Project Title: San Juan River Larval Razorback Sucker and Colorado Pikeminnow Monitoring

Bureau of Reclamation Agreement Number:

Reclamation Agreement Term:

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

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

Relationship to LRP, Study Goals and Objectives:

This work is being conducted as required by the San Juan River Basin Recovery Implementation Program Monitoring Plan and Protocol (2012). The objectives of this specific monitoring effort are identified and listed below and related to the specific tasks listed in the 2016 Long Range Plan set forth by the San Juan River Basin Recovery Implementation Program (SJRRIP).

1) Conduct larval fish sampling to determine if (Colorado Pikeminnow and Razorback Sucker) reproduction is occurring, locate spawning and nursery areas, and gage the extent of annual reproduction. (Task 4.1.2.1)

2) Document and quantify reproduction, survival, and recruitment. (Task 4.4.1.1).

3) Document and track trends in the use of specific mesohabitat types by larval Colorado Pikeminnow and Razorback Sucker. (Task 4.2.3.2).

4) Analyze and evaluate monitoring data and produce Annual Fish Monitoring Reports to ensure that the best sampling design and strategies are employed. (Task 4.1.1.2)

5) Provide detailed analysis of data collected to determine progress towards endangered species recovery in the San Juan River. (Task 5.1.1.3)

6) Identify principal river reaches and habitats used by various life stages of endangered fish. (Task 4.2.4.1)

7) Deposit, process, and secure San Juan River fish specimens, field notes, and associated data at an organized permanent repository. (Task 4.1.2.5)

8) Provide annual updates on the rate of opercular deformities found in Razorback Sucker. (Task 4.1.7.2)

9) Monitor TNC's restoration sites for the presence of endangered species and compare species composition and relative abundance of fishes captured in restoration sites to nearby control sites. (Task 4.3.2.1)

Study Background/Rationale and Hypotheses:

Surveys conducted in late 1980s documented small resident populations of Colorado Pikeminnow and Razorback Sucker in the San Juan River and the San Juan River arm of Lake Powell (Platania et al. 1991). Beginning in the mid-1990s, populations of these two species were augmented with annual hatchery stocking under the auspices of the San Juan River Basin Recovery Implementation Program (SJRRIP). Currently, populations of Colorado Pikeminnow and Razorback Sucker persist in the lowermost 180 river miles of the San Juan River (between the Animas River confluence and the inlet of Lake Powell Reservoir). Annual investigations into the early life history of Colorado Pikeminnow began in 1991 when passive drift-netting for larval and young-of-year (YOY) fish was initiated in the San Juan River. The primary objectives of the passive drift-netting study were to 1) determine the temporal distribution of San Juan River ichthyoplankton in relation to the hydrograph, 2) provide comparative analysis of the reproductive success of San Juan River fishes, 3) attempt to characterize downstream movement of ichthyoplankton, and 4) attempt to validate the presumed spawning period of Colorado Pikeminnow.

Two stationary sites were used for the drift-net surveys. The upstream site was located 4.6 miles upstream of the Mancos River confluence in New Mexico. A second site was established in Mexican Hat, UT. Over 70 river miles separated the two field stations. Results from eleven years of drift-net surveys (1991–2001) resulted in the collection of 11 larval Colorado Pikeminnow (Table 1) as well as information regarding the ichthyofaunal community of the San Juan River during the presumed spawning period of Colorado Pikeminnow (Platania et al. 2000, 2002).

In 1994, personnel from the U.S. Fish and Wildlife Service Colorado River Fishery Project (CRFP; Grand Junction, Colorado) stocked the first series of Razorback Sucker (n=672) in the San Juan River. Those fish, whose mean length and mass at the time of stocking were about 400 mm TL and 710 g, respectively, were released between Hogback, New Mexico and Bluff, Utah. In their 1995 report of activities, Ryden and Pfeifer (1996) suggested that most experimentally stocked 1994 San Juan River Razorback Sucker would achieve sexual maturity by 1996 thereby providing the potential for spawning during 1997–1998. The success of the experimental stocking study resulted in the development of a full-scale augmentation program for Razorback Sucker in the San Juan River.

At the November 1996 SJRRIP Biology Committee integration meeting, it was suggested that the Colorado Pikeminnow, larval fish drift study be expanded to document spawning of Razorback Sucker. However, because reproduction by Razorback Sucker (March-May) occurred considerably earlier than Colorado Pikeminnow (June-July), separate investigations of spawning periodicity and magnitude were deemed necessary. The initial attempt to document reproduction by Razorback Sucker stocked in the San Juan River took place in 1997. Low velocity monitoring sites were established in numerous locations adjacent to U.S. Hwy 163 and Utah State Hwy 262 (which parallels the San Juan River between Aneth and Bluff, UT) that appeared suitable for sampling with light-traps. Light-traps were set nightly in low-velocity habitats between Aneth and Mexican Hat from late March through mid-June 1997. Sampling success during the 1997 Razorback Sucker larval fish study was poor. While there were over 200 light-trap sets, those sampling efforts produced only 297 fish and larval Razorback Sucker was not present. While there were probably several variables that accounted for the poor light-trap catch rate, a principal factor was limited access to suitable habitats. A primary result of the 1997 study was the realization that being bound to specific collecting sites was an inefficient means of collecting the large number of larval fish necessary to document reproduction of a rare species.

