Identification of Razorback Sucker spawning areas in the upper San Juan River using mobile PIT tag antennas

FY2022 NEW-3 Scope of Work

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Objectives

The objectives of this proposed effort are captured under the SJRIP Long-Range Plan (2016). Specific Actions in the Long-Range Plan relate to Task 4.1.2.1 (Conduct larval fish sampling to determine if reproduction is occurring, locate spawning and nursery areas, and to gauge the extent of annual reproduction) and Task 4.4.1.1 (Document and quantify reproduction, survival, and recruitment; USFWS 2016).

Specific objectives are to:

1. Identify Razorback Sucker spawning locations using floating inflatable PIT tag antennas (Biomark).
2. Characterize habitat of identified spawning locations.
3. Validate utility of PITPASS technology for detection of spawning aggregates.
4. Confirm spawning locations (year-2) to evaluate spawning site fidelity.
5. Confirm spawning by Razorback Suckers (year-2) by using drift net samples placed immediately upstream and downstream of identified spawning areas.
6. Potentially utilize unmanned aerial vehicle (drone) surveys, underwater videography, and/or snorkeling to confirm spawning activity dependent on turbidity levels.

Introduction

Razorback Sucker *Xyrauchen texanus* are endemic to the Colorado River Basin including the San Juan River drainage. Human development and modification to hydrologic regime of western rivers resulted in extirpation of Razorback Sucker in the San Juan River and general decline throughout their historically occupied range (USFWS 2002). Currently, Razorback Suckers are federally endangered and protected under the Endangered Species Act of 1973. Augmentation efforts led by the San Juan River Basin Recovery and Implementation Program (SJRIP) resulted in the re-establishment of a Razorback Sucker population within the San Juan River. This population, which is currently estimated at 2,461–3,210 adult individuals (Schleicher et al. 2019), is entirely supported through annual stocking of large (>300 mm TL) hatchery-reared fish despite documentation of reproduction since 1998 and with rare incidence of recruitment (Zeigler and Wick 2019). Razorback Suckers are known to make long distance movements and upstream spawning migrations (Tyus and Karp 1990; Pennock et al. 2020), yet discrete spawning locations in the San Juan River have not been verified. In the Green River, UT, Razorback Suckers exhibit annual spawning site fidelity (Webber and Beers 2014). Knowledge of spawning locations is invaluable for informing management decisions related to promotion of successful Razorback Sucker reproduction and subsequent recruitment, both of which are paramount for recovery. Acquiring this information would allow direct measurement of reproductive effort, documentation of interannual variation in spawning, identification of flow requirements for inundation of spawning habitat, and evaluation of habitat requirements for spawning fish including any deficits that may exist in the San Juan River. Lastly, because Razorback Sucker recruitment is lacking in the San Juan River, documentation of spawning locations allows mitigation of larval downstream transport through informed development of low-to-zero velocity habitats near identified spawning locations to increase retention and ontogenetic development.

Spawning of Razorback Sucker is associated with the ascending limb of the spring hydrograph, peak spring discharge, and warming river temperatures. Adults congregate in riffles with cobble, gravel, and sand substrates typically in depths less than one meter (USFWS 2002). In the San Juan River, Razorback Suckers exhibit an extended spawning season from March to June or July, depending on temperature and discharge conditions, with peak spawning period typically occurring in the last 2 weeks of April and first week of May (Clark Barkalow et al. in press). In the San Juan River, spawning by Razorback Sucker was first documented in 1998 and successful spawning has occurred in each of the last 23 years (1998 – 2020; Farrington et al. 2019, Farrington et al. in preparation). In recent years, larval Razorback Suckers have been progressively documented further upstream and in 2020 were documented as far upstream as RM 184.3 near Bloomfield, NM (Farrington et al. in preparation).

In the San Juan River, large-bodied adult fishes are often sampled using raft-mounted electrofishing (Schleicher et al. 2019; Clark et al. 2018). Although electrofishing is an effective technique for capturing and studying adult fishes, electric currents may be lethal to fish embryos (Muth and Ruppert 1997; Bohl et al. 2010; Simpson et al. 2018), so attempts to document spawning aggregates of endangered fishes using this technique should be avoided. Passive integrated transponder (PIT) tag interrogation is a non-invasive method for detecting endangered fishes and is widely used in studies on long-lived fishes including Razorback Suckers. Most, it not all, Razorback Suckers in the San Juan River originated from hatcheries and have been implanted with PIT tags prior to being stocked in the San Juan River. Recent research in the San Juan River has used both stationary, passive integrated antenna (PIA), and mobile (Passive Integrate Transponder Portable Antenna SystemsS [PITPASS]; Stout et al. 2019, 2020) PIT tag antennas to non-invasively monitor endangered fishes (Durst and Franssen, 2014; Stout et al. 2019; Pennock et al. 2020).

