FY 2018 Scope of Work

To

Bureau of Reclamation

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For

Assessment of calcein mark retention and detection in hatchery-reared age-0 Colorado Pikeminnow, and their interactions with wild age-0 Colorado Pikeminnow in the San Juan River

Reporting Dates

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INTRODUCTION

The San Juan River Basin Recovery Implementation Program (SJRIP) was formed in 1992 to recover endangered Colorado Pikeminnow *Ptychocheilus lucius* and Razorback Sucker *Xyrauchen texanus* in the San Juan River. The construction of Navajo and Glen Canyon Dams and the introduction of nonnative species caused significant declines of the species in the San Juan River by the time the program was initiated, with the last known wild Colorado Pikeminnow being captured in 2000 (Ryden 2003a). In an effort to increase the abundance of Colorado Pikeminnow in the river, experimental stockings began as early as 1996 with a formal augmentation plan for the species completed in 2003 (Ryden 2003b). Currently the SJRIP is stocking age-0 Colorado Pikeminnow under the Phase II Augmentation Plan (Furr 2010), and since being initiated in 2010 over 2.4 million age-0 fish have been stocked (Furr 2016).

Although monitoring has confirmed the recruitment of these stocked Colorado Pikeminnow to adults (Schleicher 2015), as well as moderate reproduction in some years since 2003 (Farrington et al. 2017), recruitment of wild fish past the larval stage has not been documented. However, 23 wild age-0 Colorado Pikeminnows were captured during standardized small-bodied fishes monitoring in 2016 (Zeigler and Ruhl 2017). It was later estimated that 1,000s to 10,000s wild age-0 Colorado Pikeminnows were present in the river based on expected low capture probabilities and the amount of unsampled habitat available to the species. The significance of documented recruitment of wild fish past the larval stage is unequivocal for the recovery of Colorado Pikeminnow in the San Juan River.

A self-sustaining population of Colorado Pikeminnow in the San Juan River is critical to the delisting of the species; however because of low adult abundances and sporadic reproduction, augmentation is likely to continue. If wild age-0 Colorado Pikeminnow are present in the system, a method to distinguish them from hatchery-reared fish is needed so wild fish can be easily identified and tracked until maturity. Due to the small size (45 – 55 mm total length) and total number (≥ 400,000) of age-0 Colorado Pikeminnow stocked every year (Furr 2010), individual identification tags (i.e., PIT tags) are impractical. At the May 2017 SJRIP Biology Committee meeting it was determined that all age-0 Colorado Pikeminnow will be batch marked using calcein before being stocked in the San Juan River to aide in the identification of wild fish.

Calcein, which can be used to mark fish via immersion or feeding, has been successfully used to batch mark Atlantic Salmon *Salmo salar* (Mohler 1997), Rainbow Trout *Oncorhynchus mykiss* (Bart et al. 2001), Walleyes *Sander vitreus* (Brooks et al. 1994), and several other fish species (Leips et al. 2001; Honeyfield et al. 2006). The calcein binds to calcified body parts of an organism and can be detected using ultraviolet light (Elle et al. 2010). Marks are known to fade if fish are not sufficiently exposed to the calcein solution or if fish are exposed to direct sunlight for an extended period (Bashey et al. 2004; Elle et al. 2010; Marsden et al. 2014), but detection of external marks for at least 12 months after marking has been observed in some studies (Mohler 2004; Negus and Tureson 2004). Retention of marks is better on internal structures such as otoliths and vertebrae as opposed to external body structures such as fin rays, but such detections involve lethal sampling (Leips et al. 2001; Elle et al. 2010). Furthermore, calcein marking is not known to affect growth or survival (Bashey 2004), nor increase predation (Mohler et al. 2002).

