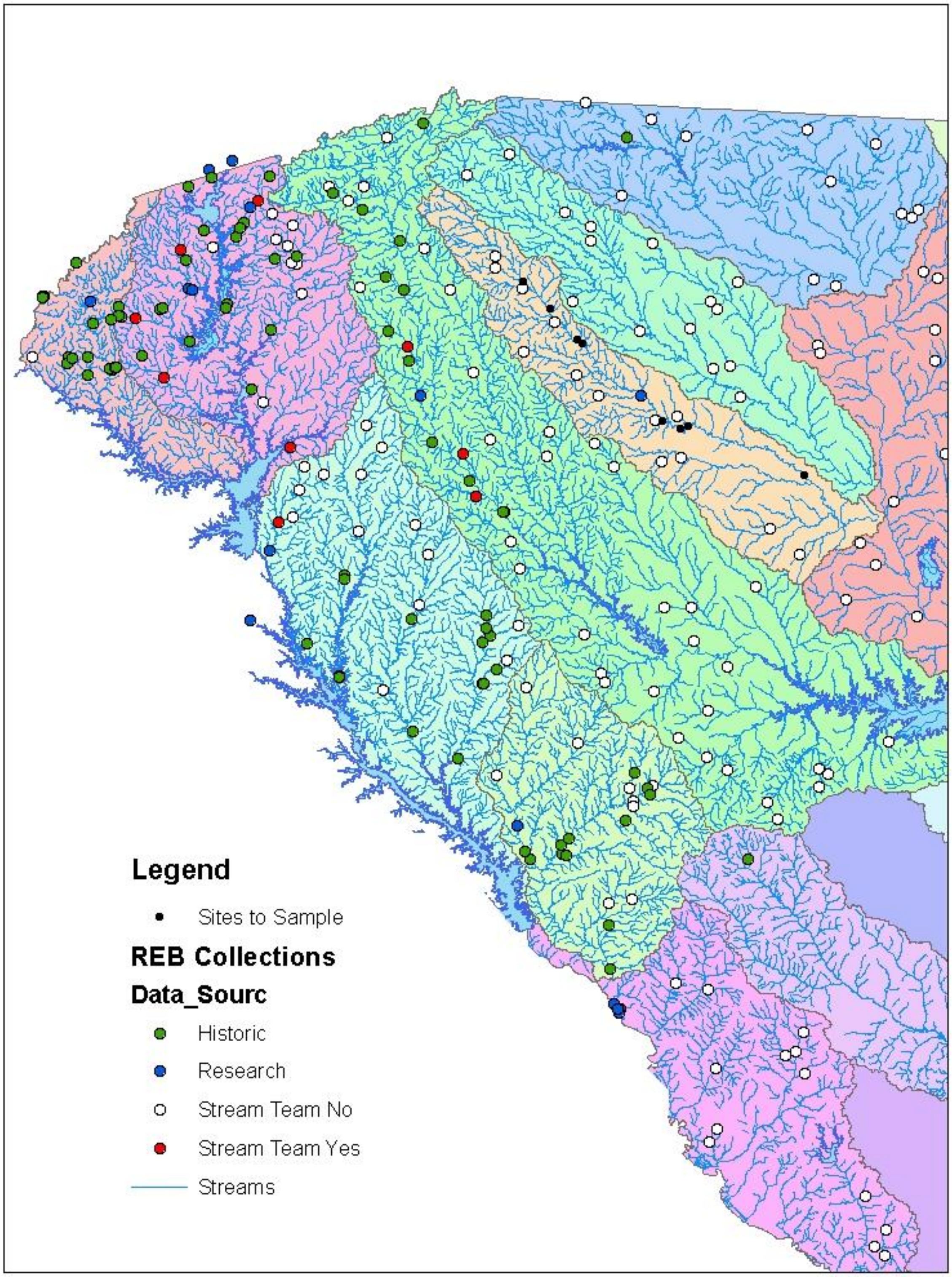


Figure 3. Tributary collections from 2004-present (Research and Stream Team) and from 1962 – 2002 (Historic). All 'Historic', 'Research', and 'Stream Team Yes' collections include redeye bass. 'Stream Team No' collections did not include redeye bass.

