Project Title
Augmenting an augmentation program: proposed egg and larval stocking of Colorado Pikeminnow in the San Juan River

Bureau of Reclamation Agreement Number:

Reclamation Agreement Term:

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

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

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

Relationship to LRP:

This project's actions are related to Long-Range Plan Task 1.1.2.1, which is to annually stock CPM and RBS and opportunistically stock any excess fish and Task 1.2.1.2, which is to identify, describe, and implement strategies for improving survival, retention, and genetic diversity of stocked CPM and RBS, including acclimation prior to stocking, size of fish stocked, time and location of stocking, physiological conditioning, predator avoidance, and brood stock genetic augmentation.

Study Background:

Recovery criteria for Colorado Pikeminnow requires reaching a minimum population of wild individuals either through augmentation or natural recruitment. In order to reach this goal, augmentation has been required because wild recruitment of Colorado Pikeminnow in the San Juan River remains low (Clark et al. 2018). Consequently, the number of fish persisting is dependent on the number of stocked fish that survive to recruitment age. The Southwestern Native Aquatic Resources and Recovery Center (Southwestern ARRC) spawns Colorado Pikeminnow during late May to early June of every year to meet stocking commitments for the San Juan River Recovery Implementation Program (SJRIP). Current annual production is approximately 12,000 age-1 fish. Grow-out of these fish to older ages requires both increased time in captivity and the need for adequate on-site rearing space. Given that hatchery space is limited, it is currently difficult to substantially increase the number of fish stocked. Moreover, captive environments can alter fish behavior and subsequent survival, therefore stocking earlier life stages may provide the opportunity to increase stocking numbers while reducing the effects of artificial environments on stocked individuals. We propose evaluating the suitability of stocking eggs and larvae for three years (FY23-25) as a management strategy to increase the number of Colorado Pikeminnow in the San Juan River. This proposed effort is in addition to the pilot approved for 2022 (Table 1).

Table 1. Proposed study timeline for stocking evaluation and subsequent monitoring. This table includes the approved pilot with an additional three years. Genetic parentage assignment will include larval samples used for Nb estimates; however, the collection of additional samples (> 120 larvae) will dictate what is necessary to complete this work in a future version of this scope of work, which is denoted as to be determined (TBD).

Sampling Event	2022	2023	2024	2025	2026	2027
Egg and Larval Stocking	X	X	X	X		
Larval Sampling	X	X	X	X		
Small-Bodied Monitoring	X	X	X	X		
Adult Monitoring		X	X	X	X	
Effective Number of Breeders (N_b)	X	X	X	X	X	X
Parentage Analysis		TBD	TBD	TBD	X	X

Annual spawning at the Southwestern ARRC usually comprises 25-30 pairs of Colorado Pikeminnow to meet the minimum genetic diversity requirements outlined for the Program. For the purposes of this project, we suggest increasing spawning to 40 pairs for the first year of the study. This relatively small increase in the number of spawned pairs should be able to conservatively produce 250,000 eggs and 250,000 larvae. While survival of these developmental stages in the San Juan River is unknown, these stocking sizes were chosen to limit hatchery demands while ensuring sizes are reasonably large to increase the likelihood of at least some individuals surviving. Future years, however, could request an increase in these numbers based on results obtained from year one.

Our proposed increase in the number of pairs spawned will also allow us to genetically track the success of each stocking treatment (i.e., eggs or larvae) as 20 unique parental pairs can be used for the egg treatment while the remaining 20 pairs would be used to stock larvae. If either treatment proves more successful than the other, then this slight increase in parental pairs will ensure genetic management considerations are met by not unintentionally skewing the number of parents that contributed to either treatment, which could reduce genetic diversity within that cohort. In addition, these genetic data would allow us to mitigate potential impacts to long-term monitoring datasets because we will be able to genetically determine wild-spawned fish from hatchery stocked eggs or larvae. For example, larval surveys currently preserve all field collections in 95% ethanol, with results being used to monitor in-stream reproduction through the presence of larval fish and through estimates of the effective number of breeders (N_b; Saltzgiver and Mussmann 2022). Genetic analysis of larval collections can determine wild-spawned fish from hatchery produced fish through parentage analysis. These determinations can then be used to estimate the proportion of hatchery fish collected in larval surveys (which will also be removed from the N_b analyses to ensure accurate estimates of annual contributions by "wild fish"). Similarly, fin clips collected from age-0 or age-1 fish captured during small-bodied or adult monitoring can be analyzed genetically to determine their origin (i.e., wild-spawned vs. egg/larval stockings).