Currently, the SJRIP Program Office is conducting a 12 month study at Southwestern Native Aquatic Resources and Recovery Center (Southwestern Native ARRC) in Dexter, NM to assess the detection and retention of calcein marks on Colorado Pikeminnow under hatchery conditions (Durst et al. 2016). Calcein marked age-0 Colorado Pikeminnow will be stocked into the San Juan River as part of the SJRIP's augmentation plan in November 2017. Although the mark may be readily identified in laboratory conditions, reliable detection of the mark in the field will be important for assessing contribution of wild and hatchery-reared fish to the population. A previous study using calcein marked Colorado Pikeminnow in the San Juan River found that the mark could not be detected under field conditions approximately one month post-stocking but could be detected under laboratory conditions (Golden and Holden 2005). No information on the marking procedure was provided in the study and faded external marks could have been caused by improper marking techniques. The adoption of calcein marking technique for hatchery-reared age-0 Colorado Pikeminnow created an information need for the SJRIP:

Information Need 1: Efficacy of calcein marking for distinguishing hatchery-reared age-0 and age-1 Colorado Pikeminnow in the field.

In addition to the need to readily identify hatchery-reared fish, the presence of wild age-0 Colorado Pikeminnow in 2016 raised questions about potential negative interactions that may occur between hatchery-reared and wild fish in the river. While little information is available on the interactions between wild and hatchery non-game endangered fish species, an abundance of literature is available for other species, in particular, salmonids. Studies have shown hatchery-reared fish to be more aggressive, compete with wild fish for food, and displace wild fish; potentially leading to deleterious effects to wild fish populations (Einum and Fleming 2001; Weber and Fausch 2003).

Hatchery-reared fish can cause significant impacts to populations of wild fish including decreased abundance (Vincent 1987; Flagg et al. 1995), reduced growth and fitness (Dewald and Wilzbach 1992; McMichael et al. 1997; Weiss and Schmutz 1999), and displacement from suitable habitat at local and reach scales (Fenderson et al. 1968; McGinnity et al. 1997; Fleming et al. 2000; Sundt-Hansen et al. 2015). The majority of these negative effects to wild fish are greatly influenced by density-dependent factors (Petrosky and Bjornn 1988; Weber and Fausch 2003), although other factors such as agonistic behaviors can occur even at low densities (Fenderson and Carpenter 1971). However, other studies have failed to produce results which indicated any competition between hatchery-reared and wild fish (Levings et al. 1986; Unwin and Glova 1997; Deverill et al. 1999; Weiss and Schmutz 1999; Skov et al. 2011), potentially due to the reduced likelihood of domesticated fish to survive in the wild (Einum and Fleming 2001). Still, even in the absence of direct competition, hatchery-reared stocks have been shown to replace, rather than enhance wild populations (Hilborn and Eggers 2000; Quinones et al. 2014); this raises the question of their utility for rebuilding depleted fish populations.

The augmentation of Colorado Pikeminnow in the San Juan River differs significantly from most stocking programs, as its initial aim was to reintroduce an essentially extirpated population and not merely enhance a depleted one. Eliminating any potential negative interactions between hatchery-reared and wild age-0 Colorado Pikeminnow will be pertinent not only for increasing the recruitment of these wild fish to adults for delisting, but also critical because wild fish are potentially reproductively superior to hatchery-reared fish (Christie et al. 2014; Clarke et al. 2016) and occupy higher trophic positions (Quinn et al. 2012; Kaeriyama et al. 2014). Continued augmentation of the Colorado Pikeminnow population with hatchery-reared age-0 fish when wild age-0 fish are present creates a second information need for the SJRIP:

Information Need 2: Ecological effects of hatchery-reared Colorado Pikeminnow on wild Colorado Pikeminnow when both are present in the system during early life stages (i.e., age-0 and age-1).

This study is designed to specifically address **Information Need 1** as described above. Field verification of the efficacy of using calcein marking to field identify age-1 hatchery-reared Colorado Pikeminnow is vitally important for assessing recovery of the species in the San Juan River while augmentation continues. If field detection of the calcein mark through the first year post-stocking is deemed insufficient to accurately identify hatchery-reared Colorado Pikeminnow in the system, then alternative batch marking techniques must be investigated. In addition to addressing **Information Need 1**, this study will also attempt to address **Information Need 2**. Elucidating any potential negative interactions between hatchery-reared and wild age-0 Colorado Pikeminnow is important for assessing the utility of the current augmentation plan. Ecological interactions between hatchery-reared and wild fish would preferably be assessed using a substitutive, or at least an additive, study design, but the stochastic nature of the San Juan River hydrograph and the improbability of obtaining sufficient replicates precludes these types of experiments. Even in the absence of definitively detecting negative effects of hatchery-reared Colorado Pikeminnow on wild age-0 Colorado Pikeminnow, the second portion of this study will provide information on habitat use, factors affecting condition, and distribution of age-0 Colorado Pikeminnow post-winter in the San Juan River.