In 1998, the Razorback Sucker larval fish sampling technique was modified to allow for collections over a larger portion of the San Juan River and capture of a considerably larger number of larval fish. Navigation of the river by use of an inflatable raft provided the opportunity to sample habitats that were formerly either inaccessible or unobservable under the constraints of the 1997 sampling protocol. The primary collecting method was sampling low-velocity habitats with a fine mesh seine (0.8 mm mesh). The seining technique yielded over 13,000 specimens between river miles 127.5 and 53.3 with most these individuals (n=9,960) being larval catostomids. Included in the 1998 larval fish catch were two larval Razorback Suckers (Table 2). The success of this project became evident as documentation of reproduction by Razorback Sucker continued in successive years. Between 1998 and 2021 there have been both spatial and temporal expansions upon this project but sampling methodology has remained relatively consistent.

The most significant change to the larval Razorback Sucker survey was the incorporation of the spawning periodicity of Colorado Pikeminnow using the same methodology. Beginning in July 2002, personnel began an active sampling regime that mirrored the sampling protocol successfully used to document reproduction by Razorback Sucker. This temporal expansion of the larval fish survey resulted in the monitoring of reproduction by the entire ichthyofaunal community.

Following the 2002 expansion of the San Juan River larval fish survey to actively sample for larval Colorado Pikeminnow, 1,734 individuals have been collected. Over 95% (n = 1,682) of all larval Colorado Pikeminnow has been collected since 2014 (Table 1). Back-calculated spawning dates, based on Colorado Pikeminnow larvae, range from 14 June to 3 August and are associated with the descending limb of spring runoff and mean river temperatures typically above 18°C.

The results of the investigation into the early life history of Colorado Pikeminnow and Razorback Sucker is a foundation of work detailing the spawning periodicity of the entire ichthyofaunal community of the San Juan River. For the endangered fishes, catch data elucidates potential spawning areas, distribution, displacement of propagules in the system and back-calculated spawning and hatching dates. This study provides the material needed by the SJRRIP for genetic analysis of Colorado Pikeminnow and Razorback Sucker which is used to generate estimates of the effective number of adult breeders for both species each year. The genetic work also allows the SRRRIP to document the occurrence and magnitude of Razorback Sucker hybridization with other catostomid species in the San Juan River. This study also provides the only monitoring of habitat restoration sites constructed by the SJRRIP and examines backwater persistence in a series of lateral washes and canyons found within critical habitat. The long-term data set (24 years) can track trends of the entire ichthyofaunal community by year, month, and river reach. The larval survey is also able to detect spawning by the some rarest of the San Juan River fishes; Colorado Pikeminnow and Roundtail Chub, *Gila robusta*.

To date larval fish monitoring has collected and identified 13,139 larval or age-0 juvenile Razorback Sucker and documented reproduction by Razorback Sucker for 24 consecutive years (Table 2). Back-calculated hatching dates derived from individual Razorback Sucker larvae indicates spawning occurs prior to spring run-off often including the ascending limb of the spring hydrograph and can persist into early summer (Farrington et al. 2017). Mean water temperatures during this period among all years ranged between 7.7–26.5°C. This minimum, maximum and subsequent range of temperatures at which successful spawning by Razorback Sucker has been documented is outside of the temperature range typically reported for this species (Bozek et al. 1990; Tyus and Karp, 1990; Muth et al. 1998; Bestgen et al. 2011).

Table 1. Summary of larval and YOY Colorado Pikeminnow collected in the San Juan River during larval drift-netting/larval seining (1991-2021) and back- calculated dates of spawning.

Year	***Sample Method***	***Study Area (River Miles)***	***N=***	***Length (TL)***	***Collection Date***	***Spawning Date***
1991-2001	Drift Netting	127.5, 53.3	11	8.5–9.2	26 Jul–03 Aug	15-18 Jul
2004	Larval Seine	141.5 – 2.9	2	14.2, 18.1	22, 26 Jul	4, 10 Jul
2007	Larval Seine	141.5 – 2.9	3	14.3-16.2	25, 27 Jul	8–13 Jul
2009	Larval Seine	141.5 – 2.9	1	25.2	27 Jul	13 Jul
2010	Larval Seine	141.5 – 2.9	5	12.6–21.4	20-23 Jul	2–6 Jul
2011	Larval Seine	141.5 – 2.9	29	10.0–21.3	20, 21 Jul, 10,11 Aug	2–27 Jul
2013	Larval Seine	147.9 – 2.9	12	14.1–28.7	17–30 Jul	1–16 Jul
2014	Larval Seine	147.9 – 2.9	312	8.5–20.8	13–28 Jul	17 Jun–5 Jul
2015	Larval Seine	147.9 – 2.9	24	8.6–9.7	28–30 Jul	11–14 Jul
2016	Larval Seine	147.9 – 2.9	548	8.8–14.7	24–28 Jul	30 Jun–12 Jul
2017	Larval Seine	180.6 – 2.9	174	9.0–21.5	23 Jul–16 Aug	16 Jun–17 Jul
2018	Larval Seine	180.6 – -17.0*	54	9.3–25.3	7–26 Jul	4–28 Jun
2019	Larval Seine	180.6 – -19.0*	300	8.9–37.0	17 Jul–19 Aug	30 Jun–3 Aug
2020	Larval Seine	188.3 – -17.0*	270	9.2–37.0	12 Jul–12 Aug	14 Jun–12 Jul
2021 ***	Larval Seine	180.6 – -19.0**	TBD	TBD	TBD	TBD

* RM -17.0 is equivalent to Lake Mile 38 in the San Juan arm of Lake Powell.
** RM -19.0 is equivalent to Lake Mile 36 in the San Juan arm of Lake Powell.
*** 2021 samples are still being processed and identified.

