



## United States Department of the Interior

### Fish and Wildlife Service

Southwestern Native Aquatic Resources and Recovery Center

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**Title:** Using Molecular Techniques to Determine Effective Number of Breeders ( $N_b$ ) for Razorback Sucker and Colorado Pikeminnow in the San Juan River

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### Introduction & Justification

Recovery plans for many endangered fishes include the production and release of hatchery reared individuals to reestablish or augment wild populations in an effort to restore self-sustaining populations. Propagation programs have been implemented with two major objectives: increase population size and avoid unnatural loss of genetic diversity (Miller and Kapuscinski, 2003). In order to meet these recovery criteria, survival, reproduction, and recruitment of wild and augmented individuals must occur at a sustainable scale. Understanding factors that limit success in achieving a self-sustaining population can be difficult to identify. Monitoring various aspects of population dynamics allows managers to implement an adaptive management approach, which provides opportunity to maximize the rate and extent of learning from current management actions to better understand how to achieve management goals.

In an effort to mitigate further loss of two federally listed species, Razorback Sucker (*Xyrauchen texanus*) and Colorado Pikeminnow (*Ptychocheilus lucius*), augmentation of captive-reared individuals into the San Juan River commenced in the mid-1990s and has continued through present day (USFWS 2005; USFWS 2015). Annual monitoring of survival, reproduction, and recruitment of augmented populations has been supported through the San Juan River Recovery and Implementation Program (SJRRIP). Survival of augmented individuals is the first component to reestablishment. Mark-recapture data on PIT tagged individuals have provided quantifiable survival estimates of stocked Razorback Sucker, which in turn has prompted additional research investigating ways to increase first-year survival (Franssen, N.R. per. comm.). Reproduction and survival of offspring to a reproductive age (recruitment) is the second step to reestablishing a self-sustaining population. Although recruitment of Colorado Pikeminnow and Razorback Sucker is limited, San Juan River larval fish surveys have documented successful reproduction of both species (Farrington et al. 2015); however, surveys do not quantify the number of individuals in the population that have reproduced each year. A successful augmentation program requires a large portion of the reestablished population to reproduce annually to maintain high levels of genetic diversity. Quantifying the number of individuals that are reproducing annually will provide insight into the augmentation program and additional management actions needed to reestablish a self-sustaining population.

Population-level reproductive success can be difficult to quantify from field studies, especially for highly fecund species where few individuals can produce a large number of offspring. There is substantial evidence that shows reproductive output depends on environmental conditions and age- or size-related differences with older/larger fish producing a greater number of eggs relative to younger fish (Lauer et al. 2005; Lambert 2008). For example, relative fecundity for Razorback Sucker is high and increases with length. In addition, batch spawning has been observed for Razorback Sucker with adults vacating a spawning area early in the season and later returning to spawn again that year (Marsh et al. 2015). Such reproductive strategies further compound the ability to determine individual contribution over a reproductive season. Consequently, understanding population-level reproductive success for long-lived, highly fecund, iteroparous species such as Razorback Sucker and Colorado Pikeminnow can be difficult to quantify. In an effort to better understand the number of individuals reproducing in the San Juan River each year (effective number of breeders,  $N_b$ ), tissues from specimens collected from annual larval fish surveys will be used to calculate  $N_b$  using molecular methods.

Compared to traditional population monitoring, molecular methods can provide information about the augmentation program that cannot be quantified from field surveys. For iteroparous species with overlapping generations  $N_b$  is an ideal metric to monitor because it requires data from a single cohort within a breeding season. In addition, it is a useful metric for understanding eco-evolutionary processes in age structure species due to its defined seasonal reproductive bouts (Waples et al. 2013; Waples et al. 2014). Single cohort,  $N_b$ , estimates have been shown to be reflective of the effective number of breeders in a reproductive season (Waples et al 2014). Obtaining  $N_b$  estimates for the endangered fishes of the San Juan River can provide information regarding recovery limitations, such as understanding if a bottleneck to recruitment is influenced by limited population-level reproduction.

Annual genetic monitoring of larval Razorback Sucker collected from Lake Mohave between 1997 and 2010 showed a significant increase in  $N_b$  over a fourteen year period (e.g.  $N_b=743$  (1998);  $N_b=49,984$  (2005)). Results suggest management actions effectively reduced the variance in population-level reproductive success, thus further reducing effects of non-neutral processes such as hatchery-induced selection, non-random survival or relaxation of selection pressures (Carson et al. 2016). Similar analyses conducted on San Juan River Razorback Sucker and Colorado Pikeminnow can be used to determine if current management actions (e.g. augmentation, habitat restoration, flow regulations) are significantly increasing reproductive success. Understanding whether or not reproduction is limited is necessary to further evaluate if other biological and/or environmental factors (i.e. population numbers, spawning habitat, or resource availability) are limiting recruitment and recovery.  $N_b$  results can provide the SJRRIP data that can provide additional insight into current management actions, which can then be incorporated into future management decisions regarding program success.

## Objectives

Using wild caught larval fish currently held at the Museum of Southwestern Biology, the specific objectives of this study are to:

1. Quantify the effective number of breeders ( $N_b$ ) for Razorback Sucker annually from 2009-2015.
  - a. Determine if results correlate with augmentation efforts.
2. Quantify the effective number of breeders ( $N_b$ ) for Colorado Pikeminnow when samples sizes for any given year are greater than 25.