LINKS TO LONG-RANGE PLAN

The SJRIP Long-Range Plan is designed to identify and implement specific actions which will contribute to the recovery of Colorado Pikeminnow and Razorback Sucker in the basin (SJRIP 2016). This project is designed to inform future augmentation plans and protocols for Colorado Pikeminnow (Tasks 1.1.1.1 and 1.1.2.2). Results from this project will also help to increase information on rearing habitat use (Task 4.1.2.2) and early success (Task 4.1.5.1) of stocked and wild age-0 Colorado Pikeminnow.

STUDY GOALS AND OBJECTIVES

The specific goals and objectives for this project include:

- Information Need 1: Efficacy of calcein marking for distinguishing hatchery-reared age-0 and age-1 Colorado Pikeminnow in the field
  - Objective 1A: Determine the difference in the detectability of the calcein mark on external structures between field and laboratory conditions
  - Objective 1B: Determine the retention of the calcein mark from pre-runoff to fall sampling
- Information Need 2: Ecological effects of hatchery-reared Colorado Pikeminnow on wild Colorado Pikeminnow when both are present in the system during early life stages (i.e., age-0 and age-1)
  - Objective 2A: Evaluate the effects of density on the condition of age-1 Colorado Pikeminnow in zero velocity habitats
  - Objective 2B: Determine the effects of the density of hatchery-reared age-1 Colorado Pikeminnow on the condition of wild age-1 Colorado Pikeminnow in zero velocity habitats

STUDY AREA

The study area for this project includes the San Juan River from Shiprock, NM (River Mile 147.8) downstream to Clay Hills, UT (River Mile 2.9). This section of river includes five different geomorphic reaches, Reach 1 through Reach 5.

SAMPLING METHODS AND DATA ANALYSIS

Calcein Marking and age-0 Colorado Pikeminnow Stocking

All hatchery-reared age-0 Colorado Pikeminnow will be calcein marked at Southwestern Native ARRC using an osmotic induction procedure developed by Mohler (2003). All fish will be placed in a 3.5% salt bath for 3.5 min and then transferred to a 1% calcein bath for 5 min. All marked fish will then be stocked following the procedures outlined in "Augmentation of Colorado Pikeminnow (*Ptychocheilus lucius*) in the San Juan River: Phase II, 2010-2020" (Furr 2010).

Pre-runoff Fish Sampling

One sampling trip will occur before spring runoff (i.e., late March or early April) in 2018, 2019, and 2020. All zero velocity habitats (i.e., backwaters and embayments) greater than 30 m^2 will be sampled when encountered. Habitats will be closed off using a block net and river mile, geographic coordinates (UTM NAD83), and water quality parameters (e.g., dissolved oxygen, conductivity, and temperature) will be recorded. A 3.0 m x 1.8 m (3.0 mm heavy duty Delta untreated mesh) drag seine will be used to repeatedly sample the backwater until no fish are captured in three consecutive seine hauls. After sampling is completed, the area of the zero velocity habitat will be determined by measuring its length and width at five equally spaced locations. Depth and substrate will be taken at 10 random locations within the habitat to determine mean and maximum depth. The percent cover (i.e., large woody debris, inundated vegetation) available within the zero velocity habitat will be estimated to the nearest 5%.