Table 2. Summary of larval and YOY of Razorback Sucker collected in the San Juan River (1998 – 2020) and back-calculated dates of spawning.

Year	***Sample Method***	***Study Area (River Miles)***	***N=***	***Length (TL)***	***Collection Date***	***Spawning Date***
1998	Larval seine Light traps	127.5 – 53.3	2	12.1, 12.7	21, 22 May	NA
1999	Larval seine Light traps	127.5 – 2.9	7	10.2–18.6	4 May–14 Jun	NA
2000	Larval seine Light traps	127.5 – 2.9	129	9.3–16.2	9 May–2 Jun	5 Apr–5 May
2001	Larval seine Light traps	141.5 – 2.9	50	10.1–28.8	16 May–14 Jun	7 Apr–7 May
2002	Larval seine Light traps	141.5 – 2.9	813	9.7–62.4	29 Apr–12 Jul	30 Mar–12 May
2003	Larval seine Light traps	141.5 – 2.9	472	9.2–37.3	16 May–18 Jun	7 Apr–15 May
2004	Larval Seine	147.9 – 2.9	41	8.7–25.9	15 May–15 Jun	15 Apr–21 May
2005	Larval Seine	147.9 – 2.9	19	10.8–25.3	14 May–3 Aug	8 Apr–18 Jun
2006	Larval Seine	147.9 – 2.9	202	8.9–22.5	23 Apr–30 May	2 Apr–16 May
2007	Larval Seine	147.9 – 2.9	200	6.7–31.9	19 Apr–26 Jul	12 Mar–21 Jun
2008	Larval Seine	147.9 – 2.9	126	8.3–18.7	21 May–21 Jun	6 Apr–22 May
2009	Larval Seine	147.9 – 2.9	272	10.1–30.2	19 May–19 Jun	7 Apr–24 May
2010	Larval Seine	147.9 – 2.9	1,251	9.4–30.0	17 May–20 Jul	14 Apr–30 May
2011	Larval Seine	147.9 – 2.9	1,065	8.6–34.2	16 May–19 Jul	28 Mar–20 May
2012	Larval Seine	147.9 – 2.9	1,778	6.1–31.8	14 May–14 Jun	19 Mar–14 May
2013	Larval Seine	147.9 – 2.9	979	9.5–70.0	17 May–18 Jul	28 Mar–26 May
2014	Larval Seine	147.9 – 2.9	612	8.8–57.6	22 Apr–17 Jul	12 Mar–21 Jun

Table 2. Summary of larval and YOY of Razorback Sucker collected in the San Juan River (1998 – 2021) and back-calculated dates of spawning (continued).

Year	*Sample Method*	*Study Area (River Miles)*	*N=*	*Length (TL)*	*Collection Date*	*Spawning Date*
2015	Larval Seine	141.5 – 2.9	1,205	9.6–22.4	19 Apr–21 May	19 Mar–28 Apr
2016	Larval Seine	141.5 – 2.9	824	9.3–48.4	21 Apr–25 Jul	14 Mar–22 Jun
2017	Larval Seine	180.6 – 2.9	360	9.2–43.0	20 Apr–26 Jul	16 Mar–30 Jun
2018	Larval Seine	180.6 – -17.0*	1,833	8.2–102.2	23 Apr–26 Jul	9 Mar–30 May
2019	Larval Seine	180.6 – -19.0**	722	9.1–37.9	25 Apr–1 Aug	18 Mar–5 Jul
2020	Larval Seine	188.3 – -17.0*	177	9.2–48.0	19 May–31 Jul	29 Mar–29 Jun
2021 ***	Larval Seine	180.6 – -19.0**	TBD	TBD	TBD	TBD

* RM 17.0 is equivalent to Lake Mile 38 in the San Juan arm of Lake Powell.
** RM 19.0 is equivalent to Lake Mile 36 in the San Juan arm of Lake Powell.
*** 2021 samples are still being processed and identified.

Project Modifications

There have been numerous modifications to the field methodology of the larval fish survey over time as well as changes in reporting priorities, protocol, and format (Table 3). The extent of the study area and aspects of the longitudinal sampling have been modified to improve spatial comparisons. The study area was expanded in 1999, 2001, 2012 and 2017 by a total of 103.5 river miles (over double the length of the original study area) to include Reach 6 through Reach 1 (Farmington, NM to Clay Hills Crossing, UT). The expansions of the study area were a result of captures of larval Razorback Sucker at the top of the previous study area boundary. Within two years (and often the following year) of each study area expansion, larval Razorback Sucker was documented in the newly expanded study area. The most recent expansion occurred in 2018 when larval fish sampling was expanded downstream of the Paiute Farms waterfall (RM -1.0) to include riverine habitat in the San Juan arm of Lake Powell. Larval Razorback Sucker larvae were collected downstream of the waterfall in 2018 and 2019. Sampling done upstream within the "Expanded Study Area" (RM 168.4–180.6, Waterflow, NM to Farmington, NM) is independent of the work proposed in this SOW with discrete sampling dates, SOW, and budget submitted to the SJRRIP each fiscal year.

Beginning in 2003, the entire study area (excluded the aforementioned Expanded Study Area and Paiute Farms waterfall reach) was sampled in single uninterrupted trips (10–12 field days per trip) rather than in two temporally discrete sections as done in previous years (1998 – 2002). Because of the increasing numbers of larval Razorback Sucker collected (as well as detailed information regarding the native fish community), the SJRRIP Biology Committee voted to elevate the larval fish surveys from an "experimental" project to a monitoring program. This change allowed for comparisons of catch per unit effort (CPUE) data with the programs monitoring activities (i.e., small-bodied fish, sub-adult and adult, habitat, etc.).