Objectives:

The objective of this study is to determine whether stocking Colorado Pikeminnow eggs and larvae is a viable way to increase augmentation of this species in the San Juan River. Success will be measured through genetic parentage assignment of Colorado Pikeminnow collected in the field.

Study Area:

The proposed stocking locations include the Phase II site at approximately river mile 135 and just above the Four Corners Bridge at approximately river mile 119. If these sites are unsuitable, alternative sites include Mosquito Point near river mile 130 and the reconstructed secondary channel near the Shiprock Bridge at approximately river mile 147 (Figure 1). These sites were chosen because they can be accessed easily, they are relatively high in the system, and they provide inflow to large secondary channels with large amounts of low-velocity habitats. These proposed sites will be evaluated approximately one week prior to stocking to ensure secondary channels are connected. If these sites are unsuitable, the alternative sites will be used (Figure 2).

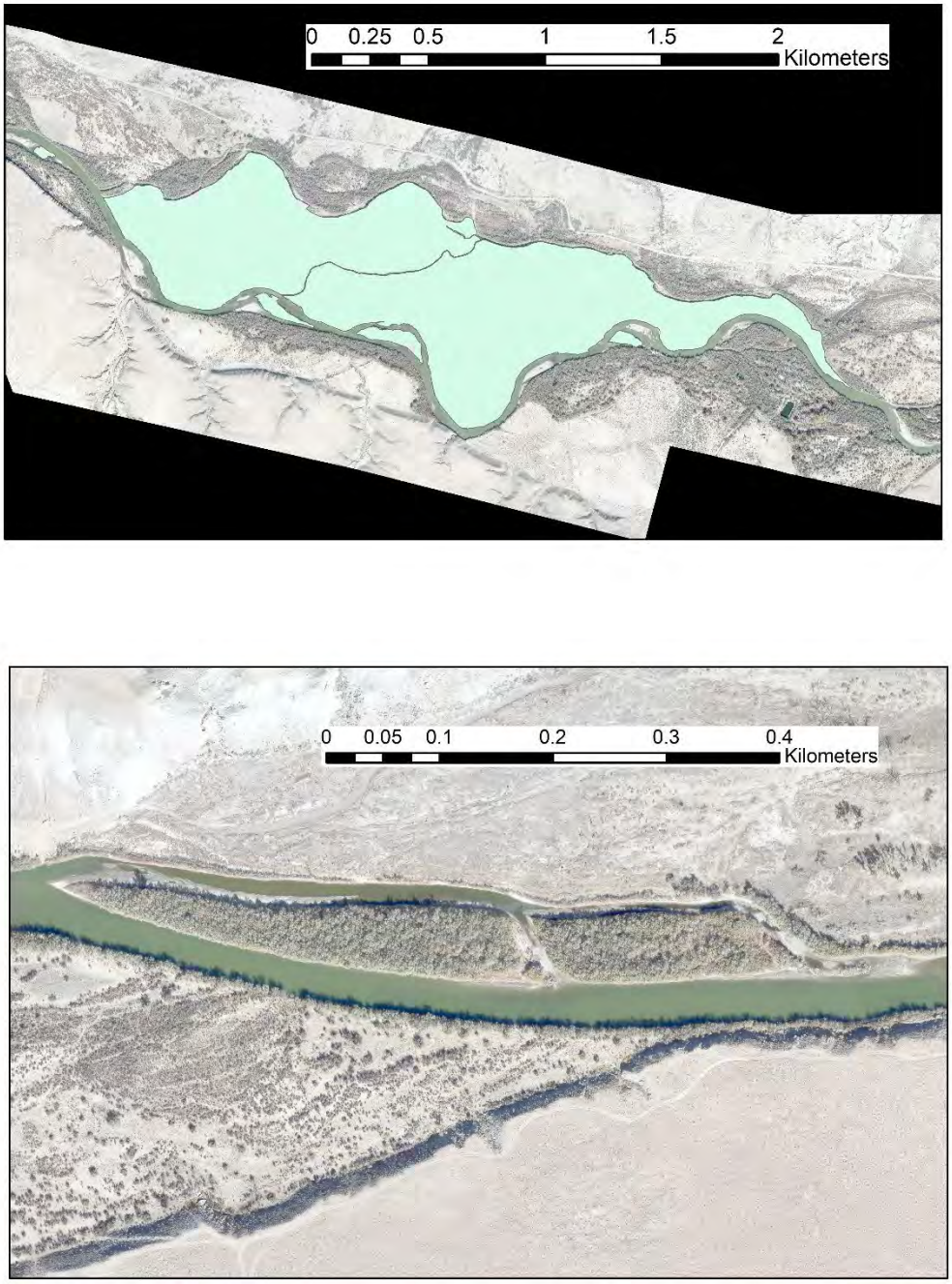

Figure 1. Aerial photography of the San Juan River. The top panel shows the Phase II site at approximately river mile 135. The green layer shows the islands in the secondary channel complex. The lower panel shows the proposed stocking site above the Four Corners Bridge at approximately river mile 119. Eggs will be placed in the upstream riffles of these sites and larvae will be stocked into the zero-velocity habitats in the secondary channels.