Although stationary PIT tag antennas are valuable for tracking fish movement past discrete locations (e.g., PNM weir) and may allude to timing of spawning migrations, they are unable to indicate the ultimate destination or verify spawning. Floating PIT tag antennas, like PITPASS are an innovative technology (Stout et al. 2019, 2020, Zentner et al. 2021) recently used in the San Juan River and can detect adult fishes at similar rates to electrofishing (McKinstry and MacKinnon 2011). With PITPASS, PIT tags can be detected at depths up to 1.0 m (Biomark) or discharge up to 999 cfs (28.3 m^3/s) in the San Juan River (Stout et al. 2019). Unlike a stationary PIA, PITPASS can detect tagged fish longitudinally across a reach of river and does not require fish swim past a set point. Further, longitudinal PITPASS surveys can be repeated in an area in short succession to build a history of detection events and interpret fish movements. Due to the high number of PIT tagged endangered fishes in the San Juan River (STReamS 03/22/2021), PITPASS may be a valuable tool for detecting congregations of fishes like those that may be present in spawning aggregates. A similar floating PIT tag antenna system has been developed for use in trout streams of Colorado (Richer et al. 2017) and has shown high detection efficiency, with greater numbers of fish detected with multiple passes.

Razorback Suckers have been spawning continuously in the San Juan River since 1998 and spawning aggregates have been observed (Ryden 2004), yet discrete spawning areas, like those documented in other locations in the Upper Colorado River basin (Bestgen et al. 2011, 2012) have yet to be verified. Detection of spawning locations in the San Juan River facilitates management decisions related to spawning by endangered fishes and recruitment of larvae to later age classes. Identification of spawning areas allows SJRIP to ensure fish can bypass potential spawning migrations barriers (USFWS 2016; Task 4.4.3.1), enable creation of spawning habitat if needed (USFWS 2016; Task 4.3.1.2) and perhaps most importantly, dictate the placement and operation of nursery habitat for the promotion of recruitment to later life stages (USFWS 2016; Task 4.4.3.1).The San Juan River is characterized by rapid rate of larval drift terminating in Lake Powell (Dudley and Platania 2000), steep gradient relative to other rivers of the upper Colorado River basin (Holden 1999), and lack of large permanent nursery habitats like those present in other rivers where Razorback Suckers exist (Modde 1996; Bestgen et al. 2011; Bestgen et al. 2012). The San Juan River habitat workshop identified a lack of persistent low velocity habitat as a likely bottleneck to Razorback Sucker recruitment and project rankings indicated a high prioritization of nursery habitat creation, like that of lateral canyons and persistent secondary channel backwaters. By identifying spawning locations, constructed nursery habitats can be more ideally located and operated to best entrain larvae to promote recruitment to later age classes.

Study Area

The study area is a 28.9 mile reach of the San Juan River between RM 188.3 (Sun Ray Park and Casino) and RM 159.4 (Buck Wheeler Farm; Figure 1). In 2020, yolked Razorback Sucker larvae were detected at RM 184.3 near Sun Ray Casino, Bloomfield, NM, the furthest upstream detection to date. Because the larvae were in the earliest stage of their development it is assumed that a spawning location was nearby. Additional early development (protolarvae and flexion-mesolarvae) Razorback Suckers were collected downstream to RM 161.2, supporting the hypothesis that a spawning area is present in the reach. The proposed study area was chosen primarily because the short river sections allow the segments to be sampled in a single day, successive detection passes may be repeated to ensure adequate data collection, and a high number of adult Razorback Suckers have been documented in this stretch (Schleicher et al. 2018).

