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

New SOW Proposal for FY2022

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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.

Introduction

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 et al., 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 (N_b) 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., *in review*). Comparing annual harmonic mean N_b estimates

for Flannelmouth Sucker ($\bar{x}_h$= 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 N_b 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 N_b 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., *in review*). 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., *in review*). 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., *in review*). 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.

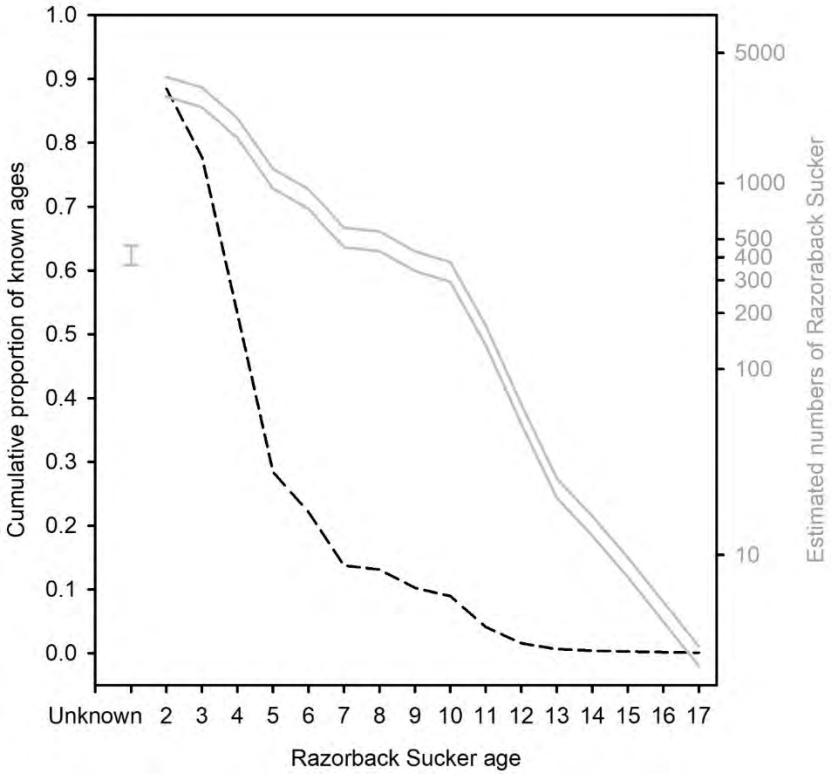

Figure 1. Cumulative proportion of stocked Razorback Sucker in 2019 at a given age and older (black dashed line) between Shiprock, NM (RM 147.9), and San Island Boat Ramp (RM 72). 88% of fish could be aged via their PIT tag history at stocking and 12% had unknown ages. Cumulative estimated numbers (lower 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. in review.

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 N_b 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 numbers 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).

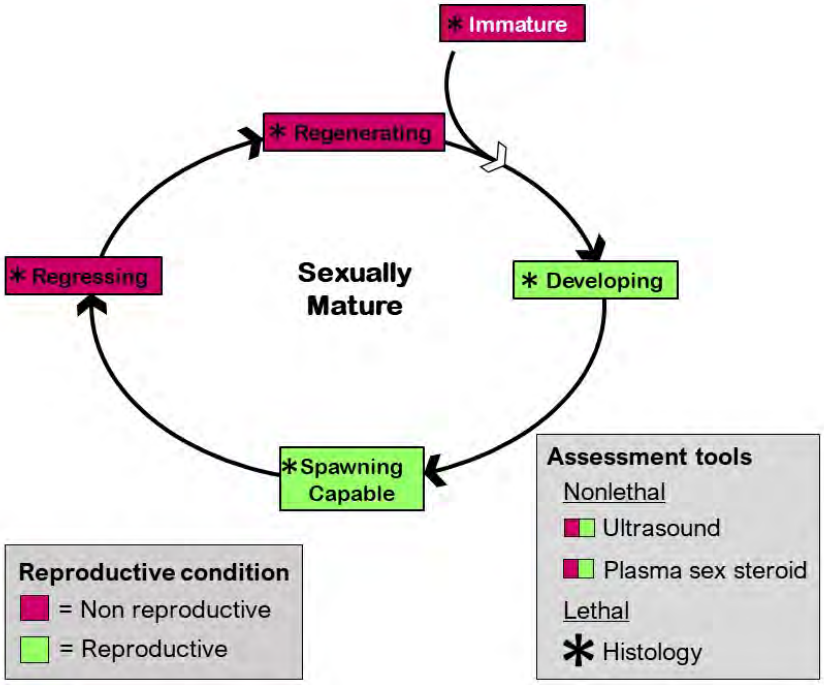

Figure 2. Diagram of sexual development stages and reproductive condition and the tools that are capable of assessing them. Ultrasound and plasma sex steroid can differentiate reproductive and non reproductive condition whereas histology can differentiate among all sexual development stages. The figure 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).