Conducting the larval Razorback Sucker and Colorado Pikeminnow surveys under this new protocol not only provided discrete reach information but also provided greater temporal resolution in respect to the longitudinal distribution of Razorback Sucker larvae and the ability to correlate potential environmental cues required by Razorback Sucker for spawning. These same advantages also apply to Colorado Pikeminnow. Disadvantages to this top to bottom approach were that the duration of the monthly sampling trips (10–12 field days) made them more subject to abiotic fluctuations (floods, flow spikes). Large flood events reduce sampling efficiency as many low velocity habitats become flooded by rising water levels thereby transporting larval and early juvenile fish downstream. In addition, large flood events have necessitated premature termination of some survey runs, reducing the temporal resolution of the single-continuous pass effort. Annually, at least one trip (an average) had to be cut short due to large flood events or low water events in the lower canyon. The abbreviated trips were subsequently resumed once conditions improved (usually 1–2 weeks later). Additional costs were incurred because of the need to return to the field to complete the sampling effort for that month.

To reduce the variability of abiotic conditions as well as gain even greater temporal resolution of the longitudinal distribution of Colorado Pikeminnow and Razorback Sucker larvae, the protocol was modified to survey the upper and lower halves of the study area simultaneously. This effort began in 2007 and utilized two fully equipped and autonomous crews (Table 3). Beginning in 2011, the September sampling trip was discontinued. Previous sampling efforts during September efforts had shown that most species had grown to a sufficient size (juvenile developmental stage) that they no longer occupied low velocity habitats. The SJRRIP Biology Committee concluded that the September larval fish survey did not provide enough information, especially with respect to catostomids, to warrant continuation.

In 2013 a new analysis of Colorado Pikeminnow and Razorback Sucker trend data was developed using mixture models (White, 1978; Welsh et al., 1996; Fletcher et al., 2005; Martin et al., 2005). Mixture models can be particularly effective at modeling ecological data with multiple zeros to estimate occurrence and abundance separately (e.g., combining a binomial distribution with a lognormal distribution). Data collection for this new approach meant each seine haul was preserved independently along with physical descriptors of each haul. Beginning in 2014, the mixture model analysis was expanded to include annual trends for many of the common species collected.

Table 3. Summary of annual projects and project modifications of the larval fish surveys from 1997 to 2021.

Year	***Sample Method***	***Study Area (River Miles)***	***Specimens Collected***	***Field Modification***	***Laboratory Modification***
1997	Light Trap Drift-nets	99 – 75	297		
1998	Larval Seine Light Trap Drift-nets	127.5 – 53.3	13,608	study area expanded; active sampling	
1999	Larval Seine Light Trap Drift-nets	127.5 – 2.9	20,711	study area expanded; upper-lower reaches sampled separately; nonsynchronous	
2000	Larval Seine Light Trap Drift-nets	127.5 – 2.9	13,549		
2001	Larval Seine Light Trap Drift-nets	141.5 – 2.9	95,629	study area expanded; upper-lower reaches sampled	
2002	Larval Seine Light Trap	141.5 – 2.9	138,602	study period expanded to September. Drift-nets no longer used.	
2003	Larval Seine Light Trap	141.5 – 2.9	112,842	upper-lower reaches sampled monthly in one uninterrupted trip (11–12 day runs)	CPUE data used for integration in reporting
2004	Larval Seine	141.5 – 2.9	160,292		Reports merged, trend data reported
2005	Larval Seine	141.5 – 2.9	109,368		
2006	Larval Seine	141.5 – 2.9	50,616		
2007	Larval Seine	141.5 – 2.9	53,084	Two rafts-two crews;	Analyzed catch with habitat data

				upper-lower reaches samples synchronous	
2008	Larval Seine	141.5 – 2.9	40,855		
2009	Larval Seine	141.5 – 2.9	72,404	Specimens preserved in 95% ethanol	
2010	Larval Seine	141.5 – 2.9	70,610		

Table 3. Summary of annual projects and project modifications of the larval fish surveys from 1997 to 2021 (continued).

Year	***Sample Method***	***Study Area (River Miles)***	***Specimens Collected***	***Field Modification***	***Laboratory Modification***
2011	Larval Seine	141.5 – 2.9	28,258	September survey dropped from the monitoring	
2012	Larval Seine	147.9 – 2.9	29,384	Study area expanded	
2013	Larval Seine	147.9 – 2.9	25,842	Individual seine hauls preserved independently	Mixture Model analysis used for trend data
2014	Larval Seine	147.9 – 2.9	20,508		Mixture Model analysis used for several common species
2015	Larval Seine	147.9 – 2.9	17,787		Multiple covariates used in all mixture models
2016	Larval Seine	147.9 – 2.9	12,973		Additional covariates used in CPM mixture models
2017	Larval Seine	180.6 – 2.9	31,587	Study Area expanded	
2018	Larval Seine	180.6 – - 17.0*	44,611	Study Area expanded downstream to include riverine habitat below the waterfall.	Fall monitoring covariate changed from raw numbers to CPUE

2019	Larval Seine	180.6 – - 19.0**	30,395		
2020	Larval Seine	188.3 – - 17.0*	47,655		
2021 ***	Larval Seine	180.6 – - 19.0**	>90,000		

* RM 17.0 is equivalent to Lake Mile 38 in the San Juan River arm of Lake Powell.

