

**FINAL REPORT**  
**South Carolina Competitive State Wildlife Grant SC-U2-F16AP00581**

South Carolina Department of Natural Resources\*  
January 1, 2017 – December 31, 2021

*\*This C-SWG grant was a partnership with Georgia and North Carolina where GA was the lead state. A final report summarizing the entire project is available.*

**Project Title:** Increasing the probability of persistence for Robust Redhorse through improved management and monitoring.

**Purpose and Objectives** - The intent of our project was to continue and improve conservation efforts for Robust Redhorse and increase the probability that populations of the species will persist across its range. Our South Carolina-specific project Tasks for each Objectives are listed below.

**Objective 1:** Determine if populations of Robust Redhorse in the Ocmulgee, Oconee, Ogeechee, Broad, Savannah, Santee, and Pee Dee river systems are self-sustaining using a combination of visual, acoustic, and genetic monitoring techniques.

**Task 1.3:** Genetic Monitoring Protocol - Archiving and genotyping of genetic samples from all populations and building/archiving parentage files for parentage analyses in the Pee Dee River stocking program. Final genetic data will be used to calculate population-specific genetic metrics and evaluate differentiation among populations.

**Task 1.4:** Detect New Spawning Sites - Targeted electrofishing during the spawning season in the Santee River.

**Objective 2:** Improve adaptive management for Robust Redhorse populations across their range and identify key management actions needed to preclude listing under the Endangered Species Act (ESA).

**Task 2.1:** Improve Data Management – Assist with development of relational databases; genetic data component.

**Objective 3:** Implement management actions and monitoring according to the results of Objectives 1 & 2 and support continued stocking of the Pee Dee River population.

**Task 3.1:** Stocking the Pee Dee River – Collecting broodfish for spawning and production, raising offspring in hatchery ponds, and releasing Phase I and II fingerlings.

## Accomplishments -

### Task 1.3 - Genetic Monitoring Protocol Implementation

For all genetic samples, we used our standard metal bead isolation protocol to extract DNA from fin clips. Following DNA isolation, samples were amplified in three multiplexed polymerase chain reactions (PCR) using our optimized suite of nine microsatellite loci (Table 1) with reaction conditions provided in Darden and Tarpey (2014). PCR products were separated and visualized on a Beckman CEQ 8000/ GenomeLab GeXP™ capillary electrophoresis system. All resulting chromatograms were scored using CEQ Fragment Analysis Software by two independent readers for quality assurance.

Table 1. Multiplex group, PCR primer ratio, fluorescent label (dye), and repeat motif for 9 loci used to genotype Robust Redhorse. The loci were originally developed by Lippe et al. (2004) for the Copper Redhorse and were multiplexed by Moyer et al. (2009) for use with the Sicklefim Redhorse.

Multiplex Group	Locus	Primer Ratio	Well-RED Dye	Repeat Motif
1	Mro268	1.5	Black	[GACA] <sub>5</sub> [GGCA] <sub>2</sub> GGTATA[TAGA] <sub>23</sub>
	Mro294	3	Green	[GATA] <sub>18</sub>
	Mro296	0.5	Blue	[ATCT] <sub>21</sub> [TTCT] <sub>11</sub>
2	Mro321	2.5	Green	[CTAT] <sub>2</sub> CTGT[CTAT] <sub>10</sub> CTGT[CTAT] <sub>12</sub> [CTGTCTAT] <sub>2</sub> CTGT[CTAT] <sub>3</sub>
	Mro336	0.8	Blue	[GATA] <sub>15</sub> [GAGAGATA] <sub>3</sub>
3	Mro212	3	Black	[GATA] <sub>19</sub>
	Mro213	3	Green	[ATCT] <sub>17</sub>
	Mro286	1.5	Blue	[ATCT] <sub>17</sub>
	Mro203	0.3	Blue	[TGAG] <sub>15</sub> [GATA] <sub>10</sub>

Broodstock genetic samples and spawning designs for the Santee drainage and Pee Dee River stocking programs continue to be archived with the SCDNR Genetics Lab in Charleston, SC. Previous simulations for known-sex parentage analysis in CERVUS consisted of 10,000 offspring and 20 candidate parents using allele frequencies generated from all prior samples of Robust Redhorse in the Savannah River. Critical delta values were determined using 99% and 99.9% confidence for the relaxed and strict criteria, respectively. All parentage analyses were run with the modal simulation file. Additionally, all individuals were genetically screened for previous capture using the identity analysis in CERVUS, with a non-exclusion probability of  $9.1 \times 10^{-15}$  for the marker suite (i.e. a very low probability of misidentifying individuals).

