Gila River Basin Native Fishes Conservation Program: Arizona Game and Fish Department's Native Fish Conservation Efforts During 2019

Cooperative Agreement R16AC00077 Between Bureau of Reclamation and Arizona Game and Fish Department Annual Report

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Table of Contents

OVERVIEW	1
PERFORMANCE MEASURES	2
GENERAL ACTIVITIES	3
PRIORITY ACTIONS	4
General methods	4
Acquire Spikedace, Loach Minnow and rare populations of other native fish (Task AZ-2003-2)).7
Muleshoe ecosystem stream and spring repatriations (Task AZ-2003-1)	9
Fresno Canyon repatriations (Task AZ-2006-1)	.14
Gila Topminnow stockings (Task AZ-2002-1)	.16
Spring Creek (Oak Creek tributary) repatriations (Task AZ-2013-1)	.31
Blue River native fish restoration (Task AZ-2002-3)	.34
Assess potential repatriation waters (Task AZ-2008-1)	.39
Aquatic Research and Conservation Center O&M (Task HA-2006-2)	.57
Expand Roundtail Chub population in Harden Cienega Creek (Task AZ-2014-1)	.45
Eagle Creek repatriations (Task AZ-2018-1)	.46
Red Tank Draw native fish restoration (Task AZ-2016-2)	.48
Sharp Spring native fish restoration (Task AZ-2016-3)	.52
Boyce Thompson Ayer Lake native fish restoration (Task AZ-2000-1)	.54
Upper Verde River Native Fish Restoration (Task AZ-2020-1)	.56
PROJECTS REMOVED FROM PRIORITY LIST	.61
FIGURES	.67
TABLES	.94
APPENDICES1	.07

OVERVIEW

The Gila River Basin Native Fishes Conservation Program (Program; previously known as the Central Arizona Project [CAP] Fund Transfer Program) was developed to partially mitigate impacts of the CAP on Threatened and Endangered native fishes of the Gila River basin. The U.S. Fish and Wildlife Service (USFWS) concluded in a 1994 biological opinion that the CAP would be a conduit for transfers of nonnative fishes and other aquatic organisms from the lower Colorado River (where the CAP originates) to waters of the Gila River basin. That opinion identified the spread and establishment of nonnative aquatic organisms as a serious long-term threat to the status and recovery of native aquatic species, following a long history of habitat loss and degradation. Impacts of nonnatives include predation, competition, hybridization, and parasite and pathogen transmission.

The 1994 USFWS opinion concluded that operation of the CAP would jeopardize the continued existence of four native Threatened or Endangered fish species: Gila Topminnow Poeciliopsis occidentalis occidentalis, Spikedace Meda fulgida, Loach Minnow Rhinichthys cobitis, and Razorback Sucker Xyrauchen texanus. The Service also concluded that the CAP would adversely modify designated critical habitat of Spikedace, Loach Minnow, and Razorback Sucker. Five reasonable and prudent alternatives were specified: 1) construction and operation of barriers to prevent the spread of nonnative fishes from the CAP to native fish habitats, 2) monitoring of nonnative fish, 3) transfer of funds to USFWS to recover natives, 4) transfer of funds to USFWS to manage nonnatives and research to support that management, and 5) inform and educate the public about native fishes and the impacts caused by nonnative fishes. The transfer of funds under reasonable and prudent alternatives 3 and 4 became known as the CAP Funds Transfer Program. In a 2001 revision of the 1994 opinion, the reasonable and prudent alternatives became conservation measures. In a 2008 revision, the newly-listed endangered Gila Chub¹ Gila intermedia and Chiricahua Leopard Frog Lithobates chiricahuensis were added to the Program as species affected by operation of the CAP, and the Santa Cruz River drainage was added to its geographic scope.

The Program is funded by the U.S. Bureau of Reclamation (Reclamation), and is directed by the USFWS and Reclamation in cooperation with the New Mexico Department of Game and Fish (NMDGF) and Arizona Game and Fish Department (Department). Reclamation began taking over administration of the funding Program from USFWS in 2015. The Department and Reclamation finalized a one-year agreement (R16AC00077) in August 2016, which was modified and extended to five years in August 2017. The Program mission is to undertake and support conservation actions (recovery and protection) for federal/state-listed or candidate fish species native to the Gila River basin by implementing existing and future recovery plans for those fishes. There are

¹ In 2016, the American Fisheries Society and the American Society of Ichthyologists and Herpetologists reclassified and merged Roundtail Chub *Gila robusta*, Gila Chub *Gila intermedia*, and Headwater Chub *Gila nigra* into one species, the Roundtail Chub.

finalized and approved recovery plans for four of the five priority species, and a draft recovery plan for the Gila Chub (U.S. Fish and Wildlife Service 1984, 1991a, 1991b, 1998, 2002, 2015). There were several draft revised recovery plans for Gila Topminnow, one of which (USFWS 1999) was posted on the USFWS Ecological Services web site. The Loach Minnow and Spikedace recovery plans are being revised.

In addition to the fish and amphibian species specified above, other species mentioned in this report include: Longfin Dace Agosia chrysogaster, Speckled Dace Rhinichthys osculus, Roundtail Chub Gila robusta, Woundfin Plagopterus argentissimus, Desert Pupfish Cyprinodon macularius, Desert Sucker Catostomus clarki, Sonora Sucker Catostomus insignis, Green Sunfish Lepomis cyanellus, Smallmouth Bass Micropterus dolomieu, Tilapia Oreochromis sp., Channel Catfish Ictalurus punctatus, Black Bullhead Ameiurus melas, Fathead Minnow Pimephales promelas, Goldfish Carassius auratus, Western Mosquitofish Gambusia affinis, Gila Trout Oncorhynchus gilae, and Brown Trout Salmo trutta. Other aquatic species mentioned include Lowland Leopard Frog Lithobates yavapaiensis, Chiricahua Leopard Frog Lithobates chiricahuensis, American Bullfrog Rana catesbeiana, Sonora Mud Turtle Kinosternon sonoriense, Northern Crayfish Orconectes virilis, Red Swamp Crayfish Procambarus clarkia, Narrow-headed Gartersnake Thamnophis rufipunctatus, and Northern Mexican Gartersnake Thamnophis eques.

This report summarizes Program work performed by the Department during 2019. For each priority action, work completed during 2019 is presented, followed by recommendations.

PERFORMANCE MEASURES

Cooperative Agreement R16AC00077 between U.S. Bureau of Reclamation and Arizona Game and Fish Department specified the following annual performance measures.

1. Complete a minimum of three repatriation stockings and one non-indigenous species control action.

Results: During 2019 Department staff completed repatriation stockings into 12 waters (Appendix 1). Also during the performance period Department staff completed nine non-indigenous species control actions: two nonnative fish removal efforts in Red Tank Draw, five in Rarick Canyon, one in the Blue River, and one in Redfield Canyon

2. Monitor fish to determine if population(s) have established at all locations where repatriations were attempted within the previous 3 to 5 years, or other period as agreed upon by the CAP Technical and Policy committees. The number of years to monitor is based on life-span and age-at-maturity of the species, and is three years for Gila

Topminnow and Desert Pupfish, and five years for Spikedace, Loach Minnow, and Roundtail Chub¹.

Results: During 2019, Department staff conducted post-stocking monitoring of 34 populations (Appendix 2): 2 Loach Minnow, 4 Spikedace, 3 Desert Pupfish, 20 Gila Topminnow, and 5 Roundtail Chub². Sites where native fish were repatriated and subsequent monitoring information indicated that the species had established populations are reported in Appendix 3.

3. Monitor to determine if non-indigenous fish have been eradicated where non-indigenous control was attempted within the previous year or other period as agreed upon by the Technical and Policy committees.

Results: During 2019, Department staff monitored four locations where nonnative fish removals have been implemented: Blue River, Redfield Canyon, Spring Creek, and Red Tank Draw.

4. Attempt to spawn all Loach Minnow and Spikedace populations held at the Department's Aquatic Research and Conservation Center (ARCC).

Results: In 2019, all Loach Minnow and Spikedace populations at ARCC spawned. ARCC produced 4,250 Aravaipa Creek Spikedace, 2,404 upper Gila River Spikedace, 1,132 Gila River Forks Spikedace, 713 Blue River Loach Minnow, 1,398 Aravaipa Creek Loach Minnow, 601 San Francisco River Loach Minnow, and 665 Gila River Forks Loach Minnow.

GENERAL ACTIVITIES

Department staff administered and managed Program projects identified in the agreement. Staff entered data into survey and stocking datasets, and checked data for accuracy. Department staff finalized the 2018 annual report, began analyzing data and drafting the 2019 annual report, and drafted the 2020 annual work plan, Environmental Assessment Checklists, and monitoring plans for the Red Tank Draw watershed and Verde River Tanks. Staff coordinated with intra-agency staff, other agencies, and private landowners to continue work on existing projects and to develop potential new projects. Staff also developed and gave oral presentations at the 2019 joint annual meeting of the Arizona and New Mexico Chapter of the American Fisheries Society and the

¹ Including populations previously classified as Gila Chub.

² Four of the populations were previously classified as Gila Chub.

Wildlife Society and the 2019 annual Desert Fishes Council Meeting. The Program specialist also hired new staff.

PRIORITY ACTIONS

General methods

Fish Stockings: The Department coordinates with USFWS about locations to stock and sources and lineages of fish to use. Fish for repatriations were collected, transported, and stocked according to Department fish collection, transport, and stocking protocols (best management practice #4; AGFD 2011), and Hazard Analysis and Critical Control Point (HACCP) practices. Fish were collected from pre-determined waters inhabited by target lineages. Fish were collected using gear appropriate for the given water; typical gear types were seines, minnow traps, or electrofishing. Fish were placed into aerated 5-gallon buckets from which they were sorted to confirm species identity and assess condition. Fish were then transferred into transport coolers (100 qt. minimum) equipped with aerators and filled with well water treated with salt and Amquel®. At the repatriation site, the fish were transferred from the transport cooler back to aerated 5-gallon buckets and carried to the stocking location. Water quality characteristics in the buckets and the stocking location were measured. Conductivity (µS), salinity (mg/L), total dissolved solids (mg/L), pH, and water temperature (°C), were measured using a Hach® Combo meter, and dissolved oxygen (mg/L) using a Sper Scientific® dissolved oxygen meter. Fish were acclimated to stocking site conditions by exchanging 25 to 50% of transport bucket water with stream water, about every 10 minutes, until bucket temperatures were within two degrees of the stream. Fish were sorted a final time to verify species identity, assess condition, and determine a final count before being released into the stream.

Data recorded for stocking included: site name, date, time of arrival and stocking, participants, type of transport container, water quality in the tanks and site (water temperature, pH, conductivity) counts of individuals stocked, condition of fish, fish behavior after release, and number of mortalities.

Fish Surveys: Backpack electrofishing was used at 100-m transects (except in the Blue River where transects are 200-m long) to survey repatriated populations of Spikedace, Loach Minnow, and Roundtail Chub¹, and to assess habitats for fish repatriations. The number of transects sampled was determined by length of target reach, with a minimum of three transects for short reaches and at least 12% of the reach length in longer streams (e.g., there were twelve 200-m transects in the 18 km of the lower Blue River). A backpack electrofisher (Smith-Root; Model 12-B) was used to electrofish upstream through each transect in a single pass. Stunned fish were netted with dip nets (tear-drop shaped, 0.43 m x 0.37 m with 2 or 3 mm mesh). At the upstream end of each major mesohabitat type (pool, run, riffle, cascade) within each transect, fish were processed and data were recorded. Captured fish were identified to species and counted. All Spikedace, Loach

¹ Including chub populations previously classified as Gila Chub.

Cooperative Agreement R16AC00077: 2019 Annual Report - Final 03/24/2020

Minnow, Roundtail Chub¹ were measured to the nearest millimeter in total length (mm TL). Other species were counted within two size classes for small bodied fishes (\leq 40 and >40 mm TL for Speckled Dace and Longfin Dace; \leq 20 and >20 mm TL for Desert Pupfish and Gila Topminnow) and three size classes for large bodied fish (<50, 50-100, and >100 mm TL; e.g. Desert Sucker, Smallmouth Bass). With the exception of topminnow and pupfish, fish less than 20 mm TL were categorized as larvae. After processing, fish were released alive just downstream from where they were captured. Data recorded for each sampling effort included: site name, site location (GPS coordinates), length of site, date, time, participants, gear type, gear settings, gear dimensions, seconds shocked, species of fish captured, size class of fish, and counts of individuals within each species-size-class category.

Minnow traps or hoop nets baited with dry Gravy Train® dog food were used to survey for Gila Topminnow, Desert Pupfish, and some Roundtail Chub¹ populations. Promar® collapsible minnow traps (0.46 m long x 0.3 m wide, with 2 mm mesh) were used for Gila Topminnow and Desert Pupfish monitoring, whereas Promar® collapsible mini-hoop nets (0.85 m long x 0.3 m diameter circular hoops, with 9 mm mesh) were used for Roundtail Chub¹ monitoring. Typically a minimum of 10 traps were set in each location. Traps were set for a minimum soak time of two hours, and fish were processed and released alive back to the location of capture. Data recorded for each sampling effort included: site name, site location (GPS coordinates), date, time, participants, gear type, gear dimensions, set and pull times for each trap set, species of fish captured, size class of fish (≤ 20 mm or >20 mm), and counts of individuals within each species-size-class category.

Evaluation of Species Establishment: The goal of repatriation efforts is to establish populations of Spikedace, Loach Minnow, Gila Topminnow and Roundtail Chub¹ to contribute to recovery of these species. A species is considered to have established (a successful repatriation) when it is reproducing to the point where it is self-sustaining (Griffith et al. 1989, Bright and Smithson 2001, Armstrong and Seddon 2007). Similarly, the Spikedace recovery plan (USFWS 1991) describes criteria for establishment with characteristics of abundance, age-class structure, and recruitment in the range of natural variation. To assess this goal, post-stocking monitoring data were collected for each repatriated species to evaluate species presence, an index of abundance, population size structure, and dispersion. Arguably, the two most important of these four measures for determining if a species has established are population size structure and an index of abundance.

The objectives of monitoring are to:

- 1. determine presence of repatriated fish species and non-native fish species;
- 2. evaluate trends in relative abundance (estimated as catch-per-unit effort) of the repatriated species, extant native fish species, and non-native piscivores;

¹ Including chub populations previously classified as Gila Chub.

- 3. evaluate size-structure of each population of fish species to detect reproduction and recruitment to the population;
- 4. determine if repatriated species have dispersed outside of the stocking area.

Presence of individuals during post-stocking monitoring is evidence that the species has persisted, but not in and of itself evidence of population establishment. Presence of juvenile fish is evidence of reproduction, and the proportion of the population that are juveniles is evidence of year-class strength. Size structure is used as an indicator of age-structure. Presence of age-0, age-1, and older size classes for several years in a row, and consistently high catch rates for several years in a row is an indication that a population has established. Capture of individuals beyond stocking locations is evidence of dispersal.

After stocking, a site is monitored for several years to determine whether or not the species has established a population. The number of years of monitoring was dependent upon species, and generally exceeded the life span of the species by at least one year. Two years may be sufficient to determine if Gila Topminnow and Desert Pupfish, which typically live only one to two years, have established a population. However, if no fish are detected in three consecutive monitoring events, the population may be considered extirpated (Weedman and Young 1995). Therefore, three years of post-stocking monitoring will be used for Gila Topminnow and Desert Pupfish. Spikedace and Loach Minnow can live three to four years, so monitoring for five years post-stocking should be sufficient to determine if the species has established a population, because all fish stocked will have died by that time. Roundtail Chub¹ typically live about seven years. However, a yearly examination of size structure for five years after stocking is likely sufficient to determine if Roundtail Chub¹ are established. Repatriated populations will be monitored periodically after establishment by one or more of the cooperators for at least 10 years to determine population persistence and viability.

Nonnative Piscivore Removal: Nonnative fishes were typically removed using traps and electrofishing, except in the Blue River, where snorkeling and spearfishing were also used (Robinson et al. 2010). A variety of traps were used, depending on habitat size: hoop nets (0.5 m diameter, 2 m long, and 6 mm mesh) and mini-hoop nets (Promar® TR-502 collapsible traps; cylindrical, 0.85 m long x 0.3 m wide, with 9 mm mesh) baited with dry dog food (Gravy Train®). Traps were dispersed throughout the targeted reach and were primarily set in pools or runs that were more than 1-m deep. Traps were set during the afternoon and retrieved 2 to 22 hours later. For backpack electrofishing, typically the entire targeted reach was shocked, and any nonnative fish captured were removed.

In the Blue River, snorkelers used spear fishing equipment (JBL Enterprises, 1.5 m polespear affixed with a three pronged, barbless, Paralyzer spear tip; or a JBL Enterprises Mini-Carbine spear fishing gun) to remove large-bodied piscivorous nonnative fish. To improve the chances of

¹ Including chub populations previously classified as Gila Chub.

Cooperative Agreement R16AC00077: 2019 Annual Report - Final 03/24/2020

sighting fish, sampling was restricted to 8:30 am to 4:30 pm when the sun was high in the sky. All pools ≥ 1 m deep in the project reach were surveyed via snorkeling, and each pool was snorkeled through three times. The following data were recorded at each pool: coordinates (UTM, NAD 83 northing and easting), reach number or name, pool number, estimated pool length (m), width (m), and depth (m), date, snorkeling start and end time for each diver, species captured, number of each species, total length (TL, mm) of each individual fish, water clarity (m distance that fish can be accurately identified; estimated with polespear), and snorkeling crew member names.

Evaluation of Nonnative Removal: There are two general goals for nonnative removals: control or eradication. For situations where there aren't any barriers to invasions of nonnative species, the goal is to control the nonnative population until barriers can be installed. When barriers to upstream invasion of nonnative fishes are in place, the goal is eradication. Multiple removals are conducted until goals are achieved. The catch of nonnative species to low levels or to zero will be evidence of control. Absence of nonnatives for five or more consecutive removal events is evidence of eradication.

Acquire Spikedace, Loach Minnow and rare populations of other native fish (Task AZ-2003-2)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 2. Maintain and operate ASU topminnow holding facility and the Aquatic Research and Conservation Center (ARCC) to support the Program's recovery efforts for imperiled fishes in the Gila River Basin through the establishment of refuge populations of genetically distinctive stocks as insurance against extinction in the wild, captive propagation for repatriation, and applied research.

Recovery Objectives:

- Spikedace recovery objective 8.1. Determine wild stocks suitable for contribution to hatchery stocks.
- Spikedace recovery objective 8.2. Collect and transfer wild stocks to suitable facility.
- Loach Minnow recovery objective 8.1. Determine wild stocks suitable for contribution to hatchery stocks.
- Loach Minnow recovery objective 8.2. Collect and transfer wild stocks to suitable facility.
- Gila Chub draft recovery plan objective 4. Establish and maintain refuge populations in protected ponds or hatcheries as appropriate.
- Gila Topminnow 1999 draft revised recovery plan objective 1.1. Maintain refugia populations of natural populations to ensure survival of the species.

<u>Background:</u> The purpose of this task is to acquire Spikedace and Loach Minnow from all extant lineages and bring them to the Department's ARCC, or another facility, for propagation and to

establish refuge populations. The goal is to have 500 adults on station for each lineage. There are few natural populations left, and they need to be protected. Removing too many fish from a wild population could negatively impact it. The number of fish to remove from a given population is a coordinated decision between USFWS and state wildlife agencies, and is usually based on estimated number of fish in the stream derived from the most recent monitoring. Typically fish are removed within a few months of the most recent monitoring. If necessary, new individuals are brought into ARCC every year to maintain the population size and genetic diversity with wild stock.

Loach Minnow (N=115, from Aravaipa Creek) were first brought into the facility in 2002, to develop propagation techniques (Childs 2004). In 2005, 35 Spikedace and an additional 27 Loach Minnow were brought in from Aravaipa Creek (Ward 2008). Spikedace and Loach Minnow brought into ARCC to establish and maintain the refuge-broodstock populations are summarized in Table 1.

<u>Results:</u> Aravaipa Creek. On October 31, 2018, Department staff communicated to USFWS that 150 Spikedace and 25 Loach Minnow needed to be collected from Aravaipa Creek to maintain the broodstocks at ARCC. Flooding caused autumn monitoring to be postponed until mid-November 2018. The USFWS discussed monitoring results with University of Arizona and BLM, and on February 1, 2019 informed the Department that 25 Loach Minnow and 60 Spikedace could be collected without adversely affecting the populations in Aravaipa Creek. On February 4, 2019, Department and The Nature Conservancy (TNC) staff collected 25 Loach Minnow and 60 Spikedace from Aravaipa Creek downstream of the TNC guest house (~ UTM 12S 556110/3638112). There were two Loach Minnow mortalities during collection. The fish were transported to Department headquarters with no mortalities and transferred to ARCC staff. Spikedace were relatively abundant and captured in nearly every seine haul. More than half (35) of the 60 Spikedace quota was captured in the first seine haul.

On March, 25, 2019, Department staff collected a total of 60 Longfin Dace (as a proxy for Spikedace and Loach Minnow) from the west side of Aravaipa Creek (UTM 12S 540362/3639692) for a fish health assessment. No pathogens or parasites of concern were detected. This health assessment was necessary to allow for collections of Spikedace and Loach Minnow in the autumn of 2019.

Monitoring in autumn 2019 by University of Arizona, BLM, and USFWS indicated that Spikedace numbers were up. On November 8, USFWS indicated that 259 Spikedace and 31 Loach Minnow could be removed from Aravaipa Creek to maintain the broodstocks at ARCC. On November 14, 2019, Department staff collected a total of 262 Spikedace and 32 Loach Minnow in nine seine hauls downstream of the first road crossing downstream from the TNC guest house (start:

556110/3638242, end: 556091/3638090). The fish were transported to ARCC, and there was one Spikedace and one Loach Minnow mortality during the process.

Blue River. The Department's ARCC staff reported that in December 2018, there were 117 Blue River Loach Minnow broodstock at ARCC, and in February 2019 indicated that 500-5000 Blue River Loach Minnow needed to be brought into ARCC to be able to stock out thousands into Eagle Creek in 2020. USFWS had not responded before the Department had left to monitor the lower Blue River, so Department staff phoned USFWS who indicated we could collect 80 Loach Minnow. On October 10, 2019, Department staff collected 80 Loach Minnow from the Blue River near Juan Miller crossing. Fish were held overnight in aerated coolers and transported to ARCC the following day. There were two mortalities during transport.

<u>Recommendations</u>: Continue to collect Spikedace and Loach Minnow from remnant populations, with goals to minimize impact on remnant population while also acquiring the number of fish necessary to maintain a refuge population of at least 500 adults. More Loach Minnow should be collected from the Blue River and brought into ARCC to attain or exceed 500 broodstock. More Aravaipa Spikedace and Loach Minnow should be brought into ARCC to maintain the broodstocks. ARCC staff should coordinate with NMDGF regarding acquiring more stock of the New Mexico lineages.

In late 2019, University of Arizona developed a new formula based on the four-year moving average and the historic mean numbers of Spikedace and Loach Minnow captured, to help determine how many Spikedace and Loach Minnow could be removed from Aravaipa Creek, each year without negatively affecting the population. This methodology should be discussed more amongst partners.

Muleshoe ecosystem stream and spring repatriations (Task AZ-2003-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 4. Remove nonnative aquatic species threats.
 - Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Spikedace recovery objective 6.3. Reintroduce Spikedace to selected reaches.
- Spikedace recovery objective 6.4. Monitor success/failure of reintroductions.
- Loach Minnow recovery objective 6.3. Reintroduce Loach Minnow to selected reaches.
- Loach Minnow recovery objective 6.4. Monitor success/failure of reintroductions.
- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila

Topminnow in suitable habitats following geographic guidelines.

- Gila Topminnow 1999 draft revised recovery plan objective 2.4 Protect habitats of reestablished or potential populations from detrimental nonnative aquatic species.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.
- Desert Pupfish recovery objective 2. Re-establish Desert Pupfish populations.
- Desert Pupfish recovery objective 5. Monitor and maintain natural, re-established, and refugia populations.
- Gila Chub draft recovery plan objective 1.3.1. Eliminate or control problematic nonnative aquatic organisms
- Gila Chub draft recovery plan objective 2. Ensure representation, resiliency, and redundancy by expanding the size and number of populations within Gila Chub historical range via replication of remnant populations within each RU.
- Gila Chub draft recovery plan objective 7. Monitor remnant, repatriated, and refuge populations to inform adaptive management strategies.

Background: The purpose of this action is to establish Spikedace, Loach Minnow, Gila Topminnow, and Desert Pupfish into various waters on the Muleshoe Ranch Cooperative Management Area. The Muleshoe CMA is located on the western slopes of the Winchester and Galiuro mountains. The various waters and stream reaches are described in Robinson et al. (2010), and Love-Chezem et al. (2015a). Fish stockings began in 2007, when Spikedace and Loach Minnow were stocked into Hot Springs Canyon and Redfield Canyon; both species were again stocked into Redfield Canyon in 2008 and 2010, and into Hot Springs Canyon each year through 2011. In 2007, Roundtail Chub¹, Sonora Sucker, and Speckled Dace were translocated upstream of a waterfall in Redfield Canyon to expand their range in that system. Gila Topminnow and Desert Pupfish were stocked into Swamp Springs Canyon (2007 and 2008), Cherry Spring Canyon (2007 and 2008), and Secret Spring (2007 and desert pupfish only in 2010). Gila Topminnow and Desert Pupfish were stocked into Headquarters Spring in 2008, and in 2010 more pupfish were added. Gila Topminnow were stocked into Wildcat Canyon in 2014 (more were moved 100 meters further upstream in Wildcat Canvon in 2017 to expand their distribution), Bass Canvon in 2014, 2015, and 2018, and Double R Canyon in 2017 and 2018. Desert Pupfish were stocked into Larry & Charlie Tank in 2009 and into Mint Spring in 2015 and 2016.

Loach Minnow have been considered established in Hot Springs Canyon since 2016 because adults have been captured consistently with evidence of recruitment each year since the last stocking in 2011. Spikedace persist in Hot Springs Canyon and are likely established because they have detected every year since the last stocking in 2012, although they are present in very low numbers.

¹ Chub in Redfield Canyon were previously classified as Gila Chub.

Wet-dry mapping data from June, 2017 suggests that there is only about 3.4 km of continuous flow with approximately 200 meters of ideal Spikedace habitat.

Both Spikedace and Loach Minnow failed to establish in Redfield Canyon. Gila Topminnow are established in Swamp Springs Canyon, Headquarters Spring, Secret Spring, and Redfield Canyon and failed to establish in Cherry Spring Canyon. Desert Pupfish are established in Larry and Charlie Tank and in Secret Spring, but failed to establish in Swamp Springs Canyon, Cherry Spring Canyon, and Headquarters Spring.

Green Sunfish control in Redfield Canyon started in 2007 and has continued every year since. Number of sunfish removed from Reaches 1 and 2 has remained low, and far more sunfish are removed from Reach 3 every year since concerted efforts began there in 2014.

Results:

Nonnative Control. During June 4-5, 2019, Department staff performed a Green Sunfish removal in Redfield Canyon. Single-pass backpack electrofishing was conducted in Reaches 1 and 2 from near the Swamp Springs confluence at the downstream end of perennial water (UTM 12S 562272/3588781) upstream to the Sunfish Barrier (563858/3589841). Staff electrofished for a total of 11,594 seconds over two days and captured 20 Green Sunfish (mean CPUE = 6.75 fish/hour). Other species captured included 968 Roundtail Chub¹, 267 Sonora Sucker, and 49 Longfin Dace. On June 5, 2019, ten mini-hoop nets were set in Reach 3 near the wilderness boundary (Figure 1) and captured 144 Green Sunfish (24-199 mm TL). An additional 25 Green Sunfish were captured by angling in Reach 3. All Green Sunfish captured were removed from the stream. Overall, a total of 189 Green Sunfish were removed from Redfield Canyon in 2019. Green Sunfish catch increased in the upper perennial section for the second year in a row (Reach 1 and the upper end of Reach 2), suggesting that movement still occurs between Reaches 3 and 1 (Figure 2). Importantly, while Green Sunfish catch increased from 2018 to 2019 in the upper perennial section, CPUE declined from 10.05 to 6.75 fish/hour and spawning was not documented. Comparison of CPUE to years prior to 2018 is difficult because multiple gear types have been used to target Green Sunfish, and annual monitoring was occurring in addition to removal efforts. Green Sunfish numbers in Reach 3 remain high, and this location is likely the source of Green Sunfish to the upper reach.

Monitoring of Repatriated Populations.

On September 12, 2019, Department, TNC, and USFWS staff monitored Loach Minnow and Spikedace in Hot Springs Canyon. Crews sampled three fixed sites and six random sites by singlepass backpack electrofishing. Sampling crews captured 50 Loach Minnow, 2 Spikedace, 123 Roundtail Chub¹, 31 Sonora Sucker, 238 Desert Sucker, 1,036 Speckled Dace, and 1,097 Longfin Dace (Table 2). Loach Minnow catch rates have fluctuated annually since 2009 due to changing environmental conditions, particularly flooding (Figure 3). Loach Minnow continue to reproduce

¹ Roundtail Chub at these locations were previously classified as Gila Chub

and recruit into this population with both juvenile and adult size classes captured in 2019 (Figure 4). Consistent annual catch rates and evidence of reproduction since the final stocking suggests Loach Minnow are established in Hot Springs Canyon. Spikedace catch rates have been consistently low with no more than four individuals captured since 2015 (Figure 5). Evidence from monitoring indicates that Spikedace are persisting and may have established a very small population: they were last stocked in 2012, lifespan is two or three years in the wild, and juvenile Spikedace have been captured as recently as 2017(Figure 6). The limited amount of presumably suitable habitat in Hot Springs Canyon could be the primary factor limiting the size of this population. Gila Topminnow were not captured in Hot Springs Canyon in 2019, however a large flood event happened earlier in the summer and may have transported most of the topminnow downstream. Gila Topminnow were never stocked into Hot Springs Canyon, but were stocked upstream in Bass and Double R canyons and at Headquarters Spring, so the occasional presence in Hot Springs Canyon is a result of topminnow dispersing downstream from these locations.

In addition to the single-pass electrofishing, three pass depletion electrofishing was performed at each of the fixed sites with block nets set at the downstream and upstream ends of each 100 meter transect. Abundance and capture probability of each species captured was estimated using a Carle-Strub method (Carle and Strub 1978). Estimated abundance of Loach Minnow at the Reach 1 fixed site was 36 fish per hundred meters with an estimated capture probability of 0.65 (Table 3). Crews failed to capture enough fish for reliable estimates of abundance and capture probability for Loach Minnow at the two most downstream fixed sites and for Spikedace at any of the three fixed sites. Number of Loach Minnow and Spikedace captured during each pass (C1, C2, C3) along with estimates of abundance and capture probability can be found in Table 3.

Crews performed a standardized habitat survey at each of the three fixed sites in an effort to quantify habitat conditions that support translocated populations of Spikedace and Loach Minnow. All habitat data were incorporated into a non-metric multidimensional scaling ordination (NMDS), similar to the methods described in Hickerson and Walters (2019). Data from Hot Springs Canyon provides reference points for habitat conditions at sites where Loach Minnow and Spikedace translocation was successful in establishing a population. These reference points can be compared to potential translocation sites to help determine which sites are more likely to support translocated populations of Loach Minnow and Spikedace. Results of the analysis are presented in Figure 7.

On September 13, Department staff monitored Gila Topminnow in upper Bass Canyon. Ten minnow traps were set near the most recent stocking location and a total of four Gila Topminnow were captured (Table 4). Reproduction previously occurred in 2016 and 2017 indicating that suitable habitat exists for reproduction within this reach. There was substantial evidence of flooding within the surveyed reach which may have contributed to the low catch. Other species

captured in upper Bass Canyon included 85 Roundtail Chub¹, eight Longfin Dace and three Speckled Dace (Table 4). A single dip net sweep resulted in the capture of six Gila Topminnow.