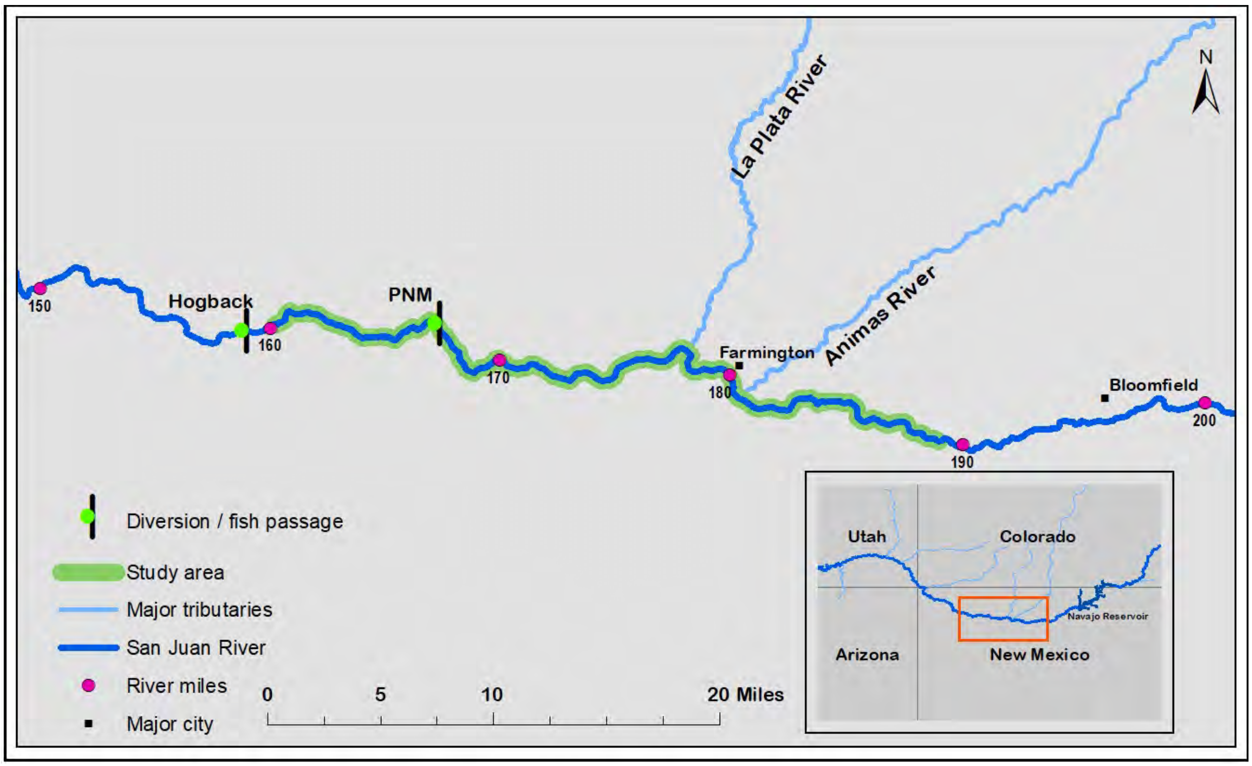

Figure 1. Proposed study area for detection of Razorback Sucker spawning locations using PITPASS.

Methods

Repeated detection passes will be performed in the study area over a three-week period from the last two weeks of April (beginning April 18) to the first week of May (Table 1.). These weeks have been identified as periods of high Razorback Sucker spawning activity (Farrington et al. 2019; Clark Barkalow et al. in press) and coincide with previous detections of early-development Razorback Suckers in the study area (Farrington et al. in preparation). Additionally, because this period is typically either before spring runoff or at the very beginning, flows are generally below the 999 cfs threshold identified by Stout et al (2019). Because Razorback Suckers typically spawn at depths less than 1.0 m (USFWS 2002), increased flow should not result in decreased detection efficiency. However, if San Juan River flow projections indicate high discharge in the spawning period, the sampling period can be adjusted to occur prior to spring runoff.

Two catarafts, equipped with Biomark Inflatable Antenna System (PITPASS), including two inflatable antennas (each 3 m x 1.2 m) and an integrated GPS and data recording system, will be used to detect PIT tags, one on river right (RR) and one on river left (RL). Each cataraft will have a PIT tag detection range of 6 m x 1.2 m to a depth up to 1.0 m and detection of unique tags is possible in milliseconds (Biomark). Catarafts will simultaneously drift near-shore at a 45° angle, each cataraft will drift on a designated side of the river (RR or RL). Because previously identified spawning bars in the upper section of the San Juan River have occurred at the upstream “nose” of an island (Bliesner and Lamarra 2007), when islands are encountered at least one cataraft will focus effort on this feature. Upon detection of a PIT tag, the data recording system automatically records latitude and longitude with relatively little error (~12 m, the total combined length of the antennas) (Biomark; Stout et al. 2019), habitat will be recorded for each detection based on observations made while the raft is drifting. Instream habitat measurements will be made at high frequency detection locations following peak spawning to prevent disruption of potential spawning activity and will be obtained at the same river stage at which Razorback Suckers were detected. Substrate will be characterized using a Wentworth scale (Wentworth 1922). Depth to embeddedness will be measured using techniques identified by Bliesner and Lamarra (2003), and velocity will be measured using a Marsh-McBirney flo-mate 2000. Additionally, at each site maximum and minimum depth (m) and water quality parameters (instantaneous temperature, dissolved oxygen, conductivity, and pH) recorded.

