Gila River Basin Native Fishes Conservation Program: Arizona Game and Fish Department's Native Fish Conservation Efforts During 2020

Cooperative Agreement R16AC00077 Between Bureau of Reclamation and Arizona Game and Fish Department Annual Report March 17, 2021

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Program
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OVERVIEW

The Gila River Basin Native Fishes Conservation Program (Program; previously known as the Central Arizona Project [CAP] Funds 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 Chub 1 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

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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 2020. For each priority action, work completed during 2020 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 2020 Department staff completed repatriation stockings into 10 waters (Appendix 1). Also during the performance period Department staff completed eight non-indigenous species control actions: three nonnative fish removal efforts in Red Tank Draw, two in Redfield Canyon, one in Harden Cienega Creek, one in Harden Cienega Creek drainage stock tanks, and one at Sweetwater Dam Pond.
- 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 2020, Department staff conducted post-stocking monitoring of 25 populations (Appendix 2): 2 Spikedace, 2 Desert Pupfish, 16 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 2020, Department staff monitored five locations where nonnative fish removals have been implemented: Rarick Canyon, Redfield Canyon, Red Tank Draw, Spring Creek, and Sweetwater Dam.

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

Results: In 2020, all Loach Minnow and Spikedace populations at ARCC spawned. ARCC produced 2,182 Aravaipa Creek Spikedace, 408 upper Gila River Spikedace, 833 Gila River Forks Spikedace, 16 Blue River Loach Minnow, 57 Aravaipa Creek Loach Minnow, 3 San Francisco River Loach Minnow, and 15 Gila River Forks Loach Minnow³.

GENERAL ACTIVITIES

Department staff administered and managed Program projects identified in the agreement. Staff developed electronic data entry forms and corresponding formatting and summary scripts, entered data into survey and stocking datasets, and checked data for accuracy. Department staff finalized the 2019 annual report, began analyzing data and drafting the 2020 annual report, and drafted the 2021 annual work plan, and Environmental Assessment Checklists. Staff coordinated with intraagency staff, other agencies, and private landowners to continue work on existing projects and to

¹ Including populations previously classified as Gila Chub.

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

³ Genetic analysis identified Gila Forks Loach Minnow as primarily Blue River Loach Minnow.

develop potential new projects. The Program specialist also hired new seasonal staff and a replacement for the Program specialist.

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, or cascade) within each transect, fish were processed and data were recorded. Captured fish were identified to species and counted. All Spikedace, Loach

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¹ Including chub populations previously classified as Gila Chub.

Minnow, and 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 (≤40 and >40 mm TL for Speckled Dace and Longfin Dace; ≤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). 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;
- 3. evaluate size-structure of each population of fish species to detect reproduction and recruitment to the population;

¹ Including chub populations previously classified as Gila Chub.

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. A single full pass is defined as electrofishing all water present from the downstream end to the upstream end of the target reach. An initial set of traps in the target reach is considered the first pass, with each reset within the same reach considered a subsequent pass.

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

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¹ Including chub populations previously classified as Gila Chub.

fishing gun) to remove large-bodied piscivorous nonnative fish. To improve the chances of 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 nonnatives across removal events will be examined, and a decrease in abundance of the target 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
 - O 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. 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 April 6, 2020, Department staff collected 61 Longfin Dace from Aravaipa Creek immediately downstream of the fish barrier for a fish health assessment. All fish were collected in two seine hauls. No pathogens or parasites of concern were detected in the subsequent fish health assessment.

On December 15, 2020, Department staff collected 49 Spikedace and 82 Loach Minnow from Aravaipa Creek, just downstream from the TNC Guest House (UTM 12S 556089/3638099). Fish were transported to ARCC with no mortalities during collection and transport.

Blue River. During June 22-24, 2020, Department staff salvaged Loach Minnow from the Blue River due to concerns about post-fire impacts from the Bringham Fire. Fish were collected by backpack electrofishing and seining both above and below Juan Miller crossing. A total of 313 Loach Minnow were salvaged and transported to ARCC to augment the existing Blue River broodstock. Unfortunately the fish were not delivered to ARCC until late Wednesday morning and there were 44 mortalities during the extended holding and transport period, with a total of 269 live fish counted by ARCC staff.