All captured fish will be held in 5 gal buckets until sampling is complete and then identified to species and enumerated. All native species except age-1 Colorado Pikeminnow will be released and all nonnative species removed from the system. Age-2+ Colorado Pikeminnows and Razorback Suckers will be measured (total length [TL] and standard length [SL]) to the nearest mm, weighed to the nearest g, and scanned for a PIT tag. A 12-mm PIT tag will be implanted in any endangered species ≥ 150 mm TL if one is not detected. All age-1 Colorado

Pikeminnow will be examined for a calcein mark on the head and fins using a handheld SE-MARK detector light (Elle et al. 2010), identified as marked or unmarked, and measured for TL and weighed. A tarp will be used to block direct sunlight and assess its usefulness for detecting marks. A subsample of up to 50 fish, but no less than 10 fish, with equal numbers of marked and unmarked fish, will be frozen using dry ice and labeled by sample site and their identified marking group for processing in the laboratory. No more than 400 age-1 Colorado Pikeminnow will be preserved in any year of sampling. We expect the number of age-1 Colorado Pikeminnow preserved in any year to be a very small proportion (about 0.01) of the entire population because capture probabilities for these fish are very low and we are sampling a very small amount of the habitat they occupy (Zeigler et al. 2017).

Fall Sampling

Age-1 Colorado Pikeminnow will be collected during annual fall small-bodied fishes sampling following the procedures as outlined in San Juan River Basin Recovery Implementation Program Monitoring Plan and Protocols (SJRIP 2012). Fish will be frozen using dry ice after being measured (TL) and weighed in the field. No more than 50 age-1 Colorado Pikeminnow will be preserved in any year of sampling.

Data Q/A and Q/C Procedures

After data collection, all original field notes will be error checked. Data will be entered into Excel spreadsheets and cross-checked with original field notes by a different biologist. Data queries will be conducted within the Excel spreadsheet to identify and rectify any additional errors.

Objective 1A: Determine the difference in the detectability of the calcein mark on external structures between field and laboratory conditions

Detectability of the calcein mark on external structures will be tested by comparing the number of field identified marked fish to the number of laboratory identified marked fish. All age-1 Colorado Pikeminnow preserved during pre-spring runoff and fall sampling will be returned to the laboratory and thawed. Each fish will be reexamined for an external calcein mark using the visual reader to confirm field identifications. Any incorrect identifications (i.e., marked or unmarked) will be noted. Fish will be blotted dry, measured (TL) to the nearest 0.1 mm, and weighed to the nearest 0.1 g. Otoliths from each preserved specimen will then be removed and examined for a calcein mark using a visual reader and any incorrect field identifications noted. A Chi-square test will be used to test if the number of fish identified as marked in the field differs from the number of fish identified as marked in the laboratory using otoliths. If the proportion of laboratory identified marked fish significantly differs ($\alpha = 0.05$) from the proportion of field identified marked fish it will be assumed that external mark loss occurred. It is highly probable that field identification will never be 100% and we propose that a mean 90% correct field identification over the three year study would be adequate for continuation of calcein as a marking technique for hatchery-reared age-0 Colorado Pikeminnow.

Objective 1B: Determine if the retention of calcein mark on external structures remains similar between the pre-runoff and fall sampling

To assess the retention of the external calcein mark, the number of correct mark identifications will be compared for fish captured in the pre-runoff and fall sampling. The mark identifications as described in *Objective 1A* will be used to address *Objective 1B*. The proportion of correct identifications from the fall sampling will be tested to determine if it is less than the proportion of correct identifications in the spring sampling. A statistically significant ($\alpha = 0.05$) difference would indicate that the number of correct external marks decreased from the pre-spring runoff sampling to the fall sampling due to external mark fading or loss.

Objective 2A: Evaluate the effects of density on the condition of age-1 Colorado Pikeminnow in zero velocity habitats

After the completion of the laboratory procedures to determine incorrectly identified marked or unmarked fish, the condition of each preserved fish will be determined by calculating the relative fat content following the procedures as outlined by Tobler (2008). The visceral organs of each fish will be removed, the fish weighed, and dried at 60°C for 3 d. Dried fish will be weighed and placed in separate vials. Lipids will be extracted using four 24 hr extractions in petroleum ether (Heulett et al. 1995; Tobler 2008). After extractions, fish will be dried and reweighed using the above procedure. Petroleum ether removes storage lipids and the difference between pre- and post-extraction weight represents relative fat content, a proxy for individual fish body condition (Tobler 2008).