On September 13, Department staff monitored Gila Topminnow in Double R Canyon. Ten minnow traps were set from the confluence with Bass Canyon (UTM 12S 571964/3579500) upstream to the most recent stocking location (571720/3579842). A total of 66 Gila Topminnow were captured with both size classes present. Other species captured in Double R Canyon included 38 Roundtail Chub, 97 Longfin Dace and 16 Speckled Dace (Table 4). Two additional dip net sweeps resulted in the capture of two Gila Topminnow.

On September 13, Department staff monitored Desert Pupfish in Mint Spring. A total of 109 Desert Pupfish were captured in five seine hauls (Table 4). Since Mint Spring is relatively small and nearly the entire pool can be sampled in one seine haul, captured fish were held in a bucket until all hauls were complete. This allowed us to estimate the abundance and capture probability of the Desert Pupfish population in Mint Spring using a multi-pass removal estimator (Carle-Strub method). Estimated abundance of Desert Pupfish was 171 ± 70.51 (estimate $\pm 95\%$ CI) with a capture probability of 0.18 \pm 0.11. Catch of Desert Pupfish in 2019 was greater than previous years (0 in 2016, 56 in 2017, 55 in 2018). Approximately 61% of the pupfish captured in 2019 were <20 mm TL, which was a greater percentage than previous years (29% in 2017, 0% in 2018), indicating that reproduction had occurred in at least two of the years since the species was first stocked. Based on the population estimate, presence of both juvenile and adult fish, and past monitoring data, Desert Pupfish are considered established in Mint Spring.

Repatriation Stockings.

Department staff did not stock any fish into Muleshoe Ranch waters in 2019.

<u>Recommendations:</u> The potential barrier to be constructed in Redfield Canyon has been canceled because State Land Department did not agree to transfer the land to Reclamation. In 2019, Department staff emailed and talked on phone with the private landowner just downstream of the Wilderness boundary to determine if permission to access their property on lower Redfield Canyon for Green Sunfish removals could be obtained. The property owners did not give a definitive answer after multiple contact attempts. Department staff will continue to contact the property owners and attempt to gain permission to access the property and remove sunfish. If permission is granted, the goal of Green Sunfish removal efforts should shift from control to eradication, and the frequency and intensity of removal efforts should be increased. If the downstream landowners do not grant permission for access, eradication of Green Sunfish in Redfield Canyon will not be feasible. The current level of removal effort (1-2 removals per year) appears to be sufficient at suppressing the sunfish population in the upper reach and should be continued until the status of the downstream population changes.

¹ Chub at these locations were previously classified as Gila Chub

Cooperative Agreement R16AC00077: 2019 Annual Report - Final 03/24/2020

As of the end of 2019, the multi-agency Muleshoe Native Fish Conservation Team had completed eight years of post-stocking monitoring in Hot Springs Canyon. Loach Minnow are considered established in Hot Springs Canyon. Spikedace numbers have decreased but persist and may have established a very small population. It is likely that there is not sufficient habitat for a robust Spikedace population in Hot Springs Canyon. Therefore, additional stockings of Spikedace are not recommended for Hot Springs Canyon unless surplus hatchery fish are available. Annual monitoring of fishes in Hot Springs Canyon should be continued, as recommended by the multi-agency team.

Monitoring of Gila Topminnow in Bass Canyon and Double R Canyon should continue until 2021 because both locations were augmented in 2018. Flooding and the presence of large piscivorous chub seems to be the main factors limiting the size of these populations.

Desert Pupfish in Mint Spring have consistently been captured since the initial stocking in 2016, and both age classes were captured in 2019 indicating the population is recruiting. Therefore, Desert Pupfish are now considered established in Mint Spring. Monitoring of Desert Pupfish in Mint Spring can be transferred to another Department program or to another agency. More Desert Pupfish should be periodically stocked into Mint Spring, Larry & Charlie Spring, and Secret Spring to maintain genetic variability.

Fresno Canyon repatriations (Task AZ-2006-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - $\circ~$ Goal 1. Identify critical streams and populations in need of protection and potential replication.
 - Goal 4. Remove nonnative aquatic species threats.
 - Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.
- Gila Chub draft recovery plan objective 2. Ensure representation, resiliency, and redundancy by expanding the size and number of populations within Gila Chub historical range via replication of remnant populations within each RU.

• Gila Chub draft recovery plan objective 7. Monitor remnant, repatriated, and refuge populations to inform adaptive management strategies.

<u>Background:</u> The purpose of this action was to establish viable populations of Gila Topminnow and Roundtail Chub¹ into Fresno Canyon after the stream was treated with rotenone in 2007 to remove Green Sunfish. After the treatment, Gila Topminnow naturally colonized from upstream Coal Mine Canyon. Gila Topminnow and Longfin Dace were also stocked in 2008. Choice of chub lineage to stock was limited to the three remnant populations in the Santa Cruz drainage: Cienega Creek, Sabino Canyon, and Sheehy Spring. Both Cienega Creek and Sheehy Spring are not replicated, but need to be, but Sheehy was the higher priority because of its very limited population size and distribution. Therefore, the stocking of Roundtail Chub¹ from Sheehy Spring was planned, but delayed until after a Habitat Conservation Plan could be completed for the private land on which Sheehy Spring is located. The Habitat Conservation Plan for San Rafael Cattle Company was completed in late 2016. Catch rates of chub in Sheehy Spring have been low (less than 90 captured) since 2011, so this small population is at risk of extirpation from stochastic events. Reproduction was evident in most years, but 2009 had the highest percentage of fish <50 mm TL (54.3%) and the highest total number of fish captured (385). It is unclear why catch rates were so much higher in 2009 than the other years.

<u>Results:</u> Department staff carried out a fish health assessment on Western Mosquitofish collected from Sheehy Spring on January 28, 2019, in preparation for an eventual translocation of Roundtail Chub¹ to Fresno Canyon or ARCC. Unfortunately the private landowner was uncomfortable with collection of Roundtail Chub¹ from Sheehy Spring in 2019. After discussions with U.S. Fish and Wildlife Service staff, the decision was made to reassess habitat in Fresno Canyon and if habitat appeared to be suitable, Roundtail Chub¹ from Cienega Creek would be translocated into Fresno Canyon. On June 18, 2019 Department staff carried out a habitat survey and documented the presence of 345 meters of surface water in about seven perennial pools. The amount of habitat would likely only support a population of 300-500 Roundtail Chub¹ population. Therefore, the agencies decided not attempt to establish Roundtail Chub into Fresno Canyon.

<u>Recommendations</u>: The Department wet-dry mapped Fresno Canyon in June 2019 and shared the information with Reclamation and USFWS. The agencies discussed the results and determined that there was insufficient habitat to sustain a population of 500 adult Gila Chub, and therefore recommended that a Gila Chub population be established elsewhere. Therefore, the Department recommends that the Fresno Canyon repatriations project be removed from the list of priority actions to be completed by the Department.

¹ Chub to be repatriated into Fresno Canyon were previously classified as Gila Chub.

Gila Topminnow stockings (Task AZ-2002-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.
- Desert Pupfish recovery objective 2. Re-establish Desert Pupfish populations.
- Desert Pupfish recovery objective 5. Monitor and maintain natural, re-established, and refugia populations.

<u>Overall Background:</u> The purpose of this action is to establish Gila Topminnow populations within historic range of the species throughout the Gila River Basin in Arizona. The target is six new establishments per year. Desert Pupfish are sometimes stocked into the same sites because the species utilize similar habitats. The Department coordinates with USFWS to determine stocking locations and appropriate donor locations and lineages. The strategy is to stock at least 500 Gila Topminnow initially or for any subsequent augmentations to establish a population. Populations are typically augmented if fewer than 100 fish are captured or observed during monitoring. After stocking, the populations are monitored at 6-months and then annually thereafter for three years after the last stocking event. If they are considered established after the third post-stocking monitoring, then the monitoring responsibilities are passed on to other Department programs or other agencies, and augmentation responsibilities are passed on to other Department programs. Monitoring techniques are consistent from year to year for a given site, and usually involve a minimum of 10 minnow trap sets per site, but dip nets or seines are sometimes used if habitat is amenable.

Fish Health Assessments During 2019:

Nabhan Pond

On January 28, 2019, Department staff collected 60 Gila Topminnow (Redrock Canyon lineage) from Nabhan Pond (UTM 12R 525253/3490484) near Patagonia, AZ for a fish health assessment. Parasites or pathogens of concern were not detected in the assessment.

SRP San Pedro Riparian Preserve

On August 12, 2019, Department staff collected 65 Gila Topminnow (Bylas Spring lineage) and 63 Desert Pupfish from the SRP San Pedro Preserve (east pond: UTM 12S 524419/ 3642886; west pond: 524346/ 3642909) for a fish health assessment. Parasites or pathogens of concern were not detected in the assessment.

Usery Park Pond

On February 11, 2019, Department staff collected 60 Gila Topminnow (Cottonwood Spring lineage) from Usery Park Pond (UTM 12S 442929/3704561) for a fish health assessment. Parasites or pathogens of concern were not detected in the assessment.

Sites Monitored or Stocked During 2019:

Arnett Creek

Background: In 1992, the Department, Tonto National Forest, and USFWS identified an opportunity to reestablish a native fish community in Arnett Creek and its tributary Telegraph Canyon. A fish barrier was built in the late 1990s, and the stream was chemically treated to remove nonnative fishes, and a few native fish were stocked. Unfortunately those fish did not establish populations, likely because too few were stocked and drought greatly reduced the amount of perennial water in the system.

The partners re-evaluated the stream in 2007, and determined that the small amount of habitat was probably only suitable for Longfin Dace and Gila Topminnow. The plan was to stock Longfin Dace first, and if they established a population, to move forward with Gila Topminnow. Longfin Dace were stocked in 2007, and they did establish. During 2010-2015, Department staff surveyed the few tanks and potential perennial reaches upstream of the proposed stocking locations and did not detect any nonnative fish.

In May 2017, Department staff collected Redrock Canyon lineage Gila Topminnow from Timbucktwo Tank and stocked 522 into Arnett Creek. Only 74 Gila Topminnow were captured during post-stocking monitoring of Arnett Creek in October, 2017. When Department staff monitored Arnett Creek in July 2018, only six juvenile Gila Topminnow were captured. Arnett Creek was nearly dry at the time of sampling with only Longfin Dace present in the initial stocking pool

Results: On July 15, 2019, Department staff monitored Gila Topminnow in Arnett Creek. A total of 10 minnow traps were set in the original stocking pool (UTM 12S 487185/3680583) with no fish captured. There were only two pools from the crossing of Telegraph Canyon Road downstream to the survey end (486397/3681075) and fish were not captured or observed in either pool. The original stocking pool was approximately 40 m long. The severe drought conditions during summer 2018, shrank the pool to less than 1 square meter of surface water, which is the most likely explanation for the absence of Gila Topminnow or Longfin Dace in 2019.

Department staff also reassessed potential habitat for Gila Topminnow in Telegraph Canyon from the confluence with Arnett Creek upstream to the end of surface water (486899/3679806), and determined 194 m of wetted habitat existed. Department staff also observed Longfin Dace throughout the wetted reach of Telegraph Canyon.

Recommendations: Gila Topminnow should not be stocked into Arnett Creek because of the lack of sufficient water during drought. However, three surveys with no Gila Topminnow are captured will be necessary to declare the species extirpated from Arnett Creek, so monitoring may continue until 2021. Perennial water in Telegraph Canyon apparently is more stable, so Gila Topminnow can be stocked into Telegraph Canyon when USFS staff complete the removal of invasive oleander in Telegraph Canyon and then monitored for at least three years to determine establishment.

Black Canyon City Heritage Park Pond

Background: Black Canyon City Heritage Park Pond is located within the Agua Fria Drainage in Yavapai County, Arizona. In 2006, the Albin Family donated 30 acres of land, which included a large pond, to Black Canyon City. The Black Canyon City Council then contacted the Department to inquire about establishing native fish populations within this man-made pond. In August 2011, Department staff stocked 3,000 Gila Topminnow and 986 Desert Pupfish into Black Canyon City Heritage Park Pond. In November 2012, Department staff stocked an additional 205 Desert Pupfish into the pond. Both Gila Topminnow and Desert Pupfish became established in the pond.

Western Mosquitofish and Tilapia were illegally stocked into the pond, and in Fall 2016 the Department and Black Canyon City decided to drawdown and dry the pond to eliminate the nonnative fish. Before the drawdown, Desert Pupfish were salvaged from the pond and held overwinter at the Department headquarters. Gila Topminnow were not salvaged because of the close similarity to Western Mosquitofish. The pond was drained, left to dry for several weeks, and refilled. In March 2017, Department staff stocked 122 of the salvaged Desert Pupfish into Black Canyon City Heritage Park Pond. In November 2017, Department staff monitored the pond and captured 622 Desert Pupfish and 3 American Bullfrogs in collapsible minnow traps (tadpoles) and seine hauls. In June 2018, Department staff collected 734 Sharp Spring lineage Gila Topminnow from Robbins Butte Wildlife Area's Stop Sign Pond and translocated the fish to Black Canyon City Heritage Park Pond. During follow up monitoring in August 2018, a total of 504 Desert Pupfish, 1,427 Gila Topminnow and 98 bullfrog tadpoles were captured.

Results: On August 1, 2019, Department staff monitored Gila Topminnow and Desert Pupfish in Black Canyon City Heritage Park Pond. Department staff set 20 collapsible minnow traps and captured 852 Desert Pupfish and 3,375 Gila Topminnow. A single seine haul resulted in the capture of an additional 306 Desert Pupfish and 2,034 Gila Topminnow. Department staff also performed six dip net sweeps and captured an additional six Desert Pupfish and 34 Gila Topminnow. More than twice as many Gila Topminnow and Desert Pupfish were captured in 2019 compared to 2018,

indicating that both species had reproduced. Approximately 94% of all Gila Topminnow and 80% of all Desert Pupfish captured were less than 20 mm TL.

Recommendations: Because Desert Pupfish were last stocked into Black Canyon City Heritage Park Pond, they should be monitored annually until at least 2020 to determine population establishment. Additional Desert Pupfish should be stocked in the pond to increase the founding population size. Gila Topminnow should be monitored until 2021, unless more are stocked. Roundtail Chub¹ can be stocked in 2020 or later, after Desert Pupfish are established.

Charlebois Spring

Background: Charlebois Spring is located in the Salt River Drainage within Tonto National Forest in the Superstition Wilderness. In June 1983, Charlebois Spring was stocked with 200 Gila Topminnow and the population persisted until 2006 before disappearing for unknown reasons. In 2015, Department staff confirmed the presence of Gila Topminnow in La Barge Canyon, roughly 7 km downstream of Charlebois Spring. It is likely that these Gila Topminnow came from Charlebois Spring and were flushed downstream during heavy rains. Since Gila Topminnow persisted at Charlebois Spring for over 20 years it was recommended that topminnow be restocked at the site. In May, 2017 a total of 622 Gila Topminnow of mixed lineage were translocated from Rio Salado Audubon Center to Charlebois Spring. In October 2018, Department staff captured a total of 983 Gila Topminnow in minnow traps and dip net sweeps.

Results: On October 31, 2019, Department staff monitored Gila Topminnow at Charlebois Spring. A total of 11 collapsible minnow traps were set in the main spring and downstream pools and captured no fish. Department staff carried out 14 dip net sweeps from the main spring down to LaBarge Canyon and failed to capture any fish. Approximately 50 Gila Topminnow were visually observed in the original stocking pool while Department staff was pulling traps, so fish are still present in the system. Charlebois Spring was not burned during the Woodbury Fire, however evidence of substantial flooding was present throughout the surveyed reach and likely contributed to the decline in topminnow abundance.

Recommendations: Gila Topminnow in Charlebois Spring should be monitored until at least 2020 to determine if they establish. Additional stockings may be warranted since the population drastically decreased in size between 2018 and 2019. Vegetation removal is recommended to reduce shade over the spring and open up the canopy for Gila Topminnow.

Edgar Canyon

Background: Edgar Canyon is a tributary of the San Pedro River that originates near Mount Bigelow in the Santa Catalina Mountains. Edgar Canyon is primarily ephemeral but has a few short intermittent and perennial reaches. The lowest perennial reach is located on Pima County

¹ Chub to be stocked into Black Canyon City Heritage Pond were previously classified as Gila Chub.

lands approximately 5 km upstream of the confluence with the San Pedro River. This perennial reach is at least 600 meters in length. Pima County conducts wet-dry mapping annually and possesses more refined data on the minimum extent of the perennial reach. Habitat in Edgar Canyon was determined to be suitable for Gila Topminnow in February, 2019.

Results: On April 18, 2019, Department and Pima County staff stocked 564 Gila Topminnow (Redrock Canyon lineage) into Edgar Canyon (UTM 12S 543140/3590495). Fish were collected from Nabhan Pond (near Patagonia) earlier in the day with four mortalities during transport. The temperature logger installed in February 2019 was read out and reset at the time of stocking.

On September 30, 2019, Department staff monitored Gila Topminnow in Edgar Canyon. A total of 10 minnow traps were set from the upper stocking location (UTM 12S 543140/3590495) downstream to near the end of perennial water (543506/3590459) and captured 560 Gila Topminnow. Three seine hauls were carried out and resulted in the capture of an additional 222 Gila Topminnow. A total of 18 dip net sweeps captured 20 more Gila Topminnow. Gila Topminnow occupied the entire perennial reach at the time of sampling with approximately 57% of all fish captured less than 20 mm TL. The temperature logger was readout and reset at the time of sampling. The Edgar Canyon population is on a promising trajectory toward establishment at this time.

Recommendations: Gila Topminnow in Edgar Canyon should be monitored annually until at least 2022 to determine if they establish. Additional fish may be stocked to enhance population establishment if deemed necessary.

Harden Cienega Creek

Harden Cienega Creek is a tributary to the San Francisco River near the New Mexico state line. Harden Cienega is inhabited by Roundtail Chub¹, Speckled Dace, Longfin Dace, Desert Sucker, and Sonora Sucker. There is about 4.4 km of perennial water extending from just above Prospect Canyon down to about 800 m above the confluence with the San Francisco River. The upper section is canyon-bound and the lower 570 m of perennial water is in a low gradient valley. Department staff assessed the habitat in lower Harden Cienega Creek and considered it suitable for Gila Topminnow and in 2015 recommended they be stocked. The Department coordinated with Apache-Sitgreaves National Forest who directed the Department to coordinate with Gila National Forest because they managed that livestock grazing allotment. The Department and Gila National Forest discussed the project in December 2018 and came up with a process to move forward.

Results: During October 15-16, 2019, Department staff translocated 631 Gila Topminnow (Bylas Spring lineage) from the SRP San Pedro Preserve into Harden Cienega Creek below the natural barrier near the mouth of the slot canyon (UTM 12S 674768/4674598). Gila Topminnow were

¹ Chub in Harden Cienega Creek were previously classified as Gila Chub.

transported via aerated cooler to Frisco Camp on the San Francisco River where they were held overnight, then the following day transported to stocking site in aerated buckets. There were 36 mortalities during transport and stocking. A temperature logger was also installed at the stocking location.

Recommendation: The topminnow in Harden Cienega Creek should be monitored for three years after the final establishment stocking. If no more topminnows are stocked to facilitate establishment, then post-stocking monitoring will be completed in 2022.

Hidden Water Spring

Background: Hidden Water Spring is located in Cane Spring Canyon, about 0.6 km upstream of the confluence with Cottonwood Creek which flows into Saguaro Lake. Gila Topminnow were first stocked into Hidden Water Spring in 1976 and 1981. Gila Topminnow were detected in 2010, but then not in 2011, 2012, or 2013. Department staff began efforts to reestablish this population in 2016 by translocating 544 Gila Topminnow (Peck Canyon lineage) from the Phoenix Zoo. In October 2017, Department staff monitored Hidden Water Spring and captured a total of 425 Gila Topminnow, 343 Longfin Dace, and 172 Lowland Leopard Frog tadpoles. During monitoring in October 2018, a total of 312 Gila Topminnow and 58 Longfin Dace were captured.

Results: On October 3, 2019, Department staff monitored Gila Topminnow at Hidden Water Spring. Department staff set 10 collapsible minnow traps and captured one Gila Topminnow and 92 Longfin Dace. Department staff also carried out 12 seine hauls and 5 dip net sweeps and captured an additional 23 Gila Topminnow, 52 Longfin Dace, and one Lowland Leopard Frog. Approximately 83% of all Gila Topminnow captured were less than 20 mm TL. An additional 20 Gila Topminnow were visually observed but not captured. There was evidence of a large recent flood with debris piled up in places up to eight feet above stream level, small trees knocked down, small willows and cattails were laid flat, and much of the habitat that was previously pools had been partially filled with sand.

Recommendations: Gila Topminnow are likely established in Hidden Water Spring, but because the population decreased so much from 2017 to 2019, we recommend it be monitored for one additional year. Additional stockings may be beneficial to augment the population in the uppermost pool (UTM 12S 459353/3717249).

Las Cienegas NCA - Bill's Wildlife Pond

Background: Bill's Wildlife Pond is located in the Gardner Canyon drainage about 2.1 km upstream of the confluence with Cienega Creek. Bill's Wildlife Pond was initially stocked with 841 Gila Topminnow (Cienega Creek lineage) in 2016. Only 18 Gila Topminnow were captured during the first monitoring in 2017, and the population was augmented with an additional 636 topminnow later in the year. In May 2018, Department staff translocated 190 Gila Topminnow

from Clyne Pond into Bill's Wildlife Pond. During monitoring in August 2018, Department staff captured a total of five Gila Topminnow but observed hundreds of Gila Topminnow that were not captured with minnow traps.

Results: On August 6th, 2019, Department staff monitored Gila Topminnow at Bill's Wildlife Pond. Department staff set 10 collapsible minnow traps, with three of the traps suspended at the top of the water utilizing floats, and captured 519 Gila Topminnow. Approximately 32% of Gila Topminnow captured were less than 20 mm TL. Department staff also noted poor water quality at site as dissolved oxygen at the shoreline of the pond at 7:33 AM was only 1.0 mg/L and five topminnow in one of the submerged traps were dead after two hours of soak time.

Recommendations: Bill's Wildlife Pond has been stocked three times and had the highest catch during monitoring 2019. The site was last stocked in 2018, therefore this population will be monitored until 2021 to determine establishment. Additional topminnow may be stocked if deemed necessary. Adding floats to the minnow traps appears to have improved capture probability for Gila Topminnow. Water quality is a potential concern for Bill's Wildlife Pond, because turbid brownish-yellow water, with low DO has been observed on multiple occasions and should be investigated. Despite these potential oxygen issues, topminnow in Bill's Wildlife Pond seem to be reproducing and increasing in abundance.

Las Cienegas National Conservation Area - Clyne Pond

Background: Clyne Pond is located in the Mud Springs Canyon drainage about 10.5 km upstream of the confluence with Cienega Creek. The pond is adjacent to a private ranch, and the rancher uses the pond to provide water to his livestock. On August 19, 2015, Department and BLM staff stocked 501 Gila Topminnow (Cienega Creek lineage) into Clyne Pond. Since Gila Topminnow was not detected during monitoring in August 2016, Department and BLM staff stocked an additional 541 Gila Topminnow on August 30, 2016. A total of 76 Roundtail Chub¹ were translocated from Cienega Creek in 2016 and 75 Roundtail Chub¹ salvaged from Cienega Creek were translocated in 2017. During monitoring in August 2017, Department staff captured 92 Gila Topminnow and 10 Chiricahua Leopard Frogs, and observed about 475 Gila Topminnow; no Roundtail Chub¹ were captured. Chub were targeted during a second monitoring trip, in August 2017, but none were captured.

In early 2018, BLM staff reported that Clyne Pond seemed likely to dry completely because of ongoing drought conditions. On May 10, 2018, Department staff attempted to salvage chub from Clyne Pond, however chub were not captured or observed. A total of 190 Gila Topminnow were salvaged and translocated to Bill's Wildlife Tank on Las Cienegas NCA. In August 2018, Department staff performed monitoring and captured a total of 12 Gila Topminnow and a single Chiricahua Leopard Frog.

¹ Cienega Creek chub stocked into Clyne Pond were previously classified as Gila Chub.

Results: On August 6, 2019, Department staff monitored Gila Topminnow and Roundtail Chub¹ at Clyne Pond. Staff set 10 collapsible minnow traps and 10 mini-hoop nets and captured 158 Gila Topminnow, and two Chiricahua Leopard Frogs. Approximately 18% of all Gila Topminnow captured were less than 20 mm TL. Several hundred Gila Topminnow were observed in the center of the pond.

Recommendations: Gila Topminnow were last stocked into Clyne Pond in 2016, and were captured in the three annual post-stocking monitoring events, so the species can be considered established. Monitoring responsibilities can shift to another Department program or another agency. More topminnow may be stocked if deemed necessary and water levels improve. Roundtail Chub¹ is likely extirpated because none were captured or observed during five successive surveys. We do not recommend any more Roundtail Chub¹ be stocked into Clyne Pond because water levels are too low during drought.

Las Cienegas NCA - Cottonwood Tank

Background: Cottonwood Tank is located in the Gardner Canyon drainage about 5.2 km upstream from the confluence with Cienega Creek. The pond is one of a set of two connected ponds, and is separated from the second pond by a berm and fence. Livestock are allowed in the north pond but are excluded from the south pond. In July, 2013, Department and BLM staff stocked 269 Desert Pupfish into the south pond. Since only 4 Desert Pupfish were captured during monitoring in August 2014, Department and BLM staff stocked an additional 177 Desert Pupfish in October 2014. During sampling in July 2015, 851 Desert Pupfish were captured. However, only 34 Desert Pupfish were captured in both 2016 and 2017. Following monitoring in 2017, the existing Cottonwood Tank population was augmented with 155 individuals. In August 2018, a total of 47 Desert Pupfish and one Chiricahua Leopard Frog were captured. Only three of the fish captured were less than 20 millimeters in length, indicating that limited reproduction was occurring in this system.

Results: On August 6, 2019, Department staff monitored Desert Pupfish in Cottonwood Tank. Department staff set 10 collapsible minnow traps, with 8 suspended above the aquatic vegetation using floats, and captured 190 Desert Pupfish (32 < 20 mm TL).

Recommendations: Desert Pupfish were last stocked in 2017, so monitoring should continue until at least 2020 to determine if they establish. Adding floats to minnow traps appears to have improved capture probability in this pond where aquatic vegetation has often made sampling difficult. All minnow traps should be floated in future monitoring efforts.

Las Cienegas National Conservation Area – Spring Water Wetland

Background: Spring Water Wetland is located just east of Cienega Creek about 0.4 km upstream of the confluence with Spring Water Canyon. Department and BLM staff translocated 674 Gila

Topminnow from Cienega Creek in May 2013. Over 8,000 Gila Topminnow were captured during monitoring in 2014, over 1,000 in 2015, and over 12,000 in 2016. In June of 2017, Department and USFWS staff salvaged 85 Roundtail Chub¹ from Cienega Creek and stocked them into Spring Water Wetland due to concerns about potential post-fire effects from the Sawmill Fire. In August 2018, Department staff captured 1,161 Gila Topminnow and 71 Roundtail Chub. The juvenile Roundtail Chub¹ size class (<50 mm TL) comprised more than 71% of the total catch (51 of 71 fish) so reproduction had occurred because only 22 individuals less than 50 mm TL were initially stocked.

Results: On August 6, 2019, Department staff monitored Roundtail $Chub^1$ in Spring Water Wetland. Staff set 10 mini hoop nets and captured 40 Roundtail $Chub^1$, with a mean size of 121 mm TL (min = 98 mm TL, max = 150 mm TL). A total of four Gila Topminnow, and 14 Sonoran Mud Turtles were also captured.

Recommendations: Gila Topminnow are considered established in Spring Water Wetland. Monitoring of Roundtail Chub¹ should continue until at least 2022 because chub were last stocked in 2017. Because the population was started with relatively few individuals, several hundred more Roundtail Chub¹ from Cienega Creek should be stocked to help the population establish and to improve the genetic diversity of the Spring Water Wetland population.

San Pedro Riparian NCA - Murray Spring

Background: Murray Spring is an east flowing tributary of the San Pedro River near Sierra Vista. A perennial reach begins about 2.8 km west of the San Pedro River and extends about 1.6 km through a cienega and has pools, runs, and glides. A wastewater treatment facility exists about 1.8 km upstream and provides groundwater input to Murray Spring. About 1.5 km upstream from the confluence with the San Pedro River, is a concrete structure that seems to act as a barrier and prevents nonnative fishes from moving upstream. Gila Topminnow and Desert Pupfish were stocked in Murray Spring in 2011, 2013, 2014 and 2017. However, neither species appeared to be establishing, as fewer than 11 topminnows were captured each year from 2012 through 2017, and fewer than six pupfish were captured each year from 2013 through 2017. Longfin Dace have increased in abundance following an unplanned translocation of 50 fish from below to above the barrier in 2013. It is possible that Longfin Dace are affecting the ability of Gila Topminnow and Desert Pupfish to establish at the site due to competition for habitat and resources. The creek is also thick with sedges and cattails which has decreased available pool habitat for Gila Topminnow and Desert Pupfish over the years. In August 2018, 57 Gila Topminnow were captured above the barrier which is the best return since monitoring began, but also the first year since 2014 that Desert Pupfish were not captured or observed.

¹ Roundtail Chub stocked into Spring Water Wetland were previously classified as Gila Chub

Results: On August 5, 2019, Department staff monitored Gila Topminnow and Desert Pupfish in Murray Spring. Department staff set 19 collapsible minnow traps and captured a total of 29 Gila Topminnow, 59 Longfin Dace, and 24 crayfish. One Gila Topminnow, five Longfin Dace and one crayfish were also captured in nine dip net sweeps. Approximately 47% of all Gila Topminnow captured were less than 20 mm TL.

Recommendations: Gila Topminnow were last stocked into Murray Spring in 2017, and therefore should be monitored until at least 2020 to determine if they establish. Since cattails have greatly increased in density and distribution in the stream bottom and very few pools are left, stream channel improvements are recommended, however, it is unknown if BLM has any plans to improve the habitat. Desert Pupfish no longer appear to be present in Murray Spring, as this is the second consecutive year they were not captured.

Mud Spring-Coronado National Forest.

Background: Mud Spring is located on the southwest slope of the Huachuca Mountains in the Sycamore Canyon drainage within the upper San Pedro River drainage on the Coronado National Forest. The pond is at 1,700 m elevation and has a surface area of about 255 m² and is about 2 m deep in the middle. The pond is fed by a spring and is on the south-facing slope of the hills, which apparently moderates winter water temperatures. The pond is surrounded by frog fence to prevent the incursion of American Bullfrog. The pond is encircled by sedges, and *Chara sp.* covers most of the bottom in the open water areas. The pond is occupied by Chiricahua Leopard Frog and is slated to be a Mexican Gartersnake repatriation site. A total of 494 Sharp Spring lineage Gila Topminnow were translocated from the captive population at Arizona State University in August, 2018. The pH in Mud Spring was high at the time of stocking.

Results: On August 14, 2019, Department staff monitored Gila Topminnow in Mud Spring. Ten collapsible minnow traps were set and captured 4,201 Gila Topminnow, and 3 Chiricahua Leopard Frog Tadpoles. Approximately 37% of all Gila Topminnow captured were less than 20 mm TL.

Recommendations: Gila Topminnow should be monitored in Mud Spring until at least 2021 to determine if topminnow establish and additional stockings should occur as necessary. The population has rapidly increased in the last year and the outlook for this population is promising.

Peterson Ranch Pond.

Background: Peterson Ranch Pond is located in Scotia Canyon (tributary to the Santa Cruz River in the San Rafael Valley) at 1,892 m elevation in the Coronado National Forest. The pond is about 670 m², has a maximum depth of about 3 m, and is fed by a spring which moderates winter water temperatures. The pond is surrounded by fencing to exclude Bullfrogs and livestock. Chiricahua Leopard Frogs, Longfin Dace (stocked in 2015), and Mexican Gartersnakes (introduced in 2018) also inhabit the pond. A total of 762 Gila Topminnow (Sharp Spring lineage) were translocated from Robbins Butte Stop Sign Tank and Swimming Pool Tank in August, 2018.