Over a two year period, we propose to 1) determine age at maturity for Razorback Sucker in the San Juan River using histology, and 2) assess nonlethal tools (plasma sex steroid concentrations and ultrasound) to assign sex and reproductive status in Razorback Sucker across age-classes. During the first year we will conduct histology on age-3, age-4, and age-5 Razorback Sucker during three sampling periods around peak spawning (i.e., mid-to-late April; Clark Barkalow et al., *in press*). Within each age, we will calculate the proportion of reproductive individuals and estimate the youngest age at which 50% of fish are reproductive. Razorback Sucker used for histology will be paired with ultrasound and sex steroids to validate these non-lethal techniques. We will apply non-lethal sampling techniques over a five-month period leading up to and during spawning season on three age-classes (age 3, age 4, ≥ age 5) as an ad-hoc approach for determining reproductive structure of this population. Finally, because fish will need to be sacrificed for histological analysis, we will also calculate gonadosomatic index (GSI) in all individuals and estimate fecundity in all spawning capable females. Our second year of sampling will be dictated by results gained during the first year and, if necessary, include euthanizing older fish (age-6, age-7) if our first year of data suggest fish are not mature by age-5.

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., *in press*). To assess temporal changes in reproductive condition of fish, we propose sampling for Razorback Sucker monthly between February–June during the first year (Table 1). We chose this temporal range to capture varying reproductive phases (i.e., immature, developing, spawning capable, regressing; Brown-Peterson et al., 2011).

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

Tentative Sampling Dates	Sex Steroids/Ultrasound (n)	Histology/GSI (n)
	age 3 / age 4 / age ≥5	age 3 / age 4 / age 5
February 7 – 11	20 / 20 / 30	NA
March 28 – April 1	20 / 20 / 30	10 / 10 / 10
April 11 – 15	20 / 20 / 30	10 / 10 / 10
April 25 – 29	20 / 20 / 30	10 / 10 / 10
May 23 – 27	20 / 20 / 30	NA
June 27 – July 1	20 / 20 / 30	NA

Razorback Sucker will be captured during six discrete passes using raft-mounted electrofishing throughout our proposed sampling period. 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). Our target sample sizes will be age-3 (n = 20), age-4 (n = 20), and ≥ age-5 (n = 30) fish from each sampling period, and we assume a 1:1 sex ratio. Individuals ≥ age 5 will have a higher sample size and will be combined into a single category because they are relatively rare in the San Juan River. 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.

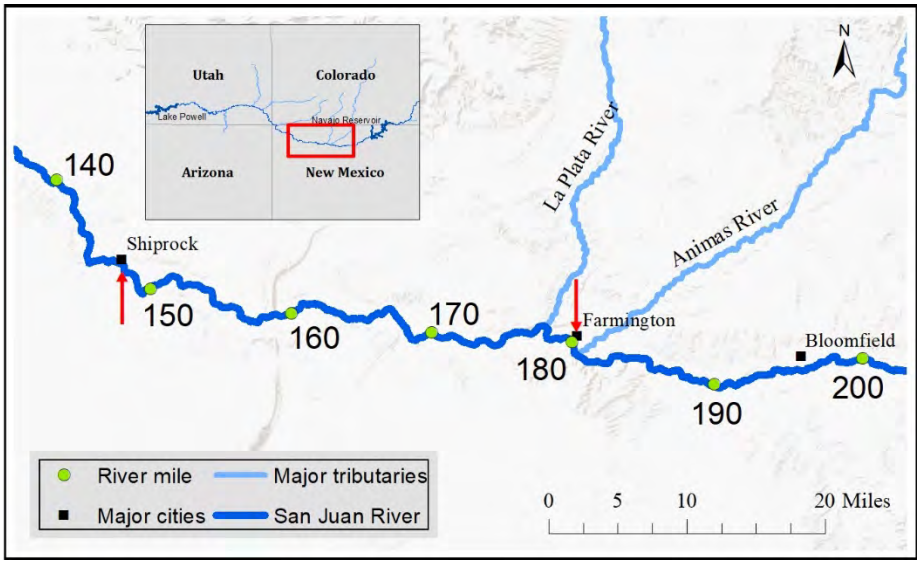

Figure 3. Map of study area assessing age at reproductive maturity of razorback sucker. Sampling will occur between Farmington, NM (river mile 180.6) and Shiprock, NM (river mile 147.9).