Over the course of this project, a total of 300 Robust Redhorse contemporary field-collected tissue samples were archived, genotyped and analyzed (Table 2). Sixty previously collected samples (2001 – 2007) from the Oconee River were genotyped for the purpose of identifying the genetic origin of the contemporary samples collected in the Ocmulgee River. Additionally, 200 Robust Redhorse tissue samples from the Pee Dee River hatchery program (Dennis Wildlife Center, SC or McKinney Lake State Fish Hatchery, NC) were analyzed to determine parental contribution and to genotype before release (Table 3). A total of 500 historic tissue samples from

a variety of collections (1998 – 2007) were transferred to SCDNR for long-term archival (Table 4).

Table 2. Total number of field-collected Robust Redhorse genotyped from January 2017 – December 2021.

River/System	Total Genotyped
Pee Dee River, NC/SC	172
Santee Cooper System, SC	105
Savannah River, SC/GA	19
Ocmulgee River, GA	4

Table 3. Hatchery-reared Pee Dee River Robust Redhorse samples genotyped and analyzed to determine parental contribution and to genotype before release. YC= Year Class

Result	Total #
Unknown/Contaminated	7
2014 YC	48
2015 YC	129
2018 YC	15
2019 YC	1

Table 4. Historic Robust Redhorse tissue samples transferred to the SCDNR Population Genetics Laboratory for long-term archival.

Source	Total #	Collection Years
<b>Hatchery</b>	<b>317</b>	
Burton Hatchery, GA	20	1998 – 1999
Campbell Hatchery, SC	10	1998
Cheraw Hatchery, SC	19	1998 – 1999
Dennis Wildlife Center, SC	100	1998 – 1999
Richmond Hill Hatchery, GA	10	2000
Walton Hatchery, GA	118	1998 – 2003
Warm Springs NFH, GA	40	2002 – 2007
<b>Wild</b>	<b>183</b>	
Altamaha River, GA	30	Unknown
Ocmulgee River, GA	4	2003 – 2004
Oconee River, GA	118	1999 – 2007
Ogeechee River, GA	1	1999
Savannah River, GA/SC	30	2002

Summary of Santee-Cooper system field collections:

Out of the 105 samples collected in the Santee-Cooper system, four samples were either missing, could not be genotyped, or were determined to be a species other than Robust Redhorse and were excluded from further evaluations. Due to incomplete broodstock genotyping (i.e. genetic samples are missing from some hatchery broodstock), some individual genotypes cannot definitively be determined as cultured or wild. Of the 101 successfully genotyped Robust Redhorse samples, 70 (69.3%) were determined to be cultured from the 2004 – 2013 year classes; 24 (23.8%) did not match to known broodstock pairs, but due to size could not be determined as wild or cultured; and 7 (6.9%) did not match known broodstock pairs, but due to size could be ruled out as cultured (Table 5).

Table 5. Genetic designations and contribution of Santee-Cooper system Robust Redhorse collected from 2017 – 2021.

Designation	Number	Contribution %
<b>Cultured (YC)</b>	<b>70</b>	<b>69.3</b>
2004	13	18.6
2005	7	10.0
2006	2	2.9
2007	1	1.4
2008	11	15.7
2009	20	28.6
2013	11	15.7
Unknown YC	5	7.1
<b>Unmatched/Unknown</b>	<b>24</b>	<b>23.8</b>
<b>Wild</b>	<b>7</b>	<b>6.9</b>
<b>Total</b>	<b>101</b>	<b>100.0</b>

Robust Redhorse were stocked in the Santee-Cooper system over a 10 year period, representing year classes from 2004 to 2013. During this project, we recaptured stocked fish from across the restoration time frame, with nearly all individual year classes recaptured. Some of the unknown/unmatched individuals could also represent the year classes from which there were no recaptures. It is a positive sign for the reestablishment of the Santee-Cooper Robust Redhorse population that all stocked year classes are persisting and frequently captured on the spawning grounds during spawning season. The capture of seven “wild” (i.e. non-stocked) younger Robust Redhorse indicates that stocked fish are not only surviving to maturity, but successfully spawning in the system. However, these wild fish were all captured in 2017 and 2018, so this population should continue to be monitored to determine if recruitment is persistent as this is critical to establish a self-sustaining population.