Recommendations: The Program Technical Committee recommended incorporating this project into the Aquatic Research and Conservation Center O&M project in the federal fiscal year (FY) 2021 work plan. Therefore, in future reports this project will be deleted and the work reported under the ARCC O&M project. Recommendations for work include continuing 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. Staff will plan and implement improved processes to better ensure that lineages do not get mixed, which will be incorporated into the hatchery management plan. Broodstock of each lineage should be injected with Visible Implant Elastomers (VIE) to give each lineage a unique mark. Tanks should be color coded with each lineage having a unique color.

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

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - o Goal 4. Remove nonnative aquatic species threats.
 - o Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - o 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.

<u>Background:</u> 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. (2015). 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. 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 were stocked into Bass Canyon in 2014, 2015, and 2018, and Double R Canyon in 2017 and 2018.

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

Both Spikedace and Loach Minnow failed to establish in Redfield Canyon. Gila Topminnow are established in Redfield Canyon.

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 April 21-22, 2020, Department staff performed the first Green Sunfish removal pass of the year in Redfield Canyon. The crew backpack electrofished 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 4,831 seconds and captured 4 Green Sunfish (mean CPUE = 2.829 fish/hour). Native fish were not counted during electrofishing because there was limited time with a two person crew and all native fish are typically counted during the June removal pass. The sunfish were captured in two of the pools that supported Green Sunfish in 2019, which indicates that sunfish have relatively strong habitat preferences within this reach.

The crew also completed one removal pass in Reach 3 (Figure 1). The crew set 12 mini-hoop nets and captured 123 Green Sunfish (59-214 mm TL). An additional 35 Green Sunfish were captured by angling with fly rods while the traps soaked.

During June 2-3, 2020, Department staff removed Green Sunfish from Redfield Canyon by completing the second full electrofishing pass from the trail access in Reach 2 upstream to the sunfish barrier. Green Sunfish were not captured during 7,400 seconds of electrofishing. A total of 660 Roundtail Chub², 186 Sonora Sucker, 58 Longfin Dace, 1 Desert Sucker and 1 Gila Topminnow were captured and returned to the stream.

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

² Chub in this location previously classified as Gila Chub.

Crews also set five mini-hoop nets in pools in Reach 1 where Green Sunfish have consistently been captured during previous removal efforts. The nets were retrieved after completion of electrofishing and one Green Sunfish was captured in a pool that has consistently harbored sunfish the last several years. Six mini-hoop nets were set overnight on June 2nd in the same pool and captured three additional Green Sunfish.

Crews also completed the second and third removal passes of the year in Reach 3. Ten mini-hoop nets were set overnight on June 2nd in the pools near the wilderness boundary. The nets were pulled the following morning with 198 Green Sunfish captured. The traps were reset for an additional two hours along with four additional minnow traps with an additional 62 Green Sunfish captured. One Green Sunfish was captured by angling the pools at the wilderness boundary while the traps soaked. Seven adult Gila Topminnow were also captured in the traps and one more was captured in a dip net sweep just upstream of the wilderness boundary, providing evidence of topminnow persistence despite the relatively high sunfish density in the Reach 3.

Overall a total of 426 Green Sunfish were removed from Redfield Canyon in 2020 (Figure 2). Green Sunfish catch in Reaches 1 and 2 in 2020 declined from 2019 which suggests that little movement occurred between Reaches 3 and 1 during the past year (Figure 2). Importantly, mean backpack electrofishing CPUE in Reaches 1 and 2 decreased for the second consecutive year (2018 = 10.05 fish/hour; 2019 = 6.75; 2020 = 1.18). The number of Green Sunfish captured in Reach 3 (418) was the most during a single year of removal efforts to date, although this could be partially due to greater removal effort than recent years (Figure 2). In addition, comparison of mean size of Green Sunfish captured in Reach 3 using a Wilcoxon rank-sum test indicates that there was a significant decrease since 2019 (W = 53869, p = <0.001; 2019 = 122.39, 2020 = 95.83; Figure 3). However, this decrease in mean length seems to be associated with in an increase in the number of juvenile Green Sunfish captured, and not a change to the size structure of the adult population which has remained relatively constant the last several years (Figure 3). Because Reach 3 is likely the source of Green Sunfish to the upstream Reaches 1 and 2, the decrease in mean size of Green Sunfish may partially explain the declining numbers of sunfish in Reaches 1 and 2, as smaller fish typically have poorer swim performance and may not be able to navigate higher flows as effectively as larger fish. Green Sunfish have not spawned in Reach 1 or 2 for several years now, and one or two removal trips a year appears to be sufficient to effectively prevent Green Sunfish from reestablishing within these reaches.