Results: On August 14, 2019, Department staff monitored Gila Topminnow in Peterson Ranch Pond. Ten collapsible minnow traps were set and captured 47 Gila Topminnow. Approximately 9% of all Gila Topminnow captured were less than 20 mm TL. Topminnow were visually abundant outside of the traps, so topminnow are likely more abundant than our data suggests.

Recommendations: Peterson Ranch Pond should be monitored annually until 2021, unless more topminnow are stocked. Additional stockings to augment the population may occur if deemed necessary. Floats should be added to all minnow traps in future monitoring efforts to improve capture probability and ensure air pockets for frogs and gartersnakes.

Rock Spring.

Background: Rock Spring is located in the Mazatzal Mountains within the Tonto National Forest about two miles west of Highway 87 near Sunflower, Arizona. The spring is in the stream bed and typically produces about a 250 m perennial stream immediately downstream; the remaining section of stream is intermittent or ephemeral (Carter and Bahm 2007). In the perennial section of the stream, two pools are located above a 1.5 m tall dam and three pools below. Longfin Dace occupy the reach below the dam, and likely dispersed into Rock Spring from Sycamore Creek. The perennial portion is fenced with a four-strand barbed wire fence to exclude livestock and protect habitat. A temperature logger was installed in the second pool below the dam on January 31, 2008, and pulled on March 24, 2011. Data from the logger indicated the second pool was dry from January through September 2010.

Gila Topminnow (middle Santa Cruz River lineage) were initially stocked in Rock Spring (above and below the dam) in 2013, and later augmented below the dam in 2014 (Frear et al. 2015). Department staff captured 49 Gila Topminnow in October 2013, 130 in August 2014 and 53 in June 2015 with both size classes captured each time (Frear et al. 2015; Mosher et al. 2016). In July 2016, 794 Gila Topminnow were captured. Gila Topminnow were not captured or observed in 2017 and only one Longfin Dace was captured. It was believed that drought had shrunk available habitat to the point where only the dace survived. Gila Topminnow were not captured or observed during monitoring in July, 2018. The absence of fish suggests that Rock Spring may have gone dry or nearly dry at least once since 2017.

Results: On March 27, 2019, Department staff monitored Gila Topminnow in Rock Spring. Department staff set 10 collapsible minnow traps and failed to capture or observe Gila Topminnow. Department staff also performed 11 dip net sweeps and failed to capture any fish. One Sonoran Mud Turtle was visually observed beneath a trap.

Recommendations: Gila Topminnow has not been detected in Rock Spring for three consecutive years and is considered extirpated from the site. Therefore, stocking and monitoring efforts will be discontinued at this site.

Sabino Canyon

Background: Sabino Canyon is located northeast of Tucson, Arizona within the Coronado National Forest and Sabino Canyon Recreation Area. Sabino Creek is a tributary to the Santa Cruz River and flows southwest through Sabino Canyon to its confluence with Tanque Verde Wash in Tucson. Sabino Creek was chemically treated in 1999 to remove nonnative Green Sunfish, and afterwards was stocked with salvaged Roundtail Chub¹ (Ehret and Dickens 2009a). In August 2015, Department, Coronado National Forest, and USFWS staff stocked 510 Gila Topminnow into Sabino Canyon Recreation Area near a location locally known as The Crack. Gila Topminnow were collected the previous day from Cienega Creek and Road Canyon Tank (Cienega Creek lineage). Only 72 Gila Topminnow were captured during the initial monitoring in June 2016, so Department and BLM staff translocated an additional 985 Gila Topminnow from Road Canyon Tank (Cienega Creek lineage) on August 30, 2016. A total of 103 Gila Topminnow were captured between the original stocking location and Tram Stop 8 during monitoring in June, 2017. In June, 2018 Department staff captured a total of 276 Gila Topminnow between the original stocking location and Tram Stop 8. Several thousand topminnow were visually observed in four pools downstream of Sabino Lake Dam. These pools were determined to be an ideal location to collect Gila Topminnow for future translocations. Habitat in a reach of Sabino Canyon located approximately 250 meters upstream from the confluence with East Fork Sabino Canyon was also reevaluated and identified as suitable for Gila Topminnow. A total of 557 Gila Topminnow were translocated from the large pools immediately below Sabino Dam to Sabino Canyon upstream of the confluence with East Fork Sabino Canyon on June 21, 2018.

Results: On May 28, 2019, Department staff monitored Gila Topminnow in Sabino Canyon from The Crack to downstream of Sabino Dam. A total of 22 collapsible minnow traps were set and captured a total of 561 Roundtail Chub², but failed to capture Gila Topminnow. Department staff also performed six dip net sweeps and captured two Gila Topminnow downstream of Sabino Dam. Following the monitoring effort, a total of 148 Roundtail Chub¹ (>100 mm TL) were collected downstream of Sabino Dam and held overnight for translocation upstream.

On May 29, 2019, Department staff monitored Gila Topminnow in Sabino Canyon just upstream of the confluence with East Fork Sabino Canyon. Department staff set 10 collapsible minnow traps and failed to capture Gila Topminnow. An additional six dip net sweeps and three seine hauls were performed with no Gila Topminnow captured or observed. Prior to the monitoring, 148 Roundtail Chub¹ collected downstream of Sabino Dam the previous day were translocated to a pool just

¹ Chub stocked into Sabino Canyon were previously classified as Gila Chub.

² Chub in Sabino Canyon were previously classified as Gila Chub.

downstream of the topminnow stocking location (UTM 12S 520836/3581045). There were no mortalities during collection or transport. Following the monitoring and stocking, an additional 148 Roundtail Chub¹ were collected from Sabino Canyon downstream of Sabino Dam and held overnight in aerated coolers for translocation to Romero Canyon.

On May 30, 2019, Department staff and volunteers from the University of Arizona stocked 148 Roundtail Chub¹ collected the previous day from Sabino Canyon, into Romero Canyon upstream of the first trail crossing (511817/3586421). The translocation effort augmented the existing population in Romero Canyon and expanded the distribution by nearly a kilometer. Roundtail Chub¹ were first established in Romero Canyon with a stocking of 120 fish in 2005, and fish dispersed downstream of the stocking location past several waterfalls.

On September 11th, 2019, Department staff conducted a visual survey downstream of Sabino Dam to determine if sufficient numbers of Gila Topminnow were available for a translocation upstream. Staff observed hundreds of Gila Topminnow.

On October 24th, 2019, Department staff collected 527 Gila Topminnow in three seine hauls from the pools immediately downstream of Sabino Dam. The fish were translocated to Sabino Canyon upstream of the confluence with East Fork Sabino Canyon (UTM 12S 520784/3581144). Unfortunately there were 177 mortalities during transport likely due transport containers not being treated with Amquel® or salt. A total of 350 Gila Topminnow were successfully stocked.

Recommendations: The Gila Topminnow population in Sabino Canyon Recreation Area does not appear to have established in the large pools near The Crack, however topminnow are relatively abundant and likely established in the pools just downstream of Sabino Dam. The low number of topminnow captured downstream of the dam in May 2019 is likely attributable to severe flooding that occurred during the previous winter. By September, several thousand topminnow were observed in this location which suggests that the topminnow population will likely be resilient to severe flood events downstream of Sabino Dam. Since the recreation area location was last stocked in 2016, this population should be considered established and the monitoring can be passed on to another Department program or external agency.

The Gila Topminnow stocked above the confluence with the East Fork were likely impacted by the severe flooding that occurred over the 2018-2019 winter. If no additional topminnow are stocked in 2020, then monitoring of this population should occur until at least 2022. If additional topminnows are stocked to establish a population, then monitoring will continue for three years after the final stocking event. Roundtail Chub¹ were also stocked just downstream in 2019 and consideration should be given to translocating Roundtail Chub¹ further upstream to Hutch's Pool near West Fork Sabino Creek. The Department also recommends a hike-through survey from the

¹Chub stocked into Sabino Canyon were previously classified as Gila Chub.

pools near East Fork Sabino down to The Crack to determine if any chub or topminnow have dispersed downstream and occupied any of the pools between the upper and lower stocking locations.

Sheepshead Canyon

Background: Sheepshead Canyon is located within the Coconino National Forest north of Cornville, Arizona. Perennial water begins below a dry waterfall about 1.8 km upstream of the confluence with Oak Creek. The perennial reach is comprised of a network of channels, pools, and wetlands that are maintained by ground water discharge from numerous springs and seeps within the drainage. A diversion ditch is located about 600 m downstream of the dry waterfall and flows southeast to private property in Cornville. A total of 819 Gila Topminnow (Middle Santa Cruz River lineage) were stocked into Sheepshead Canyon in September 2014 (336 in the pool below the dry waterfall and 483 into the pool above the diversion ditch; Mosher et al. 2016). Only two Gila Topminnow were captured during monitoring in June, 2015 and an additional 511 Gila Topminnow were stocked immediately after the monitoring (241 at the lower site and 270 at the upper site). Similarly, two Gila Topminnow were captured during monitoring in September, 2016 and an additional 216 Gila Topminnow were stocked in the upper site, 361 in the lower sites, and 79 in a middle pool located about 150 m upstream of the lower site. A total of 83 topminnow were captured and about 50 observed in the middle pool during monitoring in September 2017. Topminnow were not captured at the upper or lower stocking sites in 2017, but about 300 were observed at the upper site. A single Gila Topminnow was captured at the upper stocking site during monitoring in September, 2018 and several hundred were observed. Fish were not captured or observed at the middle pool or lower stocking site in 2018.

Results: On September 4th, 2019, Department staff set 8 collapsible minnow traps (with floats to suspend them in the water column) in the upper stocking pool and captured a total of 1,504 Gila Topminnow. Two collapsible minnow traps were set in each of the two lower pools but fish were not captured or observed. Approximately 64% of all Gila Topminnow captured were less than 20 mm TL.

Recommendations: Gila Topminnow have been captured or observed for three subsequent years since the last stocking in 2016 and should be considered established. As a result, the monitoring can be passed on to another Department program or external agency. Adding floats to minnow traps in 2019 dramatically increased the number of topminnow captured and this technique should be utilized in any future monitoring.

Tortilla Creek

Background: Tortilla Creek is located within the Salt River Drainage in the Tonto National Forest and flows into Canyon Lake near Tortilla Flat, AZ. Tortilla Creek has an established population of Gila Topminnow in the downstream reach of the creek near Tortilla Flat. Gila Topminnow in the lower reach of Tortilla Creek likely originated from a population stocked in 1982 in Mesquite Tank #2 (above Unnamed Drainage #68-B). A valve on the dam of Mesquite Tank #2 was opened, allowing it to drain and completely dry out. As a result, Gila Topminnow washed downstream and established a population in Unnamed Drainage #68-B and later dispersed into perennial pools in lower Mesquite Creek and lower Tortilla Creek. Due to the steep gradient and multiple waterfall barriers, Gila Topminnow never dispersed upstream into the upper perennial section of Tortilla Creek (about 4.3 km upstream of the confluence with Mesquite Creek). In March 2016, Department staff assessed habitat in the upper section, and deemed it suitable for Gila Topminnow. The only fish species present in the upper perennial section was nonnative Fathead Minnow, which is thought to have few negative interactions with Gila Topminnow. In June, 2017, Department staff stocked 548 Gila Topminnow (Peck Canyon lineage) into upper Tortilla Creek about 4.5 km upstream of the confluence with Mesquite Creek. A total of 829 Gila Topminnow were captured during monitoring in November, 2017. During monitoring in 2018, a total of 2,020 Gila Topminnow and 65 Fathead Minnow were captured.

Results: On October 30, 2019, Department staff monitored Gila Topminnow at upper Tortilla Creek by setting ten minnow traps. A total of 47 Gila Topminnow (> 20 mm TL) were captured in a single trap near the original stocking location (UTM 12S 467239/3708608). Topminnow were not captured in 12 dip net sweeps below the stocking pool. Department staff visually observed about 20 fish but were unable to positively identify them as either Gila Topminnow or Fathead Minnow.

The Woodbury Fire began in June 2019 and burned 123,875 acres of the Superstition Mountains including the upper Tortilla Creek watershed. Evidence of substantial flooding in Tortilla Creek with some debris up to fifteen vertical feet above the water surface was documented near the stocking location. Sediment deposition was also noted downstream of the monitoring reach. Most of the cattails, which previously made sampling this reach difficult, were broken off at ground level. However, the cattails and reeds were already starting to regenerate in some areas.

Recommendations: The Gila Topminnow population in upper Tortilla Creek should be monitored until at least 2020 to determine if they establish. Additional stockings may be warranted since the population appears to have experienced a drastic reduction from thousands of individuals to perhaps a few hundred as a result of post-fire flooding.

West Fork Pinto Creek

Background: West Fork Pinto Creek is a tributary to Pinto Creek in the Salt River Drainage within the Tonto National Forest. West Fork Pinto Creek is intermittent near Miles Ranch Trailhead; however, there is a perennial section (~150 m long based on 2017 estimates) located about 500 m downstream of the confluence with Spencer Spring Creek. This upper perennial reach consists of a series of shallow runs and pools and is inhabited by Longfin Dace. Downstream of the Miles

Ranch Trailhead, there are several large plunge pools inhabited by Longfin Dace. Further downstream is a waterfall below which Green Sunfish, Longfin Dace and Desert Sucker were detected in 2016. After three habitat assessments in 2016 and 2017, and discussions between the Department, USFS, and USFWS, the Department stocked 705 Gila Topminnow (Sharp Spring lineage) in upper West Fork Pinto Creek in May, 2017. Department staff captured 398 Gila Topminnow (238 \leq 20 mm TL) during monitoring in October, 2017. A total of nine Gila Topminnow and 23 Longfin Dace were captured during annual monitoring in July, 2018. The perennial reach was restricted to only about 30 meters of surface water at the time, largely due to ongoing drought conditions.

Results: On October 1, 2019, Department staff monitored the Gila Topminnow population and post-fire impacts in West Fork Pinto Creek. Department staff visually surveyed from the upstream stocking site (UTM 12S 491044/3700120) to 1 km downstream of Miles Ranch (494365/3700033) and failed to observe any fish. Department staff also set ten minnow traps and five mini-hoop nets downstream of Miles Ranch and failed to capture any fish.

The Woodbury Fire began in June 2019 and burned 123,875 acres of the Superstition Mountains including part of West Fork Pinto creek and the West Fork Pinto watershed. Sediment and debris were noted from the stocking site downstream to at least one kilometer below Miles Ranch. The stream channel near the original stocking location down to Miles Ranch was completely filled in with sand, with very little surface water. Where there was surface water is was mostly only 1-2 cm deep. Downstream of Miles Ranch there was continuous stream flow, however, post-fire impacts were also evident in this lower reach with substantial siltation, ash deposits and flood debris throughout.

Recommendations: Monitoring of West Fork Pinto Creek should continue until 2021 to verify that fish are extirpated from the upper perennial reach. Habitat was limited in upper West Fork Pinto Creek before the fire and this reach may not be able to support any fish for the foreseeable future unless flows scour out habitat. The lower reach of West Fork Pinto Creek downstream of Miles Ranch should be surveyed again to determine if any Green Sunfish persisted through the post-fire impacts or recolonized from Pinto Creek. The lower pools above the waterfall should be assessed in the future to determine if sufficient habitat is present for Roundtail Chub¹.

Spring Creek (Oak Creek tributary) repatriations (Task AZ-2013-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 4. Remove nonnative aquatic species threats.

¹ Chub to be repatriated were previously classified as Gila Chub.

- Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
- Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Spikedace recovery objective 6.2.5 Reclaim as necessary to remove non-native fishes.
- Spikedace recovery objective 6.3. Reintroduce Spikedace to selected reaches.
- Spikedace recovery objective 6.4. Monitor success/failure of reintroductions.
- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.
- Gila Chub draft recovery plan objective 1.3.1. Eliminate or control problematic nonnative aquatic organisms.

<u>Background</u>: Spring Creek is a tributary to Oak Creek in the Verde River drainage, and contains Roundtail Chub¹, Speckled Dace, Longfin Dace, Sonora Sucker, Desert Sucker, and Northern Mexican Gartersnake. A small diversion dam about 0.95 km upstream from the confluence with Oak Creek seemingly prevented most nonnative fishes from entering the stream above, but there are records from the 1970s and 1980s of Smallmouth Bass and Fathead Minnow. Green Sunfish were detected below the diversion dam in 2011, and in May 2014 Green Sunfish were captured 2.5 km above the dam. Department staff began removal efforts immediately and completed seven removals in June and July 2014, after which the Department's CAMP staff assumed responsibility of the removal efforts above the dam and completed an additional five days of removal in 2014, three removals in 2015, and two removals in 2016.

The purpose of this multi-agency project was to protect the existing Spring Creek population of Roundtail Chub¹ and other existing or newly established native aquatic species against possible future upstream incursion of nonnative fishes from Oak Creek and the Verde River. Establishments of Spikedace, Gila Topminnow, and possibly Loach minnow were planned.

Reclamation finished construction of a fish barrier about 1.1 km upstream from Oak Creek in April 2015. On May 11, 2015, Department staff stocked 221 Spikedace (Aravaipa Creek lineage), and on August 12, 668 Gila Topminnow (Lower Santa Cruz lineage) were stocked near Willow Point Road. During the first monitoring in September 2015, Department staff captured three Spikedace and three Gila Topminnow. During the second monitoring in September 2016, Spikedace were not captured. However, one Spikedace (died after capture) was captured during the CAMP Green Sunfish removal. Because so few Spikedace and Gila Topminnow were captured during

¹ Chub in Spring Creek were previously classified as Gila Chub.

Cooperative Agreement R16AC00077: 2019 Annual Report – Final 03/24/2020

monitoring, Department staff stocked 67 more Spikedace and 341 Gila Topminnow near Willow Point Road and an additional 347 Gila Topminnow in the large pool above the barrier in October, 2016. Few Spikedace were available from ARCC at the time of stocking in 2016, which is why so few were stocked. A total of 11 Spikedace and 207 Gila Topminnow were captured during monitoring in September, 2017. An additional 1,076 Spikedace were stocked in February, 2018 after being held in cages as part of an eDNA research study. A total of 20 Spikedace and 497 Gila Topminnow were captured during annual monitoring in September, 2018 with the first evidence of natural reproduction by Spikedace. In December, 2018 an additional 500 Spikedace were stocked near Willow Point Road.

<u>Results</u>: During September 3-4, 2019, Department staff monitored Gila Topminnow and Spikedace in Spring Creek. Department staff targeted Spikedace by electrofishing one fixed 100-meter reach and two randomly selected 100-meter reaches in Spring Creek. A total of 36 Spikedace were captured during the initial pass at each site, which is the highest catch during monitoring since stocking began in 2015 (Figure 8). Importantly, the mean size of Spikedace was 45.7 millimeters TL (min = 26 mm TL, max = 72 mm TL; Figure 9) which suggests that a majority of the fish captured were from natural reproduction rather than recaptures of fish stocked in December, 2018. In addition to the Spikedace, 245 Roundtail Chub¹, 304 Speckled Dace, 135 Desert Sucker, 45 Longfin Dace, one Gila Topminnow and 18 crayfish were captured during electrofishing (Table 5).

Three pass depletion electrofishing was carried out at the fixed site with block nets set at the downstream and upstream ends of the 100 meter reach. A total of 34 Spikedace were captured during the two additional passes. Estimated abundance of Spikedace using a Carle-Strub method was 90 fish per hundred meters with an estimated capture probability of 0.21 (Table 3; Carle and Strub 1978). Low capture probability of Spikedace was likely due to the large number of small bodied fish present within the depletion reach. Consequently, the low capture probability suggests that Spikedace may be much more abundant in Spring Creek than our sampling data suggests.

A standardized habitat survey was carried out in an effort to quantify habitat conditions that support translocated populations of Spikedace and Loach Minnow. Data collected during habitat surveys were incorporated into a non-metric multidimensional scaling ordination (NMDS) in an attempt to identify potential translocation sites most likely to support a translocated population of Spikedace or Loach Minnow (Hickerson and Walters 2019). Results of the analysis are presented in Figure 7.

Department staff targeted topminnow by setting 10 collapsible minnow traps in the large pool upstream of the barrier and two immediately downstream of the barrier and captured a total of 550 Gila Topminnow, 538 of which were >20 mm TL and 12 were \leq 20 mm TL (Table 6). Gila

¹ Chub in Spring Creek were previously classified as Gila Chub

Cooperative Agreement R16AC00077: 2019 Annual Report - Final 03/24/2020

Topminnow were visually abundant in the pool formed upstream of the diversion, and presence of young of year fish suggests that the population is reproducing.

<u>Recommendations</u>: Since Spikedace were stocked in 2018, electrofishing monitoring should continue until at least 2023. The information gained from depletion electrofishing provided better resolution on the status of the Spikedace population in Spring Creek and should continue with future monitoring. Additional stockings may occur if deemed necessary. Because of the proximity of this site to ARCC and recent advances in Spikedace rearing techniques, this may be an ideal system to evaluate factors contributing to post-stocking survival of Spikedace which could benefit translocation strategies going forward.

Because Gila Topminnow were last stocked in 2016, and catch has increased since then and there has been evidence of reproduction in the large pool above the barrier, Gila Topminnow are now considered established in Spring Creek. Future monitoring can be carried out by another Department program or another agency.

Blue River native fish restoration (Task AZ-2002-3)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 4. Remove nonnative aquatic species threats.
 - Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Spikedace recovery objective 6.2.5. Reclaim as necessary to remove non-native fishes.
- Spikedace recovery objective 6.3. Reintroduce Spikedace to selected reaches.
- Spikedace recovery objective 6.4. Monitor success/failure of reintroductions.
- Loach Minnow recovery objective 6.2.5 Reclaim as necessary to remove non-native fishes.
- Loach Minnow recovery objective 6.3. Reintroduce Loach Minnow to selected reaches.
- Loach Minnow recovery objective 6.4. Monitor success/failure of reintroductions.

<u>Background</u>: The Blue River Native Fish Restoration Project is implemented by the Department, Forest Service, Reclamation, and USFWS, with the goal of protecting and restoring the entire assemblage of native fishes within the Blue River drainage and benefiting their conservation status within the Gila River Basin (Reclamation 2010). The major components of the project are construction of a fish barrier, mechanical removal of non-native fishes, and repatriation and monitoring of federally listed warm-water fishes in the Blue River. The initial focus of the project was the lower 19 km of the Blue River, from Fritz Ranch to the confluence with the San Francisco River (Figure 11), additional efforts are taking place upstream (Figure 11). The Reclamationfunded fish barrier, located in the Blue River about 0.8 km upstream from the confluence with the San Francisco River, was completed in June 2012. Later in the same month, 539 Spikedace and 142 Roundtail Chub were stocked into the lower Blue River above the barrier (Figure 12, Figure 13). Spikedace (upper Gila River lineage) and Roundtail Chub (Eagle Creek lineage) were both acquired from ARCC, but some chub were also directly translocated from Eagle Creek. Efforts to remove non-native piscivorous fish from the lower Blue River began before barrier construction (Robinson et al. 2010) and continued annually after the barrier was installed.

During annual post-stocking monitoring, Spikedace catch rates were relatively flat during 2012 through 2014, until increasing from 2015 through 2017 (Figure 13). Following monitoring in 2015, an additional 296 Spikedace were stocked into the lower Blue River. Electrofishing catch rates for Roundtail Chub were similar to those for Spikedace, but hoop net catch rates have been more variable from year to year. Due to the low catch rates, an additional 876 Roundtail Chub were stocked into the lower Blue River 12). Relative abundance for all three species declined from 2017 to 2018 largely due to drought conditions during the winter of 2017-2018.

The ongoing mechanical removal effort appears to be effective at eradicating nonnative piscivorous fish in the Blue River (Figure 14). Nonnative fish are removed both during removal trips, and during annual post-stocking monitoring of native fishes. Catfish were the main targets of initial removal efforts and were removed by snorkeling and spearfishing. During the first removal, in June 2009, a total of 70 Channel Catfish and 4 Flathead Catfish were removed from the Blue River between Fritz Ranch and the confluence with the San Francisco River (Robinson et al. 2010). Following the 2011 Wallow Fire related fish kills and fish barrier construction, only seven Channel Catfish were captured and removed during the June 2012 removal, but one Green Sunfish was also detected (the first record in the drainage; Robinson et al. 2013). During annual monitoring in November 2012, catfish were not captured, but 106 Green Sunfish were captured and removed throughout the lower Blue River. In 2013, Department staff carried out an intensive trapping effort between Steeple Creek and Fritz Ranch to determine if Green Sunfish had dispersed upstream, but no Green Sunfish were captured (Robinson et al. 2014). In addition to the snorkeling efforts in 2014 and 2015, further removal efforts were carried out to target Green Sunfish with hoop nets and electrofishing equipment. The most recent detection of Green Sunfish was in 2016 and Channel Catfish have not been detected since 2013. In 2017, five Channel Catfish and one Green Sunfish were captured below the fish barrier by USFWS staff as part of a barrier monitoring effort. Neither species was captured below the barrier during the last survey in 2019. All large bodied nonnatives were PIT tagged and released to evaluate the barrier's effectiveness.

Native fish conservations activities in the middle Blue River (McKittrick Creek confluence upstream to The Box; Figure 11) began in 2016 when 1,194 Roundtail Chub were stocked between

The Box and Cole Flat. In 2017, Department staff monitored Roundtail Chub with 18 hoop nets set overnight in randomly selected pools, and captured 57 Roundtail Chub. Immediately following the chub monitoring, Department staff collected 448 Spikedace from the Blue River at Juan Miller crossing and translocated them to the Blue River at Cole Flat. Spikedace were held in cages as part of an eDNA study before release, which may have contributed to some post-stocking mortality. In September, 2018, Department staff electrofished ten random and two fixed 100-meter transects and captured a total of 12 Roundtail Chub, 6 Spikedace, 43 Loach Minnow and one Brown Trout. In addition, large hoop nets were set overnight in 15 randomly selected pools throughout the monitoring reach resulting in the capture of 17 more Roundtail Chub. Roundtail Chub catch declined from 2017 to 2018 despite additional electrofishing effort and juvenile Roundtail Chub were not captured within the monitoring reach. Relative abundance of Spikedace was relatively low during 2018 monitoring, an additional 291 Spikedace were translocated from the Blue River at Relative abundance of the Blue River at Relative and Miller Crossing to the middle Blue River at Cole Flat.

Lazy YJ Ranch Pond (aka Quinsler's Pond; UTM 12S 676654/3725755) was stocked with 373 Roundtail Chub (Eagle Creek lineage) from ARCC in August 2015. In September 2017, Department staff set 22 hoop nets overnight, and captured 274 Roundtail Chub with juvenile and two adult size classes present. In September, 2018 a total of 12 hoop nets were set and captured 145 Roundtail Chub, but all were greater than 140 mm TL. The private landowner began to open the outlet gates on the pond to allow Roundtail Chub to disperse into the Blue River in 2018.

<u>Results:</u> The Department completed native fish conservation actions in the lower Blue River and middle Blue River during 2019. Results for the lower Blue River are presented first.

During June 24-26, 2019, Department staff performed the annual large-bodied piscivore removal. Methods used were described in Robinson et al. (2010), and Robinson and Love-Chezem (2016). The number of individuals of each species observed in each pass was recorded. In clear pools that were too shallow to snorkel, observers walked along the shore and counted fish by species. Staff visited 125 pools or locations that were previously pools. A total of 93 pools were snorkeled, 13 pools that were too shallow to snorkel were visually observed, 16 pools had changed such that they were either run or riffle habitat, and 3 pools were dry, the latter primarily in the lowest reach. Nonnative fishes were not captured or observed in the lower Blue River. Green Sunfish have not been detected since 2016 and Channel Catfish have not been detected since 2013 (Figure 14). An estimated 2,094 Spikedace, 746 Roundtail Chub, 215 Desert Sucker, 2,008 Sonora Sucker, 863 Longfin Dace and 705 Speckled Dace were observed.

During October 8-10, 2019, Department, Reclamation and Marsh and Associates staff performed the annual fish monitoring in the lower Blue River. Electrofishing was carried out at 10 randomly selected and two fixed 200 meter transects (Table 7). More Spikedace, Loach Minnow and Roundtail Chub were captured in 2019 than any other year and all three species were captured at

all electrofishing transects for the third year in a row (Figure 12, 13, 15). Spikedace and Loach Minnow relative abundance continue to likely be mostly influenced by spring discharge, while Roundtail Chub continue to increase in relative abundance each year. All three species had strong juvenile year classes in 2019 likely due to the above average precipitation during the winter of 2018-2019 (Figure 16, 17, 18). Interestingly, 2019 is the first year with evidence of a bimodal age structure in Loach Minnow which may indicate that this population may have finally recovered from the reduction after the Wallow Fire (Figure 18).

Three pass depletion electrofishing was carried out at both fixed sites with block nets set at the downstream end of the 200 meter sampling reach and 100 meters upstream of the downstream end of the reach. A total of 158 Spikedace, 74 Loach Minnow and 39 Roundtail Chub were captured during the two additional passes at both fixed sites (Table 3). Abundance (fish per 100 meters) and capture probability of all three species was estimated using a Carle-Strub method (Carle and Strub 1978). Estimated capture probability of all three species was quite high, which suggests that backpack electrofishing alone is an effective method for monitoring these species (Table 3).

Crews performed a standardized habitat survey at both fixed sites in an effort to quantify habitat conditions that support translocated populations of Spikedace and Loach Minnow. All habitat data were incorporated into a non-metric multidimensional scaling ordination (NMDS), similar to the methods described in Hickerson and Walters (2019). Data from the Lower Blue River provides reference points for habitat conditions at sites where Loach Minnow and Spikedace translocation was successful in establishing a population. These reference points can be compared to potential translocation sites to help determine which sites are more likely to support translocated populations of Loach Minnow and Spikedace. Results of the analysis are presented in Figure 7.

On September 16, 2019, Department staff monitored the Roundtail Chub in Quinsler's Pond near the Upper Blue campground. Ten large hoop nets were set overnight and captured a total of 22 Roundtail Chub (mean TL = 179.2 mm; Figure 19), 6 Sonora Sucker and 4 Northern Crayfish. Total catch and catch rates of Roundtail Chub declined for the second year in a row, however abundance of suckers and crayfish also declined (Table 8). The low catch rates experienced in 2019 could be attributable to a number of factors including increased turbidity of the pond from recent storms, from fish emigrating from the pond into the Blue River, or low survival. Juvenile Roundtail Chub (< 100 mm TL) and hence reproduction, was evident in two of the last three years of monitoring: 2% of the catch in 2017, 0% in 2018, and 4% in 2019 (Figure 19).

During September 17-18, 2019, Department staff conducted the annual monitoring in the middle Blue River between The Box and McKittrick Creek. A total of ten random and two fixed 100meter transects were electrofished with a total of 23 Spikedace, 9 Roundtail Chub, 41 Loach Minnow, 183 Longfin Dace, 232 Desert Sucker, 162 Sonora Sucker and 310 Speckled Dace captured (Table 9). A single Narrow-headed Gartersnake was captured and released unharmed at an electrofishing site in the lower reach (666283/3710367). Roundtail Chub catch declined again from 2018 to 2019 with no evidence of reproduction to date (Figure 20, 21). However, capture efficiency was likely low due to the river being very turbid (~2-6 inches visibility) during sampling as a result of runoff from recent monsoon storms. Spikedace catch and relative abundance increased in 2019 despite the poor sampling conditions (Figure 22). Importantly, a majority of the Spikedace captured were young of year with some large adults also being captured (Figure 23).

Three pass depletion electrofishing was carried out at only the upper fixed site with block nets set at the upstream and downstream ends of the 100 meter sampling reach. Block nets could not be set at the remaining two fixed sites because of elevated flows. A total of four Roundtail Chub were captured during the two additional passes at the fixed site. Unfortunately reliable estimates of abundance and capture probability for Spikedace and Roundtail Chub could not be derived because so few fish were captured.