**Summary of Pee Dee River field collections**

Of the 172 samples archived, collected, and genotyped from the Pee Dee River between 2017 – 2021, 44 samples were recaptures of individuals. Of the remaining 125 samples, 119 (95.2%)

were determined to be cultured fish from the 2014 – 2018 year classes and 6 (4.8%) were determined to be wild (Table 6).

Table 6. Genetic designations and contribution of Pee Dee River collected Robust Redhorse from 2017 – 2021.

Designation	Number	Contribution %
<b>Cultured (YC)</b>	<b>119</b>	<b>95.2</b>
2014	65	54.6
2015	53	44.5
2018	1	0.8
<b>Wild</b>	<b>6</b>	<b>4.8</b>
<b>Total</b>	<b>125</b>	<b>100.0</b>

Robust Redhorse were stocked in the Pee Dee River for augmentation of the 2014, 2015, and 2018 – 2021 year classes. During this project, fish were recaptured from the 2014, 2015, and 2018 year classes. However, since nearly all captures of Robust Redhorse are mature fish captured during spawning season, it is not surprising that more recent stocked year classes have not yet been recaptured. The wild fish documented in the Pee Dee River were all adult fish that had not previously been captured in the system. This population should continue to be monitored to determine if the stocked fish are continuing to persist and to monitor for potential spawning by stocked individuals.

#### Summary of Savannah River and Ocmulgee River field collections:

A total of 83 Robust Redhorse samples collected from 2001 – 2018 from drainages in GA (Ocmulgee, Savannah, Oconee rivers) were genotyped during the third year of the project. The general purpose of this analysis was to determine the ancestry of the four Robust Redhorse captured in the Ocmulgee River in May 2018; two approaches were implemented to address the question – parentage and gene flow analyses.

We used CERVUS 3.0, a parentage analysis software package, to determine if any of the Oconee River Robust Redhorse could be matched as parents of the Ocmulgee River fish. Genetic data from the Oconee River fish were used to estimate population allele frequencies for each locus. Analysis was conducted with simulation files created from our Santee River Robust Redhorse project, and all fish as potential parents with sexes unknown (i.e. the software will test all possible crosses).

The analysis resulted in no parent pairs returned as perfect matches and no parent pairs with any confidence level (relaxed confidence level of 0.999). The closest possible matches had two loci mismatching. Based on these results, we determined that the Oconee River fish we genotyped were not the direct parents of the Ocmulgee River Robust Redhorse sampled in 2018.

We also used STRUCTURE 2.3.4, a Bayesian model-based algorithm software package for estimating population clusters, to determine the ancestry of the Ocmulgee River fish. We excluded recaptured fish and samples with fewer than 7 loci genotyped, leaving samples from

the Ocmulgee (n=4), Savannah (n=18), and Oconee (n=46). Analysis parameter settings included 10,000 burn-in repetitions followed by 200,000 MC repetitions, with location set as a prior, and 1–3 K (population clusters) with 3 repetitions of each K. A hierarchical approach was employed with the analyses re-run following data partitioning based on recovered genetic groups until K=1 was resolved for each cluster. STRUCTURE Harvested was used to compile resulting analyses to statistical evaluation of cluster assignments.

Results based on all sample sites indicated the presence of two population clusters (Figure 1), with the Savannah River samples being distinct from the Ocmulgee and Oconee samples (Figure 2). The Savannah River samples were removed for evaluation of potential finer scale genetic structure between the Ocmulgee and Oconee River samples. The resulting analysis suggested the most likely number of population clusters in the partitioned data set was one (Figures 3 and 4). Therefore, based on the results from STRUCTURE, we determined that the Ocmulgee River Robust Redhorse share a common ancestry with the Oconee River Robust Redhorse.