Data Collection

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). Individuals captured without a known age class (e.g., fish tagged in the river) will not be included. Presence of external sexual characteristics (e.g., tubercles, gamete expression, vent morphology) will be recorded, and a preliminary determination of sex will be made in an effort to help ensure equal sampling of sexes. 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 (n = 90 total; age 3 [n = 30], age 4 [n = 30], and age 5 [n =30]) will be sacrificed for histological analysis. Sample sizes were chosen to target approximately n = 15 of each sex. Razorback Sucker age ≥ 6 will not be euthanized the first year of the study.

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) and separated out into vitellogenic verses non-vitellogenic ovarian follicles. Each group of 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:

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

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. Absolute fecundity counts for vitellogenic primary follicles will be compared to stages of development determined histologically (e.g., primary vitellogenic, secondary vitellogenic, and tertiary vitellogenic; Brown-Peterson et al. 2011).

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. For non-lethally sampled fish, sex identified using ultrasound will be compared to genetic confirmation using a sex-linked marker (Dowling et al. 2020). 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.

Management Implications

After 25 years of stocking Razorback Sucker, 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.

Deliverables

Final annual reports will be produced by March 31 of each year and presentations of the completed work will be given to the SJRRIP Biology Committee at frequencies requested by the Program Office. All data will be quality assessed and quality checked prior to submission to the Program Office. Any equipment purchases will be stored at NMFWCO and would be available for future use by any Program participants for SJRRIP projects.

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TOTAL PROJECT COST - $203,990.77

New Mexico Fish and Wildlife Conservation Office - FY 2022 Budget

Labor Cost - Field Work (6 trips x 5 days/trip)

Position	Grade/ Step	Salary w/ benefits	Hours/ Day	Total Days	Sub-Total	
Fish Biologist	GS 9/10	$48.22	8	30	$11,572.80	
Fish Biologist	GS 11/8	$53.53	8	15	$6,423.60	
Remote Biologist	GS 9/4	$40.60	8	15	$0	$4,872.00

5 days; 60 hours per trip

Overtime Hours (weekend or >8-hour work days)

Position	Grade/ Step	Salary w/ benefits	Hours/ Day	Total Days		
Fish Biologist	GS 9/10	$65.30	4	30	$7,836.00	
Fish Biologist	GS 11/8	$53.53	4	15	$3,211.80	
Remote Biologist	GS 9/4	$54.75	4	15	$0	$3,285.00

Administrative, Reporting, Planning

Position	Grade/ Step	Salary w/ benefits	Hours/ Day	Total Days		
Fish Biologist	GS 9/10	$48.22	8	65	$25,074.40	
Supervisory Fish Biologist	GS 13/4	$74.78	8	5	$2,991.20	
Adminstrative Officer	GS 9/10	$47.35	8	5	$1,894.00	
			Total Labor		**$59,003.80**	

Travel and Per Diem * field & SNARRC

	Days	Rate		
Lodging Costs	44	$96.00	$4,224.00	$1,152.00
Per Diem (Travel Day)	22	$41.25	$907.50	$247.50
Per Diem (Full Day)	33	$55.00	$1,815.00	$495.00

Concur Fee	11		$14.50	$159.50 $43.50
			Total Travel/Per Diem	**$7,106.00**

Equipment

Vehicle Fuel	**Miles/ Qty**	**Total Miles**	**Rate**	
1 truck X 6 trips - ABQ to Shiprock, NM	450*6	2,700	$0.56	$1,512.00
1 truck x 2 trips - ABQ to Dexter, NM	450*2	900	$0.56	$504.00
Generator Fuel 15 gallons/trip x 6 trips	90		$2.85	$256.50

Field and Laboratory Supplies & Equipment

Field gear - maintenance, repair, replace (e.g., generator, dip nets, life jackets)				$3,500.00
Steroid Supplies - heparinized tubes, needles, sample shipping				$3,500.00
Histology Supplies - slides, stains, glass knives, formalin, JB-4, ethanol, etc.				$6,500.00
Misc lab supplies - gloves, tubes, pipettes, etc.				$2,500.00
Genetic supplies - tubes, etoh, extraction kit, PCR master mix, agarose				$3,000.00
Bozeman Radioimmuno Assay (RAI)	420		$80.00	$33,600.00
Ultrasound	1			$35,000.00
		Lab, Equipment & Supply Total		**$89,872.50**
Remote Biologist Savings $10,095.00				
		Total for 6 trips & lab components		**$155,982.30**
		Overhead 3%		$4,679.47
		Grand Total		$160,661.77

