Final Performance Report
South Carolina State Wildlife Grant F05AF00015 (T-9)
South Carolina Department of Natural Resources
October 1, 2004 – September 30, 2013

Project Title: Robust Redhorse Restoration and Conservation

Objective:
To establish self-sustaining populations of Robust Redhorse, *Moxostoma robustum,* suckers in the Santee River Basin using Savannah River brood stock.

A. Stock the Santee River Basin with cultured Savannah River strain Robust Redhorse (RRH).

Accomplishments: The Santee Basin was identified as a potential population establishment site because of its size, location between two identified populations, and evidence of historical RRH occurrence in the drainage. A primary consideration of this effort was to use progeny from 100 pairings to ensure that the new population would be genetically diverse. Brood stock collection was made from a numerically healthy and geographically close population of Savannah River RRH. Fish were collected during their natural spawning activities over a mid-channel gravel bar using stationary electro-fishing grids and a mobile electro-fishing boat. Eggs and milt were immediately collected from the actively spawning adults, and fertilization occurred individually between eggs from one female and milt from one male. Depending on the quantity, a female's eggs were divided between 1 and 3 males. Fertilized eggs were transported to the Bayless Hatchery for incubation and hatching. Grow-out was made in production ponds at the Dennis Wildlife Center. Spawning efforts occurred in every spring from 2004-2013 and are summarized in Table 1.

Table 1. Savannah River RRH Spring Spawning Summary.

Year	Eggs Produced	Males Spawned	Females Spawned
2004	35,000	16	6
2005	35,000	16	6
2006	25,000	16	7
2007	20,000	15	7
2008	40,000	18	6
2009	25,000	9	3
2010	30,000	15	6
2011	5,000	3	1
2012	0	3	1
2013	14,000	7	3
	Grand Totals	**118**	**46**

Of the 46 females spawned over ten years, three females have been used more than once. However, two of these incidences occurred in the 2010 year class that yielded the stocking of only 20 phase IV juveniles. The number of eggs collected from each female varied, and their contribution to subsequent juvenile stockings was not monitored. Through the 2013 grow-out and stocking season, we have stocked significant numbers of offspring from 98 individual matings, with small numbers of phase III and IV juveniles resulting from 18 additional pairings. This meets our initial genetic goals for establishing this population. Table 2 summarizes the annual details of the stocking of the nearly 72,000 juveniles. All fish stocked have been tagged with either coded wire (C) tags, pit tags (P), or were genetically identifiable (G). Juveniles were stocked in the fall at four different life stages; at 6 months and an average length of 150 mm, at 18 months and 230 mm, 30 months and 290 mm, and 42 months and 335 mm.

Table 2. The RRH stocking history of the Santee River drainage.

Year (Fall)	River	Locations	Phase I 6 Months		Phase II 18 Months		Phase III 30 Months		Phase IV 42 Months	
2004	Broad	Sandy River	9540	C						
	Broad	Below Parr Dam	9380	C						
	Wateree	Below Wateree Dam	0							
2005	Broad	Sandy River	9000	C						
	Broad	Below Parr Dam	9000	C						
	Wateree	Below Wateree Dam	5107	C	1010	C				
					1000	P				
2006	Broad	Sandy River	332	C						
	Broad	Below Parr Dam	400	C						
	Wateree	Below Wateree Dam	196	P	400	C,P	400	P		
2007	Broad	Sandy River	3000	C						
	Broad	Below Parr Dam	3000	C						
	Wateree	Below Wateree Dam	3800	C						
2008	Broad	Sandy River	444	C						
	Broad	Below Parr Dam	441	C						
	Wateree	Below Wateree Dam	498	C						
2009	Broad	Sandy River	3000	C						
	Broad	Below Parr Dam	3000	C						
	Wateree	Below Wateree Dam	3000	C						
2010	None									
2011	None									
2012	None									
2013	Broad	Sandy River	1981	G			8	P	7	P
	Broad	Below Parr Dam	1980	G			8	P	7	P
	Wateree	Below Wateree Dam	1980	G			9	P	6	P
		Total	**69,079**		**2,410**		**425**		**20**	
		Grand Total	**71,934**							

B. Survey and monitor the growth, survival, maturation, and spawning success as well as habitat use of stocked RRH in the Santee River Basin and monitor existing populations in the Savannah and Pee Dee River systems.