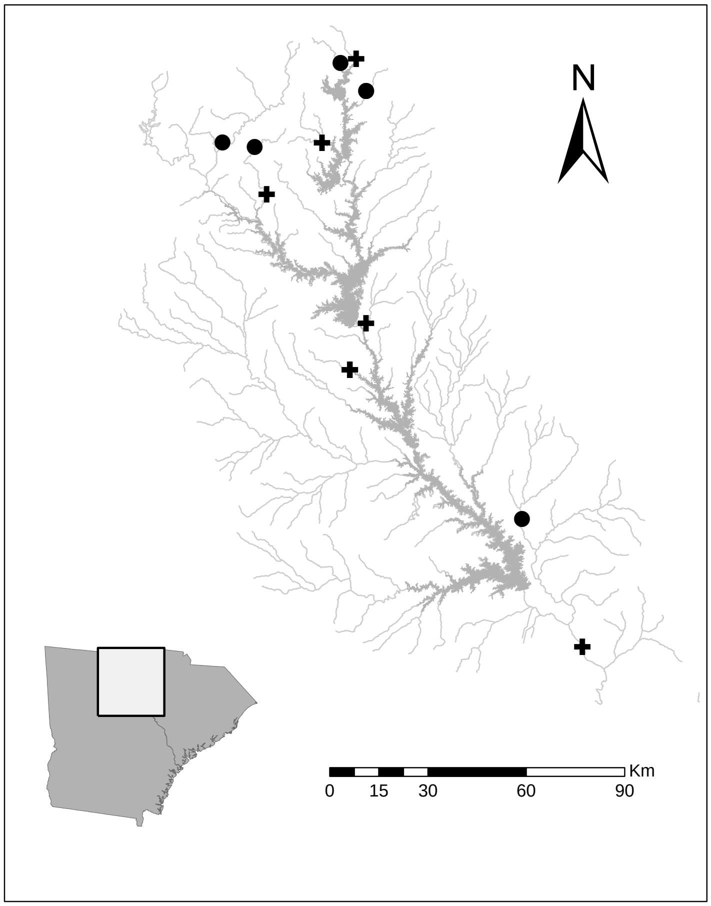


Figure 4. Spatial distribution of Savannah Basin tributary black bass collections from 2004 – 2010. Circles mark collection sites where only genetically pure redeye were collected. Crosses mark those sites where congeneric hybrids with redeye were collected. The lower most site depicted is the Augusta Shoals area of the Savannah River which produced smallmouth bass and their hybrids with redeye. All other hybrid collections were Alabama x redeye bass.

Significant Deviations: None.

4. **Genetically assay redeye bass, and four other co-distributed species from Santee drainage. Assess variation with respect to Savannah drainage populations in an examination of native or introduced status of redeye bass. Assess presence and degree of hybridization in Santee redeye bass populations.**

Activity: Upper portions of the Saluda drainage were scouted for suitable shoal habitats. Sites were limited however, and no redeye bass were collected from those that were sampled. Redeye bass were retained from several Saluda populations from SCDNR stream team sampling in 2008, and will be assayed and included in our final dataset.

Seven shoal habitat sites were identified on the Enoree River for black bass collections. This followed the first discovery of redeye bass in this portion of the Santee drainage from collection sites above and below the dam at Riverdale Mills. Redeye bass were field identified from all sites sampled (n=57). Genetic analysis confirmed species status for n = 50, while n = 7 were found to be redeye bass x Alabama spotted bass hybrids. No pure Alabama spotted bass were collected. Redeye bass were the most commonly encountered black bass (Table 4). Largemouth bass (n=3) were also collected. No smallmouth bass, or redeye x smallmouth bass hybrids were collected, though smallmouth are common in the Broad River, which the Enoree River is a tributary of.

Table 4. Species identifications for black bass collected from the Enoree River in 2010 and 2011; redeye bass (REB), redeye bass x Alabama spotted bass (REB x ASB), largemouth bass (LMB).

			Species		
Site	Site Coordinates	Date	REB	REB x ASB	LMB
Musgrove Mill	34.594096, -81.854859	12 May 2011	6	1	0
Hwy 49	34.603519, -81.910633	12 May 2011	4	1	0
Riverdale below dam	34.649847, -81.960254	11 Nov 2010	8	0	1
Riverdale above dam	“ “	11 Nov 2010	5	0	0
Hwy 418	34.80691, -82.165668	23 May 2011	25	4	0
Pelham Falls	34.853782, -82.221352	19 May 2011	1	0	0
Gibb Shoals	34.871623, -82.238625	9 Jun 2011	1	0	0
Above Gibb Shoals	34.876518, -82.245847	9 Jun 2011	0	1	2