Crews performed a standardized habitat survey at each of the upper and lower fixed sites in an effort to quantify habitat conditions that support translocated populations of Spikedace and Loach Minnow. All habitat data were incorporated into a non-metric multidimensional scaling ordination (NMDS), similar to the methods described in Hickerson and Walters (2019). Data from the Middle Blue River provides reference points for habitat conditions at sites where Loach Minnow and Spikedace translocation was successful in establishing a population. These reference points can be compared to potential translocation sites to help determine which sites are more likely to support translocated populations of Loach Minnow and Spikedace. Results of the analysis are presented in Figure 7.

On October 10, 2019, Department staff collected 100 Roundtail Chub (> 100 mm TL) near Juan Miller Crossing. Fish were transferred to two aerated cooler and translocated to the middle Blue River near Cole Flat (UTM 12S 667297/3713521) with no mortalities during transport.

<u>Recommendations:</u> Roundtail Chub and Spikedace are clearly established in the lower Blue River and continue to increase in abundance and distribution. The monitoring of this population has already proven valuable and should be continued and passed on to another entity.

Mechanical removal of nonnative piscivores from the lower Blue River has been successful, and 2019 was the sixth year in a row that catfish have not been detected. Green Sunfish have not been detected since 2016, so removals for this species should probably continue until at least 2021. Eradication of Green Sunfish from the lower Blue River should be confirmed by collecting eDNA samples throughout the surveyed reach and utilizing the newly developed Green Sunfish marker, either in 2020 or 2021.

The middle Blue River populations of Roundtail Chub and Spikedace should be monitored for five years after the final stocking to determine if they establish. If no more fish are stocked monitoring would continue through 2024 for Roundtail Chub and through 2023 for Spikedace. Additional

Spikedace and Roundtail Chub should be translocated from the lower Blue River as necessary to help establish populations.

Conservation activities in the drainage should be expanded by stocking Roundtail Chub and potentially Spikedace upstream of The Box in 2020. A survey to identify suitable pool habitats above The Box should be done before translocation. Similarly, a monitoring plan for activities above the box would be developed in to track the status of newly translocated populations.

Assess potential repatriation waters (Task AZ-2008-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 1. Identify critical streams and populations in need of protection and potential replication.

Recovery Objectives:

- Spikedace recovery objective 6.2. Identify river or stream systems for reintroductions.
- Loach Minnow recovery objective 6.2. Identify river or stream systems for reintroductions.
- Gila Topminnow 1999 draft revised recovery plan objective 2.1. Identify habitats suitable for reestablishment of Gila Topminnow.
- Gila Chub draft recovery plan objective 2.1. Prepare and protect streams appropriate for replications.

Background: The purpose of this project is to assess perennial waters in the Gila River Basin to determine if they are suitable for repatriations of Spikedace, Loach Minnow, Gila Topminnow, Roundtail Chub, or other native fishes. Assessments are restricted to waters thought to be perennial. Potential translocation sites for Spikedace, Loach Minnow, and Roundtail Chub are assessed using a standardized habitat assessment protocol (Anderson 2015). The protocol is a transect based approach within a 100 meter reach. Habitat surveys are sometimes paired with backpack electrofishing to assess fish community at sites without recent records. Potential translocation sites for Gila Topminnow can be ponds or short isolated sections of stream, so a different method is used. The vast majority of potential topminnow sites assessed are at elevations <1600 m, as recommended the draft revised recovery plan (Weedman 1999); the only exceptions are sites where temperature logger information indicates thermally stable water year-round. The total length and average width of wetted habitat is measured. Each pool or pond is measured for length, width, and maximum depth. Coves, backwaters, and other areas of potential high-flow refuge are noted. If fish survey information is lacking, traps, dip nets, or seines are typically used to collect fish community data. A summary of each of the waters assessed up to 2019 is provided in Table 10.

<u>Results:</u> A map showing locations of each of the streams assessed during 2019 is presented in Figure 24. The existing standardized habitat protocol (Anderson 2015) was updated in 2019 to include water velocity and resolve issues with inconsistent application among different staff members. Habitat assessments would ideally occur at the same time of year, however schedule demands often don't allow for that level of consistency. Information collected from standardized habitat surveys from 2015 to 2019 was incorporated into a non-metric multidimensional scaling ordination (NMDS; Hickerson and Walters 2019) to prioritize waters most likely to support a translocated population of Spikedace, Loach Minnow or Roundtail Chub (Figure 7). NMDS is useful for calculating similarities between sites in a low-dimensional, easy to visualize configuration, but the ordination does not provide conclusive information about actual habitat suitability for sites. NMDS is particularly useful for evaluating translocation site similarity for species for which habitat requirements are not well known and when multiple habitat parameters (mean embeddedness, proportion riffle habitat, etc.) may be limiting factors for populations.

Buehman Canyon. On February 14, 2019, Department, USFWS, and Pima County staff evaluated aquatic habitat in Buehman Canyon. Flow and Longfin Dace were present throughout the entire surveyed reach (UTM 12S DS: 543834/ 3586784, US: 543600/3586535). Habitat for Gila Topminnow looked optimal at the main pool (543600/3586535). A HOBO pendant temperature logger was installed in the main pool and has not yet been retrieved. Evidence of recent flooding was present throughout the surveyed reach including areas of scouring and sediment deposition. Pima County staff indicated that aquatic vegetation is typically abundant throughout the reach and that the stream goes dry approximately 100 meters upstream of the main pool in June.

Recommendations: Habitat appears to be suitable for Gila Topminnow in Buehman Canyon. The temperature logger should be retrieved to ensure winter water temperatures are sufficiently warm. Ideally, a habitat assessment would have been carried out during a drier time of the year. Unfortunately, the Pima County portion of Buehman Canyon is immediately upstream from a state trust land section so additional coordination with State Land Department is needed before topminnow can be stocked.

Citizen Canyon. On May, 2, 2019, Department and New Mexico Department of Game and Fish staff surveyed Citizen Canyon. Access was not possible near the confluence with the San Francisco River because of impassable cliffs near the canyon bottom. Access to Citizen Canyon was gained from the first unnamed tributary upstream of Bullard Canyon on the east side (confluence at UTM 12 S 682326/3682270). The route was steep and still required scrambling around several small waterfalls (20-40 ft tall) to access the canyon bottom. While the route was passable, it is still not recommended. A total of 35 Longfin Dace and 92 Speckled Dace were captured in 1,037 seconds of electrofishing within an approximately 100 meter reach near the New Mexico state line (start: 681869/3681441, end: 681977/3681533). Longfin and Speckled Dace were present throughout the surveyed reach (DS: 681869/3681441; US: 683097/3683267), but no other fishes were observed. A standardized habitat survey was not carried out at Citizen Canyon. Aquatic habitat within Citizen Canyon primarily consists of riffle habitat with clean, unembedded cobbles being the dominant

substrate type. The mean wetted width of the stream throughout the surveyed reach was less than one meter. There were several shallow pools throughout the surveyed reach, with only four having a maximum depth greater than 0.5 meters. The deepest pool (681977/3681533) was approximately 1.5 meters deep. Flow was continuous within most of the surveyed reach, with a few short intermittent sections between Bullard Canyon and Webster Canyon.

Recommendations: Suitable habitat for Loach Minnow or Roundtail Chub may exist in Citizen Canyon beyond the surveyed reach, however a second assessment is necessary to determine if there are nonnative fish present farther downstream, whether a barrier is present, how much additional habitat exists downstream, and the quality of habitat at the driest time of the year. Any future assessment should occur in June to determine the quality of habitat during drought conditions. Potential future routes to try would be coming downstream from Webster Canyon or Citizen Spring or backpacking up the San Francisco River from Frisco Camp (~16km).

East Eagle Creek. On August 20, 2019, Department staff surveyed aquatic habitat in East Eagle Creek. The single randomly selected habitat transect in the lowest kilometer of East Eagle Creek only had about 50 meters of surface water, so a habitat survey was not completed at that site. In general, very little surface water was present in East Eagle Creek. There was about 600 meters of perennial water downstream of the confluence with Salt House Creek and about 150 meters of perennial water upstream of the confluence with Dry Prong Creek.

Recommendations: East Eagle Creek does not appear to have suitable habitat for Spikedace or Loach Minnow. Speckled Dace are present in Salt House Creek, so fish likely travel upstream into East Eagle Creek from Eagle Creek during high flows periods.

Edgar Canyon. On February 14, 2019, Department, USFWS, and Pima County staff evaluated aquatic habitat in Edgar Canyon. There was approximately 600 meters of wetted stream during the visit. Wet-dry mapping by Pima County staff indicates this reach is perennial (Powell 2018). Recent flooding had deposited sand throughout the reach, with only two distinct pools present (UTM 12S: 543245/3590512; 543112/3590498). The maximum depth of the two pools was 0.4 and 0.45 meters respectively. A HOBO pendant temperature logger was installed in one of the pools (543112/3590498). Stream gradient was low with a fairly open riparian canopy, both of which should be conducive for Gila Topminnow establishment. Temperature logger data was retrieved for this site in September, however a flood on April 18th appears to have pushed the logger out of the channel, limiting the useable data to mid-February through mid-April.

Recommendations: Aquatic habitat in Edgar Canyon appeared to be suitable for Gila Topminnow which were stocked in April, 2019. A temperature logger should remain in place until all three years of monitoring are complete.

Fish Creek. On May 8, 2019, Department staff evaluated stream habitat for native fish in upper Fish Creek. Flows appeared to be intermittent from Paradise Canyon down to near Frog Spring. Staff walked the creek from the confluence with Rogers Canyon (479706/3705203) upstream to

just past Rough Canyon (480065/3705586) and documented six pools that appear to be perennial (max depth range = 0.55-1.26 m). Department staff also walked downstream of the confluence with Rogers Canyon for approximately 850 m (478984/3705377) and documented an additional five pools (max depth range = 0.82-2.57 m). Habitat between the pools largely consists of high gradient riffle and cascades. Most of the surveyed reach of Fish Creek flows through a tight canyon and velocity refuge for small bodied fish during floods would probably be limited. It appears that there is at least one effective fish barrier between the surveyed reach and lower Fish Creek because no fish were observed. Two Sonora Mud Turtles were observed downstream of Rogers Canyon, so perennial water likely exists in this reach. However, a follow up assessment during a drier year would be informative because the extraordinary amount of snowfall in the Superstition Mountains this past winter generated streamflows that are probably not representative of typical conditions.

Recommendations: The surveyed reach of Fish Creek does not appear to be suitable for Gila Topminnow but may be suitable for Roundtail Chub. The Woodbury Fire of 2019 burned much of the Fish Creek watershed a few weeks after the survey took place, so a follow up survey is required before any translocations take place.

Haunted Canyon. On March 18, 2019, Department staff evaluated Gila Topminnow habitat in Haunted Canyon near Kennedy Spring. Staff hiked from the Haunted Canyon trailhead, up through Haunted Canyon to Kennedy Spring. Flows were elevated from recent precipitation and water was present from the trailhead all the way upstream to the survey endpoint (UTM 12S 494480/3693439) approximately 1 km upstream of Tony Ranch. Kennedy Spring (494975/3693127) consists of a pool with a residual depth of 0.51 meters (max depth 0.61 meters, tail crest depth 0.1 meters), with some cascades and smaller bedrock pools downstream. The stream immediately below the spring was relatively high gradient, and the absence of established riparian vegetation other than sycamore trees suggests the stream is likely ephemeral.

Recommendations: Haunted Canyon and Kennedy Spring do not appear to be suitable for Gila Topminnow. However, suitable habitat may exist for other species like Longfin Dace and Desert Sucker in Haunted Canyon.

Ladrone Canyon. On May 15, 2019, Department staff assessed stream habitat for native fish in Ladrone Canyon, tributary to the Blue River. Two standardized habitat surveys were carried out at randomly selected 100 meter reaches. The relative similarity of Ladrone Canyon habitat sites to habitat sites assessed since 2016 can be found in Figure 7. Substrate within the survey sites was composed primarily of fines and bedrock. Crayfish were observed above the waterfall fish barrier. There was about 1,225 m of continuous flow in Ladrone Canyon above the barrier at the time of the survey

Recommendations: Stream habitat in Ladrone Canyon does not appear to be suitable for Loach Minnow but may contain habitat for other native fishes. However, because of the relatively short amount of perennial habitat, we do not recommend translocations of native fish into Ladrone Canyon.

Little Blue Creek. On July 9, 2019, Department staff collected four eDNA samples and conducted five standardized habitat surveys at randomly selected 100-meter reaches in Little Blue Creek, tributary to the Blue River. The relative similarity of Little Blue Creek habitat sites to habitat sites assessed since 2016 can be found in Figure 7. The location of wet and dry reaches was also documented within the stream. In general, Little Blue Creek appeared to be intermittent, and contained a total of 2.6 km of surface water within the 7.3 km reach surveyed from the Blue River upstream to the confluence with Dutch Blue Creek. Fish were visually abundant in most wetted sections. Flow was absent in Little Blue Creek from the confluence with the Blue River upstream approximately 2.9 km. There was approximately 1.3 km of surface water in Dutch Blue Creek from the confluence with Little Blue Creek upstream to the highest eDNA site in the Dutch Blue Box (UTM 12S 671410/3699497). There appeared to be suitable and sufficient habitat for Loach Minnow, but not for Spikedace. No Spikedace nor Loach Minnow eDNA was detected in any of the four locations where samples were collected.

Recommendations: Because Loach Minnow eDNA was not detected in any of the samples collected from Little Blue Creek and Dutch Blue Creek, it may be worthwhile to attempt translocating Loach Minnow into upper Little Blue Creek near Dutch Blue Creek. There does not appear to be a natural barrier to upstream dispersal of Loach Minnow, however intermittent reaches near the confluence with the Blue River may prevent consistent colonization of upstream reaches. The Department will discuss the proposed translocation with Apache-Sitgreaves National Forest and USFWS staff before proceeding with a translocation.

Pigeon Creek. Pigeon Creek is a tributary to the Blue River. In 2017 habitat in Pigeon Creek and its tributary Turkey Creek was assessed using an older version of the standardized habitat survey, and deemed qualitatively suitable for Loach Minnow. Habitat information from Pigeon Creek and Turkey Creek was not incorporated into the NMDS ordination because crews failed to record macrohabitat data on the back side of the datasheet. Pigeon Creek extends approximately 7.4 from the confluence with Turkey Creek downstream to the confluence with the Blue River. Because a variety of native fish were detected, and because Pigeon Creek was identified in the Spikedace and Loach Minnow Atlas Project, Department staff decided to collect eDNA samples in Pigeon Creek in an effort to detect Spikedace and Loach Minnow. On June 26, 2019, Department staff collected eDNA sample at four sites on Pigeon Creek between Bear Creek and just upstream of Turkey Creek. Loach Minnow were detected at only one of the locations: just upstream of the confluence with Bear Creek. Spikedace were not detected in any of the samples. This is the first detection of Loach Minnow in Pigeon Creek. Additionally, 11 adult Roundtail Chub were observed in a single pool (UTM 12S 663861/3683170) approximately 5.7 km upstream from the Blue River. This was the first observation of Roundtail Chub in Pigeon Creek and is the first evidence of dispersal from the lower Blue River to tributary streams.

Recommendations: Loach Minnow eDNA was detected at one of four sampling locations; just upstream of the confluence with Bear Creek. Therefore, we recommend an electrofishing survey throughout this section of Pigeon Creek to confirm the presence of Loach Minnow. Pending survey results, the Department, USFS, and USFWS should discuss whether Loach Minnow should be translocated from the Blue River into Pigeon Creek to bolster the population. We recommend that Pigeon Creek be consistently monitored for Loach Minnow and Roundtail Chub by another Department program or under the Gila River Basin monitoring contract.

Reavis Creek. On May 7, 2019, Department staff evaluated stream habitat for native fish in Reavis Creek. Staff evaluated habitat at Reavis Saddle Spring and Honeycutt Spring in the headwaters of Reavis Creek and determined that fish habitat likely did not exist at either of these locations. Flow was present in most of Reavis Creek from Honeycutt Spring down to the first large perennial pool (UTM 12S 485714/3705216). Habitat in this upper reach is mostly high gradient riffle through a canopy of small willow trees. Department staff installed a temperature logger in the first perennial pool (max depth = 1.22 m) to verify that the pool is in fact perennial and assess winter minimum water temperatures. Cold water temperatures may be of concern at this location due to the relatively high elevation (~4,900 ft). Staff identified six additional pools that appeared to be perennial downstream to near the crossing of trail 117 (max depth range = 0.8-1.25m). Habitat between the pools consisted of a mixture of low gradient riffle, glides and shallow pools. Downstream of the trail 117 crossing, the riparian vegetation becomes sparse and flows went subsurface at 485401/3707173.

Recommendations: Habitat in Reavis Creek appears to be suitable for Gila Topminnow. However, a final determination should not be made until the temperature logger is retrieved sometime in spring of 2020. The Woodbury Fire of 2019 burned a portion of the Reavis Creek watershed a few weeks after the survey took place so habitat needs to be reassessed prior to any translocations.

Salt House Creek. On August 20, 2019, Department staff surveyed aquatic habitat in Salt House Creek, tributary to East Eagle Creek. Standardized habitat surveys were carried out at two randomly selected transects from Sawmill Cabin on East Eagle Creek upstream through Salt House Creek to the confluence with Chitty Creek. The relative similarity of Salt House Creek habitat sites assessed since 2016 can be found in Figure 7. There was substantial spring input in the middle of the most upstream site (UTM 12S 648411/ 3708680) and continuous flow for about a kilometer downstream to the confluence with East Eagle Creek. Six Speckled Dace were observed and one was captured with a dip net in lower Salt House Creek, which suggests that flows are likely perennial and fish seem to be able to disperse upstream from Eagle Creek at higher flows.

Recommendations: Salt House Creek may be suitable for Loach Minnow. Speckled Dace apparently persist in Salt House Creek, but further surveys should be carried out at low flow periods to determine how much perennial water is present. A temperature logger could also be installed to determine if the stream dries and whether temperatures are suitable for Gila Trout.

Sycamore Canyon. On May 15, 2019, Department staff assessed stream habitat for native fish in Sycamore Canyon, tributary to the Blue River. Two standardized habitat surveys were carried out at randomly selected 100 meter reaches. The relative similarity of Sycamore Canyon habitat sites to habitat sites assessed since 2016 can be found in Figure 7. Unfortunately, the stream is very small (mean wetted width typically < 1 meter) and intermittent in sections. There were two sections of continuous flow at the time of the survey, one about 400 m long and the second about 1.22 km long. Sycamore Canyon may not have enough flow to support fish populations in dry years, especially Loach Minnow. However there were a few deep pools present which could potentially support Roundtail Chub.

Recommendations: Surface flow was present in less than 2 km of Sycamore Canyon during the survey, and the total length of surface flow likely declined before monsoon rains. Consequently, the Department does not recommend translocating any native fish into Sycamore Canyon.

Expand Roundtail Chub¹ population in Harden Cienega Creek (Task AZ-2014-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 1. Identify critical streams and populations in need of protection and potential replication.
 - Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

• Gila Chub draft recovery plan objective 2. Ensure representation, resiliency, and redundancy by expanding the size and number of populations within Gila Chub historical range via replication of remnant populations within each RU.

<u>Background:</u> Harden Cienega Creek is a tributary to the San Francisco River near the New Mexico state line. Roundtail Chub¹ distribution was historically limited to approximately 2 km of stream below a natural waterfall barrier. In April 2013, Department staff surveyed above the waterfall and determined that about 1.4 km of perennial water existed above the waterfall that was suitable for Roundtail Chub¹. It was recommended that chub be moved above the waterfall to expand their distribution in Harden Cienega Creek and the CAP Policy committee approved the project in February 2014. In April, 2015, a total of 102 Roundtail Chub¹ were translocated from lower Harden Cienega Creek to above the waterfall. Monitoring in 2017 and 2018 detected several hundred chub representing all size classes. In 2018, Department staff also captured five Roundtail

¹ Roundtail Chub in Harden Cienega Creek were previously classified as Gila Chub

Chub¹ below the barrier and translocated them to near the upper end of perennial water in an attempt to maintain genetic diversity. Unfortunately, during the post-stocking monitoring, Green Sunfish were detected above the barrier in both 2017 and 2018 with one removed in 2017 and two in 2018. Because Green Sunfish were captured well upstream of the barrier, it is likely that there is an upstream source of Green Sunfish in the Harden Cienega drainage.

<u>Results:</u> On October 16, 2019, Department staff captured a total of 104 Roundtail Chub¹ in five seine hauls downstream of the barrier and translocated them to near the upstream end of perennial water (UTM 12S 676720/3673459) to improve the genetic diversity of the existing population. Before releasing the chub, Department staff also monitored Roundtail Chub¹ above the barrier by setting twelve mini-hoop nets from the upper end of perennial water downstream to near the previous lower stocking location (676284/3673788). Five traps were pulled before two hours of soak time so that staff could safely return to camp before dark. A total of 103 Roundtail Chub¹ were captured with a mean length of 104 mm TL (min = 28, max = 221; Figure 25). Roundtail Chub¹ are certainly established above the barrier and have dispersed to virtually all available habitat.

Four Green Sunfish (168, 138, 152 and 130 mm TL) were captured in a pool downstream of the barrier (675592/3674293) and all but one was removed. Because Green Sunfish have been captured well upstream of the barrier, it is likely that there is an upstream source of Green Sunfish in the Harden Cienega drainage.

<u>Recommendations:</u> The upstream source of Green Sunfish in the Harden Cienega Creek drainage should be identified. There are approximately 82 tanks upstream in the drainage, with 33 located in New Mexico, so it will be necessary to work with New Mexico Department of Game and Fish to identify the source of Green Sunfish. A removal plan for Harden Cienega Creek should be developed and tank surveys and removal passes should begin in 2020. Roundtail Chub in upper Harden Cienega Creek should continue to be monitored until at least 2020.

Eagle Creek repatriations (Task AZ-2018-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 1. Identify critical streams and populations in need of protection and potential replication.
 - \circ Goal 3. Protect native fish populations from nonnative fish invasions.
 - Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.

¹ Chub in Harden Cienega Creek were previously classified as Gila Chub.

• Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Spikedace recovery objective 6.3. Reintroduce Spikedace to selected reaches.
- Spikedace recovery objective 6.4. Monitor success/failure of reintroductions.
- Loach Minnow recovery objective 6.3. Reintroduce Loach Minnow to selected reaches.
- Loach Minnow recovery objective 6.4. Monitor success/failure of reintroductions.
- Gila Chub draft recovery plan objective 2. Ensure representation, resiliency, and redundancy by expanding the size and number of populations within Gila Chub historical range via replication of remnant populations within each RU.
- Gila Chub draft recovery plan objective 7. Monitor remnant, repatriated, and refuge populations to inform adaptive management strategies.
- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.

Background: Eagle Creek is a tributary to the Gila River near Clifton Arizona, and flows across Apache-Sitgreaves National Forest, San Carlos Apache, and private lands. Native fish documented from Eagle Creek include Spikedace, Loach Minnow, Roundtail Chub¹, Speckled Dace, Longfin Dace, Desert Sucker, Sonora Sucker, and Gila Trout. However, Spikedace were last recorded in 1989 and Loach Minnow in 1997. Various nonnative fish species occupy Eagle Creek but the upper reach above the confluence with Willow Creek is now occupied by only native species. Water pumped from the Black River into Eagle Creek by Freeport McMoran for use at the Morenci Mine is a potential source of nonnative fish to the Eagle Creek drainage, particularly Smallmouth Bass. Freeport McMoran has committed to building a barrier on upper Eagle Creek above the Willow Creek confluence as part of a management plan. Reclamation is providing engineering expertise for design of the barrier. The Department plans to repatriate Spikedace and Loach Minnow once the barrier is constructed. Gila Topminnow will be considered for translocation in the reach upstream of the barrier. In 2018, Department staff developed a draft monitoring plan for Eagle Creek in preparation for barrier construction and native fish reintroduction.

<u>Results:</u> In August, 2019, Department staff collected eDNA samples at ten sites and carried out standardized habitat surveys at three sites between Honeymoon Campground and the confluence with Dry Prong Creek. Data collected during habitat surveys were incorporated into a non-metric multidimensional scaling analysis (NMDS) in an attempt to identify potential translocation sites most likely to support a translocated population of Spikedace or Loach Minnow based on current

¹ Both Roundtail Chub and the form previously classified as Gila Chub are documented in Eagle Creek.

or historical presence of Spikedace and Loach Minnow (Hickerson and Walters 2019). The relative similarity of Eagle Creek habitat sites to habitat sites assessed since 2016 can be found in Figure 7. In general, Eagle Creek habitat sites were relatively similar to sites currently occupied by Loach Minnow and Spikdeace, which is unsurprising given that both species historically occupied Eagle Creek.

Neither Spikedace nor Loach Minnow were positively detected in any of the eDNA samples. Prebarrier fish surveys were not carried out because it became clear that the barrier would not be built in 2020.

<u>Recommendations:</u> Pre-barrier fish surveys of Eagle Creek should occur in 2020 if the barrier is likely to be built in 2021. The USFWS collected eDNA samples on the San Carlos Apache lands in 2019 to determine presence and distribution of the species. We recommend that eDNA samples also be collected from the three USFS sections of Eagle Creek downstream of Sheep Wash and bounded by tribal property: a 0.6 km section near Cistern Canyon and the 4.4 km section roughly between Whitewater Canyon and Knight Canyon, and a 0.6 km section near Milkshake Spring. There are eight eDNA collection locations in these sections.

Red Tank Draw native fish restoration (Task AZ-2016-2)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 1. Identify critical streams and populations in need of protection and potential replication.
 - o Goal 4. Remove nonnative aquatic species threats.
 - Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Gila Chub draft recovery plan objective 1.3.1. Eliminate or control problematic nonnative aquatic organisms.
- Gila Chub draft recovery plan objective 7. Monitor remnant, repatriated, and refuge populations to inform adaptive management strategies.

- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 2.4 Protect habitats of reestablished or potential populations from detrimental nonnative aquatic species.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.

Background: Red Tank Draw is a tributary to Wet Beaver Creek on the Coconino National Forest. Red Tank Draw is occupied by Roundtail Chub¹, Longfin Dace, Desert Sucker, Sonora Sucker, and several nonnative species including Green Sunfish, Black Bullhead, Fathead Minnow, and Northern Crayfish. Roundtail Chub¹ inhabit a fragmented perennial reach between the USGS gage and the confluence of Rarick and Mullican Canyons. Perennial pools exist in the tributaries Rarick Canyon and Mullican Canyon that support nonnative fishes. The total perennial portion of Red Tank Draw is about 2.40 km long and is isolated from upstream invasion of nonnative fish from Wet Beaver Creek by an intermittent reach that is approximately 7.7 km long, but is likely passable during high continuous flows. The purpose of this project is to remove Green Sunfish and Black Bullhead from the Roundtail Chub¹ occupied reach, and the entire drainage above the chub occupied reach if possible. A comprehensive survey of stock tanks in the Red Tank Draw drainage above the chub occupied reach in 2017 found only Fathead Minnow occurred in the Rarick Canyon drainage (Rarick Tank and Gnat Tank). Unfortunately, Green Sunfish and Black Bullhead were detected in Mullican Place Tank in the Mullican Canyon drainage. Mullican Place Tank is immediately downstream of Bruce Place Tank which is on private property. The landowner indicated that fish were present in Bruce Place Tank, but denied access for sampling in 2017. An impassable waterfall barrier (UTM 12S 437657/3843902) was documented in Rarick Canyon in 2018. The barrier is approximately 10 meters in height and is located about 2 km upstream from the confluence with Mullican Canyon. Isolated perennial pools upstream of the barrier in Rarick Canyon were visually assessed during 2017 and 2018 and a total of 23 perennial pools were identified, with Fathead Minnow observed throughout wetted reach. Unfortunately, two Black Bullhead were also observed in a single pool upstream of the barrier in the 2018 surveys.

<u>Results:</u> A full summary of number of fish captured by removal effort in Red Tank Draw from 2016 to 2019 can be found in Figure 26. Similarly, trends in mean relative abundance of Roundtail Chub¹, Green Sunfish, Black Bullhead and Fathead Minnow by year can be found in Figure 27.

A removal plan for the Red Tank Draw drainage, including Rarick and Mullican Canyons, was drafted in March, 2019 (Hickerson and Robinson 2019). The plan outlines removal strategies and metrics for success for different portions of the drainage. In brief, the plan outlines that successful eradication in all waters will be characterized by five complete passes without any nonnative fish detected. In addition, the plan specifies that a successful suppression effort in Red Tank Draw will

¹ Chub in Red Tank Draw were previously classified as Gila Chub.

Cooperative Agreement R16AC00077: 2019 Annual Report - Final 03/24/2020

be characterized by decreasing relative abundance (CPUE) of nonnative fishes and a shift in population size structure to primarily smaller, juvenile fish with few adults present. Similarly, targets for nonnative fishes are an increasing relative abundance and a population size structure with all age classes present and evidence of reproduction. Success will be evaluated within and between years.

Rarick Canyon above the 10-m waterfall. During April 25-26, 2019, Department staff surveyed pools above the 10-m tall waterfall barrier in Rarick Canyon. They set 53 mini-hoop nets in 19 pools and captured a total of four Black Bullhead from a single pool. Four of the pools identified in Rarick Canyon in 2018 were not sampled because they were either dry or nearly dry. Department staff also snorkeled 10 pools where water clarity and depth permitted, but failed to detect any bullhead.

During June 17-19, 2019, Department staff conducted a second removal. A total of 63 mini hoop nets were set overnight in 16 pools. Three of the pools previously sampled or identified were not sampled because they were either dry or nearly dry. A total of five Black Bullhead and 95 Fathead Minnow were captured and removed from the stream. Department staff also snorkeled one pool where visibility and depth allowed but did not detect Black Bullhead.

During July 1-3, 2019, Department staff performed the third removal pass. On the first day of the removal effort, a total of 20 mini-hoop nets were set overnight in the six most downstream pools and resulted in the capture of 46 Fathead Minnow. One Swedish-style gill net was also set overnight in the pool where the majority of the Black Bullhead had been detected and resulted in the capture of one Black Bullhead. Department staff also tried angling using chicken livers but failed to capture any Black Bullhead. An additional 28 mini-hoop nets were set overnight on the second day of removals in the 11 most upstream pools and resulted in the capture of 59 Fathead Minnow.

On July 16, 2019, Department staff performed a fifth removal. A total of 20 mini-hoop nets were set in the five most downstream pools. A total of 49 Fathead Minnow were captured. Three Swedish-style gill nets were also set overnight in the pools where Black Bullhead had been detected and resulted in the capture of two Black Bullhead.

During July 22-24, 2019, Department staff performed the sixth removal in Rarick Canyon. On the first day of removal efforts, a total of six mini-hoop nets were set in the two most upstream pools and captured a total of 10 Fathead Minnow. Department staff also set 21 mini-hoop nets overnight in the four most downstream pools and captured a total of 42 Fathead Minnow. Three Swedish-style gill-nets were also set overnight in the pools where most Black Bullhead had been detected with no fish captured. Mini-hoop nets were re-set in the four most downstream pools on the second day and captured a total of 17 Fathead Minnow. The Swedish-style gill nets were also re-set and

captured a single Black Bullhead. Department staff angled in the pool where the majority of Black Bullhead had been detected but failed to capture any.

During July 29-31, 2019, Department staff performed the seventh removal effort. On the first day a total of 19 mini-hoop nets were set overnight in seven pools and resulted in the capture of 47 Fathead Minnow. Three Swedish-style gill nets were also set overnight in the pool where most of the Black Bullhead had been detected with no fish captured. The following day, Department staff reset all 19 traps and set another 8 mini-hoop nets overnight in two additional pools and captured a total of 41 Fathead Minnow. All three Swedish-style gill nets were reset and failed to capture any fish.