A second condition factor, the relative condition factor (K_n), will also be calculated for each individual fish using weights taken before visceral organs are removed. The relative condition factor will be calculated as

$$K_n = (W/W') \times 100$$

where W is the individual weight and W' is the predicted length-specific weight based on log10 transformed data (Pope and Kruse 2007). A fish which is considered to be in good condition is expected to have a K_n of 95 - 105. Weight-length relationships used for the calculation of K_n will be confined to only age-1 Colorado Pikeminnow captured during the pre-spring runoff sampling during this study. Given the expected small size range (50 – 100 mm TL) of age-1 Colorado Pikeminnow during pre-spring runoff sampling, we expect K_n to provide a valid measure of an individual fish's condition. Furthermore, K_n can be compared to the relative fat content of each individual to assess the validity of both condition metrics.

General linear models (GLMs) will be used to assess the influence of density on the condition (i.e., K_n and relative fat content) of age-1 Colorado Pikeminnow such that condition is the response variable and density is the explanatory variable. Use of GLMs will allow for the inclusion of other explanatory variables that may describe differences in condition of age-0 Colorado Pikeminnow among zero velocity habitats (e.g., density of natives and nonnatives, average depth of habitat) and between years (e.g., mean winter discharge, winter floods, mean winter water temperature).

Objective 2B: Determine the effects of the density of hatchery-reared age-1 Colorado Pikeminnow on the condition of wild age-1 Colorado Pikeminnow in zero velocity habitats.

If wild fish are present, GLMs will be used to assess the effects of the density of hatchery-reared age-1 Colorado Pikeminnow on the condition of wild age-1 Colorado Pikeminnow. It will be assumed that any fish which are not identified as marked in the laboratory are wild. The GLM will be structured such that the condition of age-1 wild fish is the response variable and the density of age-1 hatchery-reared Colorado Pikeminnow is the explanatory variable. Additional explanatory variables that may describe differences in condition of wild age-0 Colorado Pikeminnow between zero velocity habitats (e.g., density of natives and nonnatives, average depth of habitat) and between years (e.g., mean winter discharge, winter floods, mean winter water temperature) may also be included in the models.

REPORTING

Short draft annual reports with information on sampling locations and number of fish captured will be provided by 31 March 2019 and 2020 with final annual reports provided by 30 June of both years. A draft final report with information on sampling locations and number of fish captured across all years and final analyses of the data will be provided by 31 March 2021 and the completed final report by 30 June 2021. Short update presentations may be given at Biology Committee meetings in February of 2019 and 2020 and a presentation on the projects final results given at the February 2021 Biology Committee meeting and the SJRIP annual meeting in May 2021. All PIT tag data for endangered species will be provided to the SJRIP Program Office (PO) by 31 December of each year. All data collected by this SOW will be provided to the PO by 30 June 2021.

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NMDGF FY 2018 Budget

Sampling

Personnel

Tasks - Sample all zero velocity habitats on the San Juan River from Shiprock, NM (RM 147.8) to Clay Hills, UT (RM 2.9); 11 field days projected at 12 hours of work per day = 132 hours (88 hrs regular and 44 hrs overtime).

Project Leader (1)		
88 hrs regular @ $46.34/hr ($33.69/hr (base salary) + $12.66/hr (benefits))	$	4,078
44 hrs overtime @ $69.52/hr ($46.34/hr * 1.5 (time-and-a-half)	$	3,059
Project Biologist (1)		
88 hrs regular @ $37.13/hr ($26.99/hr (base salary) + $10.14 (benefits))	$	3,267
44 hrs overtime @ $55.69/hr ($37.13/hr * 1.5 (time-and-a-half))	$	2,450
Sub-total	**$**	**12,854**

Per Diem

4 days @ $85/day (standard NM in-state rate) * 2 biologists	$	680
7 days @ 115/day (standard NM out-of-state rate) * 2 biologist	$	1,610
Sub-total	**$**	**2,290**