Five species of minnow were collected from sites within the Santee, Tennessee (French Broad) and Savannah River drainages. A total of 120 individuals were captured including 47 Saffron Shiners (*N. rubricoceus*), 27 Mirror Shiners (*N. spectrunculus*), 20 Stonerollers (*C. anomalum*), 10 Whitetail (*C. galactura*) and 16 Warpaint Shiners (*L. coccogenis*). Whitetail shiners could not be captured from the Broad River collection location despite repeated attempts, therefore this taxon was not included in the statistical analyses. Mitochondrial ND2 sequences have been acquired from these individuals as a comparison to mtDNA diversity and divergence in co-distributed redeye bass. Three loci consistently amplified and were assayed for all individuals, one mitochondrial locus (ND2) and two bi-allelic nuclear loci (CAL and ITS). The Actin locus did not amplify for all individuals and sequences obtained suggested the presence of a second Actin locus – this locus was not characterized further. The ITS locus was monomorphic in most taxa surveyed and was not included in further analyses.

Polymoprhic ND2 and Calmodulin sequences were collected from the remaining four taxa, Warpaint, Mirror and Saffron shiners, and Stoneroller. Phylogenetic analysis of Stoneroller ND2 sequences suggested the presence of two taxa instead of the anticipated single monophyletic group (Figure 5). Subspecies of Stoneroller are known to exist in the Southeast, but have not been recorded from these drainages. However, two taxa are clearly evident, and the Calmodulin data do suggest hybridization might be occurring in these river systems. Because hybridization might confound the recovery of the history of a single species, stonerollers were not considered further. Similarly, phylogenetic analysis of ND2 data from mirror shiners was unusually shallow, that is very few haplotypes were recovered and very little signal was evident in the phylogenetic trees – Mirror shiners were not considered further.