During August 7-8, 2019, Department staff conducted the eighth removal in 2019. Five mini-hoop nets were set overnight in one pool and resulted in the capture of one Fathead Minnow. Three Swedish-style gill-nets were set overnight with no fish captured.

During August 27-29, 2019, Department staff conducted the ninth removal. Six mini-hoop nets were set overnight in one pool and resulted in the capture of 55 Fathead Minnow. Three Swedish-style gill nets were set overnight with no fish captured. On the second day the six mini-hoop nets were re-set and resulted in the capture of 16 Fathead Minnow. The Swedish-style gill nets were also reset with no fish captured. Department staff also surveyed Rarick Tank and Gnat Tank in the Rarick Canyon drainage to verify that Black Bullhead were absent from tanks in the drainage. In Rarick Tank, staff performed three bag seine hauls and did not capture or observe any fish. At Gnat Tank staff performed two bag seine hauls and captured more than 1,000 Fathead Minnow.

Red Tank Draw. On May 24, 2019, Department staff backpack electrofished through about 2,591 m of Red Tank Draw from the downstream end of perennial water, ending about 400 m from the upstream end of perennial water. A total of 39 Green Sunfish and 9 Fathead Minnow were captured and removed in 4,574 seconds of electrofishing effort. A total of 44 Roundtail Chub¹ were captured and returned to the stream. A total of eight mini-hoop nets were set in the large pool at the downstream end of the sampling reach and captured an additional four Green Sunfish.

On July 18, 2019, Department staff completed the first full electrofishing pass through the perennial water in Red Tank Draw in 2019. Staff shocked for a total of 3,555 seconds and captured and removed 20 Green Sunfish and two Fathead Minnow. A total of 251 Roundtail Chub¹ and 8 Desert Sucker were captured and returned to the stream.

During July 29-30, 2019, Department staff completed the second full removal pass in Red Tank Draw. A total of 131 Green Sunfish and 89 Fathead Minnow were captured and removed in 6,754 seconds of electrofishing effort. Five mini-hoop nets and three minnow traps were set in the pool at

¹ Roundtail Chub in Red Tank Draw were previously classified as Gila Chub

the downstream end of the perennial reach for a minimum soak time of 2 hours and resulted in the capture of ten Green Sunfish. A total of 550 Roundtail Chub¹ and 6 Desert Sucker were also captured and returned to the stream.

Chub translocation. On October 21, 2019, Department staff translocated Roundtail Chub¹ from Red Tank Draw to a series of three pools in Rarick Canyon upstream of the barrier falls. A total of 319 juvenile Roundtail Chub¹ were collected from Red Tank Draw during 3,879 seconds of electrofishing effort. There were four mortalities during collection and transport. An additional 20 Green Sunfish and one Fathead Minnow were captured and removed during the collection process. A total of 154 chub were stocked in pool F23 (UTM 12S 438094/3844065), 93 in pool F20 (439785/3844473) and 72 in pool F18 (440122/3844798). Department staff also placed a temperature logger in pool F18 to monitor winter water temperatures.

<u>Recommendations</u>: The Roundtail Chub¹ stocked in Rarick Canyon should be monitored for five years after the final establishment stocking. If no additional chub are stocked to facilitate establishment, then post-stocking monitoring will be completed by 2024. Roundtail Chub¹ should be translocated into additional pools further upstream in Rarick Canyon if abundance of chub in Red Tank Draw remains sufficient to allow additional collections. If possible, Roundtail Chub¹ should also be stocked into Gnat Tank with the goal of reducing or eliminating the upstream source of Fathead Minnow to Rarick Canyon. If overwinter water temperatures are sufficient, Gila Topminnow should be translocated into Rarick Canyon in 2020, pending cooperation with partner agencies.

Nonnative fish should be eradicated from the two tanks in Mullican Canyon drainage where nonnative fish are present (Mullican Place Tank and Bruce Place Tank). However, Bruce Place Tank is on private land and further attempts to engage the landowner should be pursued.

If nonnative fish cannot be eradicated from Bruce Place Tank, then eradication in Red Tank Draw would not be possible because the upstream source of nonnative fish would remain. In that situation, the goal of removal of nonnative fishes from Red Tank Draw would be to suppress the nonnative fish populations and thus benefit the native fishes. Targets for nonnative suppression should be developed based on other successful suppression and removal efforts in the literature and the region. These suppression targets should be incorporated into the removal plan for Red Tank Draw. A suppression target will help guide how much effort should be directed toward removals in any given year until the source population in Mullican Canyon can be eradicated.

Sharp Spring native fish restoration (Task AZ-2016-3)

Strategic Plan Goals:

• Preventing Extinction and Managing Toward Recovery

- Goal 1. Identify critical streams and populations in need of protection and potential replication.
- Goal 4. Remove nonnative aquatic species threats.
- Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
- Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Gila Chub draft recovery plan objective 1.3.1. Eliminate or control problematic nonnative aquatic organisms.
- Gila Chub draft recovery plan objective 2. Ensure representation, resiliency, and redundancy by expanding the size and number of populations within Gila Chub historical range via replication of remnant populations within each RU.
- Gila Chub draft recovery plan objective 7. Monitor remnant, repatriated, and refuge populations to inform adaptive management strategies.
- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 2.4 Protect habitats of reestablished or potential populations from detrimental nonnative aquatic species.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.

<u>Background:</u> Sharp Spring is a tributary to the Santa Cruz River in the San Rafael Valley, about 2 km from the U.S.A. – Mexico border, and is on San Rafael State Natural Area. Sharp Spring is perennial, and flows through a series of cienega pools; the larger pools have numbered staff gauges to help detect changes over time. Sharp Springs was historically occupied by Gila Topminnow. Nonnative Western Mosquitofish were first found in Sharp Springs in 1979. Monitoring by the Department and partners documented the disappearance of Gila Topminnow, which has not been detected since 2002. The extirpation was attributed to predation and competition with nonnative mosquitofish, and reduced flooding. The purpose of this project is to eradicate Western Mosquitofish from Sharp Spring, and then repatriate Gila Topminnow and Roundtail Chub¹. The Sharp Springs lineage of Gila Topminnow would be translocated from one or more of the replicate populations in the state. Roundtail Chub¹ from the nearby Sheehy Spring may be translocated to Sharp Spring.

During June 2013, Department staff attempted to dry the pools in Sharp Spring by pumping water out. They pumped down the two uppermost pools, but because of the large amount of fine sediment

¹ Chub in Sheehy Spring to be repatriated into Sharp Spring were previously classified as Gila Chub.

in the bottom of the pools, could not pump all of the water out. The pools partially refilled overnight, and mosquitofish were observed in the downstream pool the next morning. The effort was terminated because the pools could not be completely dried. Afterwards, other ideas for eradicating the mosquitofish were proposed including: treating with rotenone, treating with ammonia, heating the water in each pool, adding organic matter to the pools to create anoxic conditions, covering the pools with black plastic or adding dye to the pools to create anoxic conditions. The Department met with Arizona State Parks Department in January 2017 to discuss how to move forward with nonnative fish eradication in Sharp Spring. Subsequent to the meeting, Arizona State Parks stopped communicating with the Department relative to this project.

<u>Results:</u> No work was completed on this project in 2019, because Arizona State Parks never communicated with, or gave the Department permission to move forward with the project.

<u>Recommendations:</u> Department staff thinks that the most effective way to eradicate mosquitofish from Sharp Spring would be treatment with rotenone. The Department will coordinate with State Parks to see if they will approve of the project. This project should be removed from the priority list until State Parks approves moving forward with the removal.

Boyce Thompson Ayer Lake native fish restoration (Task AZ-2000-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 4. Remove nonnative aquatic species threats.
 - Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - Goal 9. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.
- Gila Chub draft recovery plan objective 1.3.1. Eliminate or control problematic nonnative aquatic organisms.
- Gila Chub draft recovery plan objective 7. Monitor remnant, repatriated, and refuge populations to inform adaptive management strategies.
- Desert Pupfish recovery objective 2. Re-establish Desert Pupfish populations.
- Desert Pupfish recovery objective 5. Monitor and maintain natural, re-established, and refugia populations.

Background: Ayer Lake at Boyce-Thompson Arboretum, near Superior, has served as a refuge for Gila Topminnow and Desert Pupfish since the 1970's. In addition, Arizona Game and Fish Department uses these Aver Lake populations to establish new populations of these two species throughout the Gila River Basin. Gila topminnow was first stocked into Ayer Lake in 1971, then in 1972, and 1978. Desert pupfish were first stocked in 1977. Nonnative fish invaded the reservoir, and so Ayer Lake was chemically treated with piscicides three times; in 1979 to remove black bullhead, in 1980 to again remove black bullhead, and in 1983 to remove Western Mosquitofish. After the third renovation, a mixed stock of Monkey Springs and Monkey Springs-Cocio Wash-Bylas Springs populations of Gila Topminnow were stocked in 1985 (USFWS 1998). However, Hedrick et al. (2001) found only alleles from Monkey Springs, so that is the lineage considered replicated in Ayer Lake. A mixed Lower Colorado River Delta stock of Desert Pupfish were acquired from Santa Clara Slough, Dexter National Fish Hatchery, and Deer Valley High School (USFWS 1993) and stocked in 1984 and 1985. During 1986 monitoring, nonnative Fathead Minnow were discovered in the pond, and have been present ever since. Red Swamp Crayfish, another nonnative species, was first observed during 1976 monitoring, and it continues to inhabit Ayer Lake. Western Mosquitofish was detected in November 2010, after which the Department discontinued using Ayer Lake as a source of Gila Topminnow for translocations. In addition to the fish species, native herptiles Sonoran Mud Turtle and Lowland Leopard Frog inhabit the lake, as do native aquatic plants such as cattails Typha spp. and hard-stem bulrush Schoenoplectnus acutus, and a wide array of aquatic invertebrates.

In 2008, Arizona State Parks staff were concerned about treating Ayer Lake with a piscicide, because watchable invertebrates (dragonflies and damselflies) would also be temporarily eradicated and the lake's water was needed for irrigation. However, in 2015, the Director of Boyce Thompson Arboretum agreed to again consider partially draining the pond and treating it to remove the nonnative species. The park was going to try to install a new well, after which the pond could be partially drained and the treatment done. Funding fell through for the new well, and as of the end of 2018, no new funding sources had been identified.

The purpose of this project is to eradicate the nonnative fishes, and if possible the nonnative crayfish from Ayer Lake and then reestablish Gila Topminnow and Desert Pupfish, and possibly establish Roundtail Chub¹.

<u>Results:</u> During 2019 Department staff communicated several times with a new member of the Board of Directors for Boyce Thompson Arboretum State Park. The board member was very interested in restarting the project to restore Ayer Lake to a native fish refugia. The board decided to remove the lake from the watering of the arboretum. The board member communicated that it would take time to obtain water storage tanks, redirect well water to them and then connect this water to the garden. Once this is done, then they could deal with the lake (drain, dredge, remove cattails, refill, install aerator, re-landscape, etc). The whole process would take many months.

¹ Chub at these locations were previously classified as Gila Chub.

<u>Recommendations:</u> Department staff will continue to communicate with Boyce Thompson State Park to determine when or if the pond will be ready to commence with native fish restoration. This project should be removed from the priority list until State Parks is ready for the Department to perform nonnative fish removal (if not done by the park via drawdown) and/or native fish stockings. If the park performs the drawdown removal, then this project should be moved to the Gila Topminnow Stockings Project.

Upper Verde River Native Fish Restoration (Task AZ-2020-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - Goal 1a. Identify critical streams and populations in need of protection and replication
 - Goal 4a. Eradicate nonnative aquatic species from a minimum of five surface waters to prepare them for repatriations of native fishes.
 - Goal 5a. Replicate Gila topminnow stocks into a minimum of 10 surface waters.
 - $\circ~$ Goal 5b. Replication each of the other priority species into a minimum of one surface water.
 - Goal 9b. Develop/identify monitoring standards as necessary to adequately evaluate fish barrier function, success and failure of eradications, and success and failure of repatriations.

Recovery Objectives:

- Spikedace recovery objective 6.2.5. Reclaim as necessary to remove non-native fishes.
- Spikedace recovery objective 6.3. Reintroduce Spikedace to selected reaches.
- Spikedace recovery objective 6.4. Monitor success/failure of reintroductions.
- Loach Minnow recovery objective 6.2.5 Reclaim as necessary to remove non-native fishes.
- Loach Minnow recovery objective 6.3. Reintroduce Loach Minnow to selected reaches.
- Loach Minnow recovery objective 6.4. Monitor success/failure of reintroductions.
- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 2.4 Protect habitats of reestablished or potential populations from detrimental nonnative aquatic species.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.
- Razorback Sucker recovery objective 1.3 Reduce adverse biological impacts
- Razorback Sucker recovery objective 2.6 Augment or reintroduce XYTE in recovery areas
- Razorback Sucker recovery objective 2.6.2.3 Monitor reestablishment and augmentation efforts

<u>Background</u>: The upper Verde River Native Fish Restoration Project is a multi-agency effort focused on protecting and restoring the native fish assemblage within the upper Verde River drainage in central Arizona. The project consists of three main components: construction of two fish barrier(s), control of nonnative fishes in the drainage, and repatriation and monitoring of federally listed and unlisted warm-water fishes. Barrier construction may begin as early as 2022. Nonnative control could commence afterwards.

<u>Results:</u> During 2019, Department staff assessed the feasibility of surveying stock tanks in the upper Verde River drainage for presence of nonnative fishes. A shapefile containing 1,266 tanks from the drainage was obtained along with National Agricultural Imagery Program (NAIP) imagery from 2013, 2015 and 2017. An automated approach was developed in Program R to classify tanks as wet or dry using normalized difference water index (NDWI) values calculated within a ten meter buffer of each of the 1,266 tank points. A subset of tanks was manually classified using the 2015 imagery for use as a validation dataset. The automated procedure correctly classified tanks as wet 65.6% of the time. Most of the inaccuracy was due to classification of dry tanks as wet. Importantly, only five of the 799 tanks included in the validation dataset were classified as dry resulting in an area under the curve (AUC) value of 0.965. Tanks were then scored based on perennial status, previous nonnative fish records, and distance to the Verde River. Tanks that were classified as dry in at least one year of imagery were given a score of zero. A total of 146 tanks received a score of 2 or greater which corresponded to the greatest risk categories. These highest risk tanks will be prioritized for surveys when surveys are initiated prior to treatment of the Verde River.

<u>Recommendations</u>: Previous tank surveys required a substantial amount of time to visit all target stock tanks. Preliminary scheduling suggests that it may take up to 12 work weeks to sample all 146 problematic tanks. Consequently, tank surveys should be initiated well before the treatment and potentially before the barrier is constructed. Information about the frequency and duration of hydrologic connections between tributary streams (Hell Canyon, Chino Valley Wash, etc.) and the Verde River should also be evaluated with trail cameras in order to better understand the risk of dispersal of nonnative fishes from stock tanks to the Verde River. Additionally, flow information including the discharge, travel times and the location of gaining and loosing reaches should be collected at least a year before treatments are planned.

Aquatic Research and Conservation Center O&M (Task HA-2006-2)

Strategic Plan Goals:

- Scientific Foundation
 - \circ $\,$ Goal 3. Improve propagation techniques for Spikedace and Loach Minnow $\,$
- Preventing Extinction and Managing Toward Recovery
 - <u>Goal 2.</u> Maintain and operate ASU topminnow holding facility and the Aquatic Research and Conservation Center (ARCC) to support the Program's recovery efforts for imperiled fishes in the Gila River Basin through the establishment of

refuge populations of genetically distinctive stocks as insurance against extinction in the wild, captive propagation for repatriation, and applied research.

Recovery Objectives:

- Spikedace recovery objective 8. Plan and conduct investigations on captive holding, propagation and rearing.
- Loach Minnow recovery objective 8. Plan and conduct investigations on captive holding, propagation and rearing.
- Gila Topminnow draft revised (1999) recovery objective 1.1. Maintain refugia populations of natural populations to ensure survival of the species.
- Desert Pupfish recovery objective 2. Reestablish Desert Pupfish populations.
- Gila Chub draft recovery plan objective 4. Establish and maintain refuge populations in protected ponds or hatcheries as appropriate.

<u>Background:</u> Reclamation funded construction of a native fish conservation facility on the grounds of the Department's Bubbling Ponds Hatchery. The main purposes of the facility were to develop propagation techniques for Loach Minnow and Spikedace, to establish refuge populations of all lineages, and to propagate fish for repatriations. A wet lab was constructed in 2000, a well was installed in 2003 to supply water to the facility, and open-air production and grow-out building was constructed in 2007. Number of Spikedace and Loach Minnow brood stock and number of fish produced and brought into ARCC each year since 2007 is presented in Table 1. See Task AZ-2003-1 (Acquire Spikedace, Loach Minnow and rare populations of other native fish) for background information on each lineage.

Other fish species were brought to the facility for similar purposes as Loach Minnow and Spikedace. Woundfin were brought to the facility in 2008 to attempt to produce offspring for stocking into the Hassayampa River. Gila Topminnow (Sharp Spring lineage) and Desert Pupfish were brought to the facility in 2009 for a competition experiment, but most were stocked out afterwards. Eagle Creek Roundtail Chub were brought to the facility in 2010 to establish a refuge population, so fish produced could be stocked into the Blue River. In 2012, the Cottonwood Springs lineage of Gila Topminnow was brought in to establish a broodstock so that fish produced could be used in repatriations. The facility holds various other species for research or educational purposes.

The facility was originally named Bubbling Ponds Native Fish Conservation Facility, but in 2015 was renamed the Aquatic Research and Conservation Center (ARCC). Beginning in 2014, Reclamation began providing funds (through USFWS) for a variety of improvements to ARCC, including a new outdoor building to hold more tanks, a new quarantine building, and new ponds.

In late 2018 ARCC staff sent a draft version of the hatchery operation manual with a complete appendix to Department Research Branch staff for additional edits. A printed version is currently available at ARCC for staff and visitors and has already become a useful reference tool.

<u>Results:</u> The Department continued to operate ARCC in 2019. The ARCC maintains refuge populations of three lineages of Spikedace (Aravaipa Creek, upper Gila River, and Gila River Forks) and four lineages of Loach Minnow (Blue River, Aravaipa Creek, San Francisco River, and Gila River Forks). Target refuge population size for each lineage is 500. In 2019, ARCC produced 6,757 Aravaipa Creek Spikedace, 2,404 upper Gila River Spikedace, 1,975 Gila River Forks Spikedace, 734 Blue River Loach Minnow, 1,415 Aravaipa Creek Loach Minnow, 704 San Francisco River Loach Minnow, and 676 Gila River Forks Loach Minnow (Table 1). At the end of the 2019 spawn season, brood counts for Spikedace lineages were: Aravaipa 523, Gila Forks 71 and Gila River 254. Loach Minnow brood counts were: Aravaipa 354, Blue River 290, Gila Forks 169 and San Francisco 231. In 2019, the numbers of Aravaipa Spikedace and Loach Minnow, respectively, brought on station were: 60 and 25 in February and 262 and 32 in November. In addition, 98 Gila River and 1 Gila Forks Spikedace, and 78 Blue River and 42 Gila Forks Loach Minnow were brought in. No San Francisco Loach Minnow nor Gila Forks Spikedace were brought in.

The ARCC also continued to maintain a small brood stock of Eagle Creek Roundtail Chub consisting of 99 individuals. These fish were not spawned in 2019 due to a lack of stocking options for offspring already on station from the previous year.

During 2018, ARCC staff started testing effects of fish density on propagation success of captive Spikedace and Loach Minnow. After the successful 2018 trials, staffed planned to conduct a second year using the exact same setup. Unfortunately, roughly 137 Aravaipa Spikedace broodstock were lost during the 2018 spawning season, and no wild Aravaipa Spikedace or Loach Minnow were brought into ARCC in autumn 2018. On February 1, 2019, 60 wild Spikedace and 25 Loach Minnow were brought on station, but by the time the quarantine period was over, it was too late to add them to the broodstock for the 2019 spawning season. Therefore, all spawning raceways were setup identically to one another at the lowest most successful density identified during 2018 with no preference given to any one lineage. The number of raceways used for each lineage was dependent on the overall brood stock size with each raceway having 32 adult fish and 13 nest sites for Loach Minnow and 34 adults for Spikedace. Loach Minnow were once again given nest sites consisting of medium sized cobbles arranged in 15-cm circles spaced 38 cm from edge of nest to edge of nest on a bed of small chip gravel. For both species, larval fish were manually removed once per week, counted and logged. Algae was carefully removed once per week to minimize the potential effects of high algal biomass on spawning. Detailed logs were kept regarding larval fish removed each week and any larval or brood mortalities. Temperature loggers were placed in each study to help identify correlations between temperature peaks and larval fish production. Lastly, a small bail of barley straw approximately 25 cm in length and 10 cm in diameter was installed in each raceway to help reduce algal growth. Overall, these proved effective

in reducing the algal growth in the raceways and thus limited the amount pulled and the potential of damaging eggs or unintentionally removing larval fish.

Physical improvements to ARCC completed in 2019 such as the installation of the 16 new 8-ft circular tanks have directly contributed to the success of the 2019 spawn season. These new holdings tanks allowed staff to lower the densities of larval holding tanks resulting in lower larval fish mortality and enhanced security from disease transfer and effects.

<u>Recommendations:</u> For 2020, ARCC staff will focus on a second year of the majority of raceways being run at the lowest density identified in 2018. This will help verify that those densities will produce consistent larval counts. Staff may also run a few replicates of Loach Minnow raceways to examine effects of nest spacing and fish densities. Staff will also complete a second year of 2019's failed attempt at paired-propagation of Loach Minnow in aquaria.

PROJECTS REMOVED FROM PRIORITY LIST

Arnett Creek repatriations. Merged into Gila Topminnow Stockings project.

Arizona trout streams Loach Minnow repatriations. Removed from list because the Department CAMP program is implementing this project.

Bonita Creek renovation and repatriations. Removed from list because project was completed in 2018.

Fish health assessments of translocation populations. Removed from the priority list and instead merged these into individual projects.

Fossil Creek repatriations. Completed in 2016.

Mineral Creek drainage renovation and repatriations. Removed until State Land Department approves of wildlife translocations on their managed lands.

Miscellaneous stock tank surveys. Removed from the priority list and merged into individual projects.

Post-repatriation evaluations. This project was removed from the priority list because post-repatriation evaluations (monitoring) are reported under each specific priority action.

Transfer Roundtail Chub¹ and Gila Topminnow to New Mexico. Removed from priority list until New Mexico is ready to request more fish.

Sands Draw repatriations. This project was removed from the priority list in 2016 until BLM has the habitat ready for fish.

West Fork Pinto repatriations. Merged into Gila Topminnow Stockings.

LITERATURE CITED

- Anderson, M. 2015. Stream habitat assessment protocol; draft. Fisheries Branch, Arizona Game and Fish Department, Phoenix.
- Arizona Game and Fish Department. 2011. Fish Collection, transport, and stocking protocol: best management practice (BMP #4). Arizona Game and Fish Department, Phoenix.
- Armstrong, D. P., and P. J. Seddon. 2007. Directions in reintroduction biology. Trends in Ecology and Evolution 23:20-25.
- Brian T. Hickerson and Annika W. Walters. 2019. Evaluation of Potential Translocation Sites for an Imperiled Cyprinid, the Hornyhead Chub. North American Journal of Fisheries Management 39(1):205-218.
- Bright, P. W., and T. J. Smithson. 2001. Biological invasions provide framework for reintroductions: selecting areas in England for pine martin releases. Biodiversity and Conservation 10:1247-1265.
- Carle, F. L. and M. R. Strub. 1978. A new method for estimating population size from removal data. Biometrics 34:621-360.
- Carter, C. and Bahm, J. 2007. Rock Spring Fish Habitat Survey, December 6, 2007. Arizona Game and Fish Department, Phoenix.
- Childs, M. R. 2004. Development of propagation techniques for loach minnow. Final Report to U. S. Bureau of Reclamation, Phoenix Area Office, Cooperative Agreement No. 02-FC-32-0100. Arizona Game and Fish Department, Phoenix.
- Crowder, C.D. and A.T. Robinson. 2015. Gila River Basin Native Fishes Conservation Program: Arizona Game and Fish Department annual report for February 6, 2014 through February 4, 2015. A Gila River Basin Native Fishes Conservation Program Annual Report for U.S. Fish and Wildlife Service Cooperative Agreement No. F14AC00148. Arizona Game and Fish Department, Nongame Wildlife Branch, Phoenix.
- Ehret, S., and B. Dickens. 2009a. Sabino Canyon survey trip report, June 3-4, 2009. Arizona Game and Fish Department, Region V, Tucson.
- Frear, L. R., R. R. Staffeldt, A. T. Robinson, and C. D. Crowder. 2015. Attempted establishment of Gila Topminnow in Rock Spring, Arizona. Annual Report to Gila River Basin Native Fishes Conservation Program, Under Task 3-75a; U.S. Fish and Wildlife Service Cooperative

Agreement No. F09AC00084. Arizona Game and Fish Department, Nongame Branch, Phoenix.

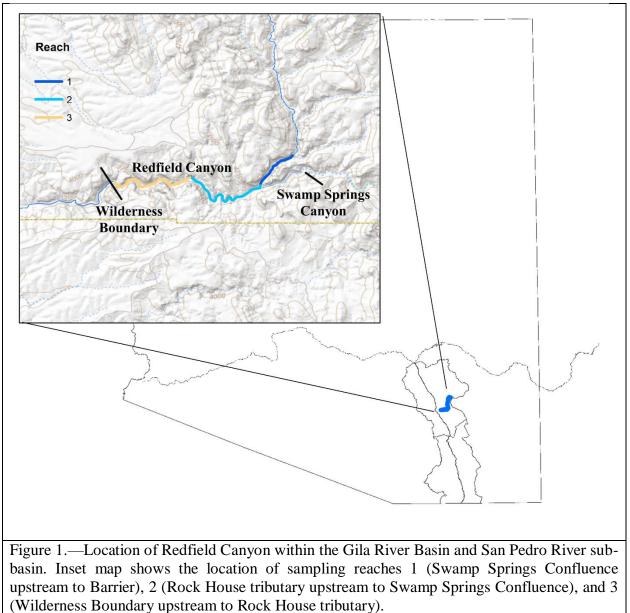
- Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science 245:477-480.
- Hedrick, P. W. 2001. Using microsatellite and MHC variation to identify species, ESUs, and MUs in the endangered Sonoran topminnow. Molecular Ecology 10(6):1399-1412.
- Hickerson, B. T., and A. T. Robinson. 2019. Red Tank Draw native fish restoration project: nonnative fish removal plan; draft. Arizona Game and Fish Department, Phoenix.
- Hickerson, B.T., and A. W. Walters. 2019. Evaluation of potential translocation sites for an imperiled cyprinid, the Hornyhead Chub. North American Journal of Fisheries Management 39(1):205-218.
- Love-Chezem, T. S., A. T. Robinson, and C. D. Crowder. 2015a. Muleshoe Cooperative Management Area native fish restoration: 2014 activities. A Gila River Basin Native Fishes Conservation Program Progress Report for Task 3-75f; U.S. Fish and Wildlife Service Cooperative Agreement No. F09AC00084. Arizona Game and Fish Department, Nongame Wildlife Branch, Phoenix.
- Mosher, K. R., T. S. Love-Chezem, and A. T. Robinson. 2016. Gila topminnow and desert pupfish stocking activities during 2015. Progress Report to Gila River Basin Native Fishes Conservation Program, Under Task 3-75a; U.S. Fish and Wildlife Service Cooperative Agreement No. F14AC00148. Arizona Game and Fish Department, Nongame Wildlife Branch, Phoenix.
- Pine, W, E., B. Healy, E. O. Smith, M. Trammell, D. Speas, R. Valdez, M. Yard, C. Walters, R. Ahrens, R. VanHaverbeke, D. Stone and W. Wilson. 2013. An individual-based model for population viability analysis of Humpback Chub in Grand Canyon. North American Journal of Fisheries Management 33(3):626-641.
- Pine, W. E., J. Caldwell, M. Allen and C. Stropki. 2017. Predicted native fish response, potential rewards and risks from flow alteration in a New Mexico arid mountain stream. River Research and Applications 33(5):641-661.
- Powell, B. 2018. Location and status of unsupplemented, foresummer surface water on Pima County Conservation Lands. Final Report. Pima County Office of Sustainability and Conservation. Tucson, AZ.

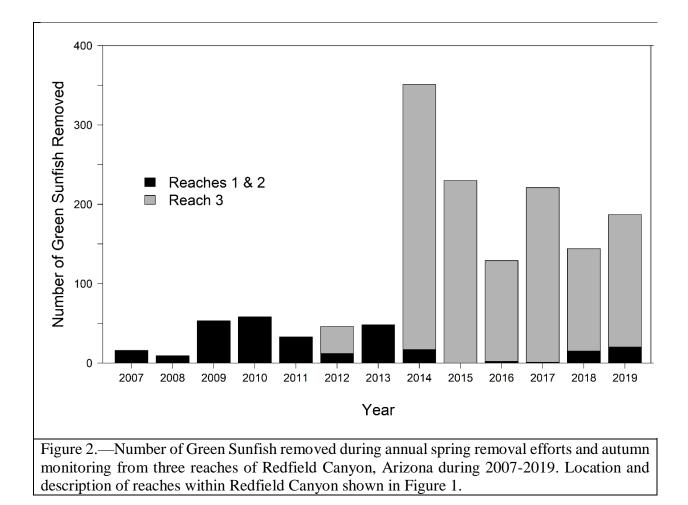
- Reclamation (U.S.D.I. Bureau of Reclamation). 2010. Final environmental assessment, Blue River native fish restoration project, Apache-Sitgreaves National Forests, Greenlee and Apache Counties, Arizona. U. S. Department of the Interior, Bureau of Reclamation, Phoenix Area Office.
- Robinson, A. T. 2007. Gila River Basin Native Fishes Conservation Program: Cooperative Agreement 2018116J808 Quarterly Report for the period April 1, 2007-June 30, 2007. Submitted to U.S. Fish and Wildlife Service, Ecological Services, Tucson, AZ. Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A. T. 2008. Gila River Basin Native Fishes Conservation Program: Cooperative Agreement 2018116J808 Quarterly Report for the period January 1, 2008---March 31, 2008. Submitted to U.S. Fish and Wildlife Service, Ecological Services, Tucson, AZ. Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A. T. 2009. Gila River Basin Native Fishes Conservation Program: Cooperative Agreement 201816J808 Quarterly Report for the period April 1, 2009-June 30, 2009. Submitted to U.S. Fish and Wildlife Service, Ecological Services, Tucson, AZ. Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A. T. 2010. Gila River Basin Native Fishes Conservation Program: Cooperative Agreement 201819J853 Draft Semi-Annual Report for the Period May 1, 2010--October 31, 2010. Submitted to U.S. Fish and Wildlife Service, Ecological Services, Tucson, AZ. Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A. T. 2011. Gila River Basin Native Fishes Conservation Program: Cooperative Agreement 201819J853 Semi-Annual Report for the Period May 1 thru October 31, 2011. Submitted to U.S. Fish and Wildlife Service, Ecological Services, Tucson, AZ.Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A. T. 2014. Gila River Basin Native Fishes Conservation Program: Cooperative Agreement F09AC00084 Draft Annual Report for the Period Nov. 1, 2012 thru February 5, 2014. Submitted to U.S. Fish and Wildlife Service, Ecological Services, Tucson, AZ. Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A. T. 2016. Gila River Basin Native Fishes Conservation Program: Arizona Game and Fish Department annual report for June 30, 2015 through June 30, 2016. A Gila River Basin Native Fishes Conservation Program Annual Performance Report for U.S. Fish and Wildlife Service Cooperative Agreement No. F14AC00148. Arizona Game and Fish Department, Aquatic Wildlife Branch, Phoenix.