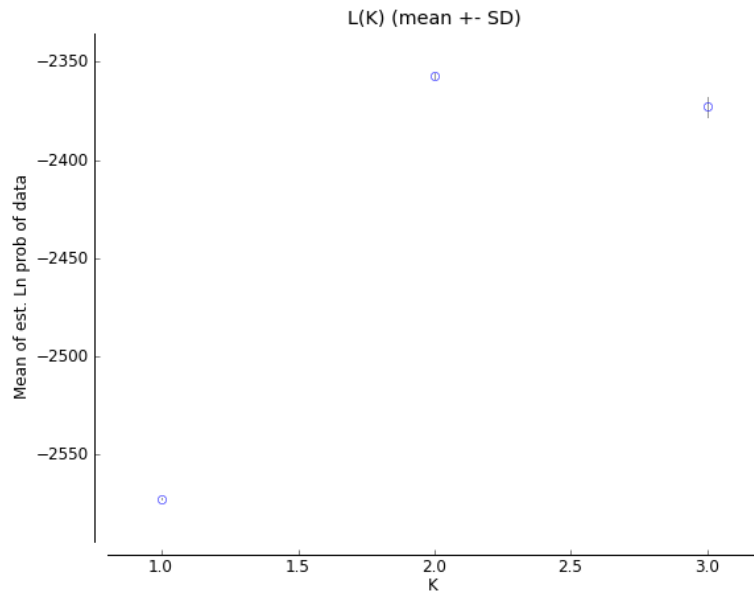


Figure 1. Mean log likelihood of multiple STRUCTURE analyses, indicating the most likely number of clusters present in the data are two.



Figure 2. The ancestry plot for K=2 showing that the Savannah River (2; red) Robust Redhorse have a distinctly different ancestry than the Ocmulgee (1; green) and Oconee River (3; green) Robust Redhorse.

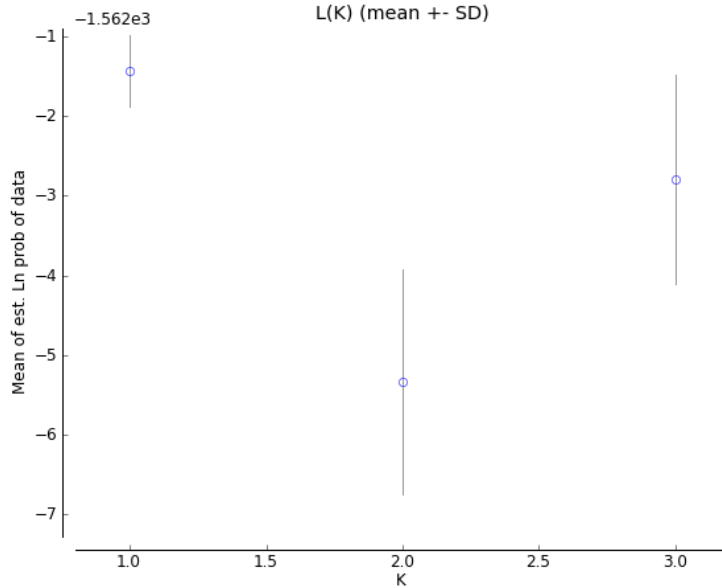


Figure 3. Mean log likelihood of multiple STRUCTURE analyses, indicating the most likely number of clusters present in the data is one.

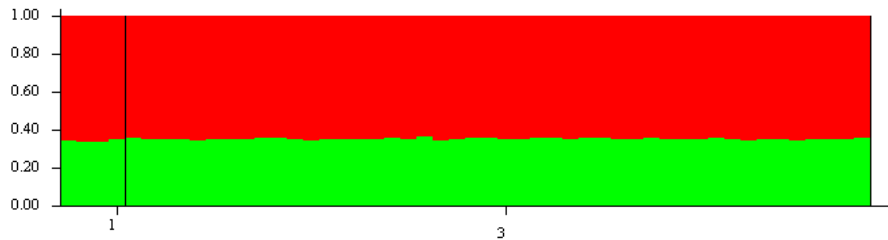


Figure 4. The ancestry plot for  $K=2$ , for illustrative purposes, showing that there is no genetic distinction between the Ocmulgee and Oconee River Robust Redhorse when the program is forced to estimate two population clusters.

#### Summary of Genetic Characterizations and Differentiation:

All genotype data generated for contemporary samples (i.e. collected during this study period), were used to calculate genetic metrics by population to compare with previously published measurements, and to re-assess the degree of genetic differentiation among populations. For recaptured individual Robust Redhorse, only a single genotype was included in analyses to ensure individual fish were not overrepresented. The total number of individuals included, average number of alleles, observed and expected heterozygosity, and inbreeding coefficient were calculated using GenAIEx 6.5 (Peakall and Smouse 2006, 2012), and effective population size was calculated using the program LDNE (Table 7). Genetic differentiation between populations was estimated by calculating pairwise  $F_{ST}$  in Arlequin 3.5.2.1 (Excoffier et al. 2005)

with significance based on 1,000 permutations (Table 8). Results for the Savannah and Ocmulgee river populations should be considered with caution due to low sample sizes.