** RM 19.0 is equivalent to Lake Mile 36 in the San Juan River arm of Lake Powell.

*** 2021 samples are still being processed and identified. Over 90,000 specimens have been identified to date.

Study Area:

The study area for this SOW encompasses the San Juan River between Shiprock, New Mexico (RM 147.9) and the Clay Hills Crossing boat landing (RM 2.9) just above Lake Powell in Utah (145.0 river miles). Five sampling efforts will take place between mid-April and late July. The upper reach of the study area, RM 147.9 to near Montezuma Creek (RM 92.2) are managed by the Navajo Nation while the reach from Montezuma Creek (RM 92.2) to Clay Hills (RM 2.9) is managed by the U.S. Bureau of Land Management (BLM).

Study Methods/Approach:

Field Work

Sampling to meet the study objectives of this SOW will be conducted in the San Juan River between RM 147.9 and RM 2.9 using sampling techniques that will provide enough fish necessary to meet study objectives. Access to the river will be gained using inflatable rafts equipped with all of the necessary equipment and provisions needed for trips of up to seven days. A day and a half is added before and after each field survey for field preparation, gear maintenance, clean up and specimen deposition. The study area will be divided into an "upper" section (Shiprock, NM, to Sand Island, UT) and a "lower" section (Sand Island, UT, to Clay Hills crossing, UT). Separate field crews will launch simultaneously in each of the two sections and proceed through their designated study area. The vehicle and raft trailer used by the field crew working in the upper section will be left at the Shiprock launch site and subsequently be shuttled to the Sand Island BLM ranger station, UT. The vehicle shuttle (with trailer) for the upper reach sampling effort was typically performed without cost by personnel from the Farmington Office of the Bureau of Indian Affairs Office. Between 2008 and 2010, personnel from the N.M. Fishery Resources Office stationed in Farmington performed this service. Beginning in 2011, ASIR personnel shuttled vehicles for the upper end crew (there is no cost for this service).

The sampling crew for the lower reach will launch from, and store their vehicle and raft trailer at Sand Island, UT, where a commercial shuttle will take the vehicle to Clay Hills crossing, UT. The cost for this service is included under the travel and per diem section of our budget.

Because crews sampling the lower section of the study area will be in a high use recreational area, reservations are required. All trips for 2023 must be scheduled by late January 2022 and submitted to the Bureau of Land Management (BLM) Office at Monticello, Utah. Designated camping permits for our lower reach sampling crews will be obtained and must be strictly adhered to in addition to other BLM-San Juan River Recreation Area regulations (i.e., low impact and pack-out policies). Low flow conditions often prevalent during the study period make several sections of the river more difficult to navigate (especially in the lower reach). Our field crews are required to render assistance to boaters stuck in rapids or otherwise in distress and report all such encounters to the appropriate BLM personnel.

Sampling efforts for larval fish will be concentrated in low velocity habitats and employ small mesh seines (1 m x 1 m x 0.8 mm) to collect fish. Individual seine hauls will be preserved independently at each site. Habitat designations will also be recorded by seine haul. Retained specimens will be placed in Whirl-paks containing 95% ethanol (EtOH) and a tag inscribed with unique alphanumeric code that is also recorded on the field data sheet. For each sample site, the lengths (to 0.1 m) of each seine haul and total number of hauls will be measures and recorded. Capture densities for seine samples will be reported as the number of fish per 100 m^2.

Native species large enough to be positively identified will be measured (standard length) and returned to the river. Post-larval endangered fish species collected during this study will be photographed, a small portion of tissue from the caudal fin clipped and retained in 95% EtOH (in the case of potential Razorback Sucker hybrids) and scanned with a PIT tag reader for the presence of a PIT tag. Specimens of sufficient size but lacking a PIT tag will be injected with a tag following the protocols established by the program (Davis 2010). All PIT tag information will be recorded in the field data sheet and subsequently forwarded to the SJRRIP for integration in the program's PIT tag database.

For each sampling locality, river mile will be determined to the nearest tenth of a mile using the most current SJRRIP Standardized Map Set. Universal Transverse Mercator (UTM) coordinates and zone will be determined with a Garmin Navigation Geographic Positioning System Instrument for each sampling locality. Mesohabitat type, length, maximum and minimum depths, water clarity (determined with a Secchi disc), and substrata will be recorded for each sampling locality. A minimum of one digital photo will also be taken of each specific habitat sampled.

Each of the six phase I River Ecosystem Restoration Initiative (RERI) sites located between river miles 132.2 and 127.2 will be the subject of repeated monthly monitoring. The goal of these collections is to detect the presence of endangered species and compare species composition and relative abundance of fishes captured in restoration sites to nearby control sites. If a site cannot be effectively sampled (e.g., too deep or swift), photos will be taken, habitat conditions noted, and no collection made. Beginning in 2011, ASIR researchers defined 15 monitoring sites located in lateral washes and canyons throughout the study area to assess persistence of backwater habitats. Monitoring sites will be visited in each survey. If suitable nursery habitat exists at the time of visitation they will be sampled. If the sites are dry or contain isolated pools, photographs will be taken, and field notes written detailing condition of the habitat. Conditions of all monitoring sites and RERI restoration sites will then be related back to discharge at time of visitation.

All collections that contain Razorback Sucker will be examined for frequency and severity of opercular deformities. The opercula are not fully developed until at least the post-flexion mesolarval stage of development. Because of this, only Razorback Sucker greater than 15 mm TL (the size at which the opercula should be fully developed) will be examined for opercular deformities. Individuals will be examined on both the left and right sides. Severity of shortening will be assessed and rated as level 0 (no opercular deformity), level 1 (slight shortening), or level 2 (severe shortening). Annual rates of opercular deformities will be plotted and compared to the rates reported by Barkstedt et al. (2018) through 2012 and those subsequently recorded.