- Robinson, A. T., C. Carter, D. Ward, and H. Blasius. 2009. Bonita Creek native fish restoration: native aquatic species salvage, chemical renovation, and repatriation of native aquatic species. Arizona Game and Fish Department, Phoenix.
- Robinson, A. T., C. Crowder, and D. Boyarski. 2010. Mechanical removal of nonnative fishes from the Blue River during June 1-3, 2009. Nongame Branch, Arizona Game and Fish Department, Phoenix, AZ.
- Robinson, A. T., C. D. Crowder, and D. B. Pearson. 2013. Blue River Native Fish Restoration Project: 2012 Annual Report. Annual Report to Gila River Basin Native Fishes Conservation Program, Task 3-42 of U.S. Fish and Wildlife Service Cooperative Agreement No. F09AC00084. Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A. T., C. D. Crowder, and D. B. Pearson. 2014. Blue River Native Fish Restoration Project: 2013 Annual Report. Annual Report to Gila River Basin Native Fishes Conservation Program, Task 3-42 of U.S. Fish and Wildlife Service Cooperative Agreement No. F09AC00084. Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A., and T. Love-Chezem. 2015. Blue River Native Fish Restoration Project: 2014 Annual Report. Annual Report to Gila River Basin Native Fishes Conservation Program, for Task 3-42 of U.S. Fish and Wildlife Service Cooperative Agreement No. F09AC00084. Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A., and T. Love-Chezem. 2016. Blue River Native Fish Restoration Project: Report of 2015 Activities. Annual Report to Gila River Basin Native Fishes Conservation Program, for Task 3-42 of U.S. Fish and Wildlife Service Cooperative Agreement No. F09AC00084. Arizona Game and Fish Department, Nongame Branch, Phoenix.
- Robinson, A. T., and K. R. Mosher. 2018. Gila River Basin Native Fishes Conservation Program: Arizona Game and Fish Department's native fish conservation efforts during 2017. An Arizona Game and Fish Department Annual Report for Cooperative Agreement No. R16AC00077 submitted to U.S. Bureau of Reclamation, Phoenix Area Office. Arizona Gameand Fish Department, Aquatic Wildlife Branch, Phoenix.
- Robinson, A. T., K. Mosher, and K. Smith. 2017. Gila River Basin Native Fishes Conservation Program: Arizona Game and Fish Department's native fish conservation efforts during 2016 and 2017 work plan. An Arizona Game and Fish Department Annual Report for Cooperative Agreement No. R16AC00077 submitted to U.S. Bureau of Reclamation, Phoenix Area Office. Arizona Game and Fish Department, Aquatic Wildlife Branch, Phoenix.

- Robinson, A. R. Timmons, D. Boyarski, and C. Crowder. 2010. Muleshoe Cooperative Management Area native fish restoration monitoring and stocking during 2009. Arizona Game and Fish Department, Phoenix.
- U.S. Fish and Wildlife Service. 1983. Gila and Yaqui Topminnow Recovery Plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico. 56 pp.
- U.S. Fish and Wildlife Service. 1991a. Spikedace Recovery Plan. Albuquerque, New Mexico. 38 pp.
- U.S. Fish and Wildlife Service. 1991b. Loach Minnow Recovery Plan. Albuquerque, New Mexico. 38 pp.
- U. S. Fish and Wildlife Service. 1998. Razorback sucker (*Xyrauchen texanus*) recovery plan. U.S. Fish and Wildlife Service, Denver, CO. 81 pp.
- U.S. Fish and Wildlife Service. 2002. Razorback sucker (*Xyrauchen texanus*) Recovery Goals: amendment and supplement to the Razorback Sucker Recovery Plan. U.S. Fish and Wildlife Service, Mountain-Prairie Region (6), Denver, Colorado.
- U.S. Fish and Wildlife Service. 2015. Gila chub (Gila intermedia) Draft Recovery Plan. U.S. Fish and Wildlife Service, Southwest Region, Albuquerque, New Mexico. 118 pp. + Appendices A-C.
- Ward, D. L. 2008. Bubbling Ponds Native Fish Propagation and Research Facility Status Update
 February 2008. Report to U.S. Bureau of Reclamation, Phoenix Area Office, by Arizona Game and Fish Department, Research Branch, Phoenix.
- Weedman, D. A. 1999. Gila topminnow, *Poeciliopsis occidentalis occidentalis,* revised recovery plan. Draft. August 1999. U.S. Fish and Wildlife Service, Phoenix, AZ.
- Weedman, D. A. and K. L. Young. 1995. Gila topminnow extirpation sites in Arizona: 1994-95 field season results. Nongame and Endangered Wildlife Program Technical Report 80. Arizona Game and Fish Department, Phoenix, Arizona.

FIGURES





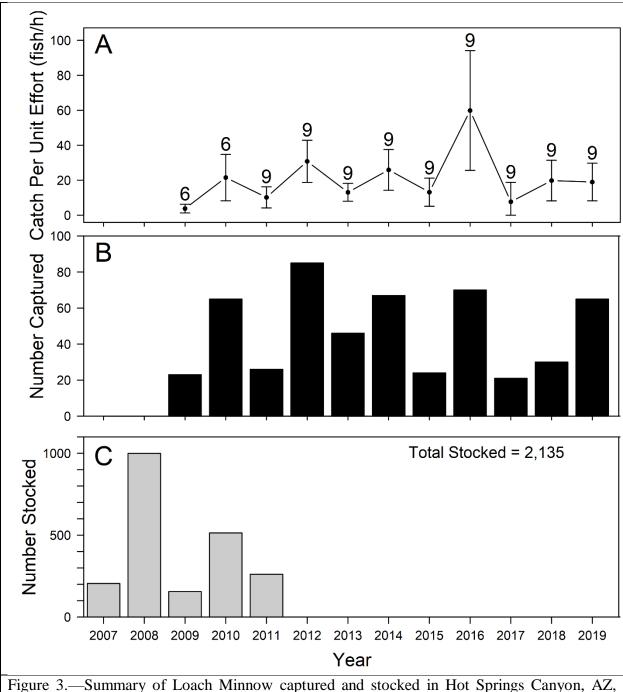
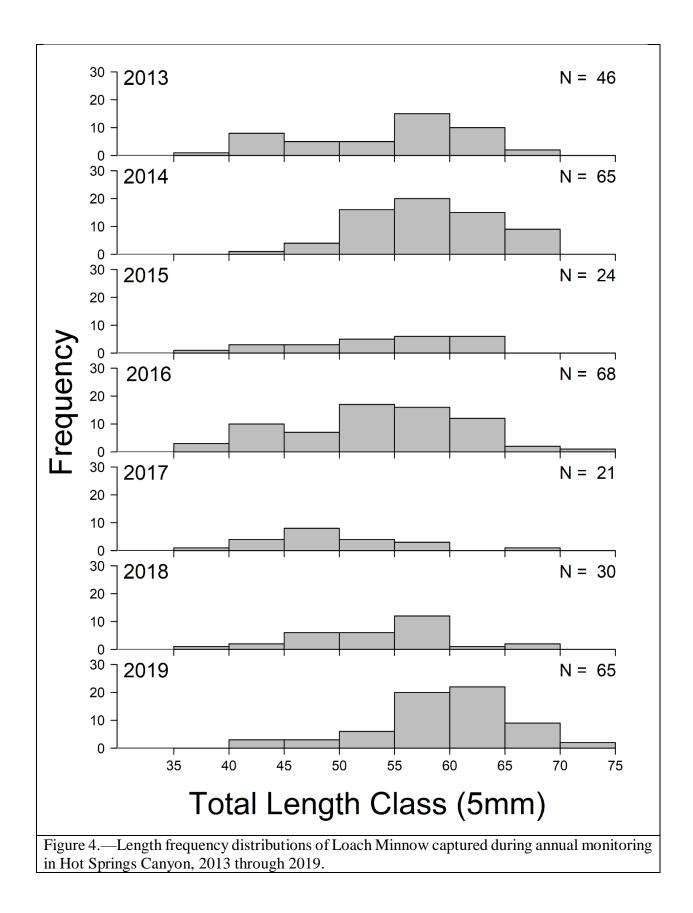


Figure 3.—Summary of Loach Minnow captured and stocked in Hot Springs Canyon, AZ, annually from 2007 to 2019 with (A) mean annual backpack electrofishing catch per unit effort (fish/hour) with standard error bars and the number of 100 meter transects sampled above the error bar, (B) total number of fish captured, and (C) total number of fish stocked.



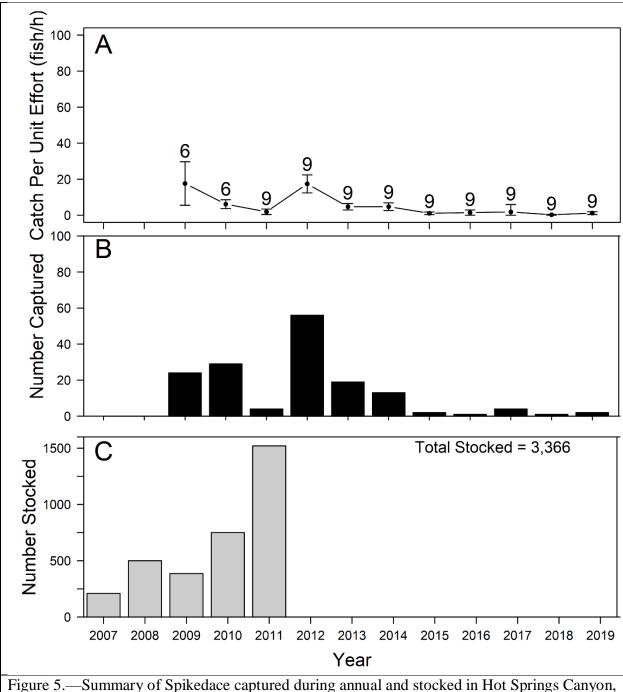
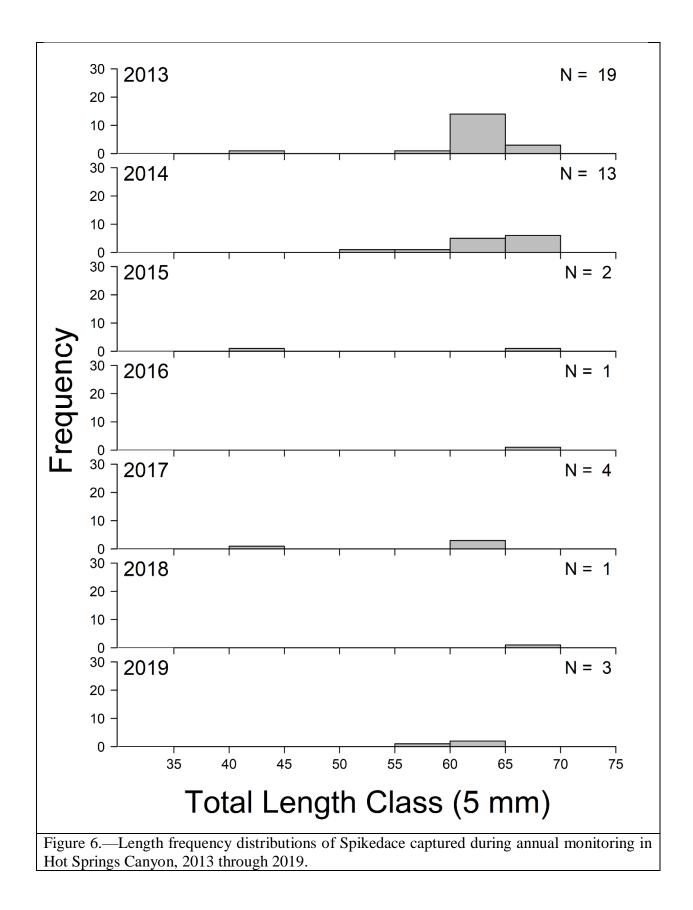


Figure 5.—Summary of Spikedace captured during annual and stocked in Hot Springs Canyon, AZ, annually from 2007 to 20189 with (A) mean annual backpack electrofishing catch per unit effort (fish/hour) with standard error bars and the number of 100 meter transects sampled above the error bar, (B) total number of fish captured, and (C) total number of fish stocked.



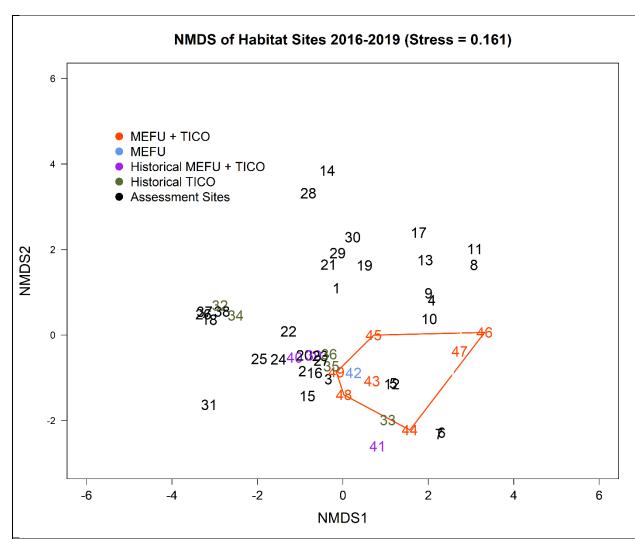


Figure 7.—Nonmetric multidimensional scaling (NMDS) ordination of 49 habitat sites assessed with the standardized stream monitoring protocol from 2016-2019. Shown are points where Spikedace and Loach Minnow are currently present (orange), sites where only Spikedace are currently present (blue), sites where Spikedace and Loach Minnow were historically present (purple), sites where only Loach Minnow were historically present (green) and potential translocation sites where Spikedace or Loach Minnow have never been present (black). The orange polygon represents the ordination space occupied by sites with current presence of Spikedace and Loach Minnow. Assessment sites that fall within the polygons have habitat conditions most similar to those sites that historically or currently support Loach Minnow and Spikedace. Numbers correspond to the following sites: Grant Creek (1), McKittrick Creek (2-3), KP Creek (4-13), Cave Creek (14), South Fork Cave Creek (15), East Turkey Creek (16-17), Foote Creek (18-20), Raspberry Creek (21-22), Dix Creek (23), Little Strayhorse Creek (24), Strayhorse Creek (32-36), Salt House Creek (37-38), Eagle Creek (39-41), Spring Creek (42), Hot Springs Canyon (43-45), Blue River (46-49).

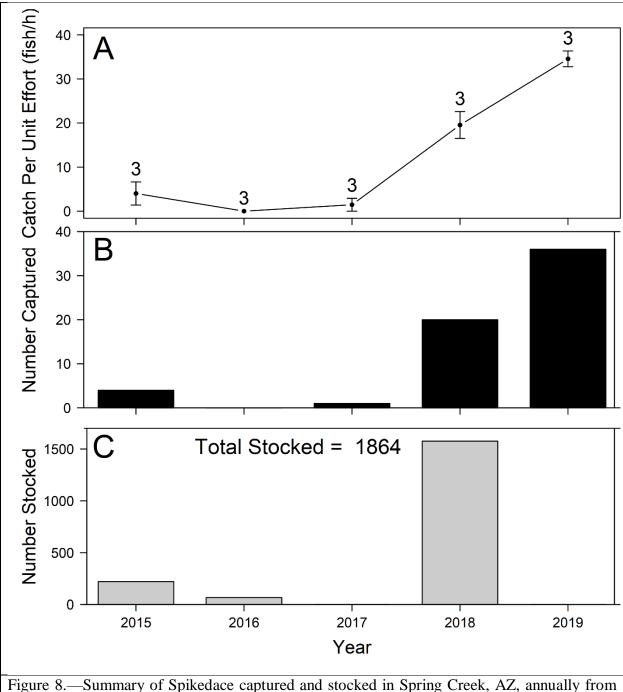
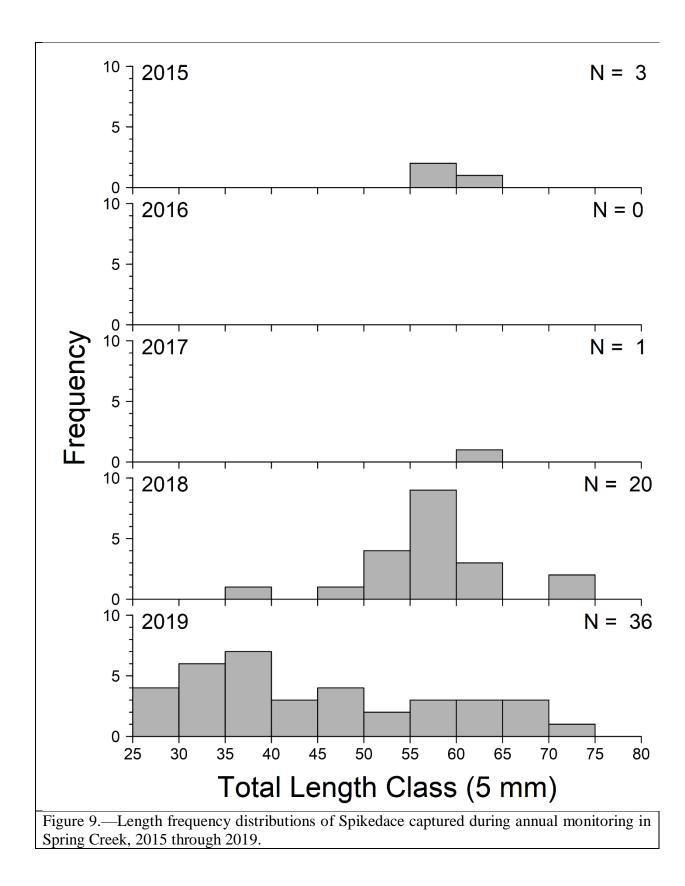


Figure 8.—Summary of Spikedace captured and stocked in Spring Creek, AZ, annually from 2015 to 2019 with (A) mean annual backpack electrofishing catch per unit effort (fish/hour), (B) total number of fish captured, and (C) total number of fish stocked.



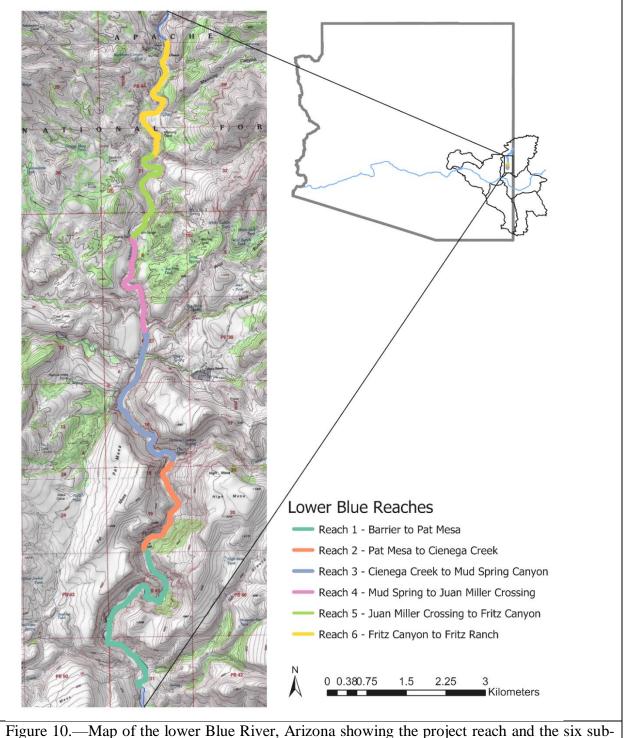
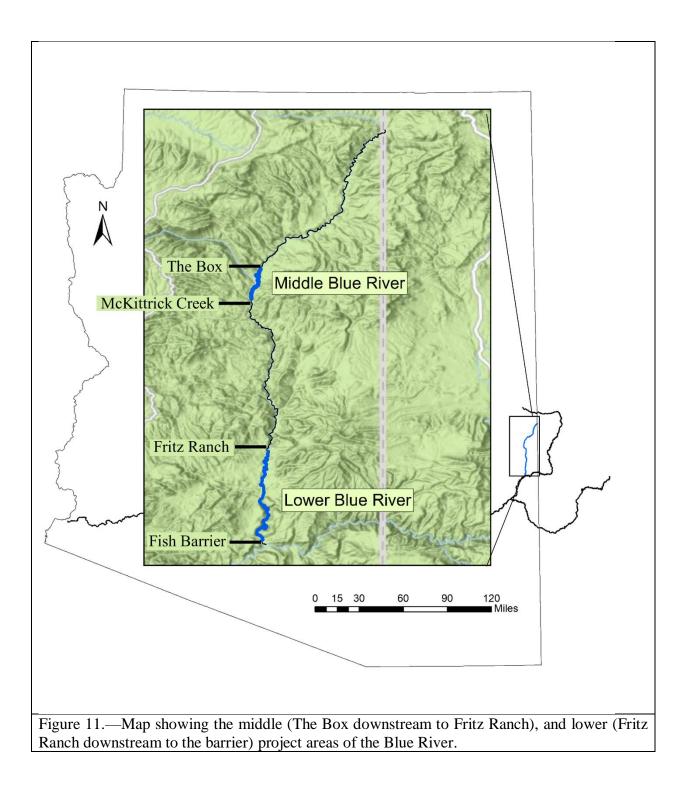
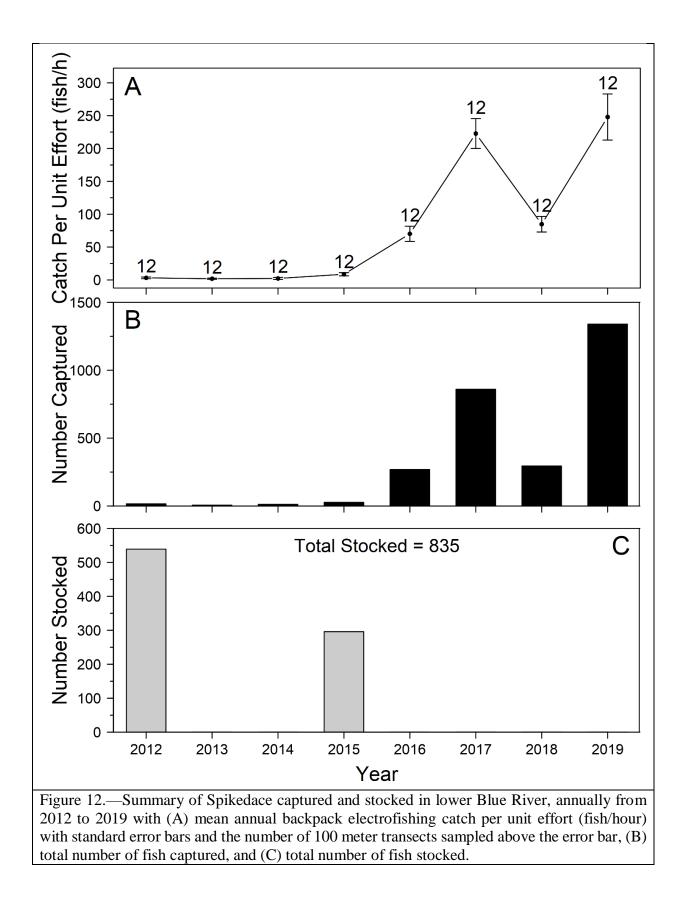
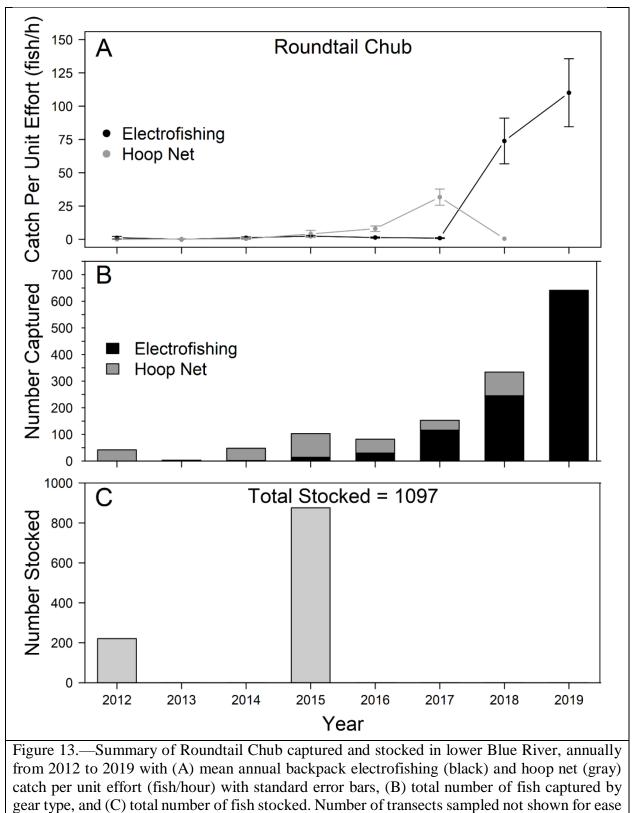


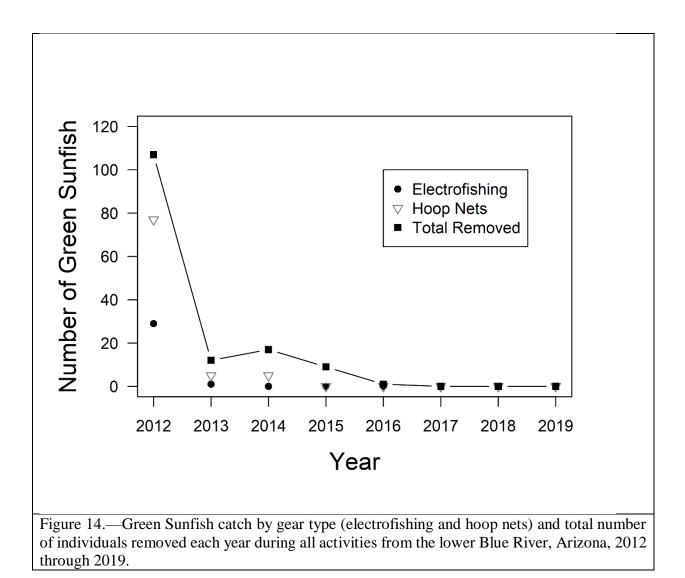
Figure 10.—Map of the lower Blue River, Arizona showing the project reach and the six subreaches; Reach 1 (Teal), Reach 2 (Orange), Reach 3 (Purple), Reach 4, (Pink), Reach 5 (Green), Reach 6 (Yellow).

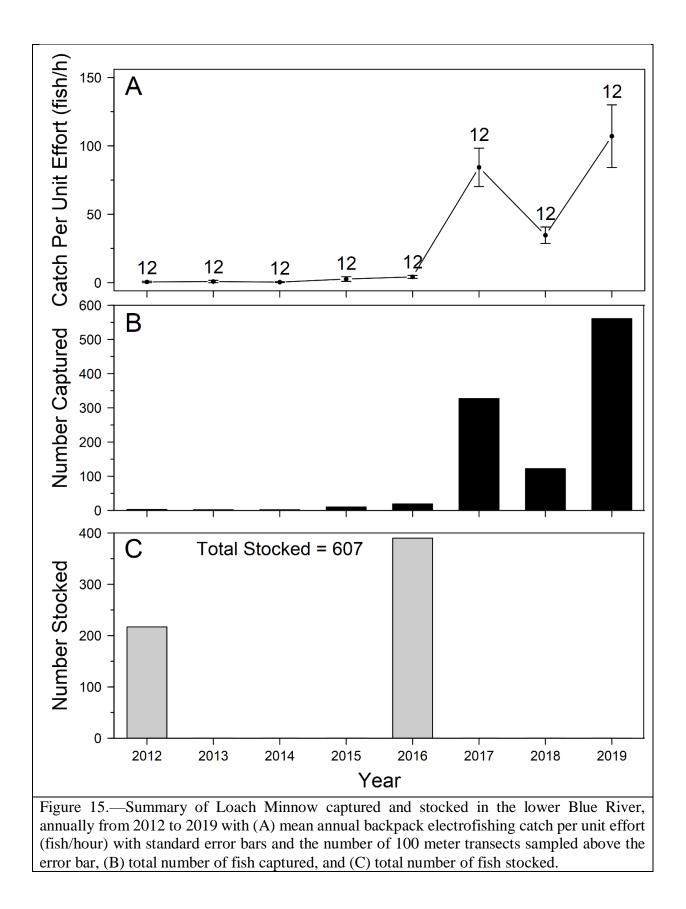


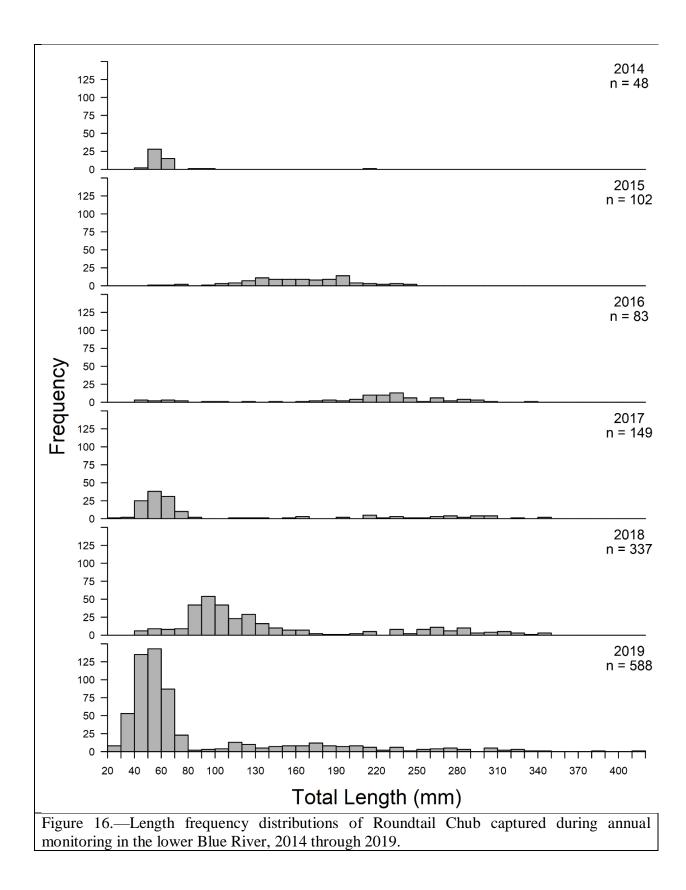


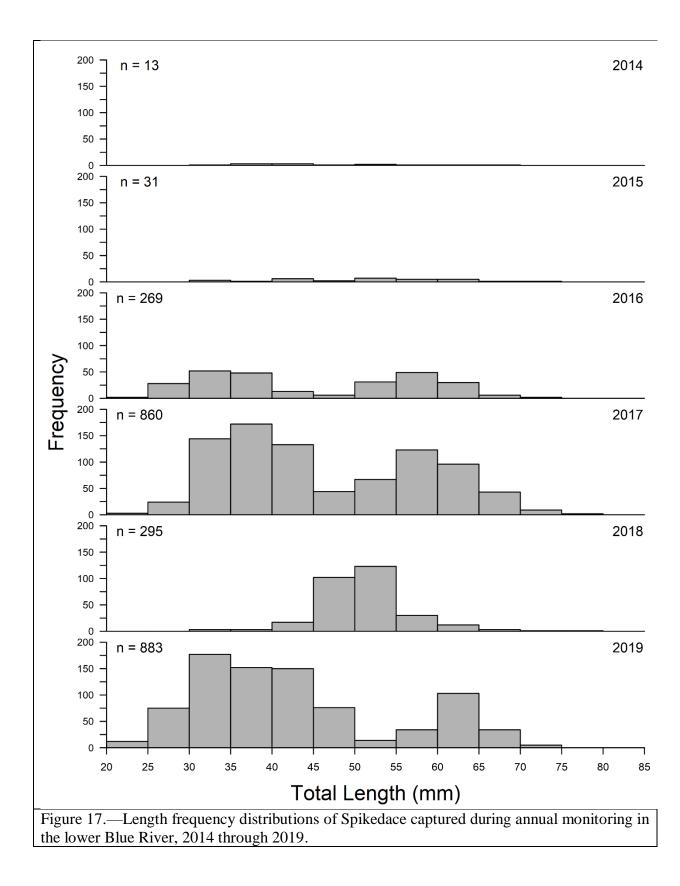


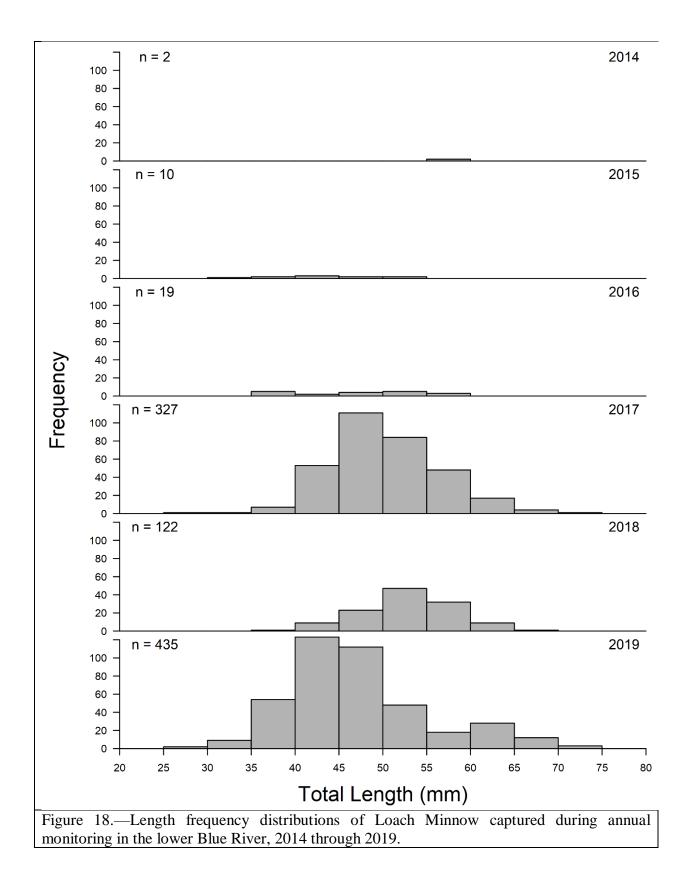
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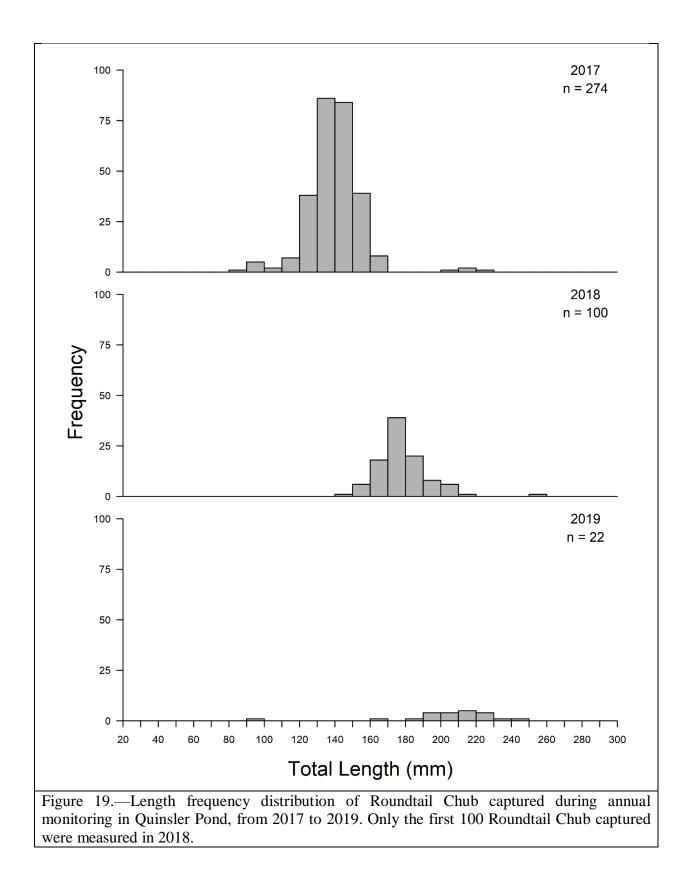