Table 7. Genetic metrics of Robust Redhorse collected 2017 – 2021 by population. The 95% parametric confidence intervals (CI) are included for contemporary  $N_e$  estimates.  $H_o$  observed heterozygosity,  $H_E$  expected heterozygosity,  $N_a$  number of alleles,  $F_{IS}$  inbreeding coefficient,  $N_e$  effective population size. \* Invalid results due to small sample size.

Population	# Ind.	$N_a$	$H_o$	$H_E$	$F_{IS}$	$N_e$ (95% CI)
Pee Dee	145	9.7	0.81	0.78	-0.03	11.0 (9.9 – 12.3)
Santee-Cooper	101	16.8	0.83	0.87	0.03	40.1 (36.1 – 44.7)
Savannah	18	10.1	0.89	0.85	-0.05	152.9 (53.4 – ∞)
Ocmulgee	4	3.8	0.83	0.61	-0.36	*

Table 8. Pairwise  $F_{ST}$  estimates (below diagonal) between populations of Robust Redhorse collected 2017 – 2021. Values of significance (above diagonal) were calculated using 1000 permutations; Bonferroni corrected critical p-values = 0.008.

	Pee Dee	Santee-Cooper	Savannah	Ocmulgee
Pee Dee		0.001	0.001	0.001
Santee-Cooper	0.109		0.003	0.001
Savannah	0.102	0.012		0.001
Ocmulgee	0.195	0.127	0.130	

Despite differences in sample size, all four populations of Robust Redhorse had similar observed heterozygosity estimates (~0.8 – 0.9), which were similar to values estimated for the Savannah and Pee Dee River populations in Darden and Tarpey (2014). Inbreeding coefficients were close to zero in all but the Ocmulgee River population, which is indicative of a lack of inbreeding and similar to previous estimates (Darden and Tarpey 2014). Given the small sample size of the Ocmulgee River population (n=4), all results should be considered with caution, including the high inbreeding coefficient estimate until additional samples can be obtained. Effective population size estimates were highest for the Savannah River population (152.9) and lowest in the Pee Dee River population (11.0), which were close to previous estimates in Darden and Tarpey (2014) (156.3 and 9.7, respectively) and are congruent with relative differences in Robust Redhorse abundance estimates from these populations.

All pairwise  $F_{ST}$  population comparisons were statistically significant. The highest level of differentiation was between the Pee Dee and Ocmulgee river populations, also the two most geographically separated. The lowest level of genetic differentiation was between the Santee-Cooper and Savannah River populations, which is logical given that the Savannah River population was used as the source population for the Santee-Cooper population restoration effort with differentiation likely the result of lingering influences of drift associated with founder effects.



#### Task 1.4 - Detection of New Spawning Sites in the Santee River

Two spawning sites in the Santee drainage population have been previously documented at the Wateree Dam tailrace and Broad River below Parr Dam. However, additional spawning habitat is expected to occur in the Upper Broad River. In conjunction with the collection of Robust Redhorse for fin clips, SCDNR conducted field surveys during spring of each year in 2017, 2018, 2019, and 2021 in an attempt to identify new spawning sites through targeted electrofishing during the breeding season. The Covid pandemic precluded our ability to conduct spring sampling in 2020. SCDNR staff spent a total of 16 field days surveying areas for new spawning locations, with efforts ranging from two to six survey days per year. Areas included in surveys included the upper Wateree River, Wateree River below Lake Wateree Dam, Congaree River between Rosewood Landing and the Blossom Street Bridge in Columbia, SC, Congaree/Broad/Saluda confluence, Upper Broad near Sandy River, Broad River's Parr Dam tail water, and various locations on Broad River below Parr Dam, near the confluence of Broad and Sandy Rivers, and near Shelton's Ferry Landing.

During 2017 surveys, 19 fish were collected in the Broad River's Parr Dam tail water and fin clips were taken from all specimens. Within this area, a new spawning location was identified approximately 1,000 feet upstream from the previously known location. Both sites were characterized by higher velocity shoots in the relatively straight channel. High turbidity precluded any visual observation of spawning behavior. However, gamete flow and the ratio mixture of flowing males and females indicate active spawning nearby.