In 2019, aerial imagery was performed in the San Juan River when water turbidity was extremely low (Lamarra and Lamarra, 2020). Aerial imagery will be evaluated prior to performing detection passes in the river to *a priori* identify cobble bars that might be candidate spawning areas (Figure 2). These cobble bars will be recorded on the river map to ensure they are monitored by PITPASS. Data will be analyzed at the completion of a detection pass (daily). Though PITPASS is valuable for detecting congregations of fish over a broad longitudinal area, because it detects PIT tags from a single moment in space, it will not be able to capture the full extent of habitat use by Razorback Suckers over a 24 h period. Therefore, after analysis of detection pass data and identification of an area of high fish density, PIA(s) (“wagon-wheel”) will be deployed to detect Razorback Suckers in suspected spawning areas over a longer timescale (24–48 hours). Temperature loggers (HOBO tidbit) will be placed simultaneously with PIAs in suspected spawning areas.

Figure 2. Example of 2019 aerial imagery showing submerged cobble.

Because the Public Service Company of New Mexico (PNM) fish passage facility is included in the lower portion of the study area there will be a record of fish detected below and successfully passed upstream of the diversion into the upstream study area. Detections at PNM will be used to evaluate the ability of successive PITPASS surveys to detect known live fish within the study area. To evaluate fish leaving the study area, we will deploy PIAs at the mouth of Animas River confluence in areas where fish would likely be detected (e.g., eddy near the thalweg).

Detection passes (with PITPASS) will be performed again in year-2 to confirm spawning sites and examine spawning-site fidelity (i.e., the same individuals being contacted in both years). Confirmation of spawning by Razorback Suckers at identified spawning areas will occur in year-2. Spawning will be confirmed using drift nets set upstream and downstream of identified spawning locations. Collection of gametes (eggs), embryos, and recently hatched larvae will be compared between upstream and downstream collections to confirm presence of the spawning area. Drift will be preserved in a 95% solution of ethanol. Larvae will be identified to species whereas eggs and embryos will be identified to family. Dependent on turbidity levels in the river, spawning sites will be confirmed visually using drone flights or through snorkel surveys. Drone flights would be operated simultaneous with detection passes to try to detect fish aggregating and would support findings from PITPASS. Snorkel surveys would occur only on suspected spawning bars. Both drone flights and snorkel surveys would rely on low turbidity (< 10 FNU) conditions to occur.

Table 1. Propose timeline for Razorback Sucker Spawning location identification in 2022 and 2023.

Year	Dates	Location	Action
2022	April 18 – 22	RM 188.3 – 159.4	PITPASS Survey (n = 4)
2022	April 25 – 29	RM 188.3 – 159.4	PITPASS Survey (n = 4)
2022	May 2 – 6	RM 188.3 – 159.4	PITPASS Survey (n = 4)
2023	April 17– 21	RM 188.3 – 159.4	PITPASS Survey (n=4)
2023	April 24 – 28	RM 188.3 – 159.4	PITPASS Survey (n=4)
2023	April 24 – 28	Spawning areas in study area	Drift nets and visual confirmation
2023	May 1 – 5	RM 188.3 – 159.4	PITPASS Survey (n=4)

Analyses

At end of the 3-week detection period, fish detections from PITPASS will be compiled and “ghost tags” removed following methods outlined in Stout et al. (2019). A heat map of Razorback Sucker density will be created by overlaying frequency of occurrence on the spatial extant of the study area. A Moran’s I test (Muska et al. 2018) will be performed to test for spatial autocorrelation (i.e., are spatial patterns significant or random) to determine significance of fish patterns. Further, a Getis-Ord Gi* (pronounced G-i-star) test will be performed to identify statistically significant hot spots (Martling et al. 2020) of PIT tag detections. Spawning areas will be determined as areas with the highest frequencies of PIT tag detections, a significant Moran’s I index and a significant Getis-Ord test statistic. Detections of PIT tags of PIAs will be examined to evaluate the number of individual fishes visiting a spawning location over a 24-hour period to determine size of spawning population of fish at each location. Pending a sufficient number of PIT tag detections, an estimate of the spawning population will be computed (Cathcart et al. 2018).