Accomplishments: Monitoring efforts were conducted in the two stocking segments of the Broad River and the Wateree and Congaree River segment (Figure 1). Data were collected with electrofishing sampling (targeted and incidental), incidental gillnet sampling, sonic telemetry, and direct observation at the Columbia Fishway. Observation of sub-adult fish began in 2008 as the males in the first stocked year class began to reach maturity and display spawning behavior. This consisted of upstream migration and the development of spawning tubercles. Duke Power collected four specimens in the Wateree Dam Tailrace during anadromous fish surveys in May of 2008, and two additional fish were collected by SCDNR personnel in the same area. Earlier that year, in March, two specimens were collected in Lake Monticello, a 2,711 hectare (6,700 acre) pump back storage reservoir, during a spring Largemouth Bass survey. They had apparently been entrained from Parr Reservoir after being stocked 33 km upstream as phase I juveniles. A late May riverine sample in this same section yielded one ripe tuberculate male and the observation of two additional specimens that could not be netted. In 2008, no observations were collected in the Broad River segment spanning Parr Dam down to the Columbia Dam due to low sampling effort. Since 2008, observation frequency of mature fish steadily increased in every stocked river segment, with a total of approximately 150 sample observations made through the fall of 2013. In addition, Robust Redhorse were first identified moving upstream through the Columbia Fishway in 2009 (Figure 2). Direct observational counts ranged from 1 in 2009 to 62 in 2013. These were sample counts which could be been reliably expand into full annual passage estimates. Spawning behavior has only been observed in the Wateree Tailrace, but this is likely an artifact of the high concentration of seasonal sampling in this small area.

Because of the low catch rates, seasonal movements of Robust Redhorse were monitored with acoustic telemetry in the upper Santee-Cooper system from 2009-2013. During this period, 27 Robust Redhorse were implanted with transmitters and monitored each year with a receiver array that consisted of at least 30 fixed receiver locations. Transmitter expulsion, tagging mortality, or transmitter failure was high for Robust Redhorse; however, the movements of 12 fish were monitored for at least one year. Those fish had large annual ranges with most fish using the entire length of the Wateree River and the majority of the Congaree River. Three fish ventured down into the upper Santee River where they potentially had access to Lake Marion, and three other fish moved above the Congaree River into the Broad and/or Saluda Rivers. Eleven of 12 fish entered the Tailrace below Wateree Dam each spring presumably to spawn. We only observed spawning aggregations below Wateree Dam; however, the movements of the one fish captured and tagged in the Congaree River, and its absence in the Wateree Dam Tailrace during spring, indicated that spawning aggregations likely occur elsewhere in this segment of the system. This conjecture is also supported by the fact that while we observed fish exhibiting spawning behavior in the Wateree Dam Tailrace, we observed significant numbers of fish ascending the Columbia Fishway. Repeated and unanimous summer occupation of the Congaree River may indicate a selection for cooler water as hypolimnetic release from Murray Dam significantly lowers Summer Saluda and Congaree River temperatures. Long-distance movement of these fish can occur relatively quickly; one fish moved downstream 124 km in 2.6 days, and there are numerous instances of fish moving more than 30 km/day.

Observation were collected on the Savannah River Robust Redhorse population during the project's ten years of brood stock collection at a spawning bar located 15 km downstream of Savannah Bluff Lock and Dam (173.3 km). Captured individuals were typically weighed, measured, fin clipped (for genetic material), examined for tags, and if untagged equipped with new PIT tags. A total of 271 RRH were collected— 223 males and 48 females. We had 74 recaptures with several multiple recaptures. The longest interval between first and last detection has been 8 years, with several fish having been picked up 3 times. One juvenile, 304mm long, was captured in the lower Savannah in 2012 by an American Shad electrofishing crew. This fish was noteworthy in that few sub-adult Robust Redhorse have ever been collected. Given these and earlier observations, the Savannah population is considered to be the most stable and healthy natural population that we have identified. Like the Congaree River, the water temperature of the Savannah River is significantly affected by cool water released from Lake Thurmond, which may positively influence the suitability of the habitat downstream.