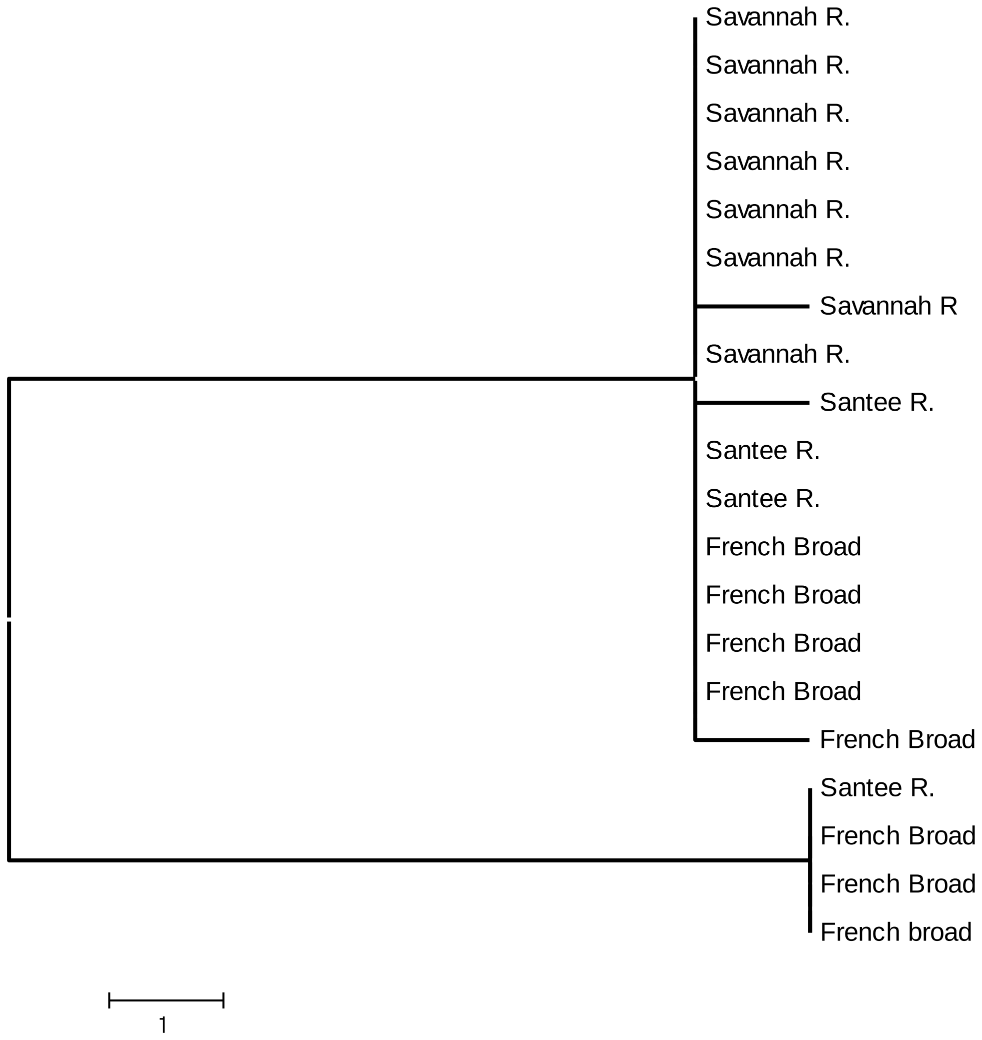
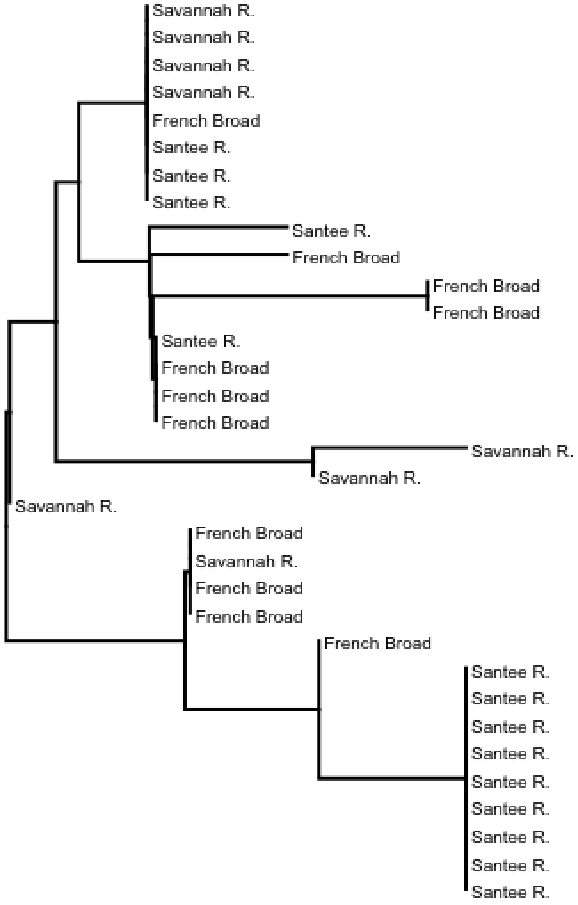
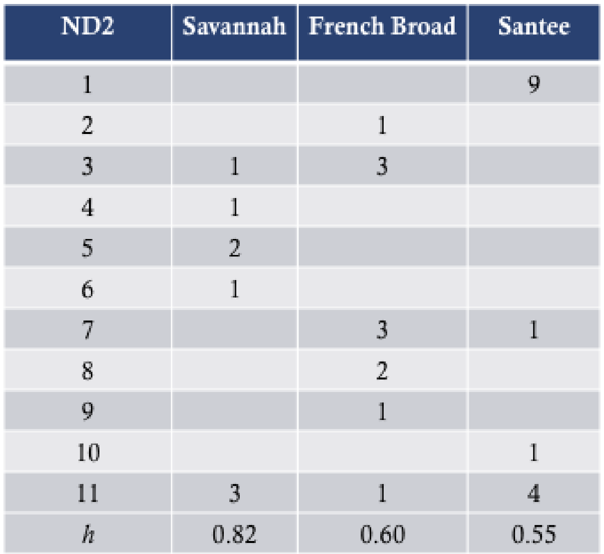


Figure 5. Neighbor-joining tree relating ND2 sequences collected from Stoneroller, *Campostoma anomalum*.

The remaining two taxa, Saffron and Warpaint shiners were sufficient variable for both ND2 and Calmodulin for meaningful comparisons to our redeye bass data. Much like redeye bass in the Saluda, divergence in these species is relatively shallow and most drainages appear to share identical or phylogenetically similar ND2 haplotypes (Figures 6 and 7). Unlike redeye bass, ND2 diversity in Saffron and Warpaint shiners was relatively high in the Santee river drainage.