New Mexico Department of Game and Fish - FY 2022 Budget

Sampling -Farmington to Shiprock, NM

Personnel

Tasks - Razorback Sucker age at sexual maturity surveys (n = 6) in the San Juan River from Farmington to Shiprock, NM; 1 day trip preperation and travel (12 hrs day) and 4 field days (12 hrs day) = 56 hours (40 hrs regular and 16 hrs overtime).

Project Leader (1; 3 surveys)		
40 hrs regular @ $47.73/hr ($34.84/hr (base salary) + $12.89/hr (benefits)) * 3	$	5,728
20 hrs overtime @ $71.60/hr ($47.73/hr * 1.5 (time-and-a-half) * 3	$	4,296
Project Biologist (1; 3 surveys)		
40 hrs regular @ $38.24/hr ($27.91/hr (base salary) + $10.33 (benefits)) * 3	$	4,589
16 hrs overtime @ $57.36/hr ($38.24/hr * 1.5 (time-and-a-half)) * 3	$	3,442
Sub-total	**$**	**18,054**
Per Diem		
24 overnight days @ $151/day (standard NM rate)	$	3,624
6 partial day per diem @ 40/day (standard NM in-state rate)	$	240
Sub-total	**$**	**3,864**
Travel		
Round-trip to Shiprock, NM – 450 miles @ $0.55/mile * six surveys	$	1,485
Sub-total	**$**	**1,485**
Animas River to Shiprock Sampling Sub-total	**$**	**23,403**
Field Equipment & Supplies		
– Raft supplies and safety equipment	$	550
– Specimen coolers	$	800
– Live wells and aerators	$	800
Sub-total	**$**	**2,150**
Sampling Sub-total	**$**	**25,553**

Data Management/Analysis and Report Preparation

Personnel

Tasks – Data management and QA/QC, data analysis and synthesis, table and graph preparation, report drafting and revision; Project Leader (80 hrs) and one Project Biologist (160 hrs each).

Project Leader (1)		
80 hrs regular @ $47.73/hr ($34.84/hr (base salary) + $12.89/hr (benefits))	$	3,818
Project Biologist (1)		
160 hrs regular @ $38.24/hr ($27.91/hr (base salary) + $10.33 (benefits))	$	6,118
Data Management/Analysis & Report Preparation Sub-total	**$**	**9,937**

FY 2022 Total

Sampling Sub-total	$	25,553
Data Management/Analysis & Report Preparation Sub-total	$	9,937
Project Sub-Total	**$**	**35,490**
IDC at 22.09 %	$	7,840
Project Total	**$**	**43,329**

Response to Comments

New 2 - **Determining age at maturity of Razorback Sucker in the San Juan River**

How can the technical aspects of this SOW be improved?

Crockett (CPW): No suggestions. Methods seem appropriate for the research question to the best of my knowledge.

Thank you.

Keith (TNC): No suggestions. Study design appears sound.

Thank you.

Larrick (UMUT): No suggestions.

Thank you.

Mazzone (Jicarilla Apache Nation): Line 258 there is a small typo.

Thank you. The correction has been made.

McKinstry (BOR): Proposal is well written and easily followed with good logic and well-referenced. The proposal is geared toward age 3, 4, and 5 yr old fish. Why not do some older fish as well? Maybe there is a problem with age 7 fish?? Why not get some fish during the waterfall sampling???? If you want large and old fish this is a great place to get them!! Might as well collect tissues for contaminants for fish that are sacrificed. Might be a relationship with lack of reproduction and contaminants. I would certainly get some fish at the waterfall since they certainly seem to want to spawn. The trouble might be getting them too early in the reproductive cycle. Could come later in April to get some that are further along. Might be nice to compare fish that come from the lake with fish that are resident in the river.