To summarize our work, we have stocked sufficient numbers of genetically unique fish to found a healthy population of Robust Redhorse in the Santee River Basin. These fish have matured and repeatedly exhibited spawning behavior. Because these fish are inherently difficult to detect from hatching to adulthood, we have yet to confirm natural reproduction. Given the genetic detection tools developed under this grant, we will be able to genetically determine the origin of any Robust Redhorse collected in the future.

Figure 1. Map of the Santee River Basin containing the three Robust Redhorse Restoration segments.

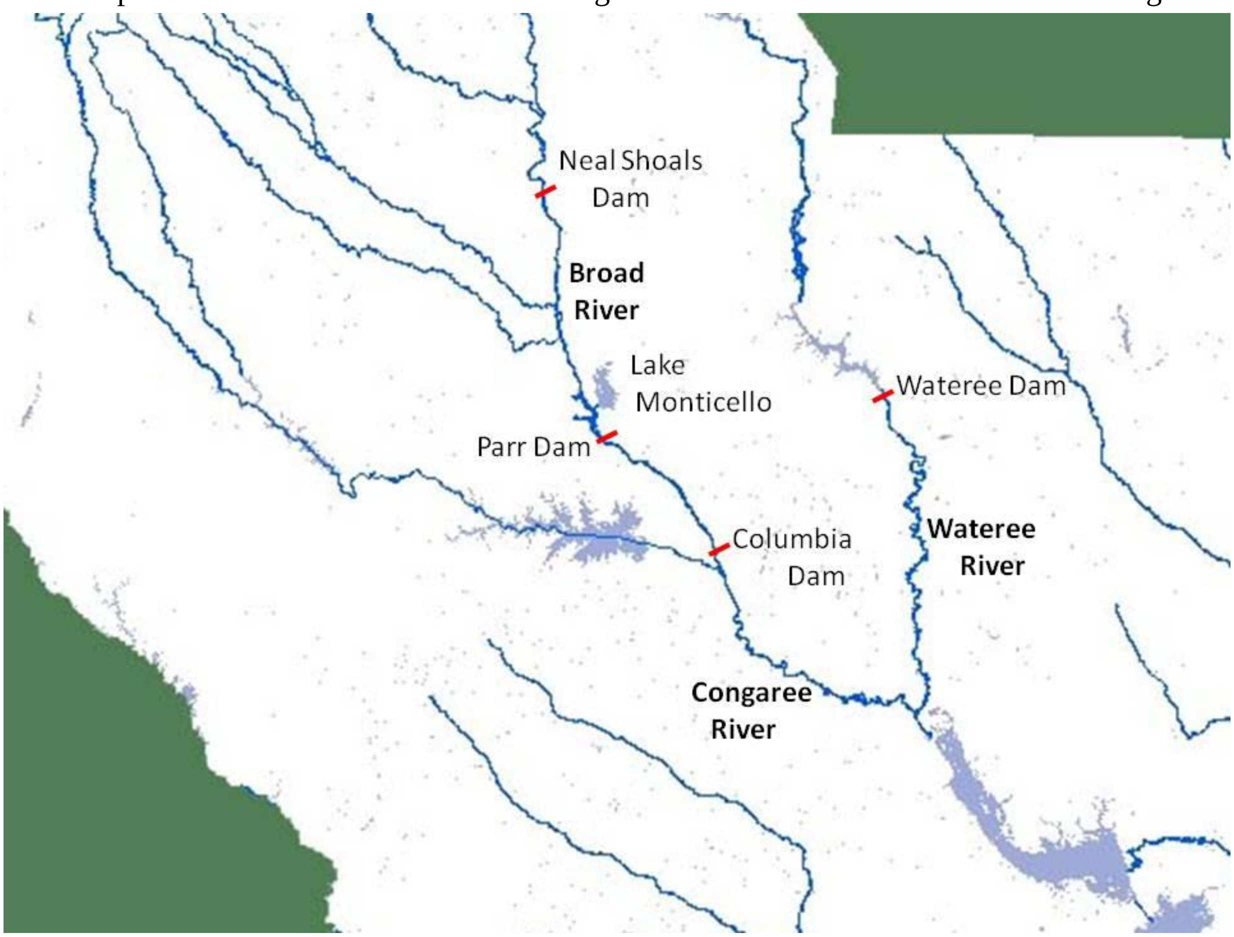

Figure 2. Adult Robust Redhorse swimming upstream through the Columbia Fishway.