Field Work, Safety

Personnel participating in fieldwork are required to successfully complete an International Rescue Instructors Association (IRIA) level 2 swiftwater rescue class and American Red Cross CPR/AED training. Type III personal flotation devices (PFD's) will be always worn by sampling personnel while working. As PFD's lose flotation capacity due to UV exposure, compression of material, and oil and grit impregnation, and since each crewmember's PFD will be used for approximately 60 days per season, the PFD's will be annually replaced. Simms Guideweight Gore-Tex waders and boots will be issued to all personnel along with 3 mm neoprene gloves (necessary in April and May). In addition to personal camping gear and rain suits, all personnel will be required to provide and use wide brimmed hats, sunscreen, and sunglasses (provided at no cost to the program).

All rafts used for this project will carry an extensively stocked first aid kit replete with items necessary for most minor medical situation. Additionally, the first aid kit will contain a suite of items (i.e., splints, neck braces, butterfly stitches) needed to address more serious medical conditions. Because ethanol is used in the preservation of specimens, several vials of eyewash solution will be incorporated into each first aid kit. First aid kits will be inventoried after each sampling trip and used and/or expired items replaced. In the upper reach of the study area, personal cell phones will be used (at no cost to the program) to contact outside parties should a medical situation arise. In the lower study area reach (canyon bound; where cell phones do not have service) an Iridium 9505-satellite phone will be provided for sampling crews. Both sampling crews will be equipped with SPOT Satellite GPS Messenger units to be used in case of an emergency.

All preservation fluids will be transported in heavy-duty LPDE carboys. Extensive exposure to UV light makes the carboys susceptible to decomposition and cracking and requires that they be inspected monthly and not used for more than two years. Safety rope throw bags will be similarly inspected and retired from use accordingly. Rafts will be equipped with raft recovery (Z-line) kits, repair kits, extra oars, and oar blades, and two spare hand pumps to help ensure that crews do not become stranded due to raft damage. Federal regulations (BLM) mandate that all boaters carry an extra PDF and emergency whistle.

Laboratory Work

Samples will be returned to the lab immediately after each field trip is completed and processed following a multi-step procedure. To maintain the larval fish in good condition (necessary to ensure accurate identification) the samples must be transferred from whirl-packs to glass jars and the field fluids replaced with new preservation fluids. Cyprinid and catostomid larvae are extremely small and transparent especially at early developmental stages. To minimize the potential loss of fish in individual seine hauls, it is best to retain the entire contents of each seine haul. A negative result of this technique is that, in addition to larval fish, whirl-pack samples usually contain considerable debris, detritus, and silt. Another important step in processing of individual samples is to separate fish from the detritus. This necessary portion of the process is labor intensive and can be quite tedious. During this process initial sorting of fish based on age class (age 0 [larvae] and age 1+) occurs. Samples that contain a large number of larval fish, especially proto or mesolarvae, often must be sorted twice to ensure all larvae are located within a sample.

After the fish are separated from the debris, personnel with San Juan River Basin larval fish identification expertise will identify individual specimens to species. Stereomicroscopes equipped with transmitted light bases (light and dark field) and polarized filters (that enhance the delineation of myomeres, pterygiophores, and fin rays) will be used to assist with the identifications. Larval fish keys are referenced to assist in species specific determinations (e.g., Contributions to a guide to the cypriniform fish larvae of the Upper Colorado River System [Snyder 1981], Catostomid fish larvae and early juveniles of the Upper Colorado River basin, Morphological descriptions, comparisons, and computer interactive key [Snyder and Muth 2004], and Identifications of larval fishes of the Great Lakes Basin [Auer 1982]). Age-0 specimens will be separated from age-1+ specimens using published literature on growth and development (Snyder 1981, Snyder and Muth 2004).

Age classes will be enumerated, measured (minimum and maximum size [mm standard length] for each species at each site), and catalogued in the Division of Fishes of the Museum of Southwestern Biology (MSB) at the University of New Mexico (UNM). Both total length (TL) and standard length (SL) of Colorado Pikeminnow and Razorback Sucker will be obtained using electronic calipers and stereomicroscope mounted micrometers. The ontogenetic stage of Colorado Pikeminnow and Razorback Sucker obtained in this study shall be determined based on the definitions provided by Snyder (1981).

Analysis

Modeling ecological data with multiple zeros can be particularly effective when using mixture models (e.g., combining a binomial distribution with a lognormal distribution) to estimate occurrence and abundance separately (White, 1978; Welsh et al., 1996; Fletcher et al., 2005; Martin et al., 2005). Long-term Razorback Sucker (1999–present) and Colorado Pikeminnow (2003–present) sampling-site density data will be analyzed using PROC NLMIXED (SAS, 2018), a numerical optimization procedure, by fitting a mixture model using the methods outlined in White (1978). Covariates specific to Razorback Sucker and Colorado Pikeminnow mixture models are in Tables 4 and 5 of this proposal. Logistic regression will be used to model the probability a site was occupied, and the lognormal model will be used to model the distribution of abundance given that the site is occupied. Models provide four parameter estimates for each year (δ = probability of occurrence, μ = mean of the lognormal distribution, σ = standard deviation of the lognormal distribution, and $E(x)$ = estimated density). Model parameter estimates will be annual plotted and compared to the long-term data set to address Objectives 2–6 of this proposal.

