# FINAL REPORT

SC-U2-F23AP00075-01
South Carolina Department of Natural Resources
January 1, 2023 – December 31, 2025

***Project Title**:* Adaptively Managing for Robust Redhorse: A Range-wide Collaboration to Address Data Gaps, Assess Potential Environmental Threats, and Implement Conservation Actions

***Purpose and Objectives:*** We plan on using an array of targeted sampling techniques to help assess the status and trend of each population of the three evolutionary significant units (ESU).

Our South Carolina-specific Tasks for each Project include:

**Project 1:** Determine status of Robust Redhorse in the Altamaha, Savannah, and Pee Dee ESUs using a combination of traditional, visual, acoustic, and genetic monitoring techniques.

- Task 1.1: Conduct Population Monitoring and Calculate Population Estimates
- Task 1.2: Conduct eDNA Surveys
- Task 1.3: Continue Genetic Monitoring Protocol

**Project 2:** Addressing identified Robust Redhorse life history data needed for accurate population modeling.

- Task 2.2: Producing and Updating Age-Length Keys for Each ESU Population
- Task 2.3: Producing a life history table for the Santee River population

**Project 3:** Support management and conservation actions as prioritized yearly by the RRCC.

- Task 3.2: Continue responsible stocking of the Pee Dee River population to meet RRCC conservation genetics stocking goals

## *Accomplishments*

Task 1.1: Conduct Population Monitoring and Calculate Population Estimates

Within the Santee River Basin, electrofishing survey efforts were conducted across 8 sampling days in 2023 (Figure 1), 6 sampling days in 2024 (Figure 2), and 5 sampling days in 2025 (Figure 3) using our standardized protocols. In total, 79 Robust Redhorse were collected during this project timeframe with 45 of those captured in the Broad River section and 34 on the Wateree River section.

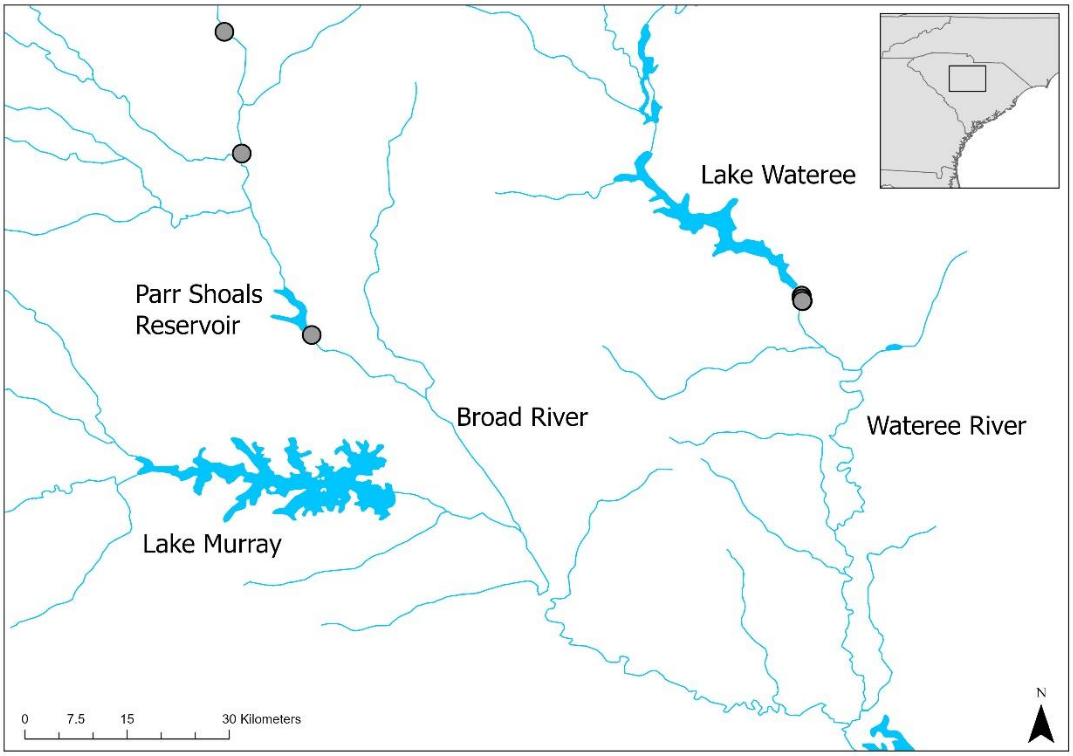


Figure 1. Collection locations of Robust Redhorse in the Santee River Basin during the 2023 collection year.

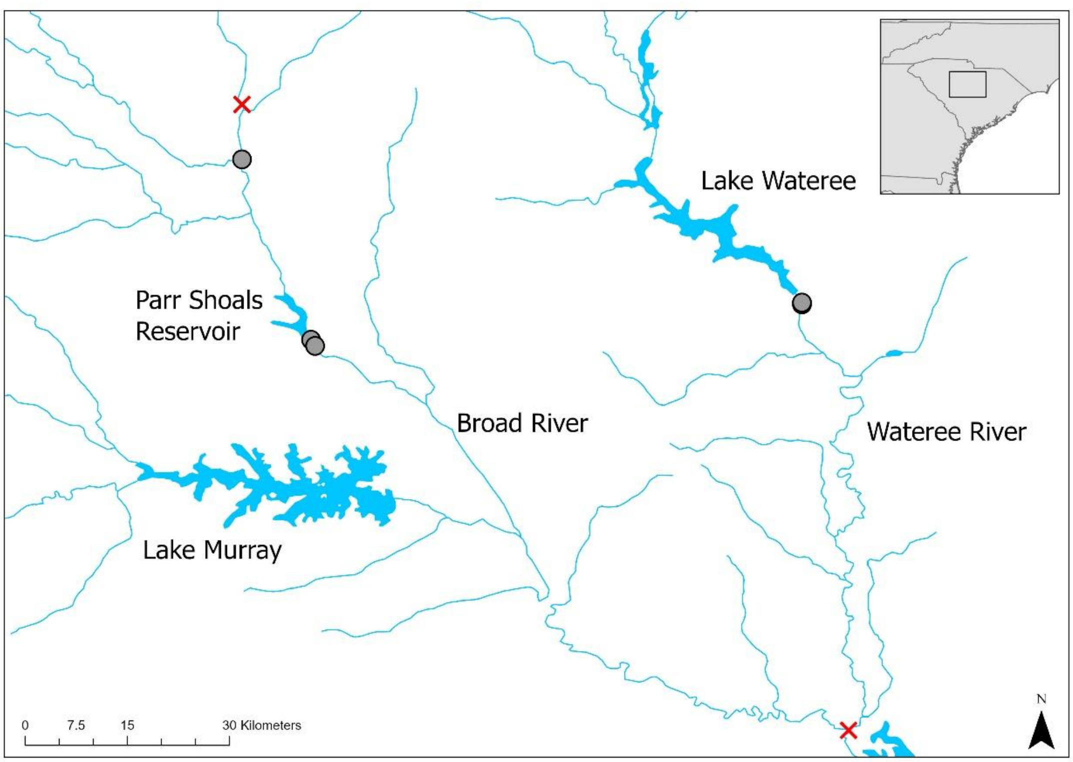


Figure 2. Collection locations of Robust Redhorse in the Santee River Basin during the 2024 collection year with gray circles representing collection points and red X's representing sampling events that failed to capture Robust Redhorse.

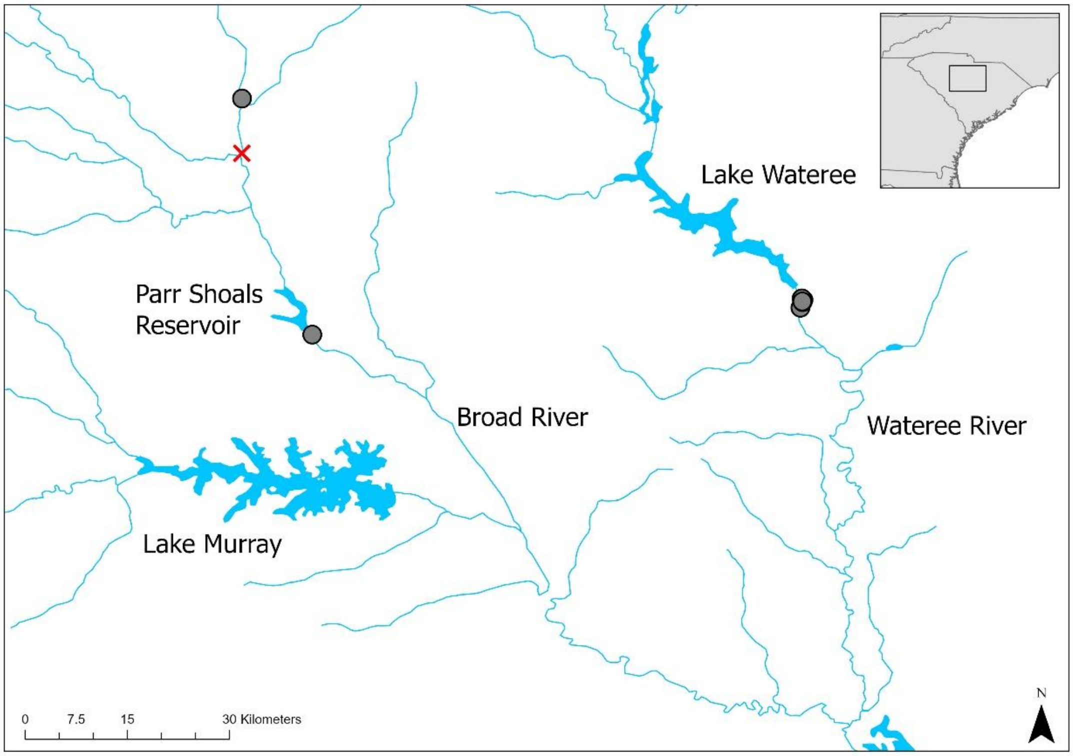


Figure 3. Collection locations of Robust Redhorse in the Santee River Basin during the 2025 collection year with gray circles representing collection points and red X's representing sampling events that failed to capture Robust Redhorse.

Additionally, 46 carpsuckers, which were field identified as Quillback, were captured incidentally during Robust Redhorse surveys. Tissue samples were collected from each individual (18 from the Broad River; 28 from the Wateree River) for archiving at the SCDNR Population Genetics Tissue Collection. Subsamples of these tissues were provided to Texas A&M for a new research project.

Within the Pee Dee River, SCDNR participated in the collaborative effort to capture adults in spawning condition during 2023 – 2025 (details are provided below for Task 3.2).

We used all Robust Redhorse collections from the Santee River Basin from 2012 – 2025 to estimate the abundance of the population. Over the project period, 258 total fish were collected with 26 representing recaptures based on matching genotypes and/or physical tags. Because captures are overwhelmingly adult fish, the Schnabel mark-recapture analysis effectively estimates the spawning adult population. Although there are more robust and complex methodologies to estimate abundance from mark-recapture data, the less complex Schnabel estimator seems most appropriate for the Robust Redhorse dataset given its small sample size and low number of recaptures. The use of more complex models can sometimes be used to avoid assumption violations. However, the frequently violated Schnabel assumption of a closed population is likely minimally violated at worst given the long lifespan of Robust Redhorse, the lack of connectivity to other populations, and that most of our project captures are adults and therefore should still provide a reliable population estimate for the Santee River Basin population. Our resulting Schnabel-estimated adult abundance of Robust Redhorse in the Santee

River Basin is 1,048 individuals (95% CI: 757 – 1702), with a Poisson distribution adjustment for the 95% CI producing a true estimate in the range of 1,006 – 1,173 adults.

Task 1.2: Conduct eDNA Surveys

Although our original project scope included conducting an eDNA survey in the Ogeechee River, GA during spring 2023, a more extensive GADOT study in the system was funded providing an opportunity to use project funds to expand the application of the eDNA tool into another system in GA. Due to the timing of notice on the GADOT study, we did not have time to pivot thoughtfully for a 2023 replacement design. Therefore, no eDNA collections occurred during 2023. During the annual RRCC meeting in December 2023, the committee discussed the need for eDNA studies in other GA river systems to replace the Ogeechee River – selecting Little River, GA. Therefore, during spring 2024 we sampled both the replacement GA system as well as the planned NC portion of the Pee Dee River.

*North Carolina:* eDNA samples were collected from two Pee Dee River sites in the Tillery Reach, NC (Hwy109 and Hwy 731 crossings) on three occasions (26 April 2024, 3 May 2024, 8 May 2024) by NCWRC and SCDNR staff (Figure 4). Eight eDNA samples and one control were collected at each site and event, for a total of 54 eDNA filters collected using our standard operating protocol.

*Georgia:* eDNA samples were collected from three sites in the Little River, GA (Martin Mill Road, Hwy 129, and Monticello Road crossings) on two occasions (30 April – 1 May 2024 and 7 – 8 May 2024) by SCDNR staff (Figure 4). Nine eDNA samples and one control were collected at each site and event, for a total of 60 eDNA filters collected using our standard operating protocol.

All collections were made using a Smith Root eDNA Sampler with 5.0 µm self-preserving filters with a maximum water volume of 5 L. Following collection, all filters were frozen immediately and kept frozen until DNA extraction and amplification.

All eDNA filter extractions and amplifications followed our SOP for Robust Redhorse eDNA processing. Extractions and amplifications were performed randomly from all 114 filters during July – September 2024 by SCDNR staff.

*Georgia collections*: For the field collection eDNA samples and controls collected in the Little River, GA there were no positive detections in any of the field or control samples.

*North Carolina collections*: For the field eDNA samples collected in the Tillery Reach, NC, there were no positive detections of Robust Redhorse; however, 38 samples across both locations and on three sampling days had non-specific amplification detected late in the real-time cycling (Figure 5). Based on our initial testing of the eDNA assay, these likely resulted from the detection of eDNA from another *Moxostoma* species, likely the ‘Carolina’ Redhorse (Figure 6). Additionally, three of the control samples collected in the Tillery Reach, NC tested positive for Robust Redhorse eDNA (Figure 7, Table 1). Sampling in the Tillery Reach, NC was done in part with a boat which had been used in the preceding days to help collect Robust Redhorse during their spawning season. Given the lack of positive detections of Robust Redhorse in the water samples during these same days, we are confident that the controls were contaminated by Robust Redhorse DNA being present in the boat, which could have been picked up directly or incidentally as gear and personnel were moved on and off the boat.

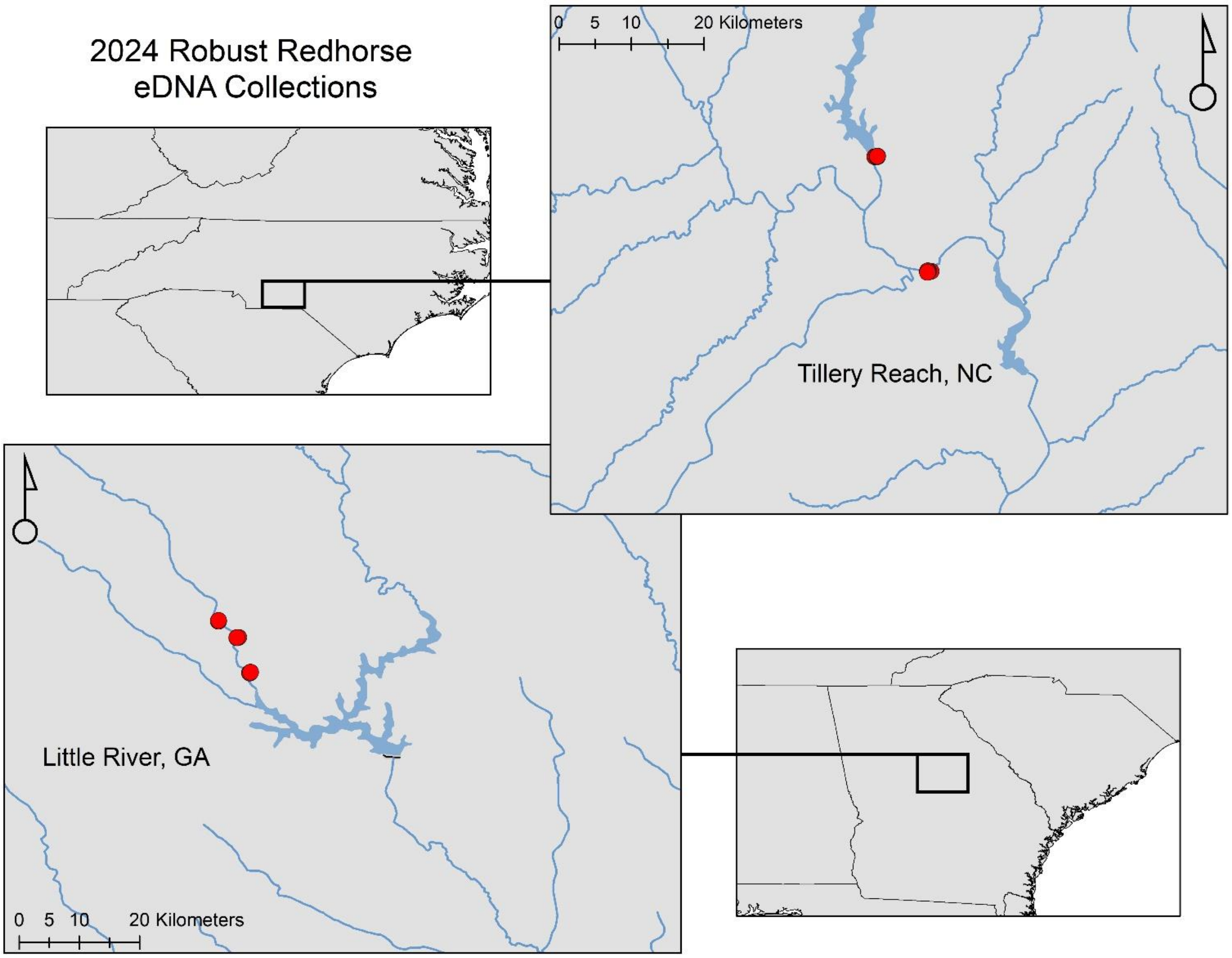

Figure 4. eDNA collection points targeting Robust Redhorse during 2024 in North Carolina and Georgia.

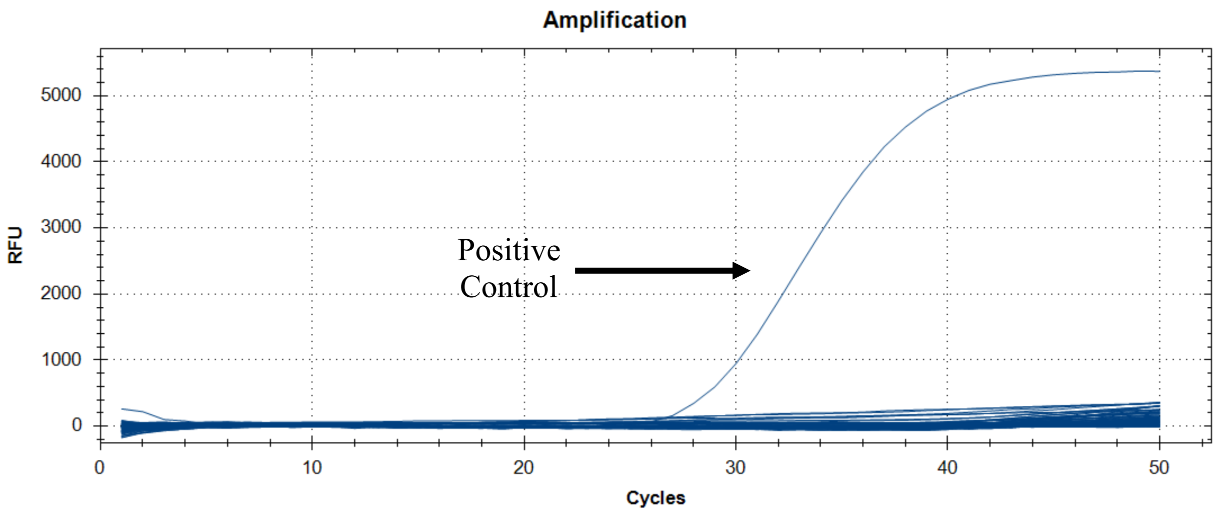


Figure 5. Example real-time PCR late cycle non-specific amplification of a likely non-Robust Redhorse *Moxostoma* species detected in the Tillery Reach, NC. A positive control included in every real-time PCR amplified to detection around 26 cycles, while the non-specific amplification occurred later and in a linear manner.

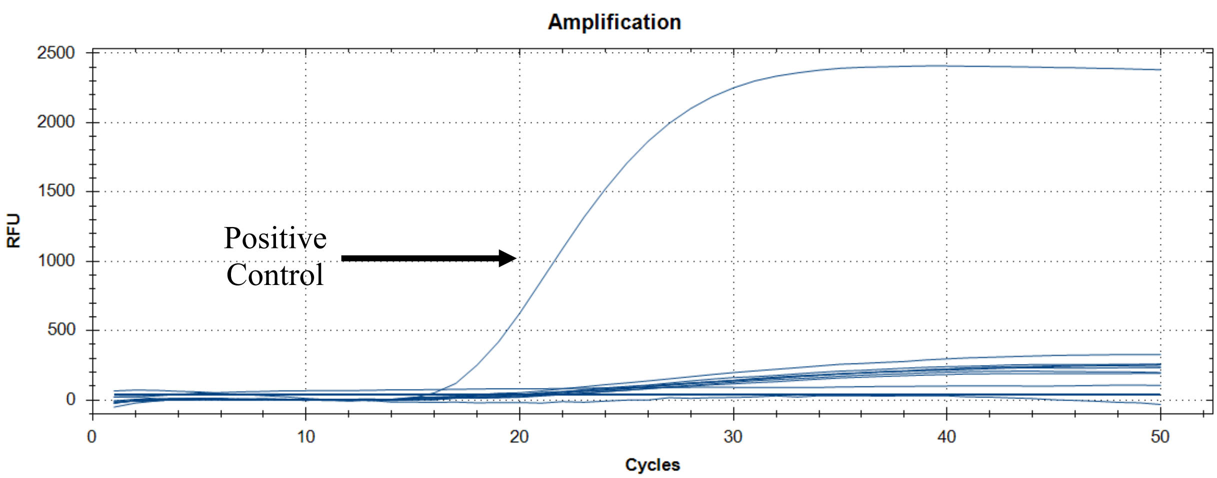


Figure 6. Example real-time PCR late cycle non-specific amplification of 'Carolina' Redhorse DNA during the Robust Redhorse eDNA assay testing process. A positive control included in every real-time PCR amplified to detection around 16 cycles, while the 'Carolina' Redhorse DNA amplified later and in a linear manner.

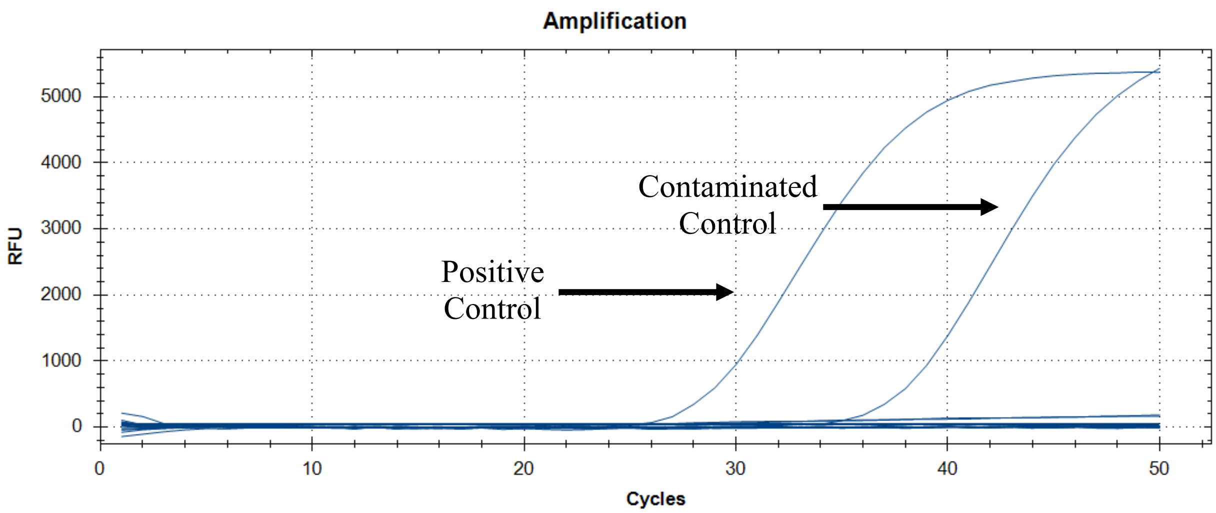


Figure 7. Example real-time result curve from a contaminated control sample from the Tillery Reach, NC. A positive control included in every real-time PCR amplified to detection around 26 cycles, while the control amplified to detection around 35 cycles.

Table 1. Control samples collected during eDNA collections for Robust Redhorse in the Tillery Reach, NC.

| Filter | Site | State | Location | Fine location | Date | Result |
|---|---|---|---|---|---|---|
| 248 | 1.C | NC | Tillery Reach | Hwy 109 | 26-Apr-24 | Negative |
| 262 | 2.C | NC | Tillery Reach | Hwy 731 | 26-Apr-24 | Positive |
| 300 | 1.C | NC | Tillery Reach | Hwy 109 | 3-May-24 | Negative |
| 305 | 2.C | NC | Tillery Reach | Hwy 731 | 3-May-24 | Positive |
| 314 | 1.C | NC | Tillery Reach | Hwy 109 | 8-May-24 | Positive |
| 319 | 2.C | NC | Tillery Reach | Hwy 731 | 8-May-24 | Negative |

Following these non-specific amplification results, samples from NC that had late-cycle amplification were tested for PCR inhibition by spiking the isolations with Robust Redhorse DNA and following the standard real-time PCR protocol. Any isolations that amplified the Robust Redhorse DNA at a later cycle than the positive control can be considered inhibited and therefore have inconclusive results. 34 of 38 tested samples showed no signs of inhibition (Figure 8), however 4 of the 8 samples collected at the Highway 109 site on 3 May 2024 showed evidence of PCR inhibition (Figure 9). It is noteworthy that all of these inhibited samples occurred at a single location on a single date. The pattern seen in these inhibited samples with spiked Robust Redhorse DNA, which only occurred in 4 of the 38 tested samples, is not the same linear amplification observed in Figure 2. Therefore, though these are inhibited, we do not feel that these patterns are inhibited Robust Redhorse detections, but instead are still likely inhibited 'Carolina Redhorse' detections.

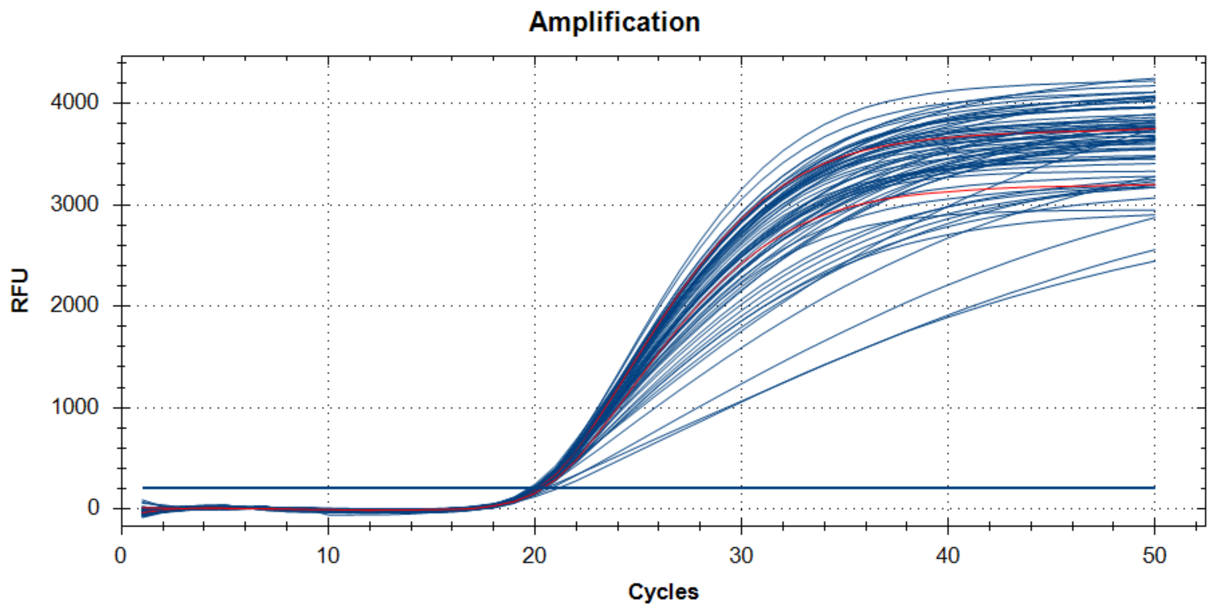


Figure 8. Real-time result curve from the inhibition test by spiking isolations with Robust Redhorse DNA. These results all pass the baseline threshold near the same cycle as the positive control samples (in red), indicating that these samples were not inhibited during the PCR.

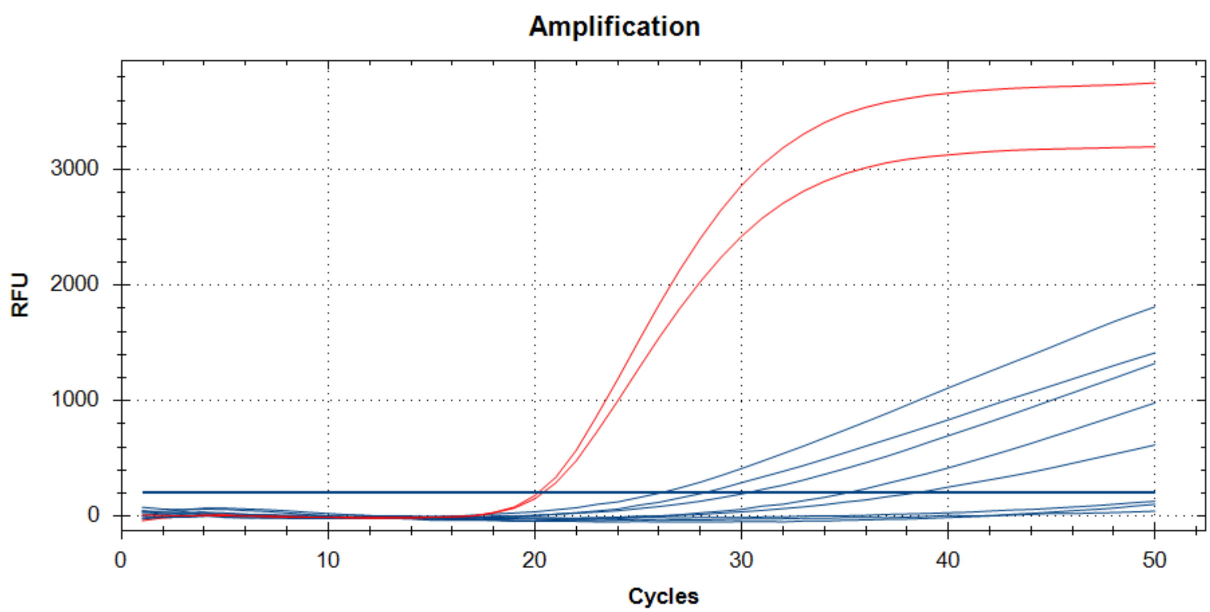


Figure 9. Real-time result curve from the inhibition test by spiking isolations with Robust Redhorse DNA. These results all pass the baseline threshold at a later cycle than the positive control samples (in red), indicating that these samples were inhibited during the PCR.

Task 1.3: Continue Genetic Monitoring Protocol

For all genetic samples, we used our standard metal bead isolation protocol, spin-column isolation protocol, or QIAcube isolation protocol (dependent upon the storage solution and number of samples being processed at once) 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 with reaction conditions provided in Darden and Tarpey (2014). PCR products were separated and visualized on a Beckman GenomeLab GeXP™ capillary electrophoresis system. All resulting chromatograms were scored using CEQ Fragment Analysis Software by two independent readers for quality assurance. During the final year of the project, we transitioned sequencing to the Applied Biosystems (ABI) SeqStudio Flex platform. We reorganized the same nine microsatellite loci into two multiplex groups to improve efficiency. We re-genotyped all 2024 and some 2023 collected Robust Redhorse samples during 2025 and standardized processing to ensure that samples resulted in identical genotypes from both sequencing methods.

Over the course of this project, a total of 376 Robust Redhorse contemporary field-collected tissue samples were genotyped, analyzed, and archived (Table 2). Note, some Pee Dee and Santee-Cooper samples collected in 2022 were genotyped in 2023 and included in these analyses to improve sample sizes and provide continuity from previous data that were collected 2017 – 2021.

Table 2. Total number of field-collected Robust Redhorse from January 2022 – December 2025 genotyped during the project period.

| River/System | Total Genotyped |
|---|---|
| Pee Dee River, NC/SC | 216 |
| Santee-Cooper System, SC | 80 |
| Savannah River, SC/GA | 36 |
| Broad River, GA | 35 |
| Ocmulgee River, GA | 9 |
| Total | 376 |

**Summary of Santee River Basin field collections**

Due to incomplete genetic tracking (i.e. missing broodstock genotypes), it is not possible to determine if some fish were of ***Wild*** or ***Cultured*** origin based on genetics alone. Our earliest stocked fish (2004 year class (YC)) could have recruited to adulthood and successfully spawned over 10 years ago, meaning that their ***Wild***-spawned offspring would now have reached the asymptotic portion of the growth curve of Santee River Basin Robust Redhorse (Figure 10). Simultaneously, our youngest stocked fish without complete genetic tracking (2010YC) would also be near the asymptote of the growth curve. Therefore, we conservatively applied a cut-off of 550 mm total length (TL) for the designation between newly recruited ***Wild***-spawned fish and older ***Unmatched*** fish for which we were unable determine their origin.

Of the 80 Robust Redhorse collected in the Santee-Cooper System during this study, there were 30 fish that genetically matched back to genotyped broodstock pairs and were designated as ***Cultured***. These included matches to the 2004, 2005, 2008, 2009, and 2013 year classes. Continued survival and collection of ***Cultured*** fish across many ages is a promising sign for the Santee River Basin population, including 1 2004YC fish that was 21 years old at the time of collection.

There were 4 small (<550 mm TL) fish captured during the study that did not genetically match to any parents and were designated as ***Wild***, representing the first documentation of ***Wild*** fish in the Santee River Basin since the 2018 collection year. The total lengths of these four fish were 498, 510, 519, and 523 mm, and all were captured in the Wateree River during 2025.

Of the remaining 46 Robust Redhorse, none could be matched genetically to known broodstock pairs in the Santee River Basin. All of these fish were large enough (>550 mm TL) that it cannot be determined if they are of ***Cultured*** or ***Wild*** origin. These were therefore designated as ***Unmatched*** fish of an unknown year class.

As it is becoming increasingly more difficult to use total length as a means of differentiating ***Wild*** spawned from ***Cultured*** Robust Redhorse, we have begun using the ***Unmatched*** designation. Documenting trends of the contribution of ***Unmatched*** to the annual collection records of Robust Redhorse may be a way to indicate some sign of increasing natural recruitment to the Santee River Basin, as it seems less likely to continue catching ‘new’ adult fish that were previously stocked than it is to begin catching ‘new’ ***Wild*** spawned fish. There has been a general increase in the percentage of ***Unmatched*** fish to the annual catch of Robust Redhorse in the Santee River Basin from 2016 – 2025 (Figure 11).

Table 3. Genetic designations and contribution of Santee-Cooper System Robust Redhorse collected from 2022 – 2025.

| Designation | | Number | | Contribution (%) | |
|---|---|---|---|---|---|
| ***Cultured*** YC | | 30 | | 37.5 | |
| | 2004 | | 8 | | 26.7 |
| | 2005 | | 3 | | 10.0 |
| | 2008 | | 4 | | 13.3 |
| | 2009 | | 8 | | 26.7 |
| | 2013 | | 7 | | 23.3 |
| ***Unmatched*** (> 550 mm TL) | | 46 | | 57.5 | |
| ***Wild*** (< 550 mm TL) | | 4 | | 5.0 | |
| Total | | 80 | | 100 | |

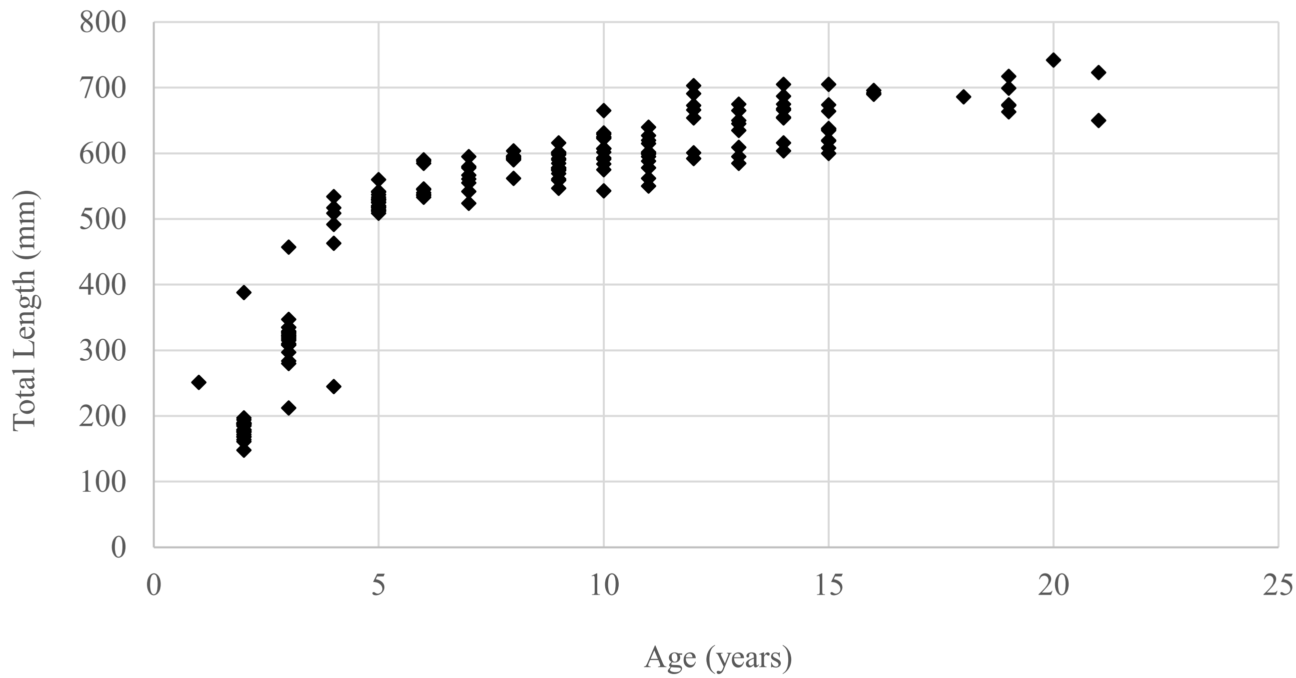


Figure 10. The length-at-age data of all known-age Robust Redhorse ever collected in the Santee River Basin.

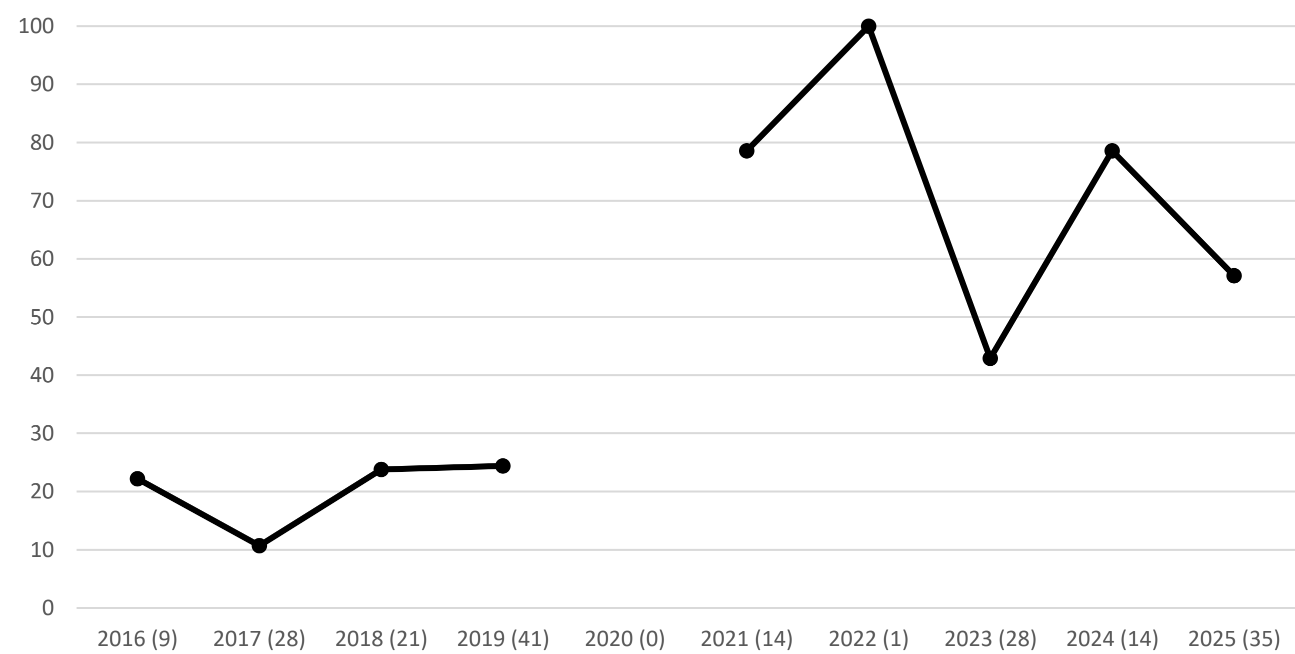


Figure 11. Percentage of Robust Redhorse designated as ***Unmatched*** by sampling year in the Santee-Cooper System, with the annual sample size in parentheses.

**Summary of Pee Dee River field collections**

Of the 216 Robust Redhorse collected in the Pee Dee River during this study, 195 genetically matched to genotyped broodstock pairs and were designated as ***Cultured***. These included matches to the 2014, 2015, 2015/2018, and 2018 – 2024 year classes. Continued survival and collection of ***Cultured*** fish across many ages is a promising sign for the Pee Dee River population.

A high hatchery contribution (***Cultured*** fish; Table 4), which ranged annually from 81.1% in 2025 to 100% in 2022 is consistent with an initially low population size and continued stocking of the Pee Dee River. The 21 ***Wild*** fish in the current dataset were of moderate size (328 – 737 mm TL) that roughly overlaps with the size of 2 – 10 year old Robust Redhorse in the Pee Dee River (Figure 12). The smaller sized ***Wild*** fish indicate natural recruitment has been occurring in recent years.

Table 4. Genetic designations and contribution of Pee Dee River Robust Redhorse collected from 2022 – 2025.

| **Designation** | | **Number** | **Contribution (%)** |
|---|---|---|---|
| ***Cultured*** YC | | 195 | 90.3 |
| | 2014 | 45 | 23.1 |
| | 2015 | 31 | 15.9 |
| | 2015 / 2018 | 6 | 3.1 |
| | 2018 | 15 | 7.7 |
| | 2019 | 34 | 17.4 |
| | 2020 | 39 | 20.0 |
| | 2021 | 7 | 3.6 |
| | 2022 | 11 | 5.6 |
| | 2023 | 3 | 1.5 |
| | 2024 | 4 | 2.1 |
| ***Wild*** | | 21 | 9.7 |
| Total | | 216 | 100 |

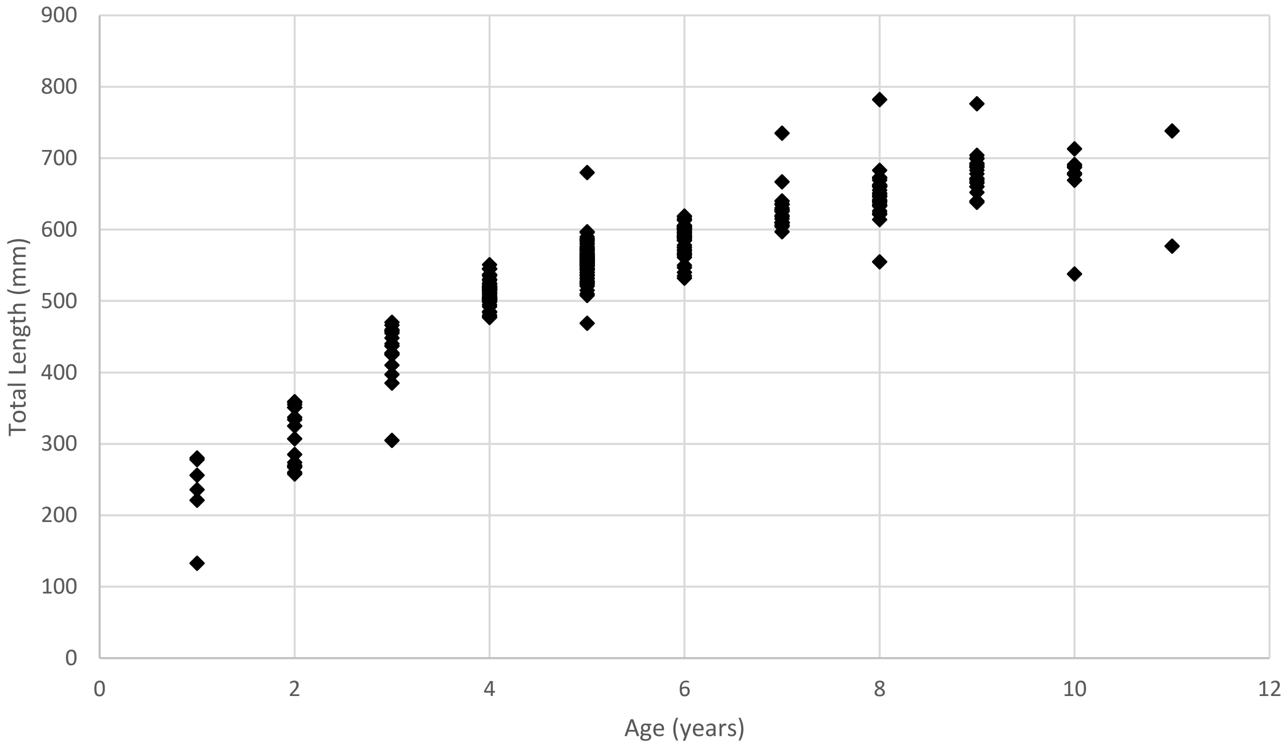


Figure 12. The length-at-age data of all known-age Robust Redhorse ever collected in the Pee Dee River.

**Summary of GA field collections**

From August 2023 – June 2025, 80 fin clip samples were collected and genotyped from Robust Redhorse collected in Georgia rivers: 36 from the Savannah River; 35 from the Broad River; and 9 from the Ocmulgee River. Although Robust Redhorse were previously stocked in some Georgia rivers, tissue samples from production broodstock were unavailable. Therefore, these samples were successfully genotyped and used for genetic health assessments but could not be used for parentage analyses. However, during the current project, an archived set of Robust Redhorse tissue samples and DNA isolations were obtained from a retired researcher. We believe that at least some of the missing broodstock tissue samples are included in the sample set; however, the samples are still being individually curated and compared with previously documented broodstock data. The new sample lot includes 3 samples from the Ocmulgee River, ~100 adult samples from the Oconee River, 9 adult samples from the Savannah River, 26 adult samples from the Pee Dee River, and 1000s of larvae/fingerling samples from multiple locations. Previously, we obtained and archived tissue samples from Georgia that included 107 samples from the Oconee River, 4 from the Ocmulgee River, 30 from the Altamaha River, 1 from the Ogeechee River, 30 from the Savannah River, and 100s of hatchery-reared larvae/fingerling samples. Future efforts will focus on identifying and genotyping the most germane tissue samples in these lots, which hopefully include all of the broodstock used in Georgia thereby allowing parentage analysis of Robust Redhorse in those systems.

**Summary of genetic metrics**

All genotype data generated for contemporary samples (i.e. collected during the current study period), were used to calculate genetic metrics by population to compare with previously published assessments, and re-assess the degree of genetic differentiation among Robust Redhorse populations. For recaptured individuals, 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 GenAlEx 6.5 (Peakall and Smouse 2006, 2012), and effective population size was calculated using the program $LDN_E$. 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. Results for the Ocmulgee River population should be interpreted with caution due to low sample size.

The Bayesian clustering program Structure 2.3.4 (Pritchard et al. 2000) was used in a hierarchical manner, separating unique genetic clusters to further evaluate finer scale structure. For these analyses, we used all individual (i.e. recaptures and duplicates were removed) genotyped Robust Redhorse from the following rivers: Ocmulgee, Oconee, Savannah, Broad-GA, Santee, and Pee Dee.

These analyses can sometimes identify family structure, if present, rather than population level structure. We ran the Structure analyses three ways to understand potential influences of family structure on our interpretation. The first included all individual fish. For the second, we identified full-sibling pairs based on genetic parentage assignments for the Santee and Pee Dee populations and randomly reduced each sibling group to one representative. We recognize the approach underestimates family structure due to the lack of wild family identifications as well as incomplete parentage records in the Santee population. For the third, we used the program Colony 2.0.6.2 (Jones and Wang 2010) to identify full-sibling families and randomly reduced each family to one representative. The settings for Colony analyses included polygamous breeding, long run length, weak prior, update allele frequencies, no genotyping error, FPLS likelihood, and high precision.

For all analyses, we used 20,000 burn-in and 200,000 MC repetitions, across a range of possible clusters (*K* starting at a maximum of 6) and 5 iterations each. We ran all analyses with and without capture location as a prior. Structure Selector (Li and Liu 2018) was used to identify which *K* best fit the data for each analysis.

Despite differences in sample size, all five populations of Robust Redhorse had similar observed heterozygosity estimates (~0.75 – 0.86), which were similar to previous values from 2017 – 2021 (Table 5) and those 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 are similar to previous estimates (Darden and Tarpey 2014) and indicative of a lack of inbreeding. Given the small sample size of the Ocmulgee River population (n=9), all results should be interpreted 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 (104.1) and lowest in the Broad River, GA (22.1; an isolated branch of the Savannah River that received stocked fish as a refugial population) and Pee Dee River population (24.2), which has increased since previous estimates from 2017 – 2021 and the estimates in Darden and Tarpey (2014). These effective population size estimates are congruent with relative differences in Robust Redhorse abundance estimates from these populations.

Table 5. Genetic metrics of Robust Redhorse collected 2017 – 2021 and 2022 – 2025 by population, with any recaptured individuals removed. The 95% parametric confidence intervals (CI) are included for contemporary *Ne* estimates. $H_O$ observed heterozygosity, $H_E$ expected heterozygosity, $N_a$ number of alleles, $F_{IS}$ inbreeding coefficient, $N_e$ effective population size. * Cautious interpretation warranted due to small sample size.

| Population | # Ind. | $N_a$ | $H_o$ | $H_E$ | $F_{IS}$ | $N_e$ (95% CI) |
|---|---|---|---|---|---|---|
| Pee Dee 2017 – 2021 | 145 | 9.7 | 0.81 | 0.78 | -0.03 | 11.0 (9.9 – 12.3) |
| Pee Dee 2022 – 2025 | 211 | 9.0 | 0.76 | 0.76 | 0.01 | 24.2 (21.7 – 26.9) |
| Santee-Cooper 2017 – 2021 | 101 | 16.8 | 0.83 | 0.87 | 0.03 | 40.1 (36.1 – 44.7) |
| Santee-Cooper 2022 – 2025 | 72 | 13.8 | 0.86 | 0.86 | 0.00 | 58.9 (50.3 – 70.0) |
| Savannah 2017 – 2021 | 18 | 10.1 | 0.89 | 0.85 | -0.05 | 152.9 (53.4 – ∞) |
| Savannah 2022 – 2025 | 36 | 12.4 | 0.87 | 0.86 | -0.01 | 104.1 (67.8 – 203.6) |
| Broad-GA 2022 – 2025 | 34 | 76 | 0.78 | 0.75 | -0.04 | 22.1 (16.8 – 30.1) |
| Ocmulgee 2017 – 2021 | 4 | 3.8 | 0.83 | 0.61 | -0.36 | -6.0 (14.4 – ∞)* |
| Ocmulgee 2022 – 2025 | 9 | 5.6 | 0.78 | 0.70 | -0.11 | 35.2 (9 - ∞)* |

All pairwise $F_{ST}$ population comparisons were statistically significant, except for the comparison between the Ocmulgee River and Broad River, GA (Table 6). The Oconee River, which meets the Ocmulgee River to form the Altamaha River, was the source of the broodstock that produced offspring which were then stocked in the Broad River. The highest level of differentiation was between the Pee Dee and Ocmulgee River populations, also the two most geographically separated, which was the same pattern observed in samples collected 2017 – 2021. The lowest level of genetic differentiation was between the Santee-Cooper and Savannah River populations (0.007), 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.

Results from Colony identified 140 groups of at least two full-siblings and 68 groups of at least three full-siblings (Figure 13). The ten largest groups of full-siblings were limited to the Santee-Cooper and Pee Dee populations, which is unsurprising given their stocking history, and included up to 36 full-siblings. There were 15 cases of inter-basin full-siblings, but all were logical given the connectivity of systems and history of fish movements/stockings: 1 pair from the Ocmulgee and Broad-GA; 2 from the Oconee and Broad-GA; 3 from the Oconee and Ocmulgee; and 9 from the Savannah and Santee.

Our Structure analyses indicated a single tier of genetic clustering was present in the data. The initial analysis represented range-wide population structure with three distinct clusters, one cluster of the Ocmulgee, Oconee, and Broad-GA, one of the Savannah and Santee, and one of the Pee Dee (Figure 14). Each of these clusters were then analyzed independently in Structure, with each analysis indicating a single cluster present in each data set. There were some indications in individual runs that there may have been some sub-structure present, but it was not consistently present among types of analysis (with or without location as a prior; with or without siblings included) or across values of *K*. One example includes an outlier group of 8 fish from the Broad-GA that clustered separately in some runs. On closer inspection, we discovered that six of these were identified as a full-sibling family in our Colony analysis, which were not identified/removed by parentage analysis in the first two analyses. Most likely, these subsequent hierarchical Structure analyses were detecting latent family grouping among samples. Once full-sibling families were removed for our third and most stringent Structure analysis, we did not observe any substructure patterns. Therefore, the consensus single-tier analysis represents the true range-wide population structure of Robust Redhorse. The observed population structure patterns follow known movement/stocking histories of these systems, as described in the preceding paragraph discussing $F_{ST}$ results.

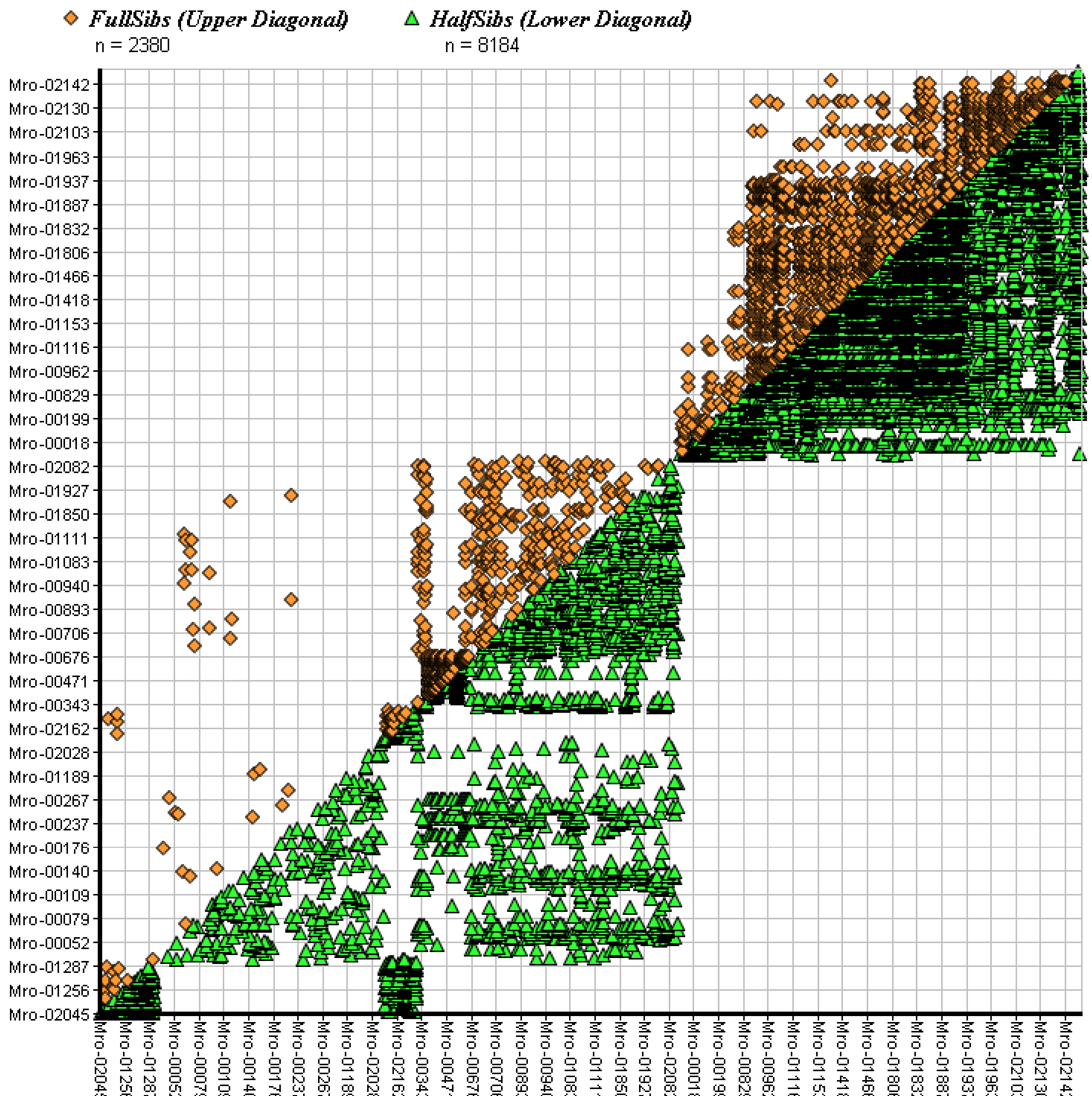


Figure 13. Pairwise full-sibling (above diagonal) and half-sibling (below diagonal) pair of Robust Redhorse, organized by populations, as identified by Colony. Starting in the lower left corner, the Ocmulgee, Oconee, Savannah, Broad-GA, Santee, and Pee Dee rivers were included.

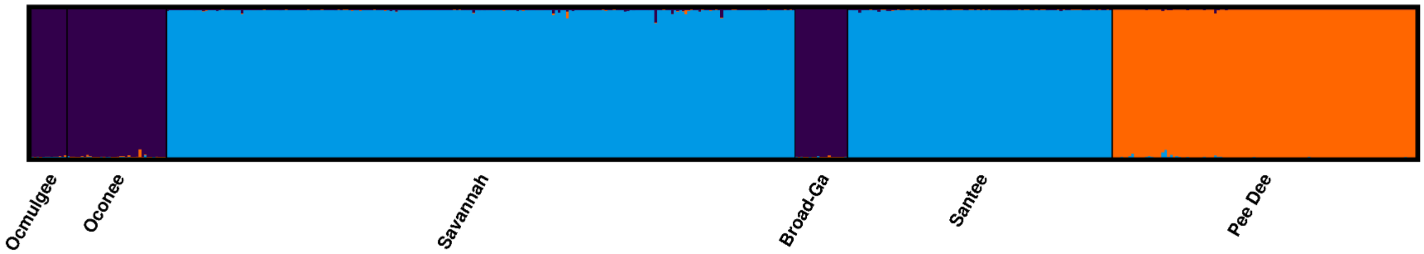


Figure 14. Results from the range-wide Structure evaluation of Robust Redhorse, showing ancestry for $K$ = 3 genetic clusters.

Table 6. Pairwise $F_{ST}$ estimates (below diagonal) between populations of Robust Redhorse collected 2023 – 2025. Values of significance (above diagonal) were calculated using 1000 permutations; Bonferroni corrected critical p-values = 0.005.

| | PeeDee | Santee-Cooper | Broad-GA | Savannah | Ocmulgee |
|---|---|---|---|---|---|
| PeeDee | | 0.001 | 0.001 | 0.001 | 0.001 |
| Santee-Cooper | 0.129 | | 0.001 | 0.003 | 0.001 |
| Broad-GA | 0.165 | 0.106 | | 0.001 | 0.077 |
| Savannah | 0.130 | 0.007 | 0.105 | | 0.001 |
| Ocmulgee | 0.177 | 0.118 | 0.014 | 0.112 | |

Table 7. 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 | |

Task 2.2: Producing and Updating Age-Length Keys for Each ESU

All length data are recorded for fish as they are captured. Once successfully genotyped, fish with matching broodstock parents can be assigned an age (apart from some Santee River Basin individuals as discussed in Task 1.3). These length-at-age data are maintained in a comprehensive database that is updated annually. During the last year of our project, compiled data (Figure 10 and Figure 12) were analyzed to produce comprehensive age-length keys for the recently stocked ESUs (Pee Dee and Santee-Cooper rivers) for comparison across populations.

Task 2.3: Producing a Life History Table for the Santee River Population

Robust Redhorse were stocked in the Santee River Basin from 2004 – 2013 (Table 8), with approximately 0.2% of those fish recaptured over the course of the restoration and monitoring efforts to date. Age could be determined for most hatchery reared fish based on parentage analysis results. Robust Redhorse were sampled in the Santee River Basin from 2012 – 2025. From 2012 – 2025, 256 samples were collected from the Broad, Catawba, Congaree, Saluda, Santee, and Wateree Rivers within the Santee-Cooper River Basin. The Broad River accounted for 180 Robust Redhorse samples, followed by 63 from the Wateree River (Figure 15). Only 13 total samples were collected from the other four rivers, often occurring as bycatch during surveys targeting other species. An additional 45 hatchery-reared Robust Redhorse that were tagged and measured before stocking were included in our analysis to fill data gaps for fish under 350 mm TL and under 5 years old.

Detection of coded wire tag (CWT), passive integrated transponder (PIT) tags, and/or genotype matches to known broodstock crosses helped determine the origin of some captured Robust Redhorse throughout sampling (Table 9). The majority (150 of 256; 58.6%) of captured fish were determined to be of hatchery origin. Due to loss or migration of CWT and PIT tags and incomplete broodstock genotypes due to missing samples, not all captured fish were reliably identified as ***Cultured*** or ***Wild***. A total of 91 fish were deemed ***Unmatched***. The ***Wild*** designation was assigned to a total of 15 fish with representation from each river except the Catawba. Our assumption that fish < 550 mm TL are too small/young to have been in the system since the last stocking event in 2013 was supported by von Bertalanffy age and growth modeling (Figure 16).

The maximum recorded age, weight, and length, von Bertalanffy age and growth parameters, and estimated weight given total length are provided in Table 10 and Figure 17. Apart from the yet to be stocked hatchery fish included in some of the analysis to fill in data gaps, the sample was comprised of mostly older, larger fish (Figures 18 and 19), as much of the sampling took place on or near spawning habitat during the spawning season when Robust Redhorse are most susceptible to our sampling techniques. These new data are critical baseline information for the Robust Redhorse populations and can serve as a reference to which future measurements can be compared to monitor conservation actions or natural population changes.

Table 8. Number of Robust Redhorse stocked from each year class and the number recaptured throughout sampling from 2013 – 2025. Only fish of known age using genetics, CWT, or PIT tags were included in the life history analysis.

| Year Class | # Phase I | # Phase II | # Phase III | # Phase IV | Total # Stocked | # Captured | % Captured |
|---|---|---|---|---|---|---|---|
| 2004 | 18920 | 2010 | 400 | 0 | 21330 | 32 | 0.15% |
| 2005 | 23107 | 400 | 0 | 0 | 23507 | 23 | 0.10% |
| 2006 | 928 | 0 | 0 | 0 | 928 | 2 | 0.22% |
| 2007 | 9800 | 0 | 0 | 0 | 9800 | 3 | 0.03% |
| 2008 | 1383 | 0 | 0 | 0 | 1383 | 20 | 1.45% |
| 2009 | 9000 | 0 | 0 | 0 | 9000 | 50 | 0.56% |
| 2010 | 0 | 0 | 0 | 20 | 20 | 0 | 0.00% |
| 2011 | 0 | 0 | 25 | 0 | 25 | 0 | 0.00% |
| 2013 | 5941 | 0 | 0 | 0 | 5941 | 21 | 0.35% |
| Total | 69079 | 2410 | 425 | 20 | 71934 | 151 | 0.21% |

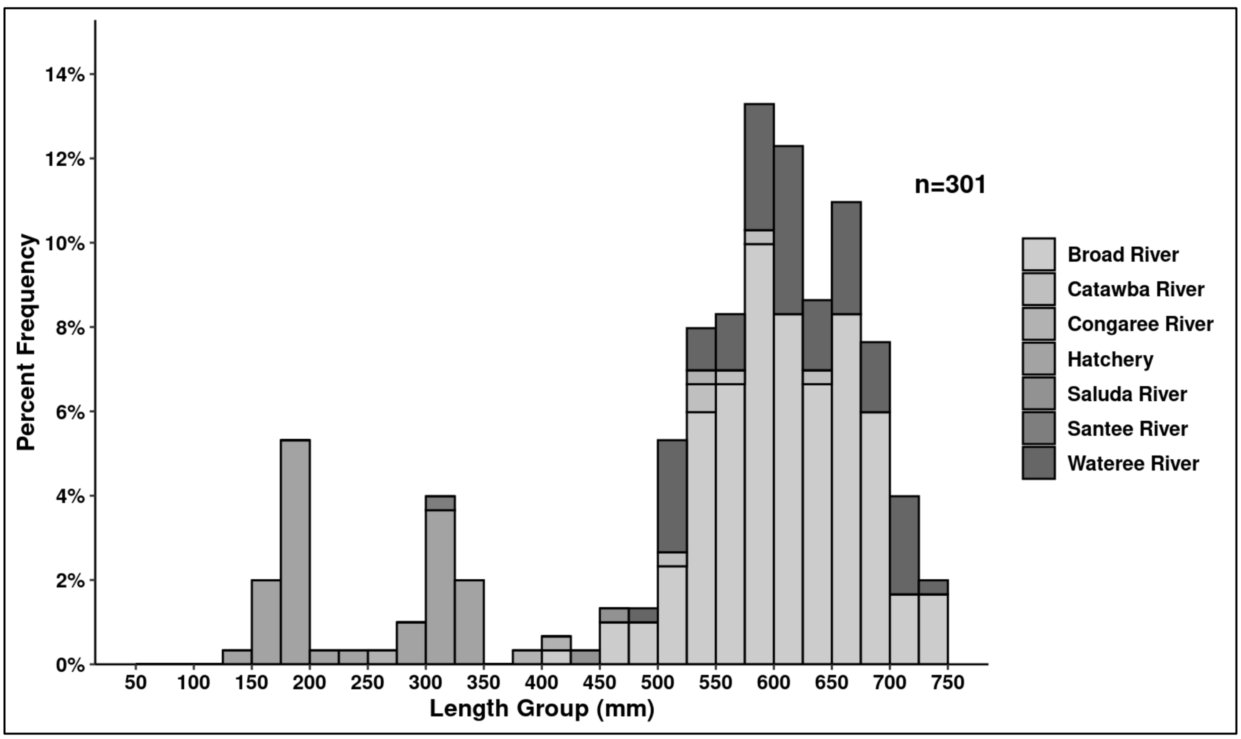


Figure 15. Length frequency histogram of Santee River Basin Robust Redhorse separated by river of capture. Hatchery designation indicates juvenile hatchery fish that were measured prior to stocking.

Table 9. Number of ***Cultured***, ***Unmatched***, and ***Wild*** Robust Redhorse captured in each river of the Santee River Basin that were included in the life history analysis.

| River | ***Cultured*** | ***Unmatched*** | ***Wild*** | Grand Total |
|---|---|---|---|---|
| Broad | 108 | 65 | 7 | 180 |
| Wateree | 36 | 23 | 4 | 63 |
| Catawba | 3 | 3 | 0 | 6 |
| Congaree | 2 | 0 | 2 | 4 |
| Saluda | 1 | 0 | 1 | 2 |
| Santee | 0 | 0 | 1 | 1 |
| Total | 150 | 91 | 15 | 256 |

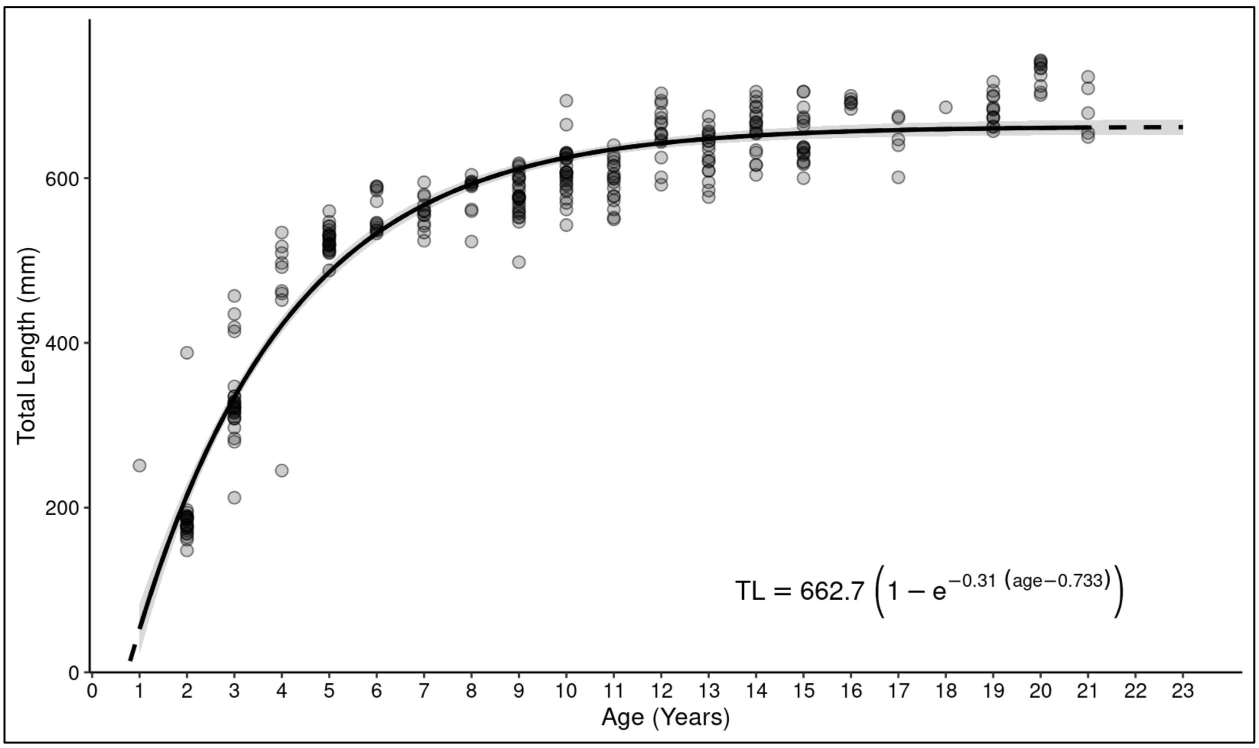


Figure 16. Von Bertalanffy growth curve of Santee River Basin Robust Redhorse including hatchery fish.

Table 10. Life table for Santee River Basin Robust Redhorse. Predicted weight equation uses total length in millimeters to predict weight of Robust Redhorse in the Santee River Basin.

| Parameter | Value |
|---|---|
| Max Total Length (mm) | 743 |
| Max Weight (g) | 8380 |
| Max Age (years) | 21 |
| L-infinity (mm) | 662.1 |
| K | 0.334 |
| t0 (years) | 1.437 |
| Predicted Wt (g) | $10^{-6}(TL)^{3.4148}$ |

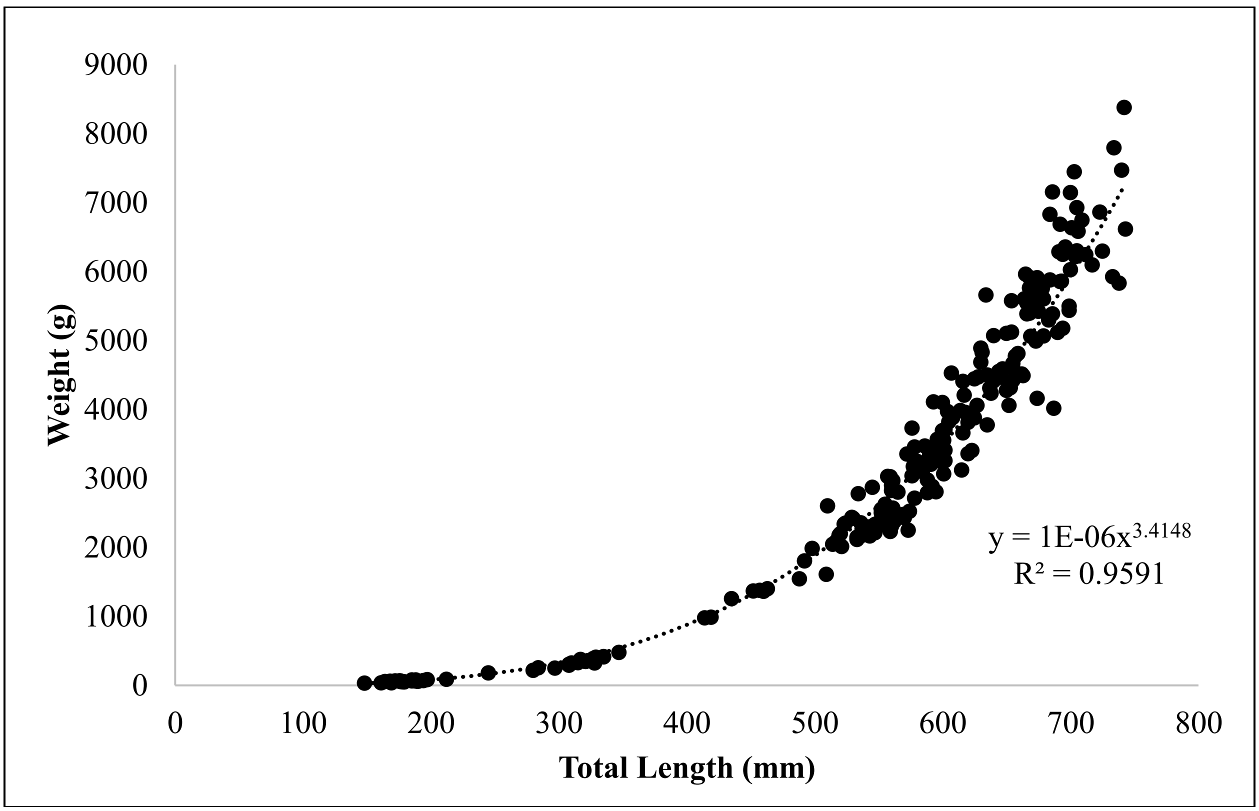


Figure 17. Length-weight relationship of Santee River Basin Robust Redhorse.

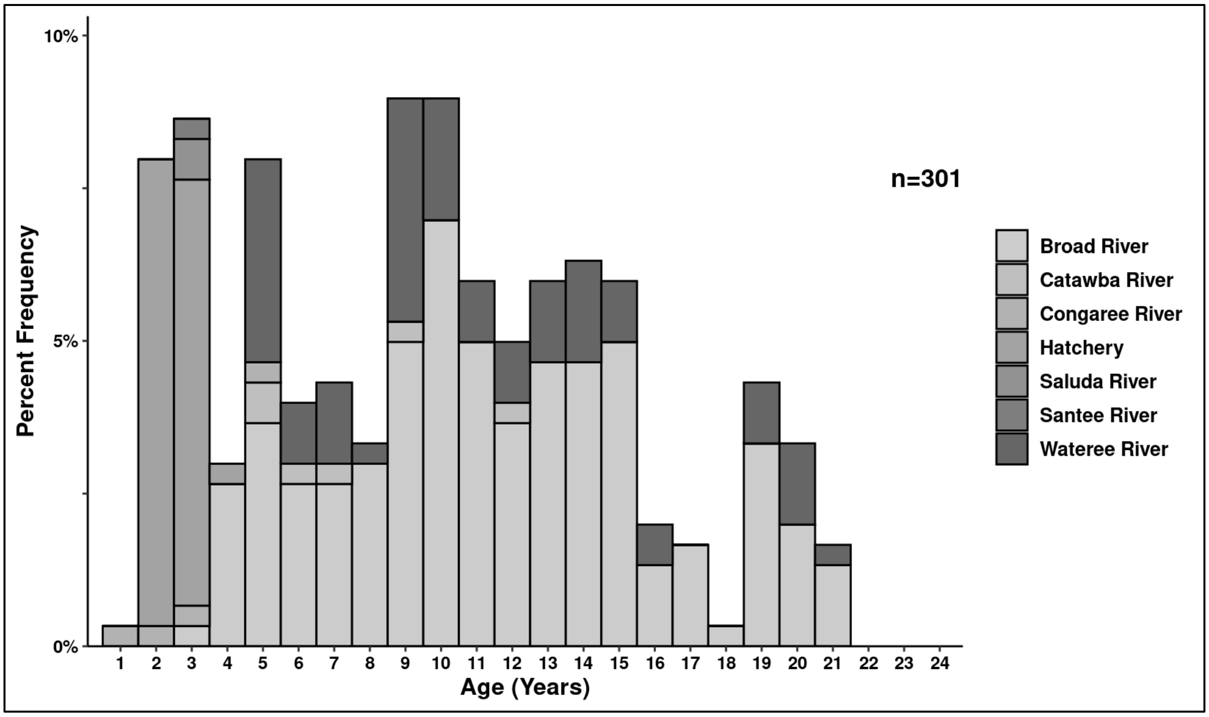


Figure 18. Age frequency histogram of Robust Redhorse captured in the Santee River Basin separated by river of capture, including fish whose unknown age was estimated using the updated known age at length key.

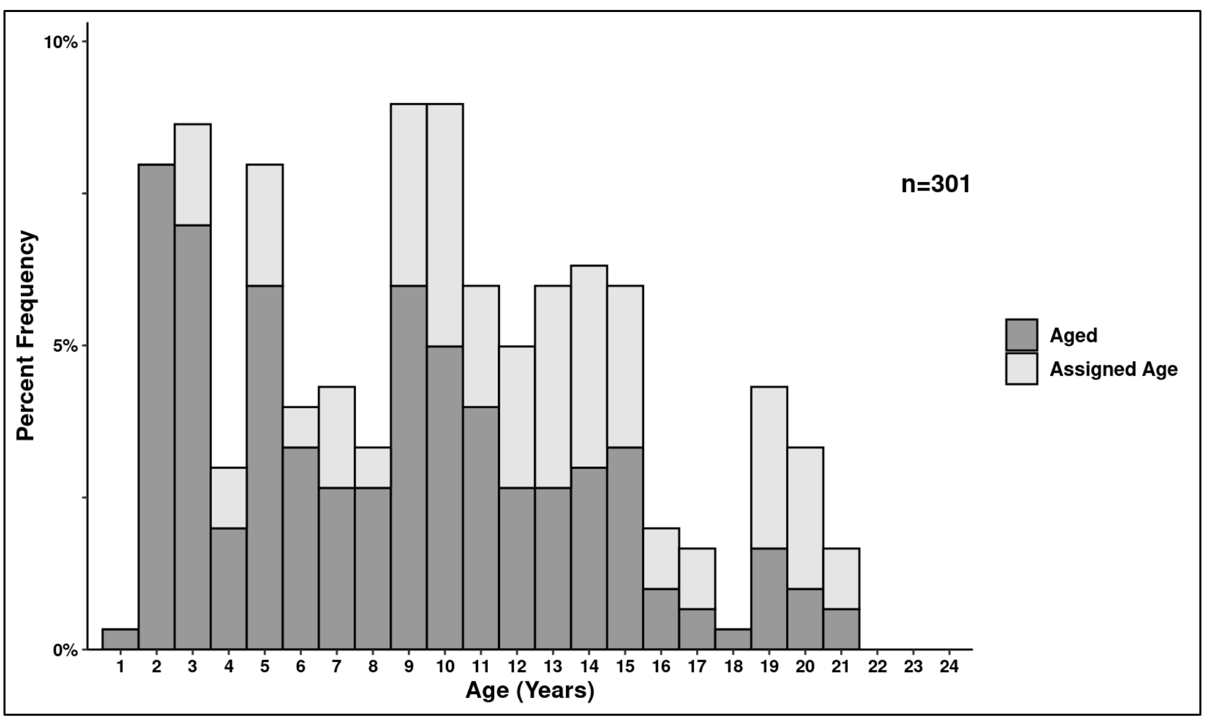


Figure 19. Age frequency histogram of Robust Redhorse captured in the Santee River Basin separated by known age and assigned age. Fish with an unknown age were assigned an age using the updated known age at length key.

Task 3.2: Continue responsible stocking of the Pee Dee River population to meet RRCC conservation genetics stocking goals

SCDNR staff assisted NCWRC staff in broodstock collection, spawning, and production of Robust Redhorse to contribute to the ongoing responsible stocking of the Pee Dee River populations for the 2023 – 2025 production years. Full details of production activities are reported by NCWRC.

***Significant deviations –*** Outside of the modification to the eDNA sampling location in Georgia previously addressed in the Year 1 interim report, no significant deviations occurred in the project period.

***Estimated Federal Cost –*** All federal and match funds for the project have been fully expended.

***Recommendations:*** Close the South Carolina portion of the grant.

***Prepared by:*** Daniel Farrae and Tanya Darden