C. Inform and educate the public about the relevance of our efforts to re-establish and conserve RRH in South Carolina.

Accomplishments: During the past ten years, our staff has included our RRH studies in all appropriate public outreach efforts. An outreach Power Point presentation was produced and given over 15 times. In addition, a table-top display and poster were constructed and used at an additional 10 events. Staff has addressed the need for display specimens at the Charleston Aquarium on several occasions. A video segment was produced by the DNR and was aired on ETV; it remains available for viewing on the Department's website.

D. Restoration and conservation effort administration:

Accomplishments: Staff members have had annual meeting of the Robust Redhorse Conservation Committee (RRCC) over the life of this study. The meetings have been attended by approximately 45 researchers representing 13 cooperators from Georgia, South Carolina and North Carolina. Study results from across the region are presented and discussed. Conservation and recovery were coordinated among the agencies and organizations involved. Staff members have been actively engaged in cross-agency research that would aid in the re-establishment of the species in the Santee Basin. Staff have also participated in RRCC Executive Committee meetings during the project period and held leadership positions within the organization.

E. Development of baseline genetic data for the Savannah population and build a foundation for future evaluation of ongoing re-establishment within the Santee River System.

Accomplishments: As a standardized suite of genetic markers had not been developed for Robust Redhorse, we began by optimizing and multiplexing 10 microsatellite markers that have amplified Robust Redhorse samples in Moyer's and Bernatchez's labs. All loci were tested for Hardy-Weinberg equilibrium (HWE), linkage disequilibrium, and null alleles. Basic molecular locus diversity indices including number of alleles, allelic size range, and gene diversity were calculated.

Baseline genetic characterization for the Savannah River was evaluated based on archived samples collected from 2001-2010 (n~200). Basic genetic characterization of the Savannah River population included calculating allelic and genotypic diversities as well as generating estimates of effective population size (N_e). A total of 56 archived samples from the Pee Dee River, collected since 2001, were used for a similar genetic characterization. A comparison of the Pee Dee and Savannah Rivers was conducted to document the degree of genetic separation. Analyses to evaluate the occurrence of recent bottlenecks in each river were also conducted.

Finally, we evaluated the capabilities of the marker suite for future parentage analysis. All spawning and production records for Robust Redhorse in the Savannah River formed the basis of the simulations. A maximum likelihood parentage approach, as implemented in the software package CERVUS, was used to provide a statistical evaluation of parentage, taking into account mutation rates and population allele frequencies. Simulations were performed to determine the average parent-pair and identity non-exclusion probabilities for the loci suite. All necessary broodstock files were generated so that parentage analysis can be conducted as needed in the future for individuals collected in the Santee River system.

Overall, our genetic evaluation indicates substantial levels of genetic structure between the Savannah and Pee Dee Rivers as indicated by the high R_{ST} value and distinct allele frequency distributions, with a high number of private alleles in each system. The detection of significant genetic differentiation between the Savannah and Pee Dee Rivers is congruent with both Wirgin's estimation that genetic divergence between these systems occurred 1.5 million years ago (DeMeo 2001) based on mitochondrial control region sequence data and his unpublished preliminary microsatellite evaluation of these systems (Wirgin et al. 2001). Collectively, these results support the current management of rivers as distinct population segments and should be continued, as suggested by Wirgin et al. (2004), due to the importance of genetic composition for future evolutionary growth of the species.

Both the Pee Dee and Savannah populations show high within-population diversity as well as low levels of inbreeding as compared to the average genetic diversity measured for freshwater fishes (0.54, DeWoody and Avise 2000). Additionally, *M. robustum* population diversity estimates from both rivers are in the upper range of those reported for other *Moxostoma* species (0.63 in Black Redhorse, *M. duquesnei*, Reid et al. 2008a; 0.72 in River Redhorse, *M. carinatum*, Reid et al. 2008b; 0.76 in Shorthead Redhorse, *M. macrolepidotum*, Reid et al. 2008b; 0.77 in Copper Redhorse, *M. hubbsi*, Lippe et al. 2006; and 0.85 in Sicklefin Redhorse, *M. sp.*, Moyer et al. 2009).