Management implications

Despite spawning by Razorback Suckers annually since 1998, spawning areas have not been located in the San Juan River as they have in other rivers of the upper Colorado River basin. Identification of spawning areas could allow these areas to be effectively managed in a way that promotes spawning and recruitment by larval fishes to encourage the creation of a self-sustaining population. Additionally, because larval fishes in the San Juan River drift in the water column during development, identifying upstream-most spawning locations may also identify fish that have a better chance of being retained in the river (i.e., entrained in nursery habitat) due to distance from Lake Powell and the Paiute Farms Waterfall.

Though this research is being targeted toward Razorback Suckers due to the large number of tagged adults (STReaMS, 03/22/2021; Schleicher 2019) and large number of larvae produced annually (Farrington et al. 2020), information about Colorado Pikeminnow *Ptychocheilus lucius* spawning locations is also lacking. Following proof-of-concept, this methodology could be applied to Colorado Pikeminnow or can be expanded to other areas of the river.

Deliverables Timeline

A presentation will be prepared to report results of this study at the February 2023 Biology Committee meeting. A draft report will be presented to the San Juan River Basin Biology Committee for review by 31 March 2023. Upon receipt and incorporation of written comments, a final report will be produced by 30 June 2023 and submitted to the Program Office along with electronic copies of the data. Finally, this work will result in the likely development of a peer-reviewed manuscript and Swimming Upstream article in 2023.

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FY2022 Budget - NMDGF

Sampling - San Juan River PIT-Pass surveys

Personnel

Tasks - Determining Razorback Sucker spawning locations via PIT-Pass surveys (n = 2) in the San Juan River from RM 188.3 to 195.4; 1 day trip preperation (8 hrs day) and 4 field days (12 hrs day) = hours (40 hrs regular and 16 hrs overtime).

Project Biologist (1; 1 survey)			
40 hrs regular @ $38.24/hr ($27.91/hr (base salary) + $10.33 (benefits))		$	1,530
16 hrs overtime @ $57.36/hr ($38.24/hr * 1.5 (time-and-a-half))		$	918
Project Leader (1; 1 survey)			
40 hrs regular @ $47.73/hr ($34.84/hr (base salary) + $12.89 (benefits))		$	1,909
16 hrs overtime @ $71.60/hr ($47.73/hr * 1.5 (time-and-a-half))		$	1,146
	Sub-total	**$**	**5,502**
Per Diem			
8 overnight days @ $151/day (standard NM in-state rate)		$	1,208
2 partial day perdiem @ $40/day (standard NM in-state rate)		$	80
	Sub-total	**$**	**1,288**
Travel			
Round-trip to Farmington, NM – 370 miles @ $0.55/mile * two surveys		$	407
Farmington to Waterflow – 45 miles @ $0.55/mile * 8 trips		$	198
	Sub-total	**$**	**605**
	Field Survey Sub-total	**$**	**7,395**
Field Equipment & Supplies			
– Personnel gear (waders, boots, wetsuits, masks, snorkel)		$	700
– Equipment maintenance and repair.		$	500
	Sub-total	**$**	**1,200**
	Sampling Sub-total	**$**	**8,595**

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 (50 hrs) and one Project Biologist (180 hrs each).

Project Leader (1)			
50 hrs regular @ $47.73/hr ($34.84/hr (base salary) + $12.89/hr (benefits))		$	2,387
Project Biologist (1)			
180 hrs regular @ $38.24/hr ($27.91/hr (base salary) + $10.33 (benefits))		$	6,883
	Data Management/Analysis & Report Preparation Sub-total	**$**	**9,270**

FY 2021 Total

Sampling Sub-total		$	8,595
Data Management/Analysis & Report Preparation Sub-total		$	9,270
	Project Sub-Total	**$**	**17,865**
IDC at 22.09 %		$	3,946
	Project Total	**$**	**21,811**

FY2022 Budget - ASIR

Project Title: New FY 2022 SOW Identification of Razorback Sucker spawning areas in the upper San Juan River using mobile PIT tag antennas

<u>Personnel</u>

<u>Field Data Collection (RM 188.3–159.4)</u>

Trip 1: April 18–22, 2022

Fisheries Biologist II (1 staff x 5 days x 12 hr/day at $77.85/hr)........................	$	4,671.00
Fisheries Technician I (1 staff x 5 days x 12 hr/day at $38.93/hr)........................	$	2,335.80

Trip 2: April 25–29, 2022

Fisheries Biologist II (1 staff x 5 days x 12 hr/day at $77.85/hr)........................	$	4,671.00
Fisheries Technician I (2 staff x 5 days x 12 hr/day at $38.93/hr)........................	$	4,671.60

Trip 3May 2–6, 2022

Fisheries Biologist II (1 staff x 5 days x 12 hr/day at $77.85/hr)........................	$	4,671.00
Fisheries Technician I (1 staff x 5 days x 12 hr/day at $38.93/hr)........................	$	2,335.80

<u>Office Work (Data analysis, Report writing)</u>

Fisheries Biologist II (30 staff days/sampling year x 8 hr/day at $77.85/hr)....	$	18,684.00

<u>SJRBRIP Meetings</u>

Three meetings/year required: 2 days/meeting.