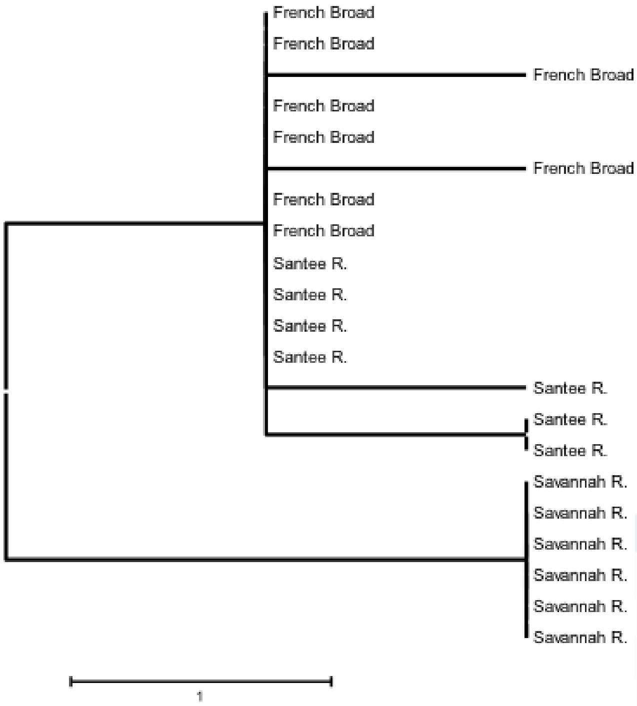
These patterns are mirrored somewhat by variation surveyed at the nuclear Calmodulin locus (Fig. 8 and 9). Although redeye bass are monomorphic in the Santee River, both Saffron and Warpaint shiners segregate for at least two alleles in this drainage and both alleles occur at appreciable frequencies.





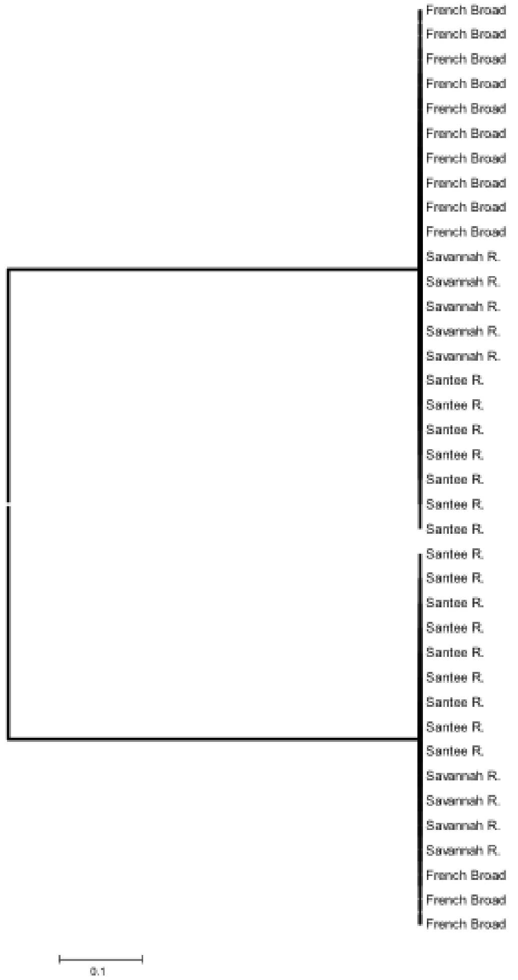
ND2	Savannah	French Broad	Santee
1			9
2		1	
3	1	3	
4	1		
5	2		
6	1		
7		3	1
8		2	
9		1	
10			1
11	3	1	4
h	0.82	0.60	0.55

Figure 6. Neighbor-joining tree relating ND2 sequences collected from Saffron Shiners (*Notropis rubricroceus*).



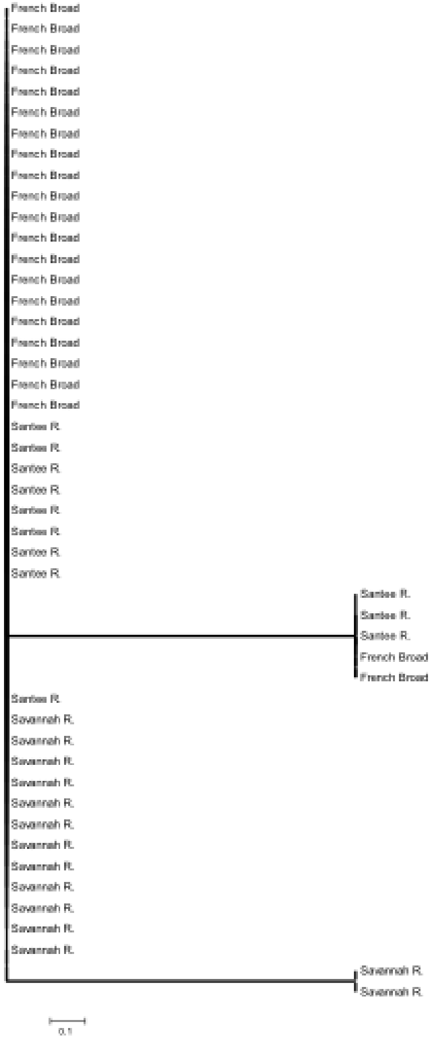
ND2	Savannah	French Broad	Santee
1	6		
2			2
3			1
4		6	4
5		1	
6		1	
h	0.00	0.51	0.67