Monitoring of Repatriated Populations. On September 15, 2020, Department staff monitored Gila Topminnow in upper Bass Canyon. Thirteen collapsible minnow traps were set between the road crossing and the most recent stocking location. A total of 2 Gila Topminnow, 302 Roundtail Chub¹, and 2 Speckled Dace were captured. One dip net sweep failed to capture any fish. The crew also performed three seine hauls and captured 51 Gila Topminnow, 77 Roundtail Chub¹, 2 Sonora Sucker, and 26 Speckled Dace. Nearly all of the topminnow in Bass Canyon were captured in a single seine haul in a side channel pool. Of the 53 topminnow captured, 45 were juveniles (< 20 mm TL) while 8 were adults (≥ 20 mm TL), which suggests more reproduction is occurring than in previous years.

While relatively few topminnow were captured, more were captured this year than during any monitoring effort since 2016 (2017 n = 25, 2018 n = 3, 2019 n = 10). The extent of surface water in the surveyed reach was greatly reduced compared to the last few years of monitoring during the same time period. The topminnow may have responded positively to the lack of typical summer flooding and the resulting low water conditions in 2020.

On September 15, 2020, Department staff monitored Gila Topminnow in Double R Canyon. Ten collapsible minnow traps were set between the confluence with Bass Canyon (UTM 12S 571964/3579500) upstream to the most recent stocking location (571720/3579842). A total of 10 Gila Topminnow, 74 Roundtail Chub¹, 35 Longfin Dace and 12 Speckled Dace were captured. An additional 15 Gila Topminnow were captured in three dip net sweeps. Staff also performed two seine hauls and captured 193 Gila Topminnow, 38 Roundtail Chub¹, 9 Longfin Dace and 191 Speckled Dace. Of the 218 topminnow captured, 159 were juveniles (< 20 mm TL) while 59 were adults (\geq 20 mm TL), which suggests fairly strong reproduction. The topminnow catch during this monitoring effort was by far the most since the first stocking of Double R Canyon in 2017 (2018 n = 0, 2019 n = 68). The topminnow seem to be responding positively to the lack of typical summer flooding and the resulting low water conditions in 2020. In addition, Double R Canyon seems to have fewer large adult chub than Bass Canyon, and the lack of potential predators may partially explain why the topminnow population is doing better in this stream.

Repatriation Stockings. On May 22, 2020, Department staff augmented the existing populations of Spikedace and Loach Minnow by stocking a total of 300 Loach Minnow and 333 Spikedace in Hot Springs Canyon at the confluence with Wildcat Canyon (UTM 12S 569185/3580087). The fish were collected from ARCC on May 21st and held overnight at Department HQ. Fish were then transferred to aerated buckets for a short UTV ride and a hike to the stocking location. There was one Spikedace mortality during transport and stocking. About 100 individuals of each species were PIT tagged on May, 20, 2020 by Kansas State researchers. Fish were stocked at the confluence with Wildcat Canyon to better accommodate Kansas State researchers because they had to carry over 400 pounds of gear from the pipeline road down to the stream in order to set up antennas. Fall annual monitoring efforts by Marsh and Associates during September, 2020 captured 30 Loach Minnow and failed to detect Spikedace (Shollenberger et al. 2021).

Recommendations: The potential barrier to be constructed in Redfield Canyon has been abandoned because State Land Department did not agree to transfer the land to Reclamation. In 2020, Department staff again contacted the private landowner just downstream of the Wilderness boundary to determine if permission could be obtained to access their property on lower Redfield Canyon for Green Sunfish removals. The landowners did not give a definitive answer during multiple phone conversations. Based on phone conversations, a proposed work plan was drafted and provided to the landowners in an email. However, there was no response from the landowners after multiple follow-up attempts.

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¹ Chub in this location previously classified as Gila Chub.

Department staff will continue to contact the landowners 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.

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.