Additional samples (i.e., each seine haul preserved individually) were taken between 2013 and 2021 to increase the overall sample size and provide supplemental information on habitats (i.e., habitat type and habitat location) to address Objectives 3 and 6 of this proposal. Field sampling efforts occurred in nine habitat types (backwater [BW], cobble shoal [CS], eddy [ED], embayment [EM], pool [PO], pocketwater [PW], run [RU], sand shoal [SS], and slackwater [SW]). These habitat designations follow those used by the SJRRIP as defined in Bliesner et al. (2008). Additionally, four categories were assigned to habitat depending on where the sample was taken. Shoreline (SH) indicates all samples taken along the land-water interface, open-water (OP) indicates samples taken away from the shoreline, and mouth (MO) or terminus (TR) indicates samples taken from those locations within a backwater or embayment.

Habitat-specific density data (i.e., providing information on habitat type and habitat location) have only been available since 2013. These data provide information on the specific habitat features used by Razorback Sucker and Colorado Pikeminnow. Habitat-specific density data are also analyzed using PROC NLMIXED (SAS, 2018), using the same methods outlined previously, to assess differences among models. A simplified list of five habitats (BW, EM, RU, LV [combining CS, PW, SS, and SW], and NZV [combining ED and PO]) is used for the purpose of statistical analysis since several habitats shared nearly identical low velocity (LV) or near zero velocity (NZV) conditions. General linear models will be used to incorporate covariates to model δ, μ, and σ. Covariates considered to model habitat-specific density data are year, reach, habitat type, and habitat location. Random effects models are used with the joint binomial and lognormal likelihood to provide random errors for the Site*Year combinations. Bivariate normal errors with mean zero and covariance are assumed for each Site*Year combination. A random error will be added to the logit of the binomial parameter δ, and a second random error was added to the log of the μ lognormal parameter. Adaptive Gaussian quadrature as described in Pinheiro and Bates (1995) is used to integrate out these random effects in fitting the model using the SAS NLMIXED procedure. Goodness-of-fit statistics (logLike and AIC_C) are generated to assess the relative fit of data to various models. The approach used to analyze habitat data between 2013 and 2021, and scheduled for use in 2022, will be used in 2023 to further elucidate fish and habitat relationships and fulfill Objectives 2, 3, 5 and 6 of this proposal.

The results in the annual report will pertain almost exclusively to age-0 fish (i.e., age-1+ are not “larval fish” and are not the focus of this effort, they are not included in analysis). The exception to this will be age-1+ augmented Colorado Pikeminnow. Capture data for all Colorado Pikeminnow are analyzed using mixture-models and trend data reported. The number of all other fish age-1+ collected during the study will be presented as an Appendix.

Hatching dates of Razorback Sucker larvae are calculated by subtracting age (in days) from collection date. Age is determined using the formula:

$$age = -2.783 + 2.841(SL) + 0.073(AprilQ) + 0.012(Rkm) - 0.999(T_{coll}) + 0.034(GDD_{coll})$$

where SL is standard length (mm), *AprilQ* is mean April discharge (m^3/s), *Rkm* is river kilometer at which fish were collected, T_{coll} is the temperature on the collection date, and GDD_{coll} is the cumulative growing degree days on collection date (Clark Barkalow et al., 2021). Incubation time is calculated using the formula $y = 1440.3e^{-0.109x}$ (Bozek et al., 1990) where y is incubation time (in hours) and x is the mean daily temperature (°C) on the hatching date. Spawning date is then calculated by subtracting age and incubation time from collection date

Hatching dates of larval Colorado Pikeminnow are calculated by subtracting age (in days) from collection date and age is determined using the formula:

$$age = -42.086 + 26.196(lnSL) - 1.080e^{-03}(JulyQ) - 5.480e^{-03}(Rkm)$$

where *lnSL* is the natural log of standard length in millimeters, *JulyQ* is mean July discharge (m^3/s), and *Rkm* is the river kilometer at which the larva was collected. Incubation time is calculated using the formula $y = 315.42e^{-0.05(T\,hatch)}$ where y is incubation time (in hours) and x is the mean daily temperature (°C) on the hatch date. The spawning date is then calculated by subtracting age and incubation time from the collection date (Clark Barkalow et al., 2021).

Hatching and spawning dates for both endangered species will then be compared with the discharge and temperature data during that period within the study area to fulfill Objective 5 of this proposal.

This study will be initiated prior to spring runoff and completed during mid-summer (late July or early August). Daily mean discharge and temperature (mean, maximum, and minimum) during the study period is acquired from U.S. Geological Survey Gage (# 09379500) near Bluff, Utah and Four Corners Bridge (#09371010).

Table 4. Covariates used in mixture models for Razorback Sucker.

Covariate	Description
Year	The calendar year in which the larval survey occurred.
Reach	Each of the 5 geomorphic reaches (5–1) within the study area.
Mean March, April, and May temperature.	Daily mean temperature data was taken from USGS gage 09379500 near Bluff, Utah.
Mean March, April, and May discharge.	Daily mean discharge data (cfs) was taken from USGS gage 09379500 near Bluff, Utah.
Cumulative # stocked	The number of Razorback Sucker stocked during the period between 1998 and the year prior to the larval survey year. (e.g., 70,000 fish stocked between 1998 and 2020 would be used as a covariate for 2021 larval capture data).
Fall monitoring captures.	Number of adult Razorback Sucker collected per hour of electrofishing effort. Fish collected during a given year were used as a covariate for larval captures during the following larval survey year (i.e., 1+ overwinter periods).