The Upper Broad survey yielded one flowing female, which appeared to be post spawn, and one immature male. While captured in close proximity, their capture location cannot be considered to be a potential spawning site at this time. Turbidity and physical characteristics of this Piedmont river, rock shoals and depth continues to hinder identification of specific spawning locations. However, one of the specimens collected was identified as a wild-spawned fish, indicating that successful recruitment has occurred in the segment of the restoration area.

During 2018 surveys, 20 Robust Redhorse were collected, with 19 captured from the Broad River's Parr Dam tail water and one in the Congaree/Broad/Saluda confluence area. Fin clips were taken from all specimens. High turbidity precluded any visual observation of spawning behavior. However, gamete flow and the ratio mixture of flowing males and females indicate active spawning in the Broad River's Parr Dam tail water. Additionally, three specimens collected were identified as wild-spawned fish, indicating that successful recruitment has occurred in the segment of the restoration area.

Thirty-four individuals were collected during 2019 surveys with 22 Robust Redhorse collected at the Parr Tailrace site, six at the Sandy River site, five at the Shelton's Ferry site, and one at the Wateree Dam/Tailrace site. Tissue samples were taken from 33 of the 34 collected individuals and submitted for parentage analysis. No visual confirmation of actively spawning individuals was documented in 2019; however, all sites where Robust Redhorse were collected included individuals that appeared to be recently spawned or were actively flowing (males and females) upon collection. The lone exception to this was the one individual (female) collected from Wateree tailrace. Although this individual did not display characteristics of a recently or actively spawning fish, prior documentation has shown this location to be a known spawning site.

During 2021 surveys, 14 individuals were collected with 11 Robust Redhorse collected above Shelton's Ferry and three below Neal Shoals Dam. As with all prior collection years, no Robust Redhorse were collected while surveying the Congaree River. Tissue samples were taken from all collected individuals and submitted for parentage analysis. No visual confirmation of actively spawning individuals was documented in 2021; however, all sites where Robust Redhorse were collected included individuals that appeared to have recently spawned or had actively flowing males or females upon collection. Although none of the collected individuals were determined as having a wild-spawned designation, previous collections at nearby sites have produced wild fish.

### Task 2.1 – *Improve Data Management*

Management of long-term data sets developed from past Robust Redhorse studies has been complicated because of the diversity of study purposes as well as the complexity of data. For example, genetic monitoring requires linking information about broodstock, broodstock progeny, and tissue samples, all of which could have been collected by different institutions. Similarly, Robust Redhorse capture information can vary from very coarse catch-per-unit effort data to detailed capture history and growth information for individuals. In the past, substantial effort has been expended to manage these disparate data sets in Microsoft Excel. Efforts during the current project have resulting in the development of an Access relational database for range-wide Robust Redhorse research activities; the existence of a centralized data repository eliminates duplicative data entries, allows for more complex data queries, and prevents accidental errors introduced through sorting and copying. SCDNR staff have assisted the Georgia Department of Natural Resources (GADNR) lead on this task throughout the project period. Specifically, we have migrated all of our Robust Redhorse collection and genetic data holdings into an Access database and have coordinated with the GADNR lead to integrate of the genetics components into the main Robust Redhorse Access database. Additionally, we have assisted with coordination of data providers for QA/QC and clarification of existing field collection data during our annual Robust Redhorse Conservation Committee meetings as well as with follow-up communications.

### Task 3.1 – *Stocking in the Pee Dee River*

Due to the extremely small Pee Dee River Robust Redhorse estimated population size, a cooperative stocking program began with spawning and production at North Carolina Wildlife Resources Commission's (NCWRC's) McKinney Lake Fish Hatchery and SCDNR's Dennis Wildlife Center Fish Hatchery. Responsible genetic guidelines for Robust Redhorse reintroduction and supplementation in the Pee Dee River were previously developed by Moyer and Darden (2013) and incorporated into the augmentation program. Annual population augmentation was initiated in the fall of 2014 with the release of Phase I fingerling (6 months old) Robust Redhorse downstream of Blewett Falls dam and has continued with both Phase I and Phase II fingerlings (18 months old) since.