Fisheries Biologist II (6 staff days/year x 8 hr/day at $77.85/hr)........................	$	3,736.80

Personnel Subtotal	$	45,777.00
GSA General and Administrative Fee (IFF)..	$	343.33
Personnel (Field, Lab, Office): .... Total	$	**46.120.33**

<u>Materials and Supplies</u>

Passive integrated antenna (Wagon Wheel- Borrowed from PO)........................	$	0.00
PITPASS cataraft n=2 (Borrowed from Bureau of Reclamation*)*........................	$	0.00
Materials and Supplies...Total	**$**	**0.00**

<u>Travel and Per Diem</u>

<u>Field sampling effort</u>

Travel - Albuquerque, NM to Farmington, NM (364 mi x 4 trucks x $0.56/mi).	$	815.36
Travel - Farmington, NM to Waterflow, NM (32 mi x 12 trips x $0.56/mi).........	$	215.04
Lodging - (2 staff x 2 trips x 4 days + 3 staff x 1 trip x 4 days at $96/day*)*..........	$	2688.00
Per diem – (2 staff x 2 trips x 5 days + 3 staff x 1 trip x 5 days at $55/day)........	$	1925.00
Travel and Per Diem (Field) .... Subtotal	**$**	**4828.04**

<u>Travel and Per Diem (Meeting)</u>		
Lodging - (1 staff x 2 days X 3 days at $102.00- room with others*)*........................	$	0.00
Per diem – (1 staff x 2 days x 3 trips at $71/day- in kind contribution)...............	$	0.00
Meeting travel and Per Diem... Subtotal	**$**	**0.00**
Travel and Per Diem: .... TOTAL	**$**	**4828.04**

<u>2022 Project Totals</u>

Personnel..Total	**$**	**<u>46,120.33</u>**
Materials and Supplies ....Total	**$**	**<u>0.00</u>**
Travel and Per Diem...Total	**$**	**<u>4,828.04</u>**
New 2022 SOW ....GRAND TOTAL	**$**	**<u>50,948.37</u>**

FY2022 Budget – TOTAL

NMDGF .... SUB TOTAL	**$**	**<u>21,811.00</u>**
ASIR .... SUB TOTAL	**$**	**<u>50,948.37</u>**
Total, all PIs ....GRAND TOTAL	**$**	**<u>72,759.37</u>**

Response to comments

Crockett (CPW): No suggestions; seems rigorous.
RESPONSE: Thank you.

Keith (TNC): It would be helpful to provide more information on methods and approach to drone and snorkel surveys, including turbidity levels at which these methods would be feasible.

RESPONSE: Our main method of confirming spawning will be with drift nets, visual confirmation will only occur if turbidity is low enough, <10 FNU. Turbidity tends to be relatively low during the proposed sampling period in the San Juan River, for example between 18 April 2021 and 8 May 2021 median turbidity was 16 FNU (USGS Farmington, NM Gage # 09365000), with a range of 7 – 986 FNU. According to USGS, 16 FNU corresponds to water clarity of about 2 feet (https://or.water.usgs.gov/will_morrison/secchi_depth_model.html). At 10 FNU, clarity increases to nearly a meter, so drone flights and snorkel surveys would rely on this value. Drone surveys, if conducted, would be used to evaluate fish avoiding detection by swimming away from the boat. Snorkel surveys, if conducted, would allow for confirmation of fish counts and potentially for observation of spawning activity.

Larrick (UMUT): No suggestions.

Mazzone (Jicarilla Apache Nation): This scope could benefit from: A description of what type of data will be collected to characterize spawning bar features [Cobble size, velocities, max/min depth, etc.] If I overlooked this aspect of the scope please accept my apology in advance. **The project could benefit from:** As the upstream end of the proposed sampling area is at the upper known extent of RZ spawning on year two deploying thermographs at identified sites might provide some useful data on site specific temperatures of pre-spawning aggregations and spawning activity. If recruitment of egg to larvae is a possible issue, dissolved oxygen at sites might also be relevant and/or noting silt inundations?

RESPONSE: Thank you for the helpful suggestions. Descriptions of habitat measurements have been added to the methods section. In addition to habitat measurements at suspected spawning sites, we intend to install HOBO temperature loggers in conjunction with PIAs at sites with high numbers of detections.