Table 5. Covariates used in mixture models for Colorado Pikeminnow.

Covariate	Description
Year	The calendar year in which the larval survey occurred.
Reach	Each of the 5 geomorphic reaches (5–1) within the study area.
Mean June and July temperature.	Daily mean temperature data was taken from USGS gage 09379500 near Bluff, Utah.
Mean June and July discharge.	Daily mean discharge data (cfs) was taken from USGS gage 09379500 near Bluff, Utah.
Cumulative # stocked	The number of age-0 Colorado Pikeminnow stocked during the period between 1998 and five years prior to the larval survey year. (e.g., 100,000 fish stocked in 2016 would be used as a covariate for 2021 larval capture data).
Fall monitoring captures 400+ mm TL.	Number of Colorado Pikeminnow greater than 400 mm TL collected per hour of electrofishing effort. Fish collected during a given year were used as a covariate for larval captures during the following larval survey year (i.e., 1+ overwinter periods).

Reporting and Permitting

Beginning in 2004, data from the two San Juan River larval fish surveys (Razorback Sucker and Colorado Pikeminnow) were analyzed collectively and presented in a single report. This provided a single picture of the reproductive activities of the entire ichthyofaunal community in the San Juan River employing the same criterion as other monitoring programs. The report will be disseminated as outlined by the program office.

In addition to the annual report of the study provided to the SJRRIP, permit reports summarizing fish collecting activities and specimens captured are also required annually under scientific collection permits provided by the U.S. Fish and Wildlife Service, New Mexico Department of Game and Fish, Navajo Nation Department of Fish and Wildlife, and Utah Division of Wildlife Resources. The aforementioned permit reports include (at a minimum) site localities, GPS coordinates, fish collected, and deposition history of retained specimens. An annual report of activities is a BLM (Monticello Field Office) requirement under our access permit to the San Juan River below San Island (Bluff, UT) and designated camps in the lower reaches of the river. Annual "Mussel-free" permits are also acquired by all project trip leaders who sample in the Utah portion of the San Juan River and Glen Canyon National Park.

Task Description, Deliverables and Schedule:

Meetings

In addition to participating in multiple conference calls, researchers are required to attend three meetings annually and report on annual monitoring projects. The two pre-set annual meetings (February and May) require researchers present PowerPoint presentations outlining the results and that year's findings. Each meeting lasts about three days (which includes travel time).

Products

Tasks and products from this study will be completed within the timeline outlined in Table 6. A draft report of the 2023 larval Razorback Sucker and Colorado Pikeminnow sampling activities will be prepared and submitted to the SJRRIP Program Coordinator for distribution to the San Juan River Basin Biology Committee for review by 31 March 2024. Upon receipt of written comments, that report will be finalized and disseminated to members of the San Juan River Basin Biology Committee by 30 June 2024 to meet Objective 5 of this proposal. Electronic copies of the 2023 collection data will be transferred to the San Juan River database manager. Fish collected from this study will be curated in the Division of Fishes, Museum of Southwestern Biology (MSB), Department of Biology, at the University of New Mexico under a MSB contract with the SJRRIP to fulfill Objective 7 of this proposal. Original field notes will be retained in the Division of Fishes and collection information electronically stored in a permanent MSB database program. These data and any maps generated from them will be available to the San Juan River Basin Biology Committee electronically and via hard-copy reports.

Table 6. Annual cycle for conducting, analyzing, and reporting data from the larval fish surveys.

Tasks	2023												2024					
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN
Conduct 5-10 surveys. Collect fish from San Juan River to characterize reproduction				X	X	X	X	X										
Conduct sampling at habitat restoration sites				X	X	X	X	X										
Prepare specimens for accession into museum archival collection.				X	X	X	X	X	X	X	X	X	X					
Collect data to compare spawning success among reaches and across years				X	X	X	X	X										
Record water quality, locality data, and habitat attributes.				X	X	X	X	X										
Characterize spawning, quantify effort and habitat, and identify specimens				X	X	X	X	X	X	X	X	X						
Perform mixture-model analysis and correlate covariates to sample results												X	X					
Identify upstream extent of spawning areas.												X						
Prepare endangered species larvae for genetic analysis													X	X	X			
Provide information to other SJRIP projects.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prepare annual reports detailing survey results.											X	X	X	X	X	X	X	X
Provide all data in electronic format												X						X

Participate in SJRIP workshops and meetings and assess progress towards recovery							X				X			X			X	
Coordinate field efforts with SJRIP staff and other SJRIP researchers	X	X	X	X	X	X	X	X					X	X	X	X	X	X

Budget Summary:

FY Year	*Office and laboratory labor*	*Materials and supplies*	*Equipment*	*Travel and per diem*	*Total*
2023	$ 227,717.04	$ 2,216.24	$ 9,988.66	$ 19,917.80	$ 259,839.74
2024	$ 232,271.38	$ 2,260.56	$ 10,188.43	$ 19,917.80	$ 264,638.17
2025	$ 236,916.81	$ 2,305.78	$ 10,392.20	$ 19,917.80	$ 269,532.59
2026	$ 241,655.14	$ 2,351.89	$ 10,600.05	$ 19,917.80	$ 274,524.88
2027	$ 246,488.25	$ 2,398.93	$ 10,812.05	$ 19,917.80	$ 279,617.02
Total	$ 1,185,048.62	$ 11,533.40	$ 51,981.39	$ 99,589.00	$ 1,348,152.41

Reviewers: Not applicable.

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