During the first spring of our project (2017), partners in the Robust Redhorse Conservation Committee's (RRCC's) Yadkin-Pee Dee Technical Working Group—including NCWRC, Duke Energy, NC State University, SCDNR, and the SC Aquarium—continued annual cooperative

sampling and population monitoring for Robust Redhorse in the Pee Dee River downstream of Blewett Falls dam. The total electrofishing effort (pedal time) in this reach was 68.3 hours. Nineteen Robust Redhorse were collected during targeted sampling; none of which were ovulating females and as a result, no spawning of captive fish occurred in 2017. Sperm was collected from one wild adult male and cryopreserved at the USFWS Warm Springs Technology Center for potential use in future production years to increase genetic diversity of hatchery offspring. Both NCWRC's McKinney Lake Fish Hatchery and SCDNR's Dennis Wildlife Center Fish Hatchery retained 50 individuals each from the 2015 propagation, which are being grown to sexual maturity in hatchery ponds. Appropriate genetic screening has been conducted to provide hatchery guidance prior to spawning to maximize offspring genetic diversity.

During the second spring of our project (2018), partners in the RRCC's Yadkin-Pee Dee Technical Working Group continued annual cooperative sampling and population monitoring for Robust Redhorse in the Pee Dee River downstream of Blewett Falls dam. The total electrofishing effort (pedal time) in this reach was 48.8 hours. Thirty-two individual Robust Redhorse were collected during targeted sampling; two of which were ovulating females that were able to be spawned by hatchery personnel. About 4,000 Robust Redhorse fry were produced between the two hatcheries and were stocked in ponds for Phase I and Phase II grow-out. As a result, 2,024 Phase I fingerlings were stocked in the Great Pee Dee River on 1 November 2018. Half of these fingerlings were released at Cheraw (SC) and the other half were released at Jones Creek Shoal (NC). Phase II fish were retained for additional grow-out and later harvest. Sperm was collected from several wild adult males and cryopreserved at the USFWS Warm Springs Technology Center for potential use in future production years to increase genetic diversity of hatchery offspring. NCWRC's McKinney Lake Fish Hatchery and SCDNR's Dennis Wildlife Center Fish Hatchery have continued to rear a total of 78 individuals from the 2015 propagation year class, which are being grown to sexual maturity in hatchery ponds.

During the spring of 2019, partners in the RRCC's Yadkin-Pee Dee Technical Working Group continued annual cooperative sampling and population monitoring for Robust Redhorse in the Pee Dee River downstream of Blewett Falls dam. The total electrofishing effort (pedal time) in this reach was 95.81 hours. A total of 53 individual Robust Redhorse were collected during targeted sampling; two of which were ovulating females that were able to be spawned by hatchery personnel. The collected fish represented 12 among year recaptures, 3 new wild fish, 32 Phase I fish from the 2014 Year Class (YC), 4 Phase II fish, and 8 within year recaptures. About 12,800 Robust Redhorse fry were produced between the two hatcheries and were stocked in ponds for Phase I and Phase II grow-out. As a result, 5,075 Phase I fingerlings were stocked in the Great Pee Dee River on 13 November 2019. Approximately half of these fingerlings were released at the Hwy. 74 Landing and the other half were released at the Diggs Tract Landing near Jones Creek Shoal. An additional 126 Phase II fish from the 2018 YC were stocked by boat at Jones Creek Shoal on the same date. McKinney Lake Hatchery held back 700 Phase I fingerlings at their facility for Phase II/III grow-out. The Dennis Wildlife Center lost nearly 2,000 Phase II Robust Redhorse from their ponds during the 2018-2019 winter months due to bird (cormorant) predation. NCWRC's McKinney Lake Fish Hatchery continues to hold approximately 64 individuals from the 2015 propagation year class, which are being grown to sexual maturity in hatchery ponds.

Due to Covid pandemic restrictions, only NCWRC and Duke Energy were permitted to participate during the spring of 2020 which involved a single day of sampling on 29 April 2020. The total electrofishing effort (pedal time) of this sampling event was 6.15 hours and resulted in a total of 10 individual running/ripe Robust Redhorse being collected during targeted sampling. All fish collected were genotyped and determined to be of hatchery origin (three 2014 YC Phase I, six 2015 YC Phase I, and one 2015 YC Phase II), with two of these fish being ovulating females that were able to be spawned by hatchery personnel. Of the two females spawned, one was a 2015 year class fish that was stocked as a Phase I, while the other was a hatchery-reared 2015 year class fish that was stocked as a Phase II from the Dennis Wildlife Center.