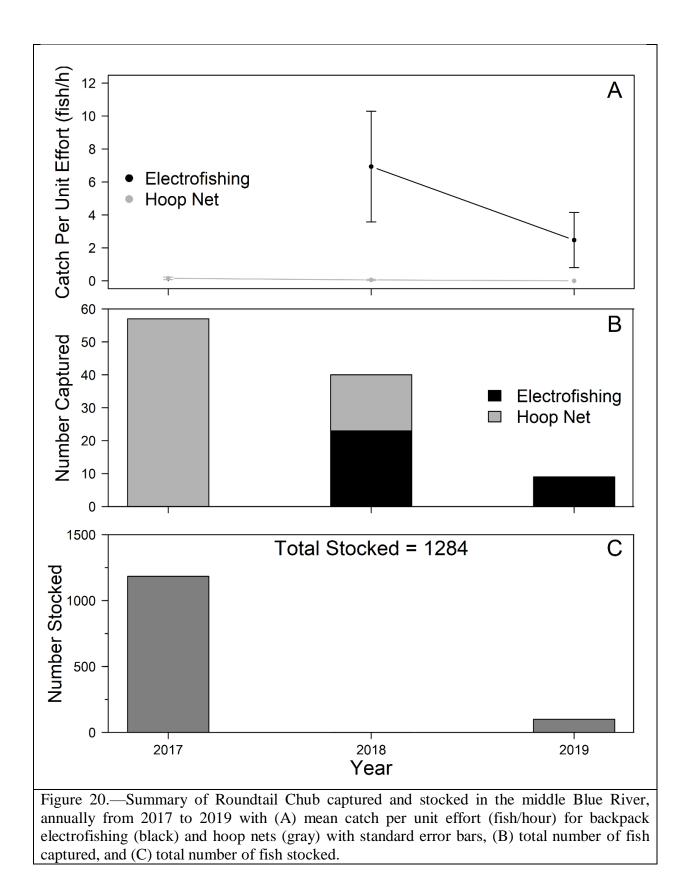


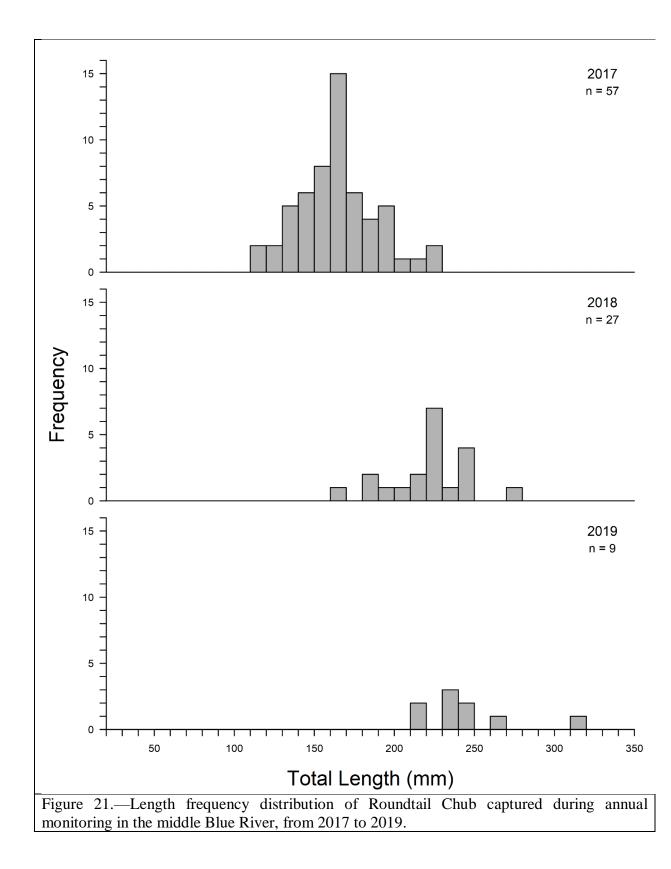


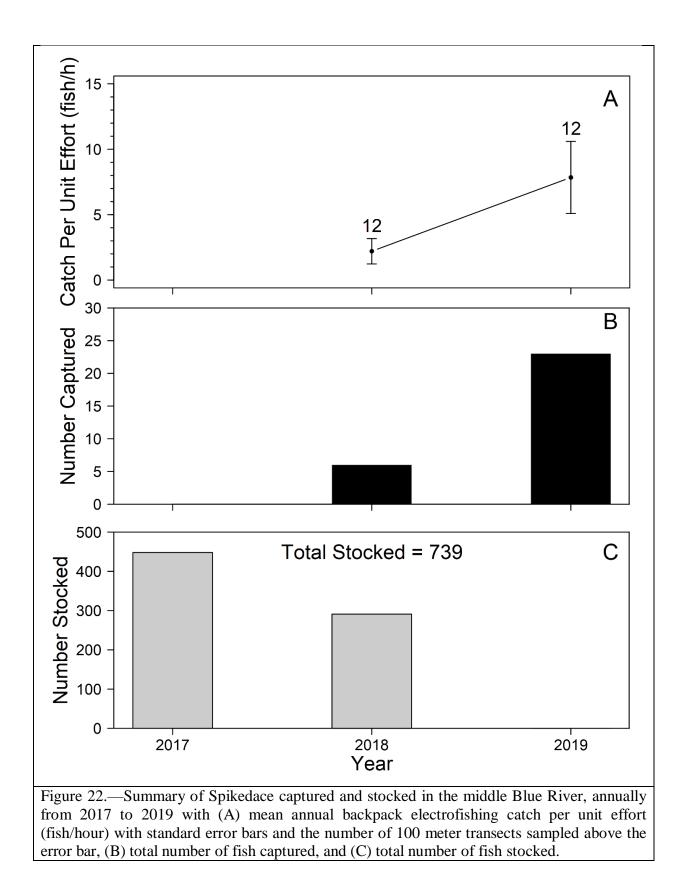


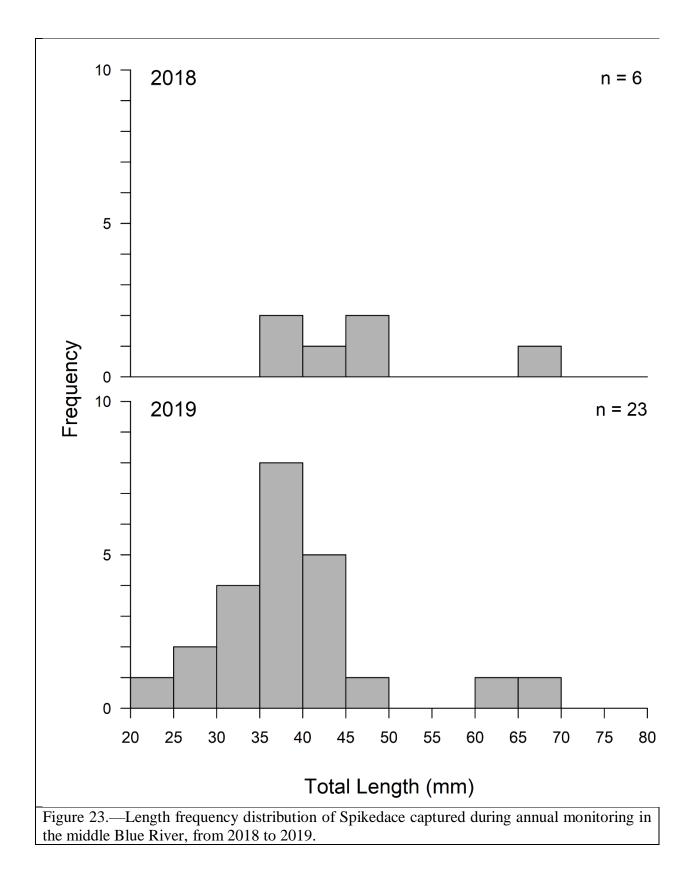












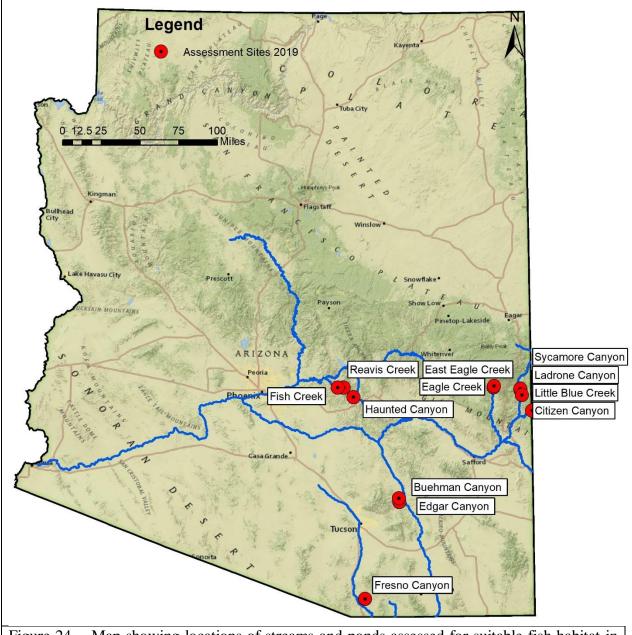
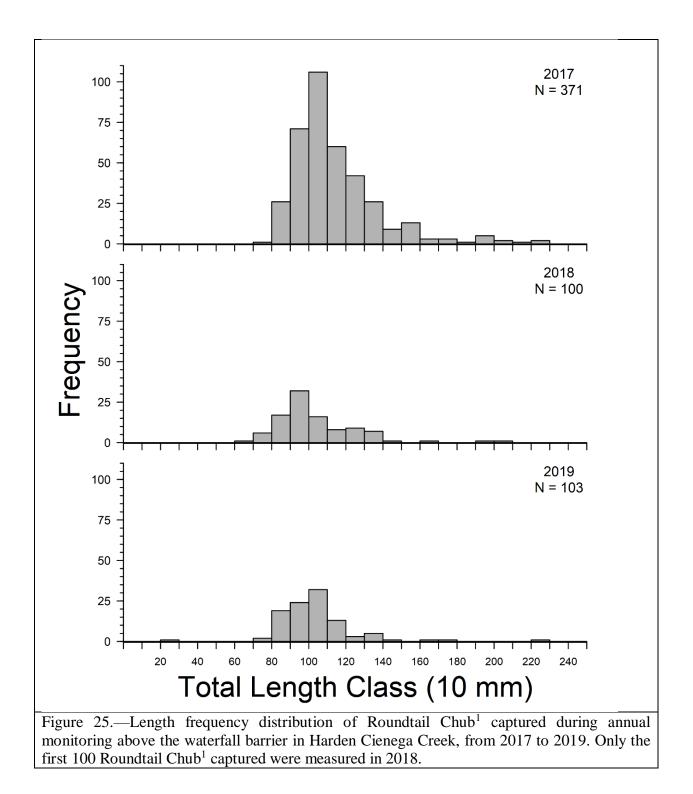
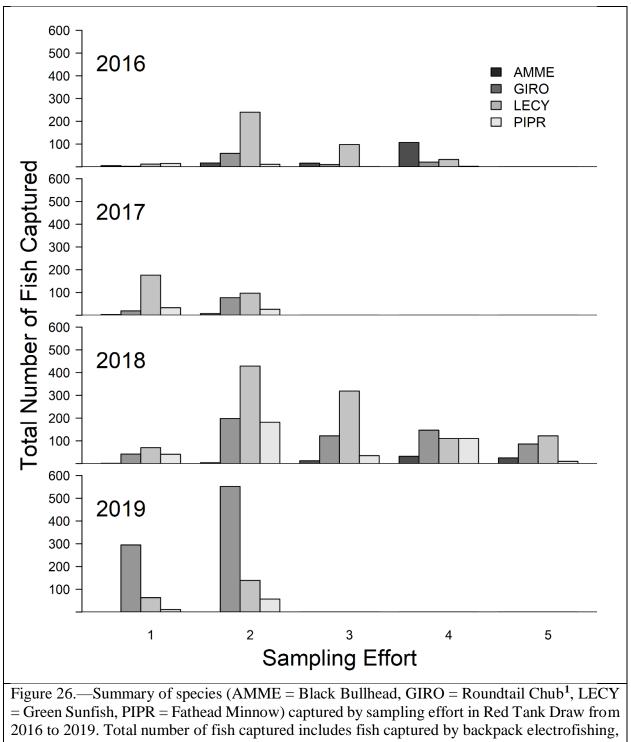


Figure 24.—Map showing locations of streams and ponds assessed for suitable fish habitat in the Gila River basin in 2019.



¹ Roundtail Chub in Harden Cienega Creek were previously classified as Gila Chub



mini-hoop nets, minnow traps and angling.

¹ Chub in Red Tank Draw were previously classified as Gila Chub.

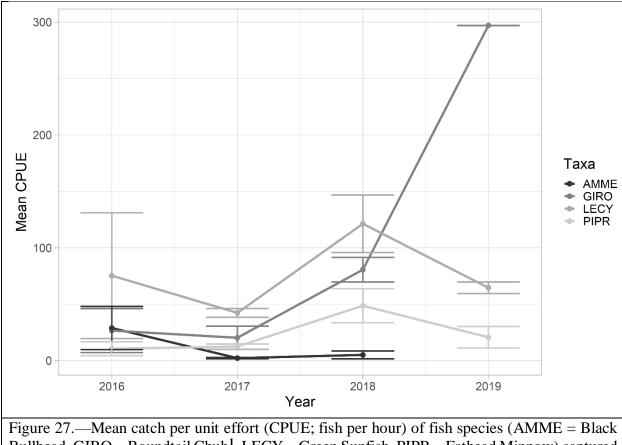


Figure 27.—Mean catch per unit effort (CPUE; fish per hour) of fish species (AMME = Black Bullhead, GIRO = Roundtail Chub¹, LECY = Green Sunfish, PIPR = Fathead Minnow) captured by backpack electrofishing in Red Tank Draw from 2016 to 2019.

¹ Chub in Red Tank Draw were previously classified as Gila Chub.

Cooperative Agreement R16AC00077: 2019 Annual Report – Final 03/24/2020

TABLES

Table 1.— Summary of species lineages held at the Aquatic Research and Conservation Center from 2007 through 2019. Data for each lineage includes, the number acquired from the wild (#A), number of broodstock (#B), number of offspring produced (#P), number of offspring stocked (#S). Numbers stocked do not include fish transferred to New Mexico. Taxa codes are Loach Minnow (TICO), Spikedace (MEFU), and Roundtail Chub (GIRO); XX indicates missing information. A total of 143 upper Gila River TICO were acquired in 2007, but did not survive.

Taxa	Extant Lineage/Stream		2007 ¹	2008^{2}	2009 ³	2010^{4}	2011^5	2012	2013 ⁶	20147	2015 ⁸	2016 ⁹	2017^{10}	2018	2019
MEFU	upper Gila River, NM	#A	640		148										
		#B		XX	XX	XX	XX	XX	558	380	392	531	267	159	254
		#P		740	165	2555	539	1300	XX	1000	296	0	384	352	2404
		#S		448	165	545		539			296		327	0	
	Gila River Forks	#A			17	250	148			XX				1	
		#B			17	267	XX	XX	277	250	204	138	122	83	71
		#P			0	379	0	800	700	300		0	1183	195	1132
		#S											1000	0	
	Aravaipa Creek	#A	258		220		XX		XX	26	150	80	160		322
		#B		XX	XX	XX	XX	XX	463	480	412	262	382	331	523
		#P		1650	410	5993	4663	3471	XX	221	35	120	1347	3214	4250
		#S		1600	386	2954	4663	3471			221	67		2234	
TICO	Gila River Forks	#A			48	100	434			61			110	145	
		#B				XX	XX	XX	78	57	81	96	128	97	169
		#P			0	0	0			250		220	7	1207	665
		#S											159	0	
	San Francisco R., NM	#A							41						
		#B								27	119	215	314	318	231
		#P								500		26	177	1627	601
		#S											243	0	
	Blue River	#A	71	50	91		27					12		223	80
		#B		XX	XX	XX	150		202	180	245	214	156	117	290
		#P		670	22	164	722		1500	288		426	47	6	713
		#S		678		156		217	310	288		390		0	

Taxa	Extant Lineage/Stream		2007^{1}	2008^{2}	2009 ³	2010 ⁴	2011 ⁵	2012	2013 ⁶	20147	2015 ⁸	2016 ⁹	2017^{10}	2018	2019
	Aravaipa Creek	#A	254		110		XX		XX	48	50	200	100		57
		#B		XX	XX	XX	XX		379	340	316	297	490	439	354
		#P		3250	274	1623	1035		951	0		265	305	1848	1398
		#S		4003	156	1561	527		951					0	
GIRO	Eagle Creek	#A			79	78									
		#B					XX	XX	85	85	85	101	99	99	
		#P					149			1500	2000	0	57	0	
		#S						221			876	1194	0	0	

1. Robinson 2007; Ward 2008.

2. Robinson 2008

3. Robinson 2009

4. Robinson 2010

5. Robinson 2011

6. Robinson 2014

7. Crowder and Robinson 2015

8. Robinson 2016

9. Robinson et al. 2017

10. Robinson and Mosher 2018

Table 2.—Summary of fish captured during single-pass backpack electrofishing at nine 100-m transects in Hot Springs Canyon within three survey reaches on September 12, 2019. Shown for each reach is the number of transects sampled (N), number of fish captured (#Ind), the mean relative abundance (number of fish captured per hour of electrofishing effort; #Ind/h) and standard error of mean relative abundance (SE).

Reach	N	Statistic	Loach Minnow	Roundtail Chub ¹	Spikedace	Desert Sucker	Longfin Dace	Sonora Sucker	Speckled Dace
3	3	#Ind	1	39	i	65	372	5	300
		#Ind/h	1.00	44.91		66.57	433.50	5.02	343.11
		SE	(1.00)	(20.15)		(57.09)	(8.48)	(5.02)	(38.08)
2	3	#Ind	24	46		49	208	9	206
		#Ind/h	33.94	67.09		70.90	292.99	12.51	290.90
		SE	(21.97)	(42.93)		(30.70)	(88.16)	(12.51)	(63.35)
1	3	#Ind	25	38	2	124	517	17	530
		#Ind/h	32.40	48.17	3.12	156.29	832.04	20.63	824.08
		SE	(24.35)	(39.92)	(1.67)	(129.36)	(109.09)	(20.63)	(223.63)
Total	9	#Ind	50	123	2	238	1097	31	1036
		#Ind/h	22.45	53.39	1.04	97.92	519.51	12.72	486.03
		SE	(9.74)	(16.30)	(0.63)	(39.58)	(80.80)	(6.67)	(97.25)

¹ Roundtail Chub at this location previously classified as Gila Chub

Table 3.—Three-pass depletion estimates of abundance for Loach Minnow, Spikedace and Roundtail Chub per 100 meters at fixed sites during annual monitoring in 2019. Included is the number of fish caught in each pass (C1, C2, C3), Carle-Strub three pass abundance estimate (N), 95% confidence interval of the abundance estimate (N 95% CI), estimated capture probability (p), and 95 % confidence interval of estimate of capture probability (p 95% CI).

Stream	Site	Species	C1	C2	C3	Ν	N 95 % CI	р	<i>p</i> 95% CI
Hot Springs Cyn	R 1 Fixed	TICO	22	10	3	36	32.4 - 39.59	0.65	0.46-0.83
Hot Springs Cyn	R 1 Fixed	MEFU	1	0	1	NA	NA	NA	NA
Hot Springs Cyn	W-N3 Fixed	TICO	0	2	0	NA	NA	NA	NA
Spring Creek	Fixed-02	MEFU	13	24	10	90	0 -183.29	0.21	0 - 0.49
Blue River (L)	Fixed-R5	GIRO	39	22	13	88	70.06-105.94	0.45	0.28-0.62
Blue River (L)	Fixed-R5	MEFU	87	29	14	136	128.39-143.61	0.63	0.54-0.73
Blue River (L)	Fixed-R5	TICO	39	6	2	47	45.90-48.10	0.82	0.71-0.93
Blue River (L)	Fixed-R6	GIRO	7	1	3	11	8.51-13.49	0.61	0.26-0.97
Blue River (L)	Fixed-R6	MEFU	161	49	66	340	298.86-381.14	0.43	0.34-0.51
Blue River (L)	Fixed-R6	TICO	100	36	30	188	168.96-207.03	0.51	0.40-0.61
Blue River (M)	Fixed-4	GIRO	2	1	2	NA	NA	NA	NA
Blue River (M)	Fixed-4	TICO	1	0	1	NA	NA	NA	NA

Table 4.—Total number of fish captured (#Ind) and relative abundance (fish per trap hour; #Ind/h)
in minnow traps in Bass Canyon and Double R Canyon on and relative abundance (fish per square
meter seined) in Mint Spring on September 13, 2019 at Muleshoe Ranch CMA, Arizona. N
indicates the number of minnow traps set or seine hauls.

Stream	N	Statistic	Gila Topminnow	Roundtail ¹ Chub	Desert Pupfish	Longfin Dace	Speckled Dace	Total
Bass			1		1			
Canyon-	10	#Ind	4	85		8	3	100
Upper								
		#Ind/h	1.90	11.39		3.81	0.72	1.84
		SE	(0.00)	(6.09)		(0.00)	(0.24)	(3.23)
Double R Canyon	10	#Ind	66	38		97	16	217
5		#Ind/h	6.93	3.11		9.25	2.66	5.53
		SE	(2.80)	(0.95)		(4.03)	(1.19)	(2.26)
Mint Spring	5	#Ind			109			109
		#Ind/h			2.81			2.81
Church in these la		SE			(0.32)			(0.32)

¹ Chub in these locations were previously classified as Gila Chub.

		Roundtail		Desert	Gila	Longfin	Speckled
Transect	Statistic	chub ¹	Spikedace	Sucker	Topminnow	Dace	Dace
Random-15	#Ind	65	13	7	1		57
	#Ind/h	170.43	34.09	18.35	2.62		149.45
Random-06	#Ind	69	10	17			29
	#Ind/h	218.85	31.72	53.92			91.98
Fixed-2	#Ind	111	13	111		45	218
	#Ind/h	323.30	37.86	323.30		131.06	634.95
Total	#Ind	245	36	135	1	45	304
	#Ind/h	237.53	34.56	131.86	0.87	43.69	292.13
	SE	(31.90)	(1.27)	(68.07)	(0.62)	(30.89)	(121.77)

Table 5.—Summary of fish captured at three 100 meter electrofishing transects in Spring Creek during annual monitoring during September 3, 2019. Shown is the number of fish captured in each transect (#Ind), and the number of fish captured per hour of electrofishing effort (#Ind/h), and the overall mean and standard error of the mean catch rate.

Table 6.—Summary of fish captured in minnow traps near the barrier at Spring Creek during annual monitoring on September 4, 2019. Included is the number of traps (N), number of individuals captured (#Ind), mean relative abundance (fish captured per net hour; #Ind/h), and standard error of mean relative abundance (SE).

					Northern
N	Statistic	Gila Topminnow	Roundtail Chub ¹	Speckled Dace	Crayfish
12	#Ind	550	674	18	3
	#Ind/h	16.84	15.86	0.71	0.26
	SE	(4.86)	(1.96)	(0.19)	(.01)

¹ Chub in these locations were previously classified as Gila Chub.

Reach	N	Statistic	Loach Minnow	Roundtail Chub	Spikedace	Desert Sucker	Longfin Dace	Sonora Sucker	Speckled Dace
2	2	#Ind #Ind/h	45 49.20	184 201.23	246 268.96	235 257.03	59 64.49	203 221.99	124 135.59
		SE	(14.18)	(35.12)	(93.88)	(69.05)	(55.73)	(5.33)	(17.41)
3	2	#Ind	84	213	262	216	71	142	86
		#Ind/h SE	80.78 (26.44)	218.68 (87.84)	258.39 (10.56)	217.84 (45.20)	71.29 (11.32)	142.22 (18.65)	87.77 (29.62)
4	2	#Ind	121	121	252	180	103	103	97
		#Ind/h SE	150.91 (71.92)	151.48 (31.74)	315.85 (40.69)	224.49 (107.29)	127.94 (97.37)	128.41 (64.71)	120.77 (72.36)
5	3	#Ind #Ind/h SE	92 76.10 (9.40)	92 73.95 (8.07)	194 160.19 (26.13)	324 278.37 (61.10)	52 43.24 (20.42)	172 137.69 (17.27)	96 78.58 (8.71)
6	3	#Ind #Ind/h SE	219 205.97 (45.44)	31 27.79 (8.70)	386 363.59 (64.79)	515 504.86 (163.05)	132 128.21 (34.60)	106 103.48 (40.63)	215 195.45 (20.18)
Total	12	#Ind #Ind/h	561 117.33	641 120.67	1340 271.48	1470 312.37	417 86.81	726 142.40	618 125.86
		SE	(20.87)	(23.65)	(27.43)	(48.87)	(18.04)	(15.71)	(16.03)

Table 7.—Summary of fish captured at 12, 200-meter electrofishing transects in the lower Blue River during annual monitoring from October 8-10, 2019. Shown for each reach is the number of transects sampled (N), number of fish captured (#Ind), the mean relative abundance (number of fish captured per hour of electrofishing effort; #Ind/h) and standard error of mean relative abundance (SE).

Table 8. Summary of fish captured in hoop nets in Quinsler's Pond during annual monitoring from September 17-18, 2019. Shown
for each year is the number of hoop nets set (N), number of fish captured (#Ind), the mean catch per unit effort (#Ind/h) and standard
error (SE) of mean CPUE.

Year	Statistic	Speckled dace	Longfin dace	Roundtail chub	Species Desert sucker	Sonora sucker	Unidentified minnow	Crayfish	Total fish
2017	#Hoops	22	22	22	22	22	22	22	22
2017	#Hoops #Ind	0	22	274	6	72	1	186	376
	#Ind/h	Ū	.70	8.46	.18	2.21	.03	5.66	11.59
	SE		(0.42)	(3.02)	(0.08)	(0.67)	(0.03)	(1.25)	(3.43)
2018	#Hoops	12	12	12	12	12	12	12	12
	#Ind	2	49	145	3	101	0	56	300
	#Ind/h	0.11	2.71	8.10	0.17	5.64		3.10	16.73
	SE	(0.11)	(2.60)	(3.38)	(0.17)	(1.77)		(0.87)	(4.66)
2019	#Hoops	10	10	10	10	10	10	10	10
	#Ind	0	0	22	0	6	0	4	28
	#Ind/h			1.50		0.41		0.27	1.91
	SE			(0.71)		(0.29)		(0.15)	(0.93)

Table 9.—Summary of fish captured within each survey reach at 12, 100-meter electrofishing transects in the middle Blue River during annual monitoring from September 17-18, 2019. Shown for each reach is the number of transects sampled (N), number of fish captured (#Ind), the mean relative abundance (number of fish captured per hour of electrofishing effort; #Ind/h) and standard error of mean relative abundance (SE).

			Loach	Roundtail		Desert	Longfin	Sonora	Speckled
Reach	Ν	Statistic	Minnow	Chub	Spikedace	Sucker	Dace	Sucker	Dace
1	5	#Ind	28		15	87	122	101	143
		#Ind/h	23.61		12.22	77.10	102.06	80.36	120.66
		SE	(4.37)		(3.89)	(61.99)	(35.83)	(29.50)	(13.86)
2	4	#Ind	8	6	2	61	7	23	47
		#Ind/h	8.61	4.96	1.98	61.38	6.15	21.15	50.76
		SE	(2.41)	(4.96)	(2.00)	(30.90)	(3.59)	(14.10)	(20.69)
3	3	#Ind	5	3	6	84	54	38	120
		#Ind/h	6.51	3.28	8.35	119.77	87.26	51.28	147.76
		SE	(5.16)	(1.69)	(8.35)	(26.91)	(57.83)	(24.49)	(33.49)
Total	12	#Ind	41	9	23	232	183	162	310
		#Ind/h	14.33	2.47	7.84	82.53	66.39	53.35	104.13
		SE	(2.95)	(1.54)	(2.54)	(25.16)	(20.89)	(14.14)	(14.80)

Table 10.—Waters assessed during 2014 through 2019 to determine suitability for native fish repatriations, showing coordinates (NAD 83 UTM, zone 12S) of the upstream and downstream points for each reach assessed, the estimated length of perennial water within the assessed reach at the time of the survey, and the species for which the water was considered be suitable for.