Thank you. We agree with the interest in examining older individuals (e.g., age 7) and, if necessary, plan on doing so in the second year of the study. However, for the first year of the study we chose to limit the age of fish included for histological analysis to age 5 because of concerns with euthanizing older, and rarer, individuals from the population. If our first year of data suggests that Razorback Sucker are not mature by age 5 (i.e., <50% of individuals are mature), then we will increase our target age in the second year. But, if our first year of data suggests that Razorback Sucker reach maturity by age 4, then we will have prevented euthanizing these older fish from the population. For sex steroid and ultrasound work, fish older than age 5, if collected, will be included to determine if they are reproductive (i.e., reproductive fish are mature, but a non-reproductive fish may be immature or mature). We have worked to clarify this in lines 163-166.

We think evaluating Razorback Sucker at the Piute Farms Waterfall (i.e., Waterfall) is an interesting comparison that we would consider pursing in the second year of sampling. However, for our first year we chose to limit sampling to the San Juan River because our question is specific to the population of Razorback Sucker that inhabit the San Juan River. Given that fish that make migrations to the Waterfall likely experience different environmental conditions than fish in the river, we chose to limit our sampling to the San Juan River population for the first year of the study. In addition, we have concerns about limiting collections to fish that are actively making seasonal migrations and the potential effect that could have on skewing estimates of age at maturity. For example, our initial discussions included using the PNM fish passage to collect fish that are attempting to move upstream during the spring; however, our concern was that those fish may not represent a random sample of Razorback Sucker in the San Juan River. This potential effect on age at maturity is why we limited our collection method to electrofishing. While it is likely that we will collect migrating fish, we hope this approach reduces potential biases by also capturing individuals that are not actively migrating in the spring.

Nonetheless, we agree that including the Waterfall as a collection site has the potential to be very informative. If we can validate non-lethal sampling techniques (i.e., sex steroid and ultrasound) in the first year, then this could be a useful technique to evaluate the reproductive structure of Razorback Sucker at the Waterfall.

Finally, we agree with collecting tissues for contaminate testing and will do that outside of this scope. We are also working with Manuel Ulibarri at the Southwestern ARRC to coordinate potential fish health testing on euthanized Razorback Sucker. While we will be limited in what we can reasonably achieve without compromising data for this study, we are open to other research needs or ideas to maximize data collected from euthanized animals.

Miller (Southern Ute Indian Tribe): The scope of work is well written and provides adequate detail for technical review. The background and purpose of the project are well supported by the literature cited in the proposal. Page 181: Lines 195 – 202: The methods state that Razorback Sucker age will be validated in real time by scanning PIT tags and cross referencing against the STReaMS database. I recommend including a statement that any captured fish without a confirmed age would not be included in the experiment. I recommend that the fish used for the analysis should be limited to only those stocked with a PIT tag and not include any fish tagged after capture in the river.

Thank you for your recommendation. We have included this in lines 201-202.

Schleicher (USFWS R6): Line 226: 90 fish is an alarming number to be euthanized in one year. Not to mention that these fish will be collected when predicted to be spawning. In a population that has potential bottleneck with spawning numbers as it is, this seems excessive. Is there a reason why 15 males and 15 females need to be taken from each age-class? Can a smaller sample of 5 males and 5 females be taken?

We understand the concern of euthanizing endangered animals, especially for long-lived species. That is why we did our best to limit euthanizing fish at a maximum age of 5 and why we kept our sample sizes relatively small. At our current sample size of 15 per age-class per sex (e.g., 15 age-3 females), each individual represents 6.7% of the sample. If we reduce that to 5 fish per age-class, then each individual represents 20% of the sample. We do not think a sample size of n = 5 would provide any confidence in our ability to interpret results. For example, if we find 2 age-5 fish that are mature and 3 that are immature, then it will be difficult to conclude if this accurately represents the population or is attributable to low sample sizes. We think that a sample size of 15 is the minimum needed for this first year of the study.

Warren (Peer Reviewer): In methods are you assuming that all ova in ovary subsections ate at the same stage of development? Most fishes show a gradation in development at any given time during the spawning season (e.g., Early Maturing, Maturing, Mature, Late Maturing). If you count all ova, then you will overestimate “absolute” fecundity. If you count every ova regardless of developmental stage that might represent potential fecundity but most females don’t “empty” the ovary in a spawning season; some eggs simply reabsorb. Since you are sacrificing these fishes I would like to see the ova staged according to development in order to get as much

information as possible. Later on you do indicate ova will be measured. Perhaps it should be introduced in the section about processing ovaries.
Also if you capture a female that has already spawned one or more times you will miss those eggs in your counts.