## Study Area

San Juan River larval surveys are conducted along a 140 mile section between Shiprock, NM, and Clay Hills, UT. Samples collected for genetic analyses will represent the spatial and temporal distribution of larval fish collections in this section.

## Methods

Approximately 1,200 larval samples will be examined. Larval fish collections from 2009-2015 were preserved in 95% ethanol and are currently stored in the Museum of Southwestern Biology. Prior to 2009, samples were preserved in 10% formalin and are not viable for genetic analyses. Tissue subsamples from the posterior portion of each specimen will be collected from 120 Razorback Sucker for each year from 2009-2015. The anterior portion of all specimens will be saved for otolith studies. Spawning dates are back-calculated based on ontogenetic stage (Farrington et al. 2015). Genetic sampling will strive to comprise a minimum of three (N=40) or four (N=30) spawning periods, depending on available data, to ensure samples are not collected from a single spawning bout. In addition, samples collected during each spawning period will cover the relative abundance and distribution of larval collections to reduce the potential bias caused by the collection of siblings at any one site. Collections for Colorado Pikeminnow are far more limited than Razorback Sucker. Three years have reasonable sample sizes to quantify the effective number of breeders. For 2011 and 2015, approximately 30 larval Colorado Pikeminnow were collected, and all specimens will be included in the analyses for these two years. In 2014, over 300 larval Colorado Pikeminnow were collected; thus, genetic sampling will follow the same protocol used for Razorback Sucker.

Genomic DNA will be extracted from tissues following standard protocols used at Southwestern ARRC. Fourteen microsatellite loci developed for evaluating the genetic diversity of Razorback Sucker broodstock (Wilson 2012) will be amplified for Razorback Sucker. If necessary, additional loci developed by Dowling and Marsh (2011) will be added to the data set. Twenty polymorphic microsatellite loci developed and screened at Southwestern ARRC will be amplified for Colorado Pikeminnow (Martin et al. 2015). Samples will be processed on an ABI 3130xl Genetic Analyzer and scored using GeneMapper™ 4.0 software (Applied Biosystems). A second researcher will perform a 10% quality assurance/quality control of samples to ensure accuracy. Departures from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) will be tested using Genepop v4.2 (Raymond and Rousset 1995), and alpha (0.05) will be adjusted for multiple comparisons using the Benjamini and Yekutieli (2001) method false discovery rate (Narum 2006). Effective number of breeders will be calculated using NeEstimator v.2 (Do et al. 2014) for each cohort. Although, NeEstimator v.2.01 is more commonly used to calculate effective population size ( $N_e$ ) per generation of a species, the same linkage disequilibrium method LDNE (Waples and Do 2008) can be applied to calculate  $N_b$  which is defined by a cohort within a single breeding season. Results will provide a seven year data set that can be compared annually to estimates of population size, environmental variables, and stocking efforts.

### Schedule:

Completion of genetic analysis  
Final Report

June 1, 2017  
September 30, 2017

### Intended Method of Information Dissemination:

Dissemination of the results will include a final report and presentation of project results at the Upper Basin/San Juan Researcher's meeting.

## Budget Narrative

The requested project funds are used to purchase replacement items as needed. Example items in each materials/supplies category: Extractions – sample tubes, DNA extraction kits, sample storage plates, ethanol; PCR Reactions – polymerase, oligonucleotides, buffer, magnesium, PCR plates, plate sealing film, primers; Genetic Analyzer Costs – size standard, buffer, polymer, reaction plates, formamide, capillary array; Other – filtered tips, gloves, tubes, solution basins, storage boxes.

Unlike other projects, purchases of supplies are not kept for one project alone. Supplies are kept on hand and used as projects are started. Many of the items have expiration dates, so as a project is started in our work flow we utilize inventory we have on hand. Project funds are used to replace what is used and needed at that point in time. Over the years we have estimated about how much of each item is used per sample and calculated a per sample cost. For example, the array in the genetic analyzer (\$1,000/per array) will last for a few thousand samples so we use it for multiple projects until we need to replace it. It does not make sense to replace it after every project. Likewise, we purchase items such as plastics (tips, plates, tubes) and ethanol in bulk to reduce prices, so again these are shared across projects but we have estimated how much is used per sample. If purchased in small quantities for a single project prices would go up. In doing things this way, sharing across projects and purchasing in bulk, money is saved, efficiency is increased, and waste decreased.

## Detailed Spending Plan

### 1. PERSONNEL

#### A. Laboratory Work

1 Bio/Geneticist (GS 9; 240 hours -3.0 pay periods) @\$34.05/hr \$8,172

#### B. Report Writing

1 Bio/Geneticist (GS13; 130 hours -1.63 pay periods) @\$42.15/hr \$5,480

Subtotal Personnel \$13,652

### 2. MATERIALS/SUPPLIES

A. Extractions \$3,061

B. PCR Reactions \$15,465

C. Genetic Analyzer Costs in kind contribution

D. Other (tubes, tips, etc.) \$8,238

Subtotal Supplies \$26,764

**Total \$40,416**

Southwestern ARRC Utilities

-Electrical, (approx. 4,259 KW/h @ .34569 per KW/h) = \$1,233

Administrative and Overhead Costs Regional Office @ 3% \$2,462

**Project Total FY2017 \$44,111**

**Project Total FY2018 \$0**

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