			Upstream		Downstream			Perennial	
Date	Water Name	Basin	Easting	Northing	Easting	Northing	Elevation (m)	Length (m)	Suitable for Species
04/01/2014	South Fork Deadman Crk	Verde	452891	3770077	450817	3772961	1105	4400	RHCO, MEFU, GIRO, POOC
04/01/2014	Deadman Creek	Verde	450829	3773116	450780	3772923	1100	600	GIRO, POOC
04/14/2014	Bonita Creek - upper	Upper Gila	637499	3647178	636206	3649943	1160	5875	RHCO, MEFU, POOC
10/16/2014	Copper Creek - upper	Agua Fria	415294	3783300	414957	3784056	1440	500	maybe POOC, GIRO
03/10/2015	Reimer Spring	Agua Fria	410156	3811873	410268	3812368	1350	250	POOC
03/10/2015	Indian Creek	Agua Fria	413535	3798878	413325	3798872	1285	220	POOC
03/17/2015	Seven Springs	Salt	421594	3758300	420324	3758595	1025	1600	MEFU, POOC, GIRO, RHOS, CACL
03/18/2015	Lime Creek - upper	Verde	421976	3771582	423842	3769530	910	1300	POOC, GIRO
03/23/2015	Towel Creek	Verde	434879	3807874	431708	3808163	1060	50	maybe AGCH, RHOS
03/24/2015	Cottonwood Creek	Salt	487568	3723472	487595	3724000	715	600	POOC
03/24/2015	Rock Creek - upper	Salt	471383	3730666	471381	3730670	1160	1300	maybe AGCH, GIRO
03/24/2015	Rock Creek - lower	Salt	475856	3731040	476451	3730776	830	700	AGCH, POOC
04/21/2015	Turkey Creek	Agua Fria	389109	3792493	388400	3790285	1180	4000	AGCH
04/22/2015	Wilson Spring	Agua Fria	415381	3815195	415355	3815206	1560	20	POOC
04/22/2015	Little Ash Creek	Agua Fria	406593	3805271	404663	3805086	1160	>1300	POOC and GIRO
07/27/2015	Copper Creek - lower	Agua Fria	414957	3784056	414324	3784428	1365	32	POOC
02/19/2016	Bishop Creek	Agua Fria	401782	3789224	403890	3788175	1020	30	Maybe POOC

02/27/2016 Grapevine Canyon	Agua Fria	412756	3766285	412770	3766280	1130	500	POOC, GIRO
03/07/2016 Tortilla Creek	Salt	467373	3708578	464233	3710019	550	500	POOC
03/15/2016 South Fork Sheep Creek	Verde	448077	3754778	446914	3756529	940	100	POOC
04/12/2016 Ash Creek	Upper Gila	607829	3632197	607789	3632123	950	?	Maybe AGCH
04/12/2016 Deadman Creek	Upper Gila	611373	3623016	611398	3623118	1390	?	Maybe GIRO
06/05/2016 Home Tank Draw	Verde	452192	3827223	452117	3826994	1695	25	None
06/08/2016 Russell Spring	Verde	430492	3831022	429941	3831305	1060	59	None
06/28/2016 Sabino Canyon	Santa Cruz	520661	3579809	520551	3579167	990	700	GIRO, CACL, RHOS
07/06/2016 Mesquite Spring	Verde	429471	3816410	428902	3815864	925	4	None
07/06/2016 Cottonwood Spring	Verde	429239	3816482	429063	3816016	930	0	None
07/06/2016 Doren's Defeat Spring	Verde	438093	3810597	436636	3811691	1220	15	None
07/06/2016 Willow Spring	Verde	438429	3811400	436636	3811691	1220	10	None
07/06/2016 Big Willow Spring	Verde	437993	3811651	437803	3811414	1255	6	None
07/26/2016 Long Gulch Artesian	Salt	487919	3732399			695	10	None
10/20/2016 West Fork Pinto Creek	Salt	493978	3699996	495059	3700174	1010	1070	POOC, GIRO, CACL
11/2/2016 Reavis Creek	Salt	484483	3710381	484521	3711190	940	500	Maybe POOC, GIRO
02/22/2017 Copper Creek	Agua Fria	414532	3784291	414339	3784419	1365	250	Might dry; Maybe POOC
03/27/2017 West Fork Pinto Creek	Salt	491038	3700111	491607	3700234	1085	700	GIRO if remove sunfish
03/28/2017 Haunted Canyon	Salt	494989	3694636	499072	3695799	1000	100	Mostly dry; maybe POOC
05/25/2017 Mule Spring	Salt	499294	3693737	499384	3693766	1120	100	POOC
06/13/2017 Sabino Canyon-upper	Santa Cruz	519538	3582136	520672	3579822	1050	400	POOC, GIRO, maybe CACL, RHOS

06/13/2017 Double R Canyon	San Pedro	571778	3579977	571730	3579864	1230	120	POOC
07/10/2017 Cave Creek	Upper Gila	666262	3526586	673178	3529739	1525	>100	RHOS
07/10/2017 South Fork Cave Creek	Upper Gila	668492	3524164	671370	3527216	1620	>200	RHOS
07/11/2017 North Fork Cave Creek	Upper Gila	665086	3529901	665185	3529976	2085	>100	Maybe ONGI
07/11/2017 East Turkey Creek	Upper Gila	664767	3531454	668015	3533601	1705	>350	RHOS, maybe GIRO
07/17/2017 Foote Creek	Blue	671461	3723300	671684	3719141	1720	4700	Maybe TICO
07/18/2017 Raspberry Creek	Blue	662652	3710016	664946	3708831	1590	2800	TICO near waterfall
07/27/2017 Bonita Creek	Upper Gila	635217	3653338	635728	3651703	1145	2000	TICO
08/28/2017 Pigeon Creek	Blue	661479	3682954	663116	3683347	1360	2500	TICO
08/29/2017 Turkey Creek (Pigeon)	Blue	662599	3683742	662715	3683641	1370	250	Maybe TICO, GIRO
10/12/2017 Buehman Canyon	San Pedro	543564	3586521	544076	3586841	920	700	POOC, maybe GIRO
10/12/2017 Bullock Canyon	San Pedro	541290	3582592	541463	3582742	1010	250	POOC, maybe GIRO
06/08/2018 Sabino Canyon	Santa Cruz	520841	3581050	520871	3581138	1100	250	GIRO, POOC, CACL, RHOS
07/11/2018 Romero Canyon	Santa Cruz	512923	3585921	511644	3586741	1095	700	GIRO maybe POOC
07/31/2018 Dix Creek	Upper Gila	671783	3673462	671727	3674884	1200	1500	TICO
07/31/2018 Sevenmile Wash	Upper Gila	533278	3716984	532785	3716643	1250	0	None
08/13/2018 Strayhorse Creek	Blue	658093	3705891	661090	3706885	1670	<1000	None
08/13/2018 Little Strayhorse Creek	Blue	656688	3706854	658922	3706360	1730	<500	None
08/28/2018 Thomas Creek	Blue	665388	3696655	668363	3695868	1390	400	None
09/25/2018 Gardner Canyon	Santa Cruz	523036	3508454	523897	3508488	1610	500	Maybe POOC
09/25/2018 Cave Creek	Santa Cruz	523048	3509620	523407	3509240	1580	300	None

09/25/2018	Sweetwater Dam	Santa Cruz	519194	3508774	519194	3508774	1730	100	POOC, GIRO
09/26/2018	Neighbor Spring	Santa Cruz	550916	3474856	550231	3474576	1540	750	POOC
09/26/2018	Temporal Gulch	Santa Cruz	518984	3498903	519186	3498737	1400	500	Maybe GIRO
10/28/2018	Hardscrabble Creek	Verde	442059	3802594	438891	3797423	820	7000	GIRO
05/02/2019	Citizen Canyon	Upper Gila	682326	3682270	681869	3681441	1400	>1000	GIRO, TICO
05/15/2019	Sycamore Canyon	Blue	670163	3703940	669803	3703494	1600	>1000	Maybe AGCH
05/15/2019	Ladrone Canyon	Blue	669769	3704075	669502	3703821	1500	1500	Maybe AGCH
07/09/2019	Little Blue Creek	Blue	671222	3697574	670600	3695176	1400	1300	AGCH, RHOS, TICO
08/21/2019	Eagle Creek	Blue	641570	3706460	641793	3705562	1500	>4000	MEFU, TICO
08/21/2019	Salt House Creek	Blue	648425	3708673	647705	3708022	1800	1000	RHOS, AGCH
08/21/2019	East Eagle Creek	Blue	642221	3707051	647536	3707913	1800	50	none

APPENDICES

Appendix 1.—Summary of native fish stocked in Arizona during 2019 by the Department under the Gila River Basin Native Fishes Conservation Program. Easting and Northing are in UTMs (NAD 83; zone 12S).

_		<i></i>					#	#
Taxa	Water Name	Site Name	Easting	Northing	Date	Lineage	Stocked	Mortalities
Desert Pupfish	Mud Springs (#18)	North Pond	454000	3734611	5/20/2019	Santa Clara Slough	131	0
Gila Topminnow	La Barge Spring		468976	3700748	4/12/2019	Mixed	154	37
Gila Topminnow	Edgar Canyon		543140	3590495	4/18/2019	Redrock Canyon	564	0
Gila Topminnow	Mud Springs (#18)	South Pond	453916	3734509	5/20/2019	Cottonwood Spring	55	0
Gila Topminnow	Cottonwood Spring (Goldfield)		453500	3706653	8/20/2019	Sharp Spring	101	0
Gila Topminnow	Harden Cienega Creek	Below Barrier	674768	3674598	10/16/2019	Bylas Spring	631	36
Gila Topminnow	Sabino Canyon	Above East Fork	520783	3581112	10/24/2019	Cienega Creek	350	177
Roundtail Chub ¹	Sabino Canyon	Above East Fork	520836	3581045	5/29/2019	Cienega Creek	148	0
Roundtail Chub ¹	Romero Canyon		511817	3586421	5/30/2019	Cienega Creek	148	0
Roundtail Chub	Blue River	Cole Flat	667297	3713521	10/10/2019	Eagle Creek	100	0
Roundtail Chub ¹	Harden Cienega Creek	Above barrier	676551	3673535	10/16/2019	Harden Cienega Creek	104	0
Roundtail Chub ¹	Rarick Canyon	F20	439785	3844473	10/21/2019	Red Tank Draw	93	1
Roundtail Chub ¹	Rarick Canyon	F23	438094	3844065	10/21/2019	Red Tank Draw	154	1
Roundtail Chub ¹	Rarick Canyon	F18	440122	3844798	10/21/2019	Red Tank Draw	72	2

¹ Roundtail Chub at this location were previously classified as Gila Chub

Cooperative Agreement R16AC00077: 2019 Annual Report – Final 03/24/2020

Taxa	Location	Date	Gear Type	Sample Size	Statistics	2019
Desert Pupfish	Black Canyon City Heritage Pond	8/1/2019	Minnow Trap	20	#Ind	849
					%YOY	79
					Mean CPUE	18.25
					SE	(1.73)
Desert Pupfish	Black Canyon City Heritage Pond	8/1/2019	Seine	1	#Ind	333
					%YOY	83
					Mean CPUE	3.17
					SE	(0)
Desert Pupfish	Black Canyon City Heritage Pond	8/1/2019	Dip Net	6	#Ind	6
					%YOY	100
					Mean CPUE	8.11
					SE	(4.13)
Desert Pupfish	Las Cienegas-Cottonwood Tank	8/6/2019	Minnow Trap	10	#Ind	190
					%YOY	17
					Mean CPUE	9.65
					SE	(2.79)
Desert Pupfish	Muleshoe CMA-Mint Spring	9/13/2019	Seine	5	#Ind	109
					%YOY	61
					Mean CPUE	1.74
					SE	(0.17)
Desert Pupfish	San Pedro Riparian NCA-Murray Spring	8/5/2019	Minnow Trap	19	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Desert Pupfish	San Pedro Riparian NCA-Murray Spring	8/5/2019	Seine	3	#Ind	0
					%YOY	0
					Mean CPUE	0

Appendix 2.—Summary of monitoring results during 2019 for the five priority species and other target native fish species that were previously stocked into various waters in the Gila River Basin Arizona.

Taxa	Location	Date	Gear Type	Sample Size	Statistics	2019
					SE	0
Desert Pupfish	San Pedro Riparian NCA-Murray Spring	8/5/2019	Dip Net	9	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Arnett Creek	7/15/2019	Minnow Trap	10	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Bass Canyon	9/13/2019	Minnow Trap	10	#Ind	4
					%YOY	50
					Mean CPUE	1.90
					SE	(0)
Gila Topminnow	Bass Canyon	9/13/2019	Dip Net	1	#Ind	6
					%YOY	0
					Mean CPUE	5.41
					SE	(0)
Gila Topminnow	Black Canyon City Heritage Pond	8/1/2019	Minnow Trap	18	#Ind	3377
					%YOY	97
					Mean CPUE	67.51
		0.11.100.1.0	a ·		SE	(8.90)
Gila Topminnow	Black Canyon City Heritage Pond	8/1/2019	Seine	1	#Ind	2185
					%YOY	89
					Mean CPUE	20.81
Cilo Tonning o	Diagle Convon City Haritage Devid	9/1/2010	Din Net	6	SE #Ind	(0)
Gila Topminnow	Black Canyon City Heritage Pond	8/1/2019	Dip Net	6	#Ind %YOY	34 100
					% YOY Mean CPUE	23.94
Cile Tonminn arr	Charlabaia Spring	10/31/2019	Minnow Trop	10	SE #Ind	(9.03)
Gila Topminnow	Charlebois Spring	10/31/2019	Minnow Trap	10	#Ind	0

Гаха	Location	Date	Gear Type	Sample Size	Statistics	2019
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Charlebois Spring	10/31/2019	Dip Net	14	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Double R Canyon	9/13/2019	Minnow Trap	10	#Ind	66
					%YOY	55
					Mean CPUE	6.93
					SE	(2.32)
Gila Topminnow	Double R Canyon	9/13/2019	Dip net	2	#Ind	2
					%YOY	100
					Mean CPUE	3.78
					SE	(1.62)
Gila Topminnow	Edgar Canyon	9/30/2019	Minnow Trap	10	#Ind	560
					%YOY	49
					Mean CPUE	36.46
					SE	(6.53)
Gila Topminnow	Edgar Canyon	9/30/2019	Seine	3	#Ind	222
					%YOY	73
					Mean CPUE	5.97
					SE	(1.05)
Gila Topminnow	Edgar Canyon	9/30/2019	Dip net	18	#Ind	20
					%YOY	85
					Mean CPUE	12.25
					SE	(2.77)
Gila Topminnow	Hidden Water Spring	10/3/2019	Minnow Trap	10	#Ind	1
					%YOY	0

Таха	Location	Date	Gear Type	Sample Size	Statistics	2019
					Mean CPUE	0.50
					SE	0
Gila Topminnow	Hidden Water Spring	10/3/2019	Dip Net	5	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Hidden Water Spring	10/3/2019	Seine	12	#Ind	23
					%YOY	87
					Mean CPUE	7.48
					SE	(3.49)
Gila Topminnow	LaBarge Spring	9/30/2019	Dip Net	13	#Ind	8
					%YOY	50
					Mean CPUE	11.71
					SE	(1.66)
Gila Topminnow	Las Cienegas-Bill's Wildlife pond	8/6/2019	Minnow Trap	10	#Ind	519
					%YOY	32
					Mean CPUE	40.00
					SE	(24.52)
Gila Topminnow	Las Cienegas-Clyne Pond	8/6/2019	Minnow Trap	10	#Ind	157
					%YOY	18
					Mean CPUE	24.82
					SE	(14.90)
Gila Topminnow	Mud Spring	8/14/2019	Minnow Trap	10	#Ind	4030
					%YOY	37
					Mean CPUE	146.17
					SE	(13.90)
Gila Topminnow	Peterson Ranch Pond	8/14/2019	Minnow Trap	10	#Ind	46
					%YOY	9
					Mean CPUE	6.13

Taxa	Location	Date	Gear Type	Sample Size	Statistics	2019
					SE	(3.28)
Gila Topminnow	Rock Spring	3/27/2019	Minnow Trap	10	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Rock Spring	3/27/2019	Dip Net	11	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Sabino Canyon	5/28/2019	Minnow Trap	22	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Sabino Canyon	5/28/2019	Dip Net	3	#Ind	2
					%YOY	0
					Mean CPUE	2.70
					SE	(0)
Gila Topminnow	Sabino Canyon	5/28/2019	Seine	10	#Ind	1
					%YOY	0
					Mean CPUE	0
01 m ·		0/5/2010		10	SE	0
Gila Topminnow	San Pedro Riparian NCA-Murray Spring	8/5/2019	Minnow Trap	19	#Ind	29
					%YOY	45
					Mean CPUE	2.63
Cilo Tonming and	Son Dodro Dinovion NCA Mumor Series	8/5/2019	Din Not	9	SE #Ind	(1.28)
Gila Topminnow	San Pedro Riparian NCA-Murray Spring	8/3/2019	Dip Net	9		1
					%YOY Moon CDUE	100
					Mean CPUE	3.60
Cile Termine	Can Dadas Diagnian NCA Munas Casing	9/5/2010	Studialet Sair -	2	SE #Lad	(0)
Gila Topminnow	San Pedro Riparian NCA-Murray Spring	8/5/2019	Straight Seine	3	#Ind	0

Таха	Location	Date	Gear Type	Sample Size	Statistics	2019
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Sheepshead Canyon	9/4/2019	Minnow Trap	13	#Ind	1504
					%YOY	64
					Mean CPUE	71.24
					SE	(14.88)
Gila Topminnow	Spring Creek	9/4/2019	Minnow Trap	12	#Ind	550
					%YOY	2
					Mean CPUE	16.84
					SE	(4.86)
Gila Topminnow	Spring Creek	9/3/2019	Backpack Electrofisher	1	#Ind	1
					%YOY	0
					Mean CPUE	0.87
					SE	(0.62)
Gila Topminnow	Tortilla Creek	10/30/2019	Minnow Trap	10	#Ind	47
					%YOY	0
					Mean CPUE	23.50
					SE	(0)
Gila Topminnow	Tortilla Creek	10/30/2019	Dip Net	12	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	West Fork Pinto Creek	7/10/2018	Dip Net	1	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	West Fork Pinto Creek	10/1/2019	Minnow Trap	10	#Ind	0
					%YOY	0
					Mean CPUE	0

Taxa	Location	Date	Gear Type	Sample Size	Statistics	2019
					SE	0
Gila Topminnow	West Fork Pinto Creek	10/1/2019	Mini-Hoop Net	5	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Loach Minnow	Blue River-lower	10/8/2019	Backpack Electrofisher	12	#Ind	561
					%YOY	18
					Mean CPUE	117.33
					SE	(20.87)
Loach Minnow	Blue River-middle	9/17/2019	Backpack Electrofisher	12	#Ind	41
					%YOY	71
					Mean CPUE	14.33
					SE	(2.95)
Loach Minnow	Hot Springs Canyon	09/18/2018	Backpack Electrofisher	9	#Ind	50
					%YOY	2
					Mean CPUE	22.45
					SE	(9.74)
Roundtail Chub	Blue River-lower	10/8/2019	Backpack Electrofisher	12	#Ind	641
					%YOY	35
					Mean CPUE	120.67
	DI D' 'IN	0.45.0010		10	SE	(23.65)
Roundtail Chub	Blue River-middle	9/17/2019	Backpack Electrofisher	12	#Ind	9
					%YOY	0
					Mean CPUE	2.47
Deurs des 1 Chrot	Handan Cianaaa	00/10/2019	Mini Haan Net	12	SE	(1.54)
Roundtail Chub	Harden Cienega	09/10/2018	Mini-Hoop Net	13	#Ind	110
					% YOY Mean CPUE	1 10.72
Roundtail Chub ¹	Los Cionagos Cluma Dand	8/6/2018	Mini Hoon Not	0	SE #Ind	(0.59)
Koundian Chub	Las Cienegas-Clyne Pond	8/0/2018	Mini-Hoop Net	9	#ING	0

Taxa	Location	Date	Gear Type	Sample Size	Statistics	2019
					%YOY	0
					Mean CPUE	0
					SE	0
Roundtail Chub	Las Cienegas-Spring Water Wetland	8/6/2019	Mini-Hoop Net	10	#Ind	40
					%YOY	0
					Mean CPUE	0.86
					SE	(0.07)
Roundtail Chub	Lazy YJ Ranch Pond	09/10/2018	Hoop Net	10	#Ind	22
					%YOY	0
					Mean CPUE	0.38
					SE	(0.04)
Spikedace	Blue River-lower	10/8/2019	Backpack Electrofisher	12	#Ind	1340
					%YOY	50
					Mean CPUE	271.48
					SE	(27.43)
Spikedace	Blue River-middle	9/17/2019	Backpack Electrofisher	12	#Ind	23
					%YOY	57
					Mean CPUE	7.84
					SE	(2.54)
Spikedace	Hot Springs Canyon	09/12/2019	Backpack Electrofisher	9	#Ind	2
					%YOY	0
					Mean CPUE	1.04
					SE	(0.63)
Spikedace	Spring Creek	9/3/2019	Backpack Electrofisher	3	#Ind	36
					%YOY	57
					Mean CPUE	34.56
					SE	(1.27)

Appendix 3.—Populations of Threatened and Endangered species repatriated under the Gila River Basin Native Fishes Conservation Program from 2007 through 2019. Estimated population size is given for those populations considered established (i.e., reproducing to the point that they are self-sustaining). Populations that have increased in numbers and continue to persist for three years after the final stocking are considered established because topminnow and pupfish begin reproducing during their first year of life. Spikedace, Loach Minnow, and Longfin Dace begin reproducing at age-1, and have a life span of about three years and can be considered established if there is evidence of reproduction and increase in population over three to four years after the final stocking. Roundtail chub begin reproducing at age-1 or age-2, and live for about eight years and require monitoring for five years after the final stocking before a relatively confident assessment of establishment can be made. The population size was estimated based on catch during the most recent monitoring and size of the stream or pond.

				Year	Population
Species	Metapopulation	Lineage	Replicated Locations	Replicated	Status/Size
Gila Topminnow	Bylas Springs	Bylas Springs	Bass Canyon (Muleshoe Ranch CMA)	2014-2018	TBD
			Bonita Creek (lower)	2008	100-499
			Bonita Creek (upper)	2010-2015	>10000
			Burro Cienega, NM	2008	1000-4999
			Double R Canyon (Muleshoe Ranch CMA)	2017-2018	TBD
			Harden Cienega Creek	2019	TBD
			Headquarters Spring (Muleshoe Ranch CMA)	2008	1000-4999
			Howard Well	2008	5000-9999
			Kei Sundt pond	2012	1000-4999
			Redfield Canyon (Muleshoe Ranch CMA)	~2009	1000-4999
			Redrock Wildlife Area Pond, NM	2010-2011	Failed
			Secret Spring (Muleshoe Ranch CMA)	2007	1000-4999
			Swamp Spring (Muleshoe Ranch CMA)	2007-2008	1000-4999
			TNC Lower San Pedro Preserve's west pond	2006	>10000
			Wildcat Canyon (Muleshoe Ranch CMA)	2014	500-999
	Upper Santa Cruz	Sharp Spring	Buckhorn Spring	2011	1000-4999
			Black Canyon City Heritage Pond	2018	TBD
			Chalky Spring	2009	Failed
			Fossil Creek	2007-2010	500-999

Population Status/Size	Year Replicated	Replicated Locations	Lineage	Metapopulation	Species
500-999	2009	Morgan City Wash	Lineage		-protect
TBD	2018	Mud Spring (Coronado NF)			
100-499	2009	Page Springs Hatchery SRP Topminnow Pond			
1000-4999	2015	Robbins Butte Stop Sign Tank			
1000-4999	2015	Robbins Butte Swimming Pool Tank			
TBD	2018	Peterson Ranch Pond			
0-99	2013	San Rafael Cattle Company Pasture #2 Pond			
B TBD	2016-2018	San Rafael Cattle Company Pasture #9 Pond			
TBD	2017	West Fork Pinto Creek			
TBD	2016	Hidden Water Spring	Peck Canyon	Lower Santa Cruz	
1000-4999	2018	International Wildlife Museum Pond			
1000-4999	2012	Phoenix Zoo Ranarium			
Failed	2013-2014	Rock Spring			
5 1000-4999	2014-2016	Sheepshead Canyon			
5 1000-4999	2015-2016	Spring Creek			
TBD	2017	Tortilla Creek (upper)			
TBD	2017	Arnett Creek	Redrock Canyon		
TBD	2019	Edgar Canyon			
500-1000	2012-2013	Walnut Spring (#392)			
Failed	2011	Ben Spring (San Pedro Riparian NCA)	Cottonwood Spr	Monkey&Cottonwood	
Failed	2011	Horse Thief Draw (San Pedro Riparian NCA)			
Failed	2009	Pemberton Pond (McDowell Mountain Reg. Park)			
500-999	2011	Usery Mountain Regional Park pond			
) TBD	2008, 2019	Cottonwood Spring (Goldfield Mountains)	Monkey Spring		
100-499	2008	Mud Spring (#18) augmentation	· - •		
Failed	2009	Spur Cross Ranch Cons. Area Solar Oasis Pond			
Failed	2009	Willow Spring (San Pedro Riparian NCA)			
B TBD	2016-2018	Bill's Wildlife Pond (Las Cienegas NCA)	Cienega Creek	Cienega Creek	
5 500-999	2015-2016	Clyne Tank (Las Cienegas NCA)	-	-	
8	2008 2009 2009 2016-201	Mud Spring (#18) augmentation Spur Cross Ranch Cons. Area Solar Oasis Pond Willow Spring (San Pedro Riparian NCA) Bill's Wildlife Pond (Las Cienegas NCA)		Cienega Creek	

Species	Metapopulation	Lineage	Replicated Locations	Year Replicated	Population Status/Size
species	incupopulation	Lineuge	Crescent Pond (Las Cienegas NCA)	2013	1000-4999
			Egret Pond (Las Cienegas NCA)	2013	5000-9999
			Empire Tank (Las Cienegas NCA)	2013	1000-4999
			Gaucho Wildlife Pond (Las Cienega NCA)	2014	1000-4999
			Little Nogales Spring (Las Cienegas NCA)	2012	Failed
			Nogales Spring (Las Cienegas NCA)	2012-2015	Failed
			Road Canyon Tank (Las Cienegas NCA)	2012	1000-4999
			Sabino Canyon (lower)	2015-2016	1000-4999
			Sabino Canyon (upper)	2018	TBD
			Spring Water Wetland (Las Cienegas NCA)	2013	5000-9999
	mixed	mixed	Charlebois Spring	2017	TBD
	mixed	mixed	Murray Spring (San Pedro Riparian NCA)	2011-2017	TBD
Desert Pupfish	Santa Clara/El Doctor	•	Bonita Creek (lower)	2008	Failed
			Bonita Creek (upper)	2010-2015	Failed
			Cinco Canyon Tank (Las Cienegas NCA)	2013	1000-4999
			Cherry Spring Canyon (Muleshoe Ranch CMA)	2007	Failed
			Cottonwood Pond (Las Cienegas NCA)	2013, 2017	500-999
			Crescent Pond (Las Cienegas NCA)	2013	500-999
			Egret Tank (Las Cienegas NCA)	2015-2016	100-499
			Empire Tank (Las Cienegas NCA)	2013	500-999
			Gaucho Wildlife Pond (Las Cienegas NCA)	2015	100-499
			Headquarters Spring (Muleshoe Ranch CMA)	2008	Failed
			Heart Pond (Las Cienegas NCA)	2013	500-999
			Horse Thief Draw (San Pedro Riparian NCA)	2011	Failed
			Howard Well	2008-2009	100-499
			Kei Sundt Pond	2010	100-499
			Larry & Charlie Tank (Muleshoe Ranch CMA)	2009	100-499
			Little Joe Spring (San Pedro Riparian NCA)	2013	1000-4999
			Mint Spring (Muleshoe Ranch CMA)	2015-2016	100-499

Species	Metapopulation	Lineage	Replicated Locations	Year Replicated	Population Status/Size
Species	in to up op when on	2	Mud Spring (#18)	2007-2009	100-499
			Murray Spring (San Pedro Riparian NCA)	2011-2014	TBD
			Nursery Tank (McDowell Mnt. Regional Park)	2010, 2019	500-999
			Pemberton Pond (McDowell Mountain Reg. Park)	2009	Failed
			Road Canyon Tank (Las Cienegas NCA)	2012	500-999
			Robbins Butte Wildlife Area Cottonwood Tank	2010	1000-4999
			Robbins Butte Wildlife Area Twin Tanks	2009	1000-4999
			Secret Spring (Muleshoe Ranch CMA)	2007-2011	100-499
			Spur Cross Ranch Cons. Area Solar Oasis pond	2009	500-999
			Swamp Spring (Muleshoe Ranch CMA)	2007	Failed
			TNC Lower San Pedro Preserve's east pond	2009	5000-9999
			Tule Creek	2007-2009	Failed
			Walnut Spring (#20)	2008	Failed
Longfin Dace		Coal Mine Canyon	Fresno Canyon	2008	1000-4999
		Hassayampa River	Arnett Creek	2007	0-99
		Hassayampa River	Telegraph Canyon	2007	100-499
		Hidden Water Spr	Rock Creek	2016	Failed
		Seven Sprs Wash	Spur Cross Ranch Cons. Area Solar Oasis pond	2008	0-99
		Tangle Creek	Fossil Creek	2008-2009	1000-4999
Loach Minnow		Aravaipa Creek	Hot Springs Canyon (Muleshoe Ranch CMA)	2007-2011	100-499
			Redfield Canyon (Muleshoe Ranch CMA)	2007-2010	Failed
			Fossil Creek	2007-2013	Failed
		Blue River	Bonita Creek (lower)	2008	Failed
			Bonita Creek (upper)	2009-2014	Failed
Spikedace		Aravaipa Creek	Fossil Creek	2007-2018	1000-4999
			Spring Creek	2015-2018	TBD
			Hot Springs Canyon (Muleshoe Ranch CMA)	2007-2011	0-99
			Redfield Canyon (Muleshoe Ranch CMA)	2007-2010	Failed
		Upper Gila River	Blue River	2012	5000-9999

Species	Metapopulation	Lineage	Replicated Locations	Year Replicated	Population Status/Size
-			Blue River (middle)	2017-2018	TBD
			Bonita Creek (lower)	2008	Failed
			Bonita Creek (upper)	2009-2010	Failed
Roundtail Chub		Cienega Creek ¹	Clyne Pond (Las Cienegas NCA)	2016, 2017	Failed
		Cienega Creek ¹	Spring Water Wetland (las Cienegas NCA)	2017	TBD
		Cienega Creek ¹	Sabino Canyon (upper)	2019	TBD
		Dix Creek ¹	Redrock Wildlife Area, NM	2010-2011	Failed
		Eagle Creek	Blue River	2012-2016	1000-4999
		Eagle Creek	Blue River (middle)	2017, 2019	TBD
		Harden Cienega ¹	Harden Cienega (upper)	2015	1000-4999
		Harden Cienega ¹	Mule Creek NM	2012-2014	TBD
		Redfield Canyon ¹	Redfield Canyon (upper)	2007	500-999
		Red Tank Draw	Rarick Canyon	2019	TBD
		O'Donnell Creek ¹	TNC Lower San Pedro Preserve's west pond	2010-2011	1000-4999
		Verde River	TNC Gila Riparian Preserve (Farm), NM	2008	Failed
Razorback Sucker		Lake Mohave	Fossil Creek	2008-2014	Failed

¹ 1 Chub in these locations were previously classified as Gila Chub.

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