McKinstry (BOR): I like this proposal. It seems like it is well-thought out and is well written. This is a unique use of a system that seems to work okay in the SJR. My biggest problem is that I'm not sure it will work as designed. I think the technique is good, but doing such a large part of the river seems difficult. Additionally, water clarity (i.e., clear water) may affect detection, especially in early spring when the fish are spawning, river flows are low, and water clarity is high.
I think I would recommend using the system but try and target some specific areas, three come to mind. 1) The spawning bar at Slickhorn. You could make several passes using a motor in this area, additionally, I would leave the system anchored to the shore or even out further from shore on the suspected spawning bar over night and during a few days (just camp right at the site). Doing this during the first week of April will likely find many fish, especially fish moved upstream of the waterfall. 2) Make several passes in the 4-corners bridge area during suspected CPM spawning. Similar to Slickhorn, I would anchor the system to shore or in shallow current

during the night and part of day you aren't floating. 3) the area in and around McElmo. This area has been identified as a possible spawning area for several years. We also have a stationary antenna just downstream of the mouth. Could you launch upstream and make several passes down through there, and also anchor the system overnight? I only suggest these things to try and increase the likelihood that it will work the first time you do it so that success can lead to more success and maybe the system can be deployed more as your original intent. In other words, try it in some areas where you think it would likely succeed, then build from there.

RESPONSE: Thank you for your thoughtful comments. We acknowledge that water clarity could affect detection rates, but the results of other low-turbidity sampling suggests fish can be detected in relatively low turbidity water. For example, during September electrofishing surveys turbidity is at a comparable level to late April/May (~ 20 FNU USGS Farmington Gage) and fish are still detected/captured. We recognize the technologies are different but given that fish are still captured with electrofishing rafts under high clarity conditions gives credit to the idea that they will not entirely avoid antennas either. A study recently published (Zentner et al. 2021) demonstrated that detections increased with greater turbidity. However, the study occurred in a clear Oklahoma stream, so the "high" turbidity scenarios within the of the study (12 FNU) is similar to the turbidity levels in the San Juan River during the sampling period. Because detections typically increased in the Zentner study at this level, we are hopeful that detections in the San Juan River would not suffer due to water clarity.

To address the second part of the comment, we are willing to take determination of the study area under advisement. Recent telemetry flights (NMGF/ KSU) have documented Razorback Suckers congregating below the San Juan River/ Animas River confluence. Combined with records of spawning bars between RM 154-156 (just downstream of the proposed study area; Bliesner and Lamarra reports from 2004 and 2005) and 2020 collections of larval fishes above the Animas confluence supports the idea that although it is not as well-known as the suspected spawning bar at Slickhorn, there are current and historical spawning bars in this area. While Slickhorn would likely provide validation of the methodology, recent research has highlighted the spawning areas near Slickhorn Canyon (Hines, Bogaard, and others) and further confirmation of a spawning bars there would not likely add to the body of knowledge surrounding spawning areas of Razorback Suckers in the SJR.

Miller (Southern Ute Indian Tribe): The Proposed SOW includes "characterize habitat of identified spawning locations" (Page 191, line 93) as an objective. The methods section includes several statements regarding habitat on page 193, lines 131-134, however, there are no details on how the habitat will be identified and what physical parameters would be measured. A detailed description of the habitat characterization and measurements are needed in the SOW. The methods further state that no physical measurements would be made until after spawning is complete. The measurements should be taken at the same discharge as in the river at the time of observation of spawning fish to replicate the hydraulics present at spawning. Further, if peak flow occurs between the time of observed spawning and the time of measurement the physical characteristics may be different due to the high flow hydraulics that can change channel bathymetry. These limitations should be acknowledged in the proposal.

RESPONSE: Thank you for the thoughtful suggestions, we have added details of which physical parameters will be measured. We have changed the timing of habitat measurements so that they will occur after peak spawning period, not after the culmination. This will hopefully enable measurements to occur at or near the same discharge as when aggregates were documented.

Schleicher (USFWS R6): Line 39: "...electric currents may be lethal to fish embryos..." Conclusions from these references suggest that electricity may be more harmful to certain embryonic stages and that effects are also based on size. The third reference sited (Simpson) looks at using electrofishing to manage nonnatives in the embryonic stage, tests were conducted with exposure times close to 30 seconds and smaller embryonic species were not significantly affected by electrofishing. If the goal of the project is to identify spawning areas, then electrofishing would be your most effective tool to do so, use of a mobile PIT antenna will require multiple passes and two years (per SOW) to determine if a sight one year was or was not a spawning aggregation. Add in that the SJ is a dynamic river system and each year may not be the same as the next nor will spawning locations, makes this long term approach less feasible than a shorter approach.