Vehicles

Round-trip to Clay Hills, UT – 950 miles @ $0.55/mile	$	523
Sub-total	**$**	**523**
Sampling Sub-total	**$**	**15,667**

Field Equipment & Supplies

Cooler @ $350	$	350
Whirlpacks (500) @ $50.00/per 500	$	50
Blocknets 2 @ $ 150	$	300
Equipment Sub-total	**$**	**700**
Sampling Sub-total	**$**	**16,367**

<u>Specimen Management</u>

<u>Personnel</u>

Tasks - Processing (sorting, identification, and data-entry) of preserved Colorado Pikeminnow samples lipid extraction; 25 days of in the laboratory at 8 hours of work per day = 200 hours.

Project Leader (1)		
200 hrs regular @ $46.34/hr ($33.69/hr (base salary) + $12.66/hr (benefits))	$	9,268
Project Biologist (1)		
200 hrs regular @ $37.13/hr ($26.99/hr (base salary) + $10.14 (benefits))		7,426
Sub-total	**$**	**16,694**

<u>Equipment & Supplies</u>

20 L Petroeum Ether @ $60 per L	$	1,200
1 Laboratory grade oven	$	1,300
Miscellaneous lab supplies	$	1,000
Sub-total	**$**	**3,500**
Specimen Management Sub-total	**$**	**20,194**

<u>Data Management/Analysis and Report Preparation</u>

<u>Personnel</u>

Tasks – Data management and QA/QC, data analysis and synthesis, table and graph preparation, report drafting and revision; Project Leader (15 hrs) and one Project Biologist (80 hrs each).

Project Leader (1)		
15 hrs regular @ $46.34/hr ($33.69/hr (base salary) + $12.66/hr (benefits))	$	695
Project Biologist (1)		
80 hrs regular @ $37.13/hr ($26.99/hr (base salary) + $10.14 (benefits))	$	2,970
Data Management/Analysis & Report Preparation Sub-total	**$**	**3,665**

<u>FY 2018 Total</u>

Sampling Sub-total	$	16,367
Specimen Management Sub-total	$	20,194
Data Management/Analysis & Report Preparation Sub-total	$	3,665
Total	**$**	**40,226**

NMFWCO FY 2018 Budget

Sampling

Personnel

Tasks - Sample all zero velocity habitats on the San Juan River from Shiprock, NM (RM 147.8) to Clay

Fish Biologist (GS 11-7)		
88 hrs regular @ $43.57/hr ($34.71/hr (base salary) + $8.86 hr (benefits))	$	3,834
44 hrs overtime @ $65.36/hr ($43.57hr * 1.5 (time-and-a-half)	$	2,876
Fish Biologist (GS 9-7)		
88 hrs regular @ $36.26/hr ($28.69/hr (base salary) + $7.57 (benefits))	$	3,191
44 hrs overtime @ $54.39/hr ($36.26/hr * 1.5 (time-and-a-half))	$	2,393
Sub-total	**$**	**12,294**

Vehicles

Round-trip to Clay Hills, UT – 950 miles @ $0.55/mile	$	523
Sub-total	**$**	**523**
Sampling Sub-total	**$**	**12,817**
Sampling Sub-total	**$**	**12,817**

Data Management/Analysis and Report Preparation

Personnel

Tasks – report drafting and revision

Fish Biologist (GS 11-7)		
40 hrs regular @ $43.57/hr ($34.71/hr (base salary) + $18.86/hr (benefits))	$	1,743
Supervisory Fish Biologist (GS 13-6)		
8 hrs regular @ $61.70/hr ($48.10/hr (base salary) + $13.60 (benefits))	$	494
Data Management/Analysis & Report Preparation Sub-total	**$**	**2,237**

FY 2018 Total

Sampling Sub-total	$	12,817
Data Management/Analysis & Report Preparation Sub-total	$	2,237
Total	**$**	**15,054**

FY 2018 Total Budget

NMDGF	$	40,226
USFWS NMFWCO	$	15,054
FY 2018 Project Total	**$**	**55,280**