We agree with the limitations in our current methods for estimating absolute fecundity. However, we are hesitant in our ability to determine different stages of development (e.g., early maturing, maturing, mature, and late maturing) through gross visualization. Nonetheless, we can parse out vitellogenic verses non-vitellogenic ova, which we will compare to the proportion of histologically determined developmental stages. Please see lines 248-250 and 256-259

We also agree with the possibility of having biased fecundity estimates if fish have already spawned. In an attempt to account for this possibility, we will look for post ovulatory follicles in histology samples. However, post ovulatory follicles can only be identified within a relatively short time period (i.e., a few days up to two weeks) post spawning and cannot serve as an absolute indicator that a fish recently spawned. We will exercise extreme caution in our estimates of absolute fecundity.

Line 263-264: recast. It reads as if the non-lethally sampled fish were not subjected to histology.

Correct. Only euthanized fish will be subjected to histology. We have worked to clarify this. Please see lines 269-272.

Zeigler (NMDGF): No Comment.

PO: It would help to describe whether there will be an effort to have females and males equally represented in sampling and if not then why?
L258 – “bad” should read “band”.

Thank you. We agree that working to obtain equal sex ratios will be important. We think this should be possible given the time of year we will be sampling by examining external sex characteristics including tubercles, gamete expression, and vent morphology. Please see lines 202-205.

We have changed ‘bad’ to ‘band’. Thank you.

What is this SOW’s contribution to recovery?

Harry Crockett (CPW): I think the contribution to recovery is well described in the “management implications” section at lines 274 ff.

Thank you.

Keith (TNC): Will help answer key questions about population bottleneck and help prioritize management actions.

We agree.

Larrick (UMUT): Addressing the question of age at maturity of RBS in the SJ river can help test hypothesis that lack of recruitment could be due to scarcity of sexually mature individuals in the river system.

We agree.
Mazzone (Jicarilla Apache Nation): This SOW provides a clear and well cited approach to gather an extremely relevant metric which is not only integral to the species recovery goals, but should also help guide future management. This seems to be a very important data set to acquire. This type of SJR specific information is critical to guiding management and policy actions in the future.

We agree.

McKinstry (BOR): There certainly seems to be a problem with reproductive output of RBS in the SJR. This project is focused on determining the size/age relationship with respect to sexual maturity. This seems like a worthy project to pursue to start determining what is limiting RBS reproductive output.

Thank you. We agree that there seems to be a problem with the reproductive output of Razorback Sucker in the San Juan River and think this could help determine if the current age structure is problematic.

Miller (Southern Ute Indian Tribe): The determination of age at maturity will assist in directing future management actions for Razorback Sucker.

We agree.

Schleicher (USFWS R6): This project aims to target information on a potential bottle neck shown by an Nb study. Management actions can be more informed with information gained from these findings.

We agree.

Warren (Peer Reviewer): Determining age at maturity and the course of ovarian development could help determine if low numbers of mature individuals are in the river or there are ample numbers but spawning success is low. I think this is basic biology that the Program needs to determine.

Thank you. We think information gained from this project is a key component in better understanding the life history of Razorback Sucker.

Zeigler (NMDGF): No Comment.

PO: The proposal investigates a potential recruitment bottleneck of the low numbers of Razorback Sucker contributing to successful annual spawning. The results of this work could have important management implications (i.e., if fish are mature at age 3-4, actions could be directed on why so few fish contribute to successful spawn. If fish are mature at age 10, actions to increase the number of older fish will be critical).

Thank you. We agree with the management implications and ideas proposed by the PO.

New Mexico CC Representative: The NMISC is concerned that the results of this study could be confounding due to the low number of RBS in the San Juan. The SOW should provide more information on how this will be addressed.

Thank you for taking the time to provide comments on our scope of work; however, we are unsure we understand the concern about our results potentially being confounded due to the low number or Razorback Sucker in the San Juan River. A recent population estimate of Razorback Sucker in the San Juan indicated that a mean estimate of 2,796 individuals (95% CI = 2,461 – 3,210) persist between Shiprock, NM and Sand Island Boat Ramp, UT. This estimate is considered to be conservative because sampling was limited to ~40% of the total range occupied by Razorback Sucker in the San Juan River. In addition, the area we will be targeting is upstream of Shiprock where Razorback Sucker abundance is relatively higher compared to downstream reaches. However, given the current age structure of the population, we do agree that it could be difficult to capture at least thirty fish that are ≥age-5. We have increased our sample size for this age category to increase our chances of contacted fish >age-5, and we have given ourselves a full week to obtain our required sample sizes. We think this should be sufficient for our target sample sizes.