RESPONSE: Electrofishing would certainly document suckers in spawning condition but given the evidence for injurious effects to early life stages or reproductive adults, one of the objectives of this study, aside from documenting spawning areas, is to develop a non-injurious method (floating PIT tag antennas) for documenting spawning congregations. In addition, we proposed using floating PIT antennas to avoid disrupting a spawning aggregation. Electrofishing would likely allow for collection of spawning fish but the act of capturing fish or merely subjecting them to electro taxis may have unidentifiable impact to fish behavior that would impact our results.

Line 161: Efforts are noted to avoid electricity as it can be harmful to eggs and embryos, one can argue that collecting and preserving these samples in ethanol is harmful as well. It appears that one evil is being traded for another.

RESPONSE: Yes, preservation of larvae or eggs is lethal, but the relative impact to a reproducing population is likely negligible. Drift netting samples a small volume of water and captures only the individuals that are drifting at that moment. If you consider the reproductive potential of a female Razorback Sucker, 30,000-100,000 eggs/fish, collecting even a small portion of that is relatively insignificant. Conversely, electrofishing has the potential to impact a reproductive female and embryos that have been deposited at a spawning bar.

Line 165: Snorkel surveys in the San Juan river will be difficult at best. I would not rely on this as a possibility as water clarity would require an individual to be directly on top of a fish.

RESPONSE: Agreed, snorkel surveys were merely mentioned as one potential option for documenting spawning, other alternatives exist.

Warren (Peer Reviewer): This SOW is well written, the design/implementation/analysis is tight, appropriate, and thoughtful. I think documenting the spawning locations of razorback suckers is long overdue, it's been discussed by the BC for years, and this work uses state of the art non-lethal, non-injurious methods to do so.

RESPONSE: Thank you.

Zeigler (NMDGF): No Comment.

PO: Moving the objectives to the front of the document may be helpful as they get lost after the introduction.

RESPONSE: Thank you for the helpful suggestion. The objectives have been moved as suggested.

Providing some data that demonstrates the floating antenna's ability to identify aggregations of fish would be beneficial (e.g., heat map of Stout et al. data, or other examples?).

RESPONSE: This is a novel technology, and it has not previously been used for to answer our proposed study questions. Because the objectives of the Stout et al. study were different, the results cannot be used to demonstrate efficacy for detecting aggregations of fish. However, in a similar raft-mounted PIT tag antenna, developed in Colorado (Richer et al. 2017), researchers were able to use multiple detection passes to detect large numbers of PIT tagged trout. While the researchers did not produce a heat map, the data that was collected would seem to support the production of a heat map. The objectives of the Richer study were different (population and abundance estimates), but the large number of fish that were detected in a small area support the use of this technology to detect aggregations.

Drift net samples seem like a lot of effort are there other ways that we could identify spawning such as collecting eggs?

RESPONSE: We acknowledge that drift netting can be a lot of effort, but it is the most effective method for confirmation of spawning. According to documents cited in the SSA report, Razorback Sucker eggs are adhesive for only a period of 3–4 hours post-fertilization. This would leave only a short period to collect them from substrate.

Please identify how effort will be adjusted in time if flows greater than the technology's detection threshold occurs.

RESPONSE: We have clarified this point in the proposal. Stout determined 999 cfs as the threshold for PITPASS because they were not only trying to detect fish in the shallows of the river (< 1.0m), where Razorback are likely to be spawning (USFWS 2002) but also in the center of the channel. However, spawning aggregations are unlikely to occur in the thalweg, so flows higher than 999 cfs are not likely to invalidate use of the technology. However, if flow predictions indicate high flows in the sampling period, it can be adjusted to occur earlier while still remaining in Razorback Sucker spawning period.

We are concerned that detections may differ in clear versus turbid water. Are there any data available that could alleviate this concern?

RESPONSE: A recent paper indicated that high turbidity did improve fish detections by mobile PIT tag antennas (Zentner et al. 2021). However, the highest turbidity level that was recorded in the study was only 12.2 FNU which is typically lower than is recorded in the San Juan River. During the proposed sampling period (last 2 weeks of April and first week of May), median FNU in 2021 was 16 FNU at the Farmington Gage, corresponding to a depth of about 2 ft. While high turbidity conditions would likely increase detection rates, Zentner's paper seems to indicate that fish would not necessarily avoid PITPASS at turbidity levels that occur in the San Juan River during the proposed study period.