Sweetwater dam nonnative removal (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
 - o Goal 4a. Eradicate nonnative aquatic species from a minimum of five surface waters to prepare them for repatriations of native fishes.
 - 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:

- Gila Topminnow draft revised recovery plan (1999)
 - o Task 2.2 (priority 1) Reestablish into suitable habitats
 - o Task 2.4 (priority 1) Protect suitable reestablishment habitats from detrimental nonnative aquatic species.
 - o Task 3 (priority 1) Monitor natural and reestablished populations and their habitats
- Gila Chub draft Recovery plan (2014)
 - Task 1.3.1 (priority 1) Eliminate or control problematic nonnative aquatic organisms
 - o Task 2.2 (priority 1) Repatriate Gila chub to new protected streams
 - o Task 3.2 (priority 2) Conduct monitoring

<u>Background:</u> In 2018, the Department's Ranid Frog Program staff reported detecting Western Mosquitofish and Common Goldfish in Sweetwater Dam pond along with mosquitofish in isolated

pools downstream in Cave Creek and East Sawmill Canyon (a tributary to Cave Creek; Figure 4). Department fisheries biologists confirmed reports of mosquitofish during follow-up surveys later that year. Sweetwater Dam is located in the Cienega Creek watershed and if either mosquitofish or goldfish made their way into Cienega Creek, they would pose a serious threat to the continued existence of the Cienega Creek remnant populations of Gila Topminnow and Roundtail Chub¹. Removals in Cienega Creek would likely be extraordinarily expensive and difficult to successfully achieve eradication. Consequently, Coronado National Forest staff attempted to pump the tank dry with trash pumps in 2019, but were unsuccessful because the pumps could not handle the viscous organic material at the bottom of the pond.

Results:

On March 17, 2020, Department staff surveyed Aliso Spring (UTM 12R 518673/3511132) and an unnamed tank (520719/3510914) in the East Sawmill Canyon drainage for presence of Western Mosquitofish. The crew carried out four bag seine hauls in the tank and did not capture or observe any fish. The crew also carried out four dip net sweeps in the concrete spring box at Aliso Spring and failed to capture or observe any fish. The results of this survey confirmed the absence of mosquitofish during visual surveys by regional staff at both locations in August 2019. The crew also visited Sweetwater Dam in an attempt to retrieve the temperature logger and observed recent evidence of anglers visiting the pond.

During June 15-17, 2020, Department staff drained Sweetwater Dam to eradicate the existing populations of Western Mosquitofish and Goldfish. The crew was able to draw down all of the free surface water with three 2" trash pumps and two 3" diaphragm pumps by about 9:30 AM on the first day of pumping, and the last live fish was observed shortly thereafter (Figure 5). As the trash pumps began to fail, the crew switched entirely to the two diaphragm pumps to remove saturated mud from the pond bottom. The diaphragm pumps were extremely effective and were able to essentially dig out a hole near the dam and water began to trickle out of the sediment and collect down to this low point. Eventually, one diaphragm pump was used and it was quickly able to pump out the mud and water that accumulated in the low point, so the pump was periodically shut off in order to allow for more mud and water to collect, before running the pump again. This process was repeated multiple times throughout the afternoon of the 16th. The pump was operated one final time on the morning of the 17th to discharge the water and mud that had collected overnight (Figure 6). No live fish were observed in the pool that had accumulated overnight, suggesting that both Western Mosquitofish and Common Goldfish were eradicated from the pond.

The intakes for the pumps had large mesh covers to minimize sucking in large objects (and larger fish) that could foul the pumps. The discharge from the trash pumps and diaphragm pumps was directed through bag seines to reduce the chances of transporting fish downstream. The stream channel downstream of Sweetwater Dam was walked at about 1400 hours on the 16th and water was observed for only about 300 m downstream. About 20 Western Mosquitofish in a shallow

pool about 30 m downstream of the dam, suggesting very few fish were able to survive the pumps and escape into the stream channel. Mosquitofish were much more abundant and broadly distributed within Cave Creek during previous surveys, so it was concluded that the risk of these fish surviving and moving downstream was very low.

On July 1, 2020, Department staff surveyed Cave Creek and Sweetwater Dam Pond to determine whether any fish survived the draining effort in June. The water level in Sweetwater Dam pond was still low and no fish were observed in 15 minutes of visual survey. The crew also walked the Cave Creek drainage from Sweetwater Spring upstream to Sweetwater Dam. Only two small pools were present and fish were not captured in several dip net sweeps or visually observed. The pools had good visibility and were only about 2 m wide at the time of sampling.

On July 30, 2020, Department staff visually inspected a bedrock pool in the stream channel downstream of Sweetwater Dam (519539/3508857) where mosquitofish were previously observed on a number of occasions. Several mosquitofish were detected and the crew was able to capture and remove eight mosquitofish with dip nets and seines. It is most likely that these fish remained in the stream channel downstream of the pond following pumping earlier in the year. Fish were not observed or captured within this reach during the previous visit following draw down of the pond, but it is possible the fish were missed.

On August 5, 2020, Department staff surveyed Sweetwater Dam and the stream channel