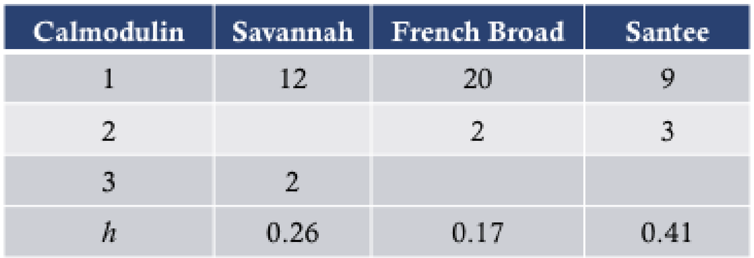
Figure 7. Neighbor-joining tree relating ND2 sequences collected from Warpaint Shiners (*Luxilus coccogenis*).



Calmodulin	Savannah	French Broad	Santee
1	5	8	17
2	13	18	15
h	0.44	0.44	0.51

Figure 8. Neighbor-joining tree relating Calmodulin sequences collected from Saffron Shiners (*Notropis rubricroceus*).





Calmodulin	Savannah	French Broad	Santee
1	12	20	9
2		2	3
3	2		
h	0.26	0.17	0.41

Figure 9. Neighbor-joining tree relating ND2 sequences collected from Warpaint Shiners (*Luxilus coccogenis*).

The Santee population of redeye bass is not genetically differentiated from populations collected throughout the upper Savannah River drainage. In contrast, Saffron and Warpaint shiner populations collected in the Savannah and Santee drainages are significantly differentiated from each other, a pattern that is quite unlike that found in redeye bass. Pairwise comparisons between individuals sampled from these two drainages, for both loci, were significantly differentiated (Table 5). Results indicate that the Santee Basin redeye bass populations evaluated here are the result of a more recent and likely human mediated introduction of fish originating from the Savannah basin.

Table 5. Estimates of population differentiation based on allelic diversity at the mitochondrial ND2 and Calmodulin loci in two species of minnow. Differentiation is based on an Analysis of Molecular Variance that partitions genetic variation to components within versus between river drainages. Significant values (**) are indicated ($P < 0.05$).

Taxon	Locus	Pairwise Comparison	Among Drainage Variance
Notropis rubricroceus	ND2		
		Savannah-French Broad	0.436**
		Savannah-Santee	0.329**
		Santee-French Broad	0.274**
Notropis rubricroceus	Calmodulin		
		Savannah-French Broad	0.565**
		Savannah-Santee	0.523**
		Santee-French Broad	0.519**
Luxilus coccogenis	ND2		
		Savannah-French Broad	0.593**
		Savannah-Santee	0.880**
		Santee-French Broad	0.107
Luxilus coccogenis	Calmodulin		
		Savannah-French Broad	0.790**
		Savannah-Santee	0.668**
		Santee-French Broad	0.737**

Significant Deviations: None.

Estimated Federal Cost (grant level): \$107,613.37 (amount of Federal funds expended to September 2010).

Recommendations: Close grant. Results obtained are a significant contribution to furthering the science and conservation of redeye bass in the Savannah Basin, and are being applied to continuing work to delineate and conserve genetically pure redeye bass populations in the Basin. Focus conservation efforts on tributary populations where pure populations of redeye bass persist, and where changes in genomic proportions over time can be effectively monitored. Identify and protect, and where possible restore high quality habitats within tributaries to support redeye bass and native Piedmont stream communities in general. Assess potential for barriers to upstream movement to protect native genomes, and potential for conservation stocking to help maintain those genomes in priority areas. Increase the public profile of redeye bass as a native species of conservation concern, a sport fish of recreational and economic value, and a species threatened by the indiscriminate movement of non native species across drainages.