Figure 2. Aerial images of two potential alternative stocking sites. The top panel is Mosquito point near RM 130 which forms a large backwater on river right during low flows. The bottom panel is the new secondary channel restoration site near Shiprock. This secondary channel is on river left indicated by the small purple shaded area. Photos are from 2020 and this site may not be connected at certain flows.

Study Methods/Approach:

We will conduct two separate stockings (Table 2). The first stocking effort will stock eggs produced from 20 of the parental pairs used for the egg stocking treatment. Eggs will be held at the Southwestern ARRC for approximately 72 hours post-spawning until they reach the ‘eye up’ stage so that estimates of viability rates of stocked eggs can be obtained (e.g., 60% of eggs were successfully fertilized). These estimates will ensure that the lack of detection of larvae from stocked eggs in the river was not related to poor fertility in the hatchery. Eggs in the ‘eye up’ stage will be picked up at the Southwestern ARRC approximately 3 days post-spawning and tempered at each proposed site. Eggs will be held in custom built egg boxes (Conley et al., 2019), which will ideally be placed on gravel substrate near a riffle to provide adequate flow for providing oxygen and preventing sediment deposits on eggs. Egg boxes will be monitored for approximately 5 days before retrieval. The second stocking treatment

will entail transporting larval fish from the Southwestern ARRC approximately 120-144 hours post-spawning, which will be tempered and released at the same stocking sites.

Table 2. Tentative timeline for Colorado Pikeminnow egg and larval stocking, field sampling and genetic analysis in 2023.

Sampling Event	Dates	PI
Larval Trip 1	mid-late April	ASIR
Larval Trip 2	mid-May	ASIR
Hatchery Spawning	late May – early June	Southwestern ARRC
Egg Stocking	late May – early June	NMFWCO
Larval Stocking	late May – early June	NMFWCO
Larval Trip 3	mid-June	ASIR
Larval Trip 4	Mid-July	ASIR
Larval Trip 5	late July	ASIR
Small-Bodied Monitoring	Fall 2023	NMDGF
Adult Monitoring	Fall 2023	GJFWCO
Genetic Analysis	TBD	Southwestern ARRC

Ideal stocking temperatures for Colorado Pikeminnow eggs and larvae is approximately 18°C, with natural temperatures in the river near this during our proposed stocking dates (Figure 3); however, the river's spring hydrology will likely determine the temperature regime experienced by the stocked eggs and larvae. In general, high spring runoff will lower water temperatures compared to a lower spring runoff. Multiple data loggers will be placed within each stocking site in order to monitor and compare stocking sites temperatures between and within years.