From these 2020 spawns, approximately 5,715 Robust Redhorse fry were produced between the two hatcheries and were stocked in ponds for Phase I and Phase II grow-out. As a result, 3,046 Phase I fingerlings were stocked in the Great Pee Dee River on 28 October 2020 at the Hwy. 74 Landing. An additional 525 Phase II fish from the 2019 year class were stocked by McKinney Lake Hatchery at the same location on the same date. The decision was made to forego PIT tagging the Phase II fish this year to avoid close human contact due to Covid-19, so these fish will only be able to be tracked genetically. McKinney Lake Hatchery held back 230 Phase I fingerlings from the 2020 year class for Phase II grow-out.

On 23 March 2020, 51 sexually mature Phase III (2015 year class) Robust Redhorse were released in the Great Pee Dee River at the Hwy. 74 Landing. These fish were raised at both McKinney Lake Hatchery and the Dennis Wildlife Center. Thirteen Phase III fish were held back at McKinney Lake Hatchery for future potential broodstock.

During the spring of 2021, partners in the RRCC's Yadkin-Pee Dee Technical Working Group continued annual cooperative sampling and population monitoring for Robust Redhorse in the Pee Dee River downstream of Blewett Falls dam. The total electrofishing effort (pedal time) in this reach was 14.61 hours. A total of 36 individual Robust Redhorse—including 22 new fish and 14 recaptures (seven Phase II 2015YC, five wild fish, and two Phase III's 2015YC)—were collected during targeted sampling, one of which was an ovulating female that was able to be spawned by hatchery personnel. This female happened to be a 2015 YC stocked fish that was originally produced at the Dennis Wildlife Center Hatchery. This female was crossed with a single male. Approximately 6,500 Robust Redhorse fry were produced between the two hatcheries and were stocked in ponds for Phase I and Phase II grow-out. McKinney Lake Hatchery did not have any survival of Robust Redhorse fingerlings at time of harvest. The Dennis Wildlife Center Hatchery harvested 2,419 Phase I fish from their production pond. As a result, 2,219 Phase I fingerlings were stocked in the Great Pee Dee River on 28 October 2021 at Diggs Tract Landing near Jones Creek Shoal. The remaining 200 Phase I fish were transported to Bud Freeman's Lab at UGA to be used for experimental eDNA studies. McKinney Lake Hatchery also harvested their 2020 year class Phase II Robust Redhorse pond on 28 October 2021, which produced 214 fish. All Phase II fish were weighed, measured and PIT tagged before being released in the Great Pee Dee River at the Hwy. 74 Bridge the same day. McKinney Lake Hatchery is still holding 11 adult Robust Redhorse from the 2015 YC in one of their ponds.

In summary, the propagation and stocking of Robust Redhorse from the McKinney Lake Hatchery and Dennis Wildlife Center has been a huge success. From 2017 to 2021, four different year classes of Robust Redhorse were produced and stocked. During this project, a

total of 13,280 Robust Redhorse were stocked in the Great Pee Dee River, including 12,364 Phase I, 865 Phase II, and 51 Phase III (adult) fish. The catch per unit effort (CPUE) has increased substantially since augmenting the population with hatchery-reared fish. Since 2016, we have consistently collected and observed more fish on the spawning grounds. To date, we have seen very little recruitment, but hopefully this will improve over the next several years as progeny from the hatchery stocked fish reach maturity and begin utilizing the spawning shoals. A conservation agreement was not reached by the conclusion of this project, so no Robust Redhorse were able to be stocked in the Tillery reach upstream of Blewett Falls dam.

***Significant deviations*** – The Covid-19 pandemic substantially impacted our project activities during the 2020. From the genetics perspective, although our lab activities were hampered due to the closure of both federal and state labs during early 2020 (and the extended effective closure of the federal lab until the spring of 2022), we were able to relocate a functional portion of our genetics lab to the state Marine Resources Research Institute (MRRI) and develop safe Covid-19 working protocols to be able to resume reduced-scale lab activities during the summer of 2020 which allowed us to complete processing of our planned workloads on schedule. Field activities were impacted due to limited staff availability and capabilities under Covid protocols, and less field effort was accomplished than planned during 2020. A full return to field capabilities occurred in 2021 for completion of all planned project activities during the final year of our project. No modifications to our project objectives were required, however a two-year, no-cost extension was provided for all partners during the life of this grant in order to complete the objectives. A reporting extension was also allowed.

***Estimated Federal Cost*** – All federally allocated fund were expended to complete the project activities, and match has been met for the project.

***Recommendation*** – Close the grant.

***Final Report prepared by:***

Daniel Farrae, Levi Kaczka, Jarrett Gibbons, and Tanya Darden  
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