All estimates of N_e differed between rivers with consistently lower estimates in the Pee Dee River, which is consistent with the suggestion of a smaller population size in the Pee Dee River (Wirgin et al. 2004). However, even the higher contemporary N_{eb} estimates for the Savannah River were lower than those estimated for the endangered *M. hubbsi* ($N_{eb(LD)}$=480, Lippe et al. 2004). Additionally, the estimates of contemporary N_{eb} are well below the goals identified in the conservation strategy for *M. robustum* in (Nichols 2003). Similar evolutionary population trends were detected in the Pee Dee and

Savannah Rivers. As no recent population bottlenecks were detected in either system, the substantial reduction from long-term to contemporary estimates likely indicates gradual population decreases over very long time periods for both *M. robustum* populations. Bottleneck detection capability is dependent on the severity of the event; however, the tests utilized here are highly robust with population reductions to N_e<10 (Luikart and Cornuet 1998); therefore, a recent bottleneck would have certainly been detected in the Pee Dee River had one occurred recently. Although interpretation of genetic data suggested a similar long term trend for *M. hubbsi* (Lippe et al. 2004), it is interesting that the long term estimates of N_e were higher in both the Pee Dee and Savannah populations of *M. robustum* (*M. hubbsi* N_e: 4,476).

Similar to other *Moxostoma* species, *M. robustum*'s life history strategy is characterized by both a long life span and overlapping generations. The maximum age reported for *M. robustum* is 27 years with reproductive maturity occurring at 4-5 years in males and 5-6 years for females, leaving approximately a 22 year reproductive window for each individual (Robust Redhorse Conservation Committee 2002). Therefore, although the very low N_{eb} estimate in the Pee Dee River population is concerning from a genetic management standpoint, their long lifespan and overlapping generations appear to result in a high potential for across-year class spawning and is likely contributing to the maintenance of high genetic diversity in light of their low effective population sizes. Although the high genetic diversity and lack of inbreeding indicators observed within both the Pee Dee and Savannah river populations of *M. robustum* would normally be indicative of populations in good genetic health with sufficient adaptive potential, it is unknown if *M. robustum*'s life history characteristics and current high genetic diversity will be capable of overcoming the negative effects of low effective population sizes in the long term. Therefore, conservative management approaches and continued monitoring of the populations is recommended as Kuo and Janzen (2004) have suggested that long life and overlapping generations could potentially mask accelerated rates of drift in small populations. The lack of demographic population history for Robust Redhorse makes it challenging to ground-truth interpretation of genetic results, and monitoring and management recommendations would greatly benefit from current demographic population estimates (particularly in the case of simulating diversity retention). The contemporary LD-based estimates of N_e for two Robust Redhorse populations provide an important benchmark for future detection of bottlenecks as it has recently been shown that LD-based estimates have the sensitivity to comparatively detect population reductions within a single generation (Antao et al. 2011). A substantial level of knowledge and intensive effort is necessary for successful recovery of protected species, and the genetic data generated during this study provides information on an important aspect of *M. robustum* biology that will be valuable in the continued monitoring and management of this species.

The microsatellites used to evaluate *M. robustum* were initially developed for other Catostomids, but have proved to be an excellent genetic tool for *M. robustum* as well. In addition to genetic characterization and monitoring of these populations, the marker suite provides a statistically robust mechanism of parentage analysis and individual identification in both rivers. Evaluation of the success of any restoration effort is dependent on the ability to identify stocked individuals, which necessitates the use of a tag or mark to distinguish hatchery from wild produced fish (Blakenship and Leber 1995). The use of molecular markers as genetic tags avoids some of the constraints and pitfalls associated with conventional tags, in that molecular markers require no additional tagging, the mark is never lost, and tag recovery is non-lethal. The archiving of all South Carolina *M. robustum* production and genetic information will allow for offspring identification of future recaptures within the Santee River during re-establishment efforts. As genotyping of Santee River offspring progresses, genetic characterization of the new population can be assessed and compared to the Pee Dee and Savannah River populations.

Total Federal Cost: $61,247.32 ($11,277.68 will revert)

Recommendations: Close the grant.

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