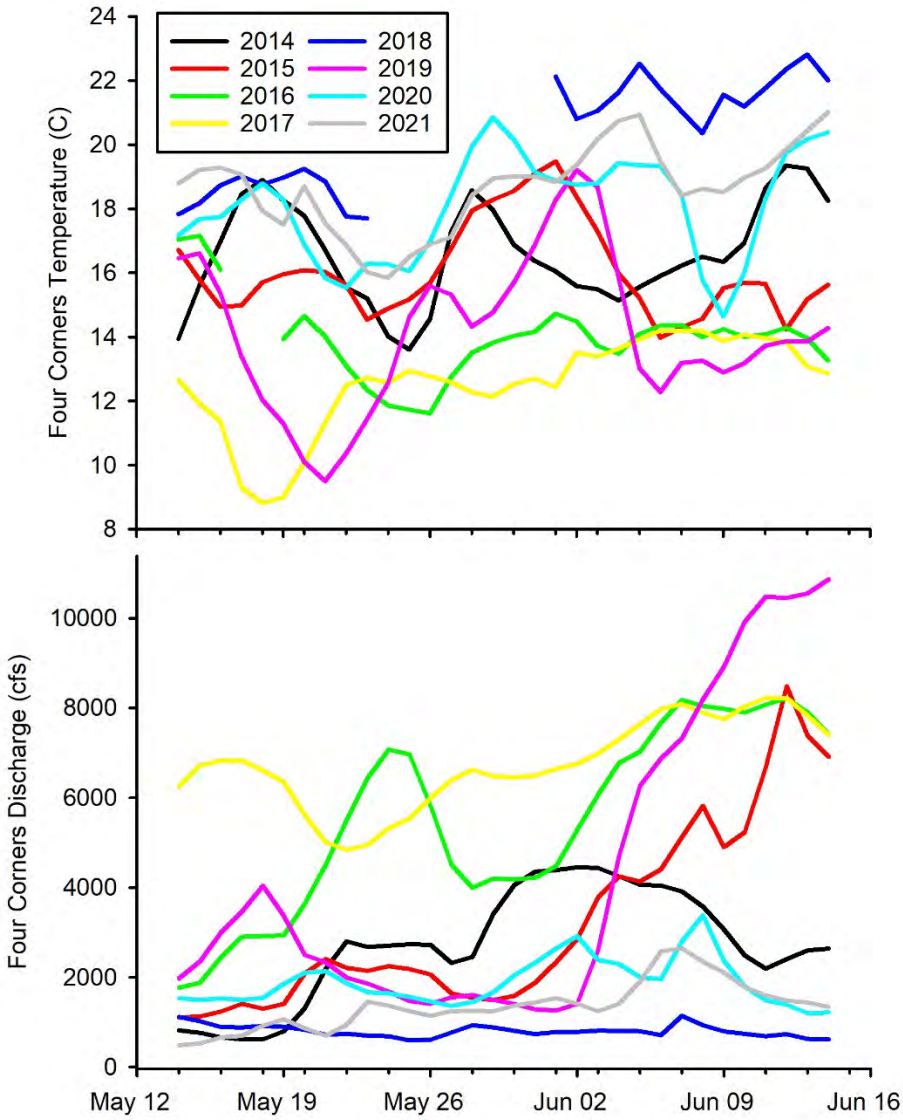

Figure 3. San Juan River discharge and temperature at the Four Corners Bridge. Ideal temperatures for eggs and larval stocked Colorado Pikeminnow are near 18°C.

Metrics to define the success of these stockings are difficult to determine. However, we assume that detection of any individuals from these stockings through the SJRRIPs annual monitoring efforts would indicate non-negligible positive effects of this action towards recovery. Identification of egg and larvae stocked individuals will be determined through genetic analysis of parental pairs and wild-caught age-0 and age-1 Colorado Pikeminnow. All broodstock parents used at time of spawning will be fin clipped and each individual cross (i.e., PIT tag and fin clip) will be recorded (e.g., F1 x M1, F2 x M2). A small subsample of larval fish from each stocking treatment will be collected and stored in 95% ethanol for both a genetic (Southwestern ARRC) and aging reference (American Southwest Ichthyological Research); samples will be evaluated if needed. For example, these samples could be used to back calculate spawning dates since hatchery spawning will likely occur prior to wild spawning; these data could be used as a precursory tool for separating wild and hatchery fish from larval collections.

Tissue samples from Colorado Pikeminnow collected during larval fish surveys (ASIR), small-bodied monitoring (New Mexico Department of Game and Fish) and adult monitoring (Grand Junction FWCO) will be used to track stocking success through genetic parentage assignment (Table 1). To do this, all hatchery parents and wild-caught age-0 and age-1 fish will be genotyped using either Single Nucleotide Polymorphism (SNPs) or microsatellites and parental reconstruction will be conducted using Sequoia (Huisman 2017). For this proposal, genetic parentage assignment can be conducted through to age-1 because field sampling will occur prior to the stocking of PIT tagged age-1 fish (October/ November). After this, however, it will be impossible to determine the difference between the stocking treatments (i.e., eggs and larvae) and tag loss of stocked age-1 fish in subsequent years (i.e., ≥ age-2). Genetic methods will be submitted as a future scope of work (e.g., FY24), as the work required will depend on field collections obtained during 2023.

Deliverables and Schedule:

Egg and larval stockings will occur during late-May to early-June for each year (Table 1). The completion of genetic analysis of collected larvae, juvenile, and broodstock fin clips is to be determined based on samples collected in the field. A formal proposal of this work will be submitted within future scopes of work (i.e., FY24). An update of results found during 2023 will be provided to the San Juan River Basin Recovery Implementation Program during the February meeting.

Budget Summary:

Table 2. Summary of office budgets for fiscal year 2023.

Fiscal Year	NMFWCO
2023	$30,166
Total	$30,166

Reviewers:

References:

Clark, S.R., Conner, M.M., Durst, S.L. and Franssen, N.R., 2018. Age-specific estimates indicate potential deleterious capture effects and low survival of stocked juvenile Colorado Pikeminnow. North American Journal of Fisheries Management, 38(5), pp.1059-1074.

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Huisman, J. 2017. Pedigree reconstruction from SNP data: parentage assignment, sibship clustering and beyond. Molecular Ecology Resources 17(5):1009-1024.

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