Project Title
Determining age at maturity of Razorback Sucker in the San Juan River

Bureau of Reclamation Agreement Number:

Reclamation Agreement Term

Note: Recovery Program FY23 scopes of work are drafted in May 2022. They often are revised before final Program approval and may subsequently be revised again in response to changing Program needs. Program participants also recognize the need and allow for some flexibility in scopes of work to accommodate new information and changing hydrological conditions.

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Category:
__ Ongoing project
X Ongoing-revised project
__ Requested new project

Expected Funding Source:
X Annual funds
__ Capital funds
__ Other *[explain]*

__ Unsolicited proposal

Relationship to LRP:
This project's actions are related to task 4.4.1.1, which is to identify and alleviate impediments to specific life and natural history processes necessary for recovery.

Study Background/Rationale and Hypotheses:

Rationale

After 25 years of stocking Razorback Sucker *Xyrauchen texanus*, managing flows from Navajo Dam, and several other conservation activities, there has yet to be any evidence for significant recruitment of the species past the age-0 life-stage in the San Juan River Basin. Moreover, larval Razorback Sucker densities, while generally increasing over time, are consistently lower than the other two native suckers. Given recent genetic analyses on larval Razorback Sucker, we think there is compelling evidence for investigating other factors that could be limiting reproductive output of this reintroduced population. Results of this proposed study have clear management implications for recovery of the species in the San Juan River. For example, if fish are found to mature at age 3 or 4, management actions could focus on why so relatively few of these individuals contribute to larval cohorts. Conversely, if fish are not mature until age 7 or 8, management should then be focused on increasing the survival of older individuals. We think addressing the question of age at maturity of Razorback Sucker in the San Juan River could go a long way toward testing the hypothesis that the lack of recruitment could be due to the scarcity of sexually mature individuals in the system.

Background

The global decline of freshwater fishes has necessitated reintroduction programs that use captive-reared individuals to recover dwindling wild populations (Brown and Day, 2002; Seddon et al., 2007). While reintroduction success can be measured by the survival and persistence of stocked individuals, recovery of populations often requires stocked fish to reproduce and recruit offspring to adults (Cochran-Biederman et al., 2015). However, the speed at which reintroduced populations can successfully reproduce and recruit will ultimately depend on species-specific life history traits that influence their population growth rates (Winemiller, 2005). In contrast to short-lived fishes with high intrinsic growth rates (i.e., opportunistic strategists; Winemiller and Rose, 1992), the ability to rapidly recover populations of 'periodic strategists,' or long-lived fishes that delay maturity, may be hindered due to their 'slow' life histories (Winemiller, 2005). While some periodic strategists can spawn annually once mature, successful recruitment only needs to occur occasionally, allowing their longevity to maximize life-time reproductive success and overall population persistence. Given naturally infrequent recruitment success of these types of fishes, understanding age at maturity may be critical in determining at what life-stage recruitment bottlenecks are occurring within reintroduced populations (e.g., low reproductive output of adults or survival of larval fish; Winemiller, 2005).

Razorback Sucker is a long lived (> 40 years) periodic life-history strategist that historically exhibited naturally variable recruitment success (McCarthy & Minckley, 1987; Kegerries et al., 2017; Albrecht et al., 2018). Long lifespans and relatively high fecundity may have allowed Razorback Sucker to bet-hedge reproductive success despite long periods of recruitment failure. While this species evolved in a dynamic and interconnected river system, largescale anthropogenic influences led to widespread declines and extirpations of Razorback Sucker throughout much of its native range (Minckley, 1983; Marsh et al., 2015; Bangs et al., 2020). The San Juan River historically harbored wild Razorback Sucker, but the population was extirpated in the 1990s (Ryden, 2005). A reintroduction program was

initiated in the mid-1990s, and since that time, thousands of hatchery-reared fish have been stocked (USFWS, 2005; USFWS, 2015). Adult (>400 mm total length [TL]) population estimates in 2019 from a large section of the river resulted in a conservative mean estimate of 2,796 individuals (95% CI = 2,461 – 3,210; Schleicher et al. 2020). In addition, wild-spawned larval Razorback Sucker have been collected annually since 1998 (Farrington et al., 2019), demonstrating successful reproduction of reintroduced fish. However, wild-spawned individuals are rarely encountered past the larval life-stage (Zeigler and Wick 2019), indicating recruitment as a significant hurdle toward recovering Razorback Sucker in the San Juan River.

Multiple hypotheses have emerged potentially explaining the lack of recruitment of wild-spawned Razorback Sucker larvae in the San Juan River, including lack of rearing habitats and nonnative fish predation. Recent genetic estimates of the effective number of breeders (Nb) and reconstructed sibling relationships of larval Razorback Sucker alternatively suggested that successful recruitment may not be limited by environmental conditions but rather by the number of reproducing adults (Diver et al., 2021). Comparing annual harmonic mean Nb estimates for Flannelmouth Sucker *Catostomus latipinnis* ($\bar{x}_h$= 3,022; 2013-2018) and Razorback Sucker ($\bar{x}_h$ = 127; 2009-2018) indicated a substantially lower number of spawning Razorback Sucker compared to the self-sustaining Flannelmouth Sucker. However, annual mean Nb estimates for Razorback Sucker increased on average by 15 per year (general linear model, slope 95% CI = 8.5 – 21.7), suggesting a relatively slow accumulation of spawning adults. Genetic reconstruction of sibling relationships among larval fish also identified considerably more full-sibling pairs of Razorback Sucker (N = 162) compared to Flannelmouth Sucker (N = 1), further corroborating Nb results of few spawning Razorback Sucker. Given the estimated population size of presumed adults in the San Juan River, it is currently unclear why so few individuals are successfully contributing to larval cohorts.

Whereas environmental conditions are affecting the survival of larval Razorback Sucker in the San Juan River, demographics of this reintroduced population could be limiting reproductive output and subsequent larval abundances. The age distribution of San Juan River Razorback Sucker is extremely right skewed, with relatively few individuals reaching older age classes (Figure 1; Diver et al., 2021). In 2019, the age structure of Razorback Sucker showed most individuals were relatively young with a rapid decline in the frequency of older fish (Schleicher et al., 2020; Diver et al., 2021). Although stockings began 25 years prior, only 53% of fish were ≥ age 4, 9% ≥ age 10, and the oldest fish was 17. However, this age structure is not unique as stocked populations of Razorback Sucker in the Colorado and Green Rivers are similarly young (Diver et al., 2021). These age structures are in sharp contrast to the only naturally recruiting population of Razorback Sucker (Lake Mead), where through 2005, 65% of individuals were ≥ 11 years (Albrecht et al., 2010). Thus, quantifying age at maturity for Razorback Sucker in the San Juan River could be important for assessing if low annual reproductive output is linked to the relatively young age of this population.

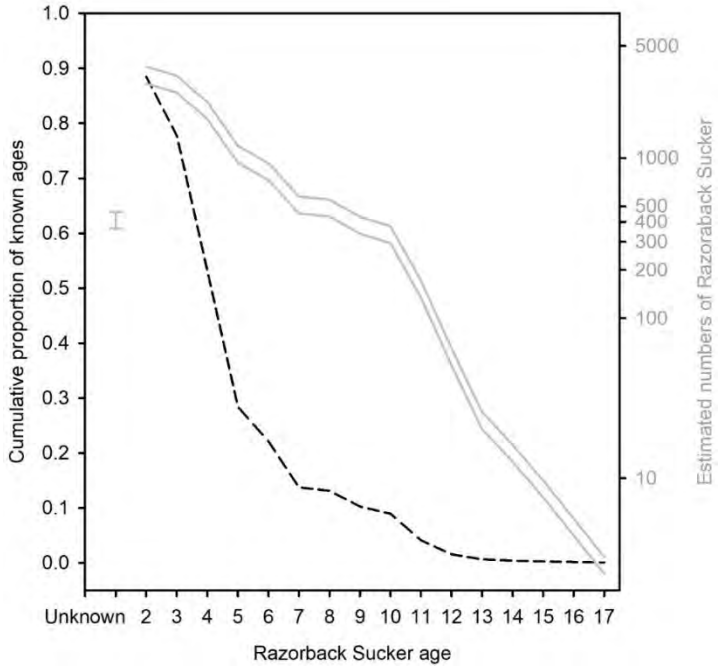

Figure 1. Cumulative proportion of stocked Razorback Sucker in 2019 at a given age and older (black dashed line) between Shiprock, NM (RM 147.9), and San Island Boat Ramp (RM 72). Eighty-eight percent of fish could be aged via their PIT tag history at stocking and 12% had unknown ages. Cumulative estimated numbers (lower and upper 95% CI) of Razorback Sucker at each age class and above (and unknown) are gray solid lines (right y-axis). Estimates are based on the lower and upper 95% CI total estimated abundance of Razorback Sucker extrapolated to the estimated age structure of fish. Figure from Diver et al. 2021.

Like many species, age at reproductive maturity for Razorback Sucker likely varies among environments (Bestgen, 1990). Hatchery-reared females can spawn as early as age 3 (Hamman, 1985), but a wider age range has been proposed for fish in the wild. Some have suggested that males can begin spawning at 2 – 4 years old and females at 3 – 5 (Dowling et al., 2014), while others stated ages 5 – 7 for both sexes (Loudermilk, 1985). Reproductive age in the San Juan River is unknown, but the consistently low and relatively stable Nb estimates may be explained by an older age of maturity (i.e., 5 – 7) than was previously assumed. The Recovery Plan for Razorback Sucker calls for 5,800 adults as part of the downlisting criteria and assumes individuals at age 4+ and >400 mm TL are reproductively mature (USFWS, 2002). Understanding population-specific age at maturity of Razorback Sucker in the San Juan River will help mangers refine conservation goals (if needed) and determine if the number of reproductive individuals in the population could be a factor limiting reproductive output.

To avoid confusion, we follow the standardized terminology for describing the reproductive phases in teleost fishes by Brown-Peterson et al. (2011). Briefly, the immature phase occurs only once in a fish's life, but once mature, each phase within the reproductive cycle can occur several times throughout their lifetime. These reproductive phases after maturity include developing, spawning capable, regressing, and regenerating (Figure 2). We simplify this terminology slightly in that we term fish that have not reached sexual maturity as "immature," fish that have reached sexual maturity as "mature," and fish that have reached sexual maturity and are in an advanced stage of gamete development as "reproductive" (i.e., in the spawning capable phase). These distinctions are important because fish in the "developing phase" may or may not actually produce viable gametes soon after becoming mature (i.e., first spawning season post maturity), which will ultimately dictate when age at

"reproduction" occurs (Junquera et al., 2003; Brown-Peterson et al., 2011). For example, in Greenland Halibut (Reinhardtius hippoglossoides), female maturity occurs by age 7 but individuals are not capable of spawning until age 10 (Junquera et al. 2003). This prolonged phase of development is not uncommon for long-lived fishes (Everson, 1994; Junquera et al., 2003), and warrants investigation in Razorback Sucker. Individuals can be classified into reproductive phases at any point in time (i.e., outside of spawning season) using histology, which will be necessary for determining age at maturity. However, because histology requires lethal sampling, we also propose using a suite of methods that can be used in the future to non-lethally quantify reproductive status of Razorback Sucker (i.e., reproductive versus non-reproductive).

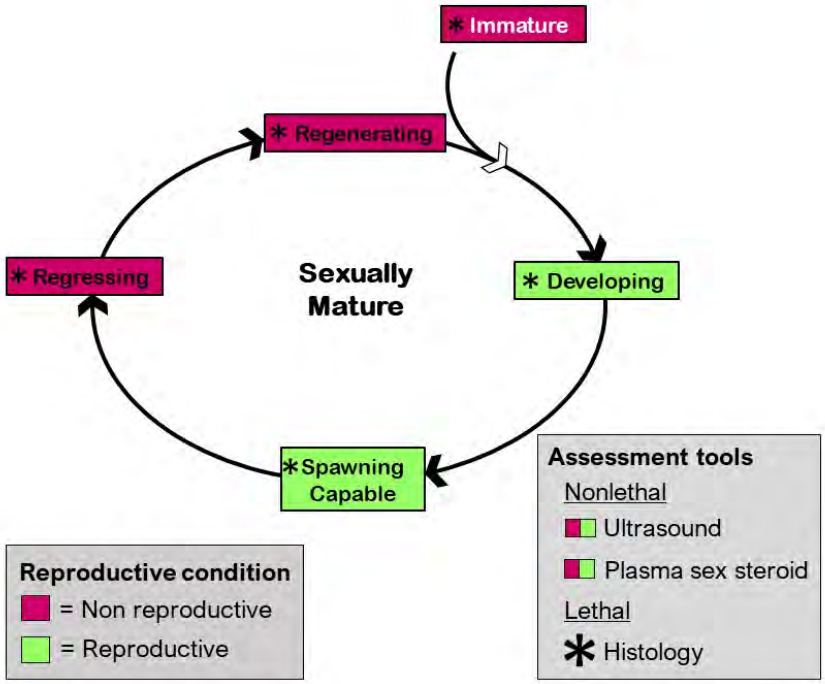

Figure 2. Diagram of sexual development stages and reproductive condition and the tools that are capable of assessing them. Ultrasound and plasma sex steroid can differentiate reproductive and non-reproductive condition whereas histology can differentiate among all sexual development stages. The figure was adapted from Brown-Peterson et al. (2011).

Because sex steroids are synthesized during gonadal development (Bangs and Nagler, 2014; Grieshaber et al., 2016; McGarvey et al., 2020), plasma sex steroids have been used to evaluate reproductive status in ray-finned fishes, including captive Razorback Sucker (Hinck et al., 2011). Quantifying these distinct patterns in steroid production can be useful in determining if individuals are reproductive and, if so, can be used to assign sex. Sex and reproductive status have also been assigned using ultrasound, which can detect morphological changes in developing gonads (Evans et al., 2004; Brizendine et al., 2018). These non-lethal tools can enable managers to determine the frequency of reproductive individuals in a population during spawning season; however, these tools must be validated using gonadal histology on reproductive individuals (Brown-Peterson et al., 2011; McGarvey et al., 2020).

Objectives:

1. Determine age at reproductive maturity for Razorback Sucker in the San Juan River using histology.
2. Validate plasma sex steroid concentrations and ultrasound as nonlethal tools for assigning sex and reproductive status (i.e., reproductive vs. non-reproductive) in Razorback Sucker.

Study Area:

In year one, we will target an upstream reach of the San Juan River from Farmington, NM (RM 180.6) to Shiprock, NM (RM 147.9) because of the relatively high abundances of Razorback Sucker within this reach (Figure 3; Franssen et al., 2016; Schleicher, 2018); however, spatial sampling in year two will depend on year one findings.

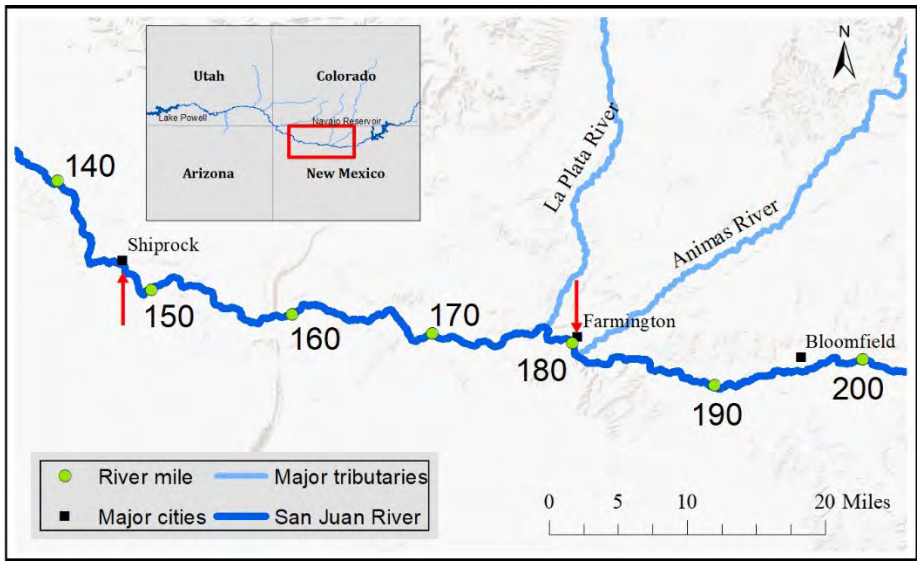

Figure 3. Map of study area assessing age at reproductive maturity of razorback sucker. Sampling will occur between Farmington, NM (river mile 180.6) and Shiprock, NM (river mile 147.9).

Study Methods/Approach:

Methods

Study Design

Razorback Sucker spawn in the San Juan River from late March to early July, with peak spawning occurring in mid-to-late April (Clark Barkalow et al., 2021). To assess temporal changes in reproductive condition of fish, we proposed sampling for Razorback Sucker monthly between February–June during the first year. We chose this temporal range to capture varying reproductive phases (i.e., immature, developing, spawning capable, regressing; Brown-Peterson et al., 2011). Depending on results obtained in year one, year two sampling will likely follow a similar schedule (Table 1).

Table 1. Tentative sampling dates and total number of samples (n) by age category assessed for each method: sex steroids, ultrasound, histology, and gonadosomatic index (GSI) for the second year of sampling.

Tentative Sampling Dates	Sex Steroids/Ultrasound (n) age 3 / age 4 / age ≥5	Histology/(GSI) (n) age 3 / age 4 / age 5
February 6 – 10	20 / 20 / 30	NA
March 27 – 31	20 / 20 / 30	10 / 10 / 10
April 10 – 14	20 / 20 / 30	10 / 10 / 10
April 24 – 28	20 / 20 / 30	10 / 10 / 10
May 22 – 26	20 / 20 / 30	NA
June 26 – 30	20 / 20 / 30	NA

Razorback Sucker will be captured during six discrete passes using raft-mounted electrofishing throughout our proposed sampling period. Target sample sizes for year one are age-3 (n = 20), age-4 (n = 20), and ≥ age-5 (n = 30) fish from each sampling period, while assuming a 1:1 sex ratio. Yet, these targeted ages and sample sizes may change in year two depending on year one results. Electrofishing settings will be turned to the lowest voltage that reliably induces electrotaxis and narcosis. Collected fish will be held in an aerated and salted livewell at low densities and processed at regular intervals.

All captured Razorback Suckers will be weighed (g), measured (standard [SL] and TL; mm) and scanned for a passive integrated transponder (PIT) tag. Any untagged fish will receive a PIT tag. The age of each captured fish will be acquired in real time by cross referencing PIT tag IDs with a previously downloaded dataset of stocked Razorback Sucker from the Species Tagging Research and Monitoring System (STReaMS). Presence of external sexual characteristics (e.g., tubercles, gamete expression) will be recorded, and a preliminary determination of sex will be made. A fin clip will be collected from each individual to genetically determine sex, if necessary.

Plasma Sex Steroids

Razorback Suckers selected for analyses will be anesthetized with tricaine methanosulfonate (MS-222). Once sedated, fish will be placed in a padded shallow tray lined with sterile operating towels, and the fish's gills will be irrigated to prevent anoxia. A sterile blood sampling needle equipped with a lithium-heparinized Vacutainer® will be used to draw blood from the caudal vein; blood draw volume will not exceed 0.1% of the fish's body weight. Blood samples will be held on ice during field collections and will be centrifuged (1,200 G for 5 min) within 8-10 hours post-sampling to obtain plasma which will be frozen immediately. Concentrations of plasma sex steroids (i.e., estradiol-17β [E2] and testosterone [T]) will be determined by radioimmunoassay following methods used in McGarvey et al. (2020) at the U.S. Fish and Wildlife Bozeman Fish Technology Center, Bozeman, Montana.

Ultrasound

Following blood collection, Razorback Suckers will be scanned using an ultrasound equipped with a linear transducer to visualize gonad morphology. We will use gonadal sonograms to determine sex, estimate reproductive status, and measure gonad (diameter and circumference) and ovarian follicle size. Fish will be oriented ventrally and scanned anteriorly starting from the urogenital pore. Sonograms will be read by trained readers and validated with both the results of histological analysis and genetic sex determination to determine accuracy of this tool**.**

Gonadosomatic Index and Histology

Sampling during peak spawning will maximize the opportunity to capture Razorback Suckers in the spawning capable phase for histology. Histology is paramount for validating the results of non-lethal techniques proposed to assess whether or not an individual is reproductively mature (i.e., steroid analysis and ultrasound; Blazer, 2002; McGarvey et al., 2020). During March and April sampling, a subset of fish (Table 2) may be sacrificed for histological analysis if either or both sexes are not found to be mature in year one sampling.

Fish selected for histology will be euthanized using an appropriate concentration of tricaine MS-222. Gonads from each individual will be excised and weighed (g) to quantify gonadosomatic index (GSI = [gonad weight / total tissue weight] × 100) for both males and females. Excised gonad diameter will be measured using digital calipers, and excised gonad circumference will be measured using a measuring tape. Two 5 cm^2 subsamples of the gonads will be taken from each gonadal lobe for histological analysis and fixed in 10% buffered formalin. Histological processing will follow the methods outlined in Sullivan-Brown et al. (2011). Briefly, ovaries will be subsectioned, dehydrated, and infiltrated and embedded using JB-4™. Embedded ovaries will be sectioned using a rotary microtome fitted with glass blades. Samples will be sectioned at 5 µm intervals and mounted on slides. Mounted sections will be stained using both hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Stained specimens will be examined under a compound microscope, and reproductive phase of fish will be described following the methods of Brown-Peterson et al. (2011).

Fecundity Estimates

Total fecundity of individual females will be estimated gravimetrically. Subsamples from the anterior, middle, and posterior sections of each ovary will be collected (total subsamples n = 6 per fish). Ovarian follicles will be placed in ethanol and counted in each subsample. Each subsample will be weighed to the nearest 0.001g. Absolute fecundity will be estimated for each female using the equation:

$$\boldsymbol{Absolute\ Fecundity} = \frac{[\sum_i \frac{\boldsymbol{O_i}}{\boldsymbol{W_i}}]}{\boldsymbol{n}} (\boldsymbol{W_{ovaries}}),$$

where O_i is the subsample ovarian follicle count, W_i is the subsample weight, n is number of subsamples, and $W_{ovaries}$ is the combined weight of both ovaries.

Genetic Confirmation of Sex

We will use DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA, USA) to extract genomic DNA from all tissues that require sex confirmation (e.g., histology excluded). Samples will be amplified using end-point PCR primers and thermal cycling conditions developed by Dowling et al. (2020). PCR products will be confirmed using gel electrophoresis; the presence of a double band determines maleness, whereas presence of a single band determines femaleness.

Analyses

To assess the accuracy of ultrasound measurements, gonad diameter and circumference measured by ultrasound will be compared to measurements made on excised gonads. The accuracy of using ultrasound to assign sex and reproductive phase will be assessed by comparing the sex and reproductive stage assignment by ultrasound to true sex determined by gonadal histology and genetic analysis from non-lethally sampled fish. Plasma sex steroids will be compared between and among sexes and reproductive status to define concentrations of T and E2 that may be used to differentiate females or males in different reproductive phases. The accuracy of ultrasound imagery to estimate GSI and ovarian follicle diameter will be determined by comparing gonad size and ovarian follicle diameter

obtained from ultrasound images to actual weights and diameter obtained from excised tissues. Age-specific relationships between GSI (both sexes), ovarian follicle diameter, and fecundity will be evaluated using general linear models.

Approach

Our second year of sampling will be dictated by results gained during the first year; thus, project activities during the second year of sampling are highly dependent on the information obtained in 2022. However, due to the timing of sampling in year one (Table 1), we will lack sufficient data to adequately develop methods needed to address additional data needs in year two. Thus, we must instead consider all possible project scenarios for year two based on potential result outcomes in year one. Ideally, year one results will be such that we 1.) confirm sexual maturity in a significant number of reproductive male and female Razorback Suckers and 2.) validate non-lethal sampling techniques (plasma sex steroids and ultrasound; Table 1). If these conditions are met, we propose using non-lethal sampling techniques to assess the basin-wide reproductive status of Razorback Sucker from Shiprock, NM to Clay Hills, UT and below the Piute Farms Waterfall during peak spawning season. If we are unable to determine age at maturity for either or both sexes and/or we are unable to validate non-lethal techniques, then we propose a number of conditional options for the second year of sampling, which will be decided once year one results have been obtained (Table 2).

Table 2. Potential year one result scenarios with proposed year two sampling scenarios

1A. Age 3, age 4, and/or age 5 female and male Razorback Sucker were found to be sexually mature and reproductive*; non-lethal techniques were validated.

> Use non-lethal sampling techniques to assess the reproductive status of Razorback Suckers basin-wide from Shiprock, NM to Clay Hills, UT and below the Piute Farms Waterfall during peak spawning.

1B. Razorback Sucker were found to be sexually mature and reproductive*; non-lethal techniques could not be validated.

> No sampling in second year; no funds requested.

2. Only age 3, age 4, and/or age 5 male Razorback Sucker were sexually mature and reproductive*; ultrasound was validated for differentiating males and females in the field.

> Collect 30 females ≥ 6 years old for histological analyses and non-lethally sample females ≥ 5 years old. Study area would likely increase to include areas downstream of Shiprock to secure enough age appropriate fish^. Sample timing would follow year one.

3. Age 3, age 4, and age 5 male and female Razorback Sucker sampled during year one were not sexually mature nor reproductive*; non-lethal techniques could not be validated.

> Collect 60 Razorback Suckers ≥ 6 years old (30 females; 30 males) for histological analysis and non-lethally sample all fish ≥ 5 years old. Study area would likely increase to include areas downstream of Shiprock to secure enough age appropriate fish^. Sample timing would follow year one.

4 Results from year one are marginal due to low sample size or number of sexually mature and reproductive males and females*; mixed results

Increase study area and number of sampling trips^.

**A statistically significant number of individuals.*

^Dependent on number of individuals contacted in 2022.

Task Description, Deliverables and Schedule:

Field sampling will be conducted between February to July 2023. A report will be given to the Program Office by May 1st, 2024, and a presentation of the completed work will be given to the SJRRIP Biology Committee during the February meeting. All data will be quality assessed and quality checked prior to submission to the Program Office.

Task Description

1. Sample Razorback Sucker in the San Juan River (February – July)
2. Prepare and conduct laboratory analyses.
3. Input and analyze data
4. Write and finalize annual report.

Schedule
Task 1: 2023
Task 2: 2023
Task 3: 2023
Task 4: 2023 and 2024

Budget Summary:

The budget for year two (FY23) will be dependent on the sampling scenario that is warranted based on year one results (Table 2). The project PI's will work to update the San Juan River Basin Recovery Implementation Program (SJRIP) Biology Committee members with 2022 preliminary project results as feasible. If the first year's results are not available or conclusive at the appropriate time for budgets to be submitted to the Program Office the PI's will develop multiple budget scenarios to aid in the development of the annual work plan.

Table 3. Office budget summary for each fiscal year 2023.

Fiscal Year	NMFWCO	NMDGF
2023	$168,013.49	$44,112.64
Total	$168,013.49	$44,112.64

Reviewers:
Recovery Program Staff and Biology Committee

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