

# SAN JUAN RIVER RECOVERY IMPLEMENTATION PROGRAM

## FY2020 ANNUAL REPORT

### **Project Title**

Estimating reproductive contribution of translocated Razorback Sucker (*Xyrauchen texanus*) in the San Juan River in 2020

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### **Abstract:**

Numerous freshwater fish species migrate across a diverse range of ecological niches. These patterns include migration for reproduction, to locate resources, or to access a more suitable climate. Large-bodied fishes of the Colorado River Basin, including Razorback Sucker (*Xyrauchen texanus*), have long been known to migrate long distances to their spawning grounds. Anthropogenic barriers (e.g. dams, weirs, etc.) impede upstream migration which decreases access to traditional spawning and rearing habitat. However, access to these habitats can be restored via translocation. This powerful management tool can promote reproduction and restore damaged or extirpated populations. Conservation translocations are widely used by natural resource managers, but must be adequately assessed to determine their effectiveness. Translocation can increase population numbers, but translocated individuals must successfully reproduce in their new habitat for the conservation measure to be considered successful. Translocation has been utilized in the San Juan River to transport Razorback Sucker above two unnatural barriers: the Piute Farms Waterfall and the Public Service Company of New Mexico (PNM) weir. Each spring, PIT tag antenna arrays detect Razorback Sucker congregating below these barriers. These aggregations suggest that the fish are trying to move upstream. In the spring of 2020, the Razorback Sucker aggregating below the waterfall were genetically sampled before being translocated upstream. We conducted parentage analysis to evaluate the reproductive contributions of the translocated Razorback Sucker by comparing their genetic profiles to those of larvae captured in 2020. None of the translocated adults were found to be parents of any larvae, indicating two possible explanations. First, it is possible that none of the translocated adults spawned successfully. Secondly, the translocated adults may have spawned, but their offspring were not sampled during larval surveys. The latter explanation seems plausible, given that larval collections were spatially and temporally limited due to COVID-19 restrictions. Future collections spanning the entire river and spawning season could be more successful at capturing contributions from translocated fish.

### **Tasks:**

1. Estimate reproductive contribution of translocated Razorback Sucker (*Xyrauchen texanus*) in the San Juan River in 2020.

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### Introduction

There are many different migration strategies in fishes. These can occur at specific times (e.g. time of day, week, or year) or at certain life stages (Lucas and Baras 2001). Numerous freshwater fish species migrate in varied spatial and temporal patterns across a diverse range of ecological niches (Brönmark et al. 2014). These patterns include migration for reproduction, to locate resources, or to a more suitable climate (Heape 1931; Lucas and Baras 2001). Migration has long been known to occur among large-bodied fishes of the Colorado River Basin, where Razorback Sucker (*Xyrauchen texanus*) and Colorado Pikeminnow (*Ptychocheilus lucius*) migrate long distances to spawning grounds (Sigler and Miller 1963; Tyus 1985; Tyus and Karp 1990; Modde and Irving 1998).

Anthropogenic barriers (e.g. dams, weirs, etc.) impede upstream migration which decreases access to traditional spawning and rearing habitat (Renaud 1997; Minckley et al. 2003; Hill et al. 2019). While habitat may be available below a dam or obstruction, there are often limitations in size or decreases in quality. These limitations often make the habitat insufficient to support a viable population and lead to severe population declines (Ligon et al. 1995). Even dams fitted with fish passages often restrict upstream movement (Jackson and Moser 2012). Smaller structures (e.g., irrigation diversion, weirs, and culverts) in tributaries also block access to spawning habitats (Moser and Mesa 2009). Habitat destruction and fragmentation of desert southwest rivers by dam construction and other impediments have been associated with the decreased ranges and declining abundances of many western fishes (Minckley et al. 2003; Minckley and Marsh 2009; Walters et al. 2013).

The Piute Farms Waterfall (PFW) and the Public Service Company of New Mexico (PNM) weir are two barriers on the San Juan River. The process of superimposition created PFW, which involved the San Juan River cutting through newly deposited sediments as water receded during a high-water year in Lake Powell (Cathcart et al. 2018). This formed a new channel for the San Juan River and led to the formation of a waterfall. PFW prevents upstream migration of Razorback Sucker from Lake Powell. In the spring, PIT tag antenna arrays detect Razorback Sucker congregating below the waterfall. This aggregation of Razorback Sucker suggests that the fish are trying to move upstream (Cathcart et al. 2018). The PNM weir, constructed in 1971 to divert water to the San Juan Power Generation Station, is another major barrier to fish passage. Originally, fish could only pass the weir at high flows. To alleviate this problem, a fish passage was constructed that traps the fish for manual transport upstream. Few fish were using the trap (Cheek 2014), so in 2018 the passage was opened to allow fish to swim freely through the structure.

Stocking has been used as a tool to mitigate the reduced abundance of many imperiled fish species (Rinne and Janisch 1995; Clarkson et al. 2005; Halverson 2008). Despite robust stocking programs, many freshwater systems show that self-sustaining populations cannot be established with dams or other barriers obstructing migrations (Marsden and Langdon 2012; Hill et al. 2019). Translocation of various species (e.g., lampreys, chubs, etc.) to suitable spawning habitats has been implemented as a tool to promote reproduction and restore damaged or extirpated populations, increase genetic variation and/or population size, move individuals away from human development, and assist migration around unnatural impediments (Spurgeon et al. 2015; Dresser et al. 2017; Mulder et al. 2017; Yackulic et al. 2021). Translocation allows for habitat connectivity so that all life stages can thrive (Jackson and Moser 2012). Conservation translocations are widely used by natural resource managers but not always assessed for effectiveness (Minckley 1995; Dresser et al. 2017; Yackulic et al. 2021). Translocation might increase population numbers, but translocated individuals must successfully reproduce in their new habitat for the conservation measure to be considered successful. The reproductive contribution of translocated individuals to future generations can be quantified through

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genetic parentage analysis, thereby determining if the conservation action is having meaningful impacts on the target population.

### **Methods**

#### *Sample Collection*

Larval fish sampling in 2020 was intended to be conducted along a 180-mile reach of the San Juan River from its confluence with the Animas River in New Mexico to Piute Farms Waterfall in Utah, but collections were limited due to COVID-19 restrictions. The first sampled location was from river mile (RM) 166.6 near PNM weir up to RM 180.6 near the Animas River confluence. The second collection location for Razorback Sucker spanned from RM 0.0 at Piute Farms Waterfall up to RM 77.0 miles near Mexican Hat. No Razorback Sucker larvae were collected below the waterfall due to all larval collections below the waterfall being truncated to August past the Razorback spawning season. Early larval stages (e.g., protolarvae to mesolarvae) were targeted during larval collections under the assumption that these individuals were from recent spawning events. All Razorback Sucker larvae collected (N = 177) were sampled for genetic testing except one which was left as a specimen voucher at the Museum of Southwest Biology (MSB), Albuquerque, NM. The larval collections were preserved in 95% ethanol making them suitable for genetic analysis. Tissue was subsampled from the posterior portion of each larval specimen while the anterior portion was retained at MSB for potential future otolith studies.

Adult Razorback Sucker were translocated from below Piute Farms Waterfall to above the falls. The intention was to translocate fish above both PFW and PNM Weir, but translocation only took place above the waterfall. There were 155 adults encountered in 2020, and fin clips were collected and sex recorded for each fish.

#### *Genetic Sampling*

Genomic DNA was extracted using NucleoSpin Tissue Kits (Macherey-Nagel, Duren, Germany). Library preparation for SNP detection followed a double digest restriction site-associated DNA (ddRAD) sequencing protocol (Peterson et al. 2012). Restriction digests of up to 1 µg genomic DNA per sample were performed in 50 µl reactions containing 5 µl New England BioLabs CutSmart Buffer and 20 units each of PstI and MspI restriction enzymes. Samples were digested at 37°C for 18-24 hours then purified using Agencourt AMPure XP beads (Beckman Coulter, Inc.). Barcoded samples (25-100 ng DNA each; standardized per library) were pooled in libraries of 48 samples following Illumina adapter ligation, then size-selected at 353-403 bp for Razorback Sucker (Bangs et al. 2018; Chafin et al. 2018) using the Pippin Prep System (Sage Science). Size-selected DNA was subjected to 12 cycles of PCR amplification using Phusion high-fidelity DNA polymerase (New England BioLabs) following manufacturer protocols. Quality checks and quantification of final libraries were performed using qPCR (NEBNext® Library Quant Kit for Illumina®; New England BioLabs) and the Agilent 2100 Bioanalyzer. All indexed libraries were pooled in equimolar amounts in sets of four to six per lane and sent for 150 base pair (bp) single-end Illumina sequencing (NextSeq 500, Whitney Genetics lab, Midwest Fisheries Center, Onalaska, WI).

#### *Data Analysis – SNPs*

The quality of the ddRAD data was assessed using FastQC v11.7 (Andrews 2010). The program fastp (Chen et al. 2018) was used to clean low-quality base calls from the restriction cut site of the

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Illumina sequences. These low-quality bases were removed from all sequences by trimming the first five bases from the 5' end of each sequence. Fastp was also used to remove adapter sequences and polyG tails, trim sequences to a maximum length of 100 bp, and discard sequences <100 bp after trimming. The quality filtering feature was disabled in fastp so that *process\_radtags* filtering options could later be applied.

The Stacks v2.6 pipeline (Rochette et al. 2019: *process\_radtags*; *denovo\_map.pl*; *populations*) was used for processing the cleaned ddRAD data using the methods of Rochette & Catchen (2017). Reads with uncalled bases or low quality scores (Phred < 10) were removed in *process\_radtags*. The option to check for intact RAD cut sites was disabled because these were removed in fastp. Data were assembled using *denovo\_map.pl* with default settings. The *populations* module was used to retain loci with a minimum minor allele frequency of 0.01. Additionally, SNPs were required to be present in at least 50% of individuals in at least two populations because we wanted loci shared among pairs of species to facilitate hybrid detection among Razorback, Bluehead (*Catostomus discobolus*) and Flannelmouth Sucker (*C. latipinnis*). Just one SNP per locus was retained.

AdmixPipe v3.0 (Mussmann et al. 2020) was used to quantify species ancestry for each sample using the program Admixture (Alexander et al. 2009). Genetic clustering values (*K*) ranged from 2 to 4, with 20 replicates performed at each *K*. AdmixPipe results were evaluated in Clumpak (Kopelman et al. 2015) to determine the optimal *K* value and identify hybrid individuals. The *populations* module was then rerun to exclude hybrid individuals, retain one SNP per locus present in at least 80% of Razorback Sucker samples, and remove SNPs with a minor allele frequency < 0.01.

All SNPs genotyped in the second run of *populations* were analyzed in Sequoia v2.3.5 (Huisman 2017) for pedigree reconstruction. Sequoia accounts for genotyping errors, overlapping or discrete generations, with or without inbreeding, and any proportion of genotyped parents. This software uses a maximum likelihood method to estimate relationships among offspring belonging to a single cohort by identifying full and half-sibling families.

### Results

A total of 165 larvae genotyped successfully as well as 147 adults. The initial analysis of the SNP data from AdmixPipe and Clumpak detected 37 hybrid larvae (Figure 1). The hybrids accounted for 22.4% of samples. No misidentified samples were detected (i.e., Flannelmouth Sucker or Bluehead Sucker mistakenly identified as Razorback Sucker). The hybrid individuals were removed from all downstream analysis. There were 18,335 SNP loci identified among the remaining Razorback Sucker (*N* = 119) larvae and adults (*N* = 147).

Sequoia did not identify any reproductive contribution of translocated Razorback Sucker to the larvae collected in 2020. However, several full-sibling relationships were identified among the larvae (Table 1). There were 14 full-sibling groups found among the larvae collected with an average of 2.07 larvae in each group. There were also full-siblings found among the adults translocated above the waterfall (Table 1), appearing to be hatchery stocked fish. There were 28 full-sibling groups found among the adults with an average of 3.14 individuals in each group.

### Discussion

With recent rapid environmental change and declining populations in many species, the role of translocation is being re-evaluated to promote persistence and resilience (Weeks et al. 2011).

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Translocation evaluations often encounter difficulties including monitoring complications, lack of funding, tracking animal behavior, quality of release habitat, and lack of public support (Berger-Tal et al. 2020). Translocations are often evaluated by measuring population establishments or population size increases (Minckley 1995), but it is important to consider additional factors in determinations of success. For translocations to be successful, translocated individuals must reproduce and genetically contribute to the population in their new habitats (Hedrick 2014). An effective way to quantify the reproductive contribution of translocated individuals is through genetic analysis which links the translocated individuals to offspring produced in the new habitat (Robinson et al. 2017; Zimmerman et al. 2019).

Our parentage analysis found that none of the adult Razorback Sucker translocated above Piute Farms Waterfall in 2020 contributed offspring to the larval sample group. There could be multiple reasons for this result. It is possible that none of the translocated adults spawned in the San Juan River, meaning that the translocation did not contribute to the population. Alternatively, the translocated individuals may have spawned, but their larvae were not encountered during collections. Collections were limited geographically and temporally throughout the 2020 collection season due to COVID-19 restrictions. This resulted in a greatly reduced number of Razorback Sucker larvae collected in the San Juan River compared to previous years. For example, 612 to 1,279 larvae were collected annually from 2010-2015, with an average of 1,166 larvae per year (Diver and Wilson 2018). Therefore, it is probable that contributions from translocated fish were missed. Not only were larval collections truncated, but translocation efforts were also limited. The intention was to translocate fish above both PFW and PNM Weir, but translocation only took place above the waterfall. Future collections that span the whole river and spawning season could be more successful at capturing contributions from translocated fish.

Sequoia was successful at finding full-sibling relationships among larvae, demonstrating that the SNPs used were capable of predicting family relationships. Sequoia also found full-sibling relationships among the translocated adults. Those relationships suggest that fish stocked in the San Juan River had moved downstream to Lake Powell and were trying to migrate back into the river. Several studies have noted that Razorback Sucker aggregate at river inflow areas of reservoirs (Albrecht et al. 2017; Cathcart et al. 2018; Pennock et al. 2020a, Pennock et al. 2020b). Pennock et al. (2020b) translocated a little over 300 Razorback Sucker that had aggregated at PFW with many of those fish only spending a short time in the San Juan River. Almost 80% of those fish were re-encountered downstream of the waterfall within a year. This migration corresponds to the known breeding season for Razorback Sucker, which suggests spawning as the intent of the upstream movement (Cathcart et al. 2018). Tracking the movement of Razorback Sucker coupled with genetic parentage analysis can help ascertain the motives behind these movement patterns.

Continuing to translocate Razorback Sucker above obstacles in the San Juan River could potentially aid the recovery of the species. However, this will only be true if the translocated fish reproduce in the San Juan River. The most concrete way to establish the contribution of those fish is to continue genetically monitoring the translocated fish and the larvae produced each year so that contributions to reproduction can be quantified (Schwartz et al. 2007; Pacioni et al. 2020). Currently it appears that the 2021 collections will be a better indicator of translocated individuals' potential contributions. Collections in 2021 were not restricted due to COVID-19, with over 300 adults being translocated over PFW and the PNM weir and over 2,000 larvae morphologically identified as Razorback Sucker. Once analysis is conducted on these samples, it is much more likely that any contributions from translocated individuals will be detected if these fish have successfully spawned in the San Juan River.

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### **Signed:**

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**Table 1.**

The number of full-sibling groups detected in the Razorback Sucker (*Xyrauchen texanus*) larvae collected in the San Juan River 2020 and adults translocated above Piute Farms Waterfall.

<b>Sample Group</b>	<b>Full-Sibling Groups</b>	<b>Mean Individuals per Group</b>	<b>Standard Deviation</b>
Larvae	14	2.07	0.25
Adults	28	3.14	1.53

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**Figure 1.** The results of an Admixture analysis conducted in AdmixPipe and visualized in CLUMPAK for single nucleotide polymorphisms in Razorback Sucker (*Xyrauchen texanus*) larvae collected from the San Juan River in 2020. Each individual is represented by a single vertical bar, with the proportion of color in each bar representing the estimated proportion of ancestry attributed to each of three genetic clusters. Orange represents Razorback Sucker, purple represents Bluehead Sucker (*Catostomus discobolus*), and blue represents Flannelmouth Sucker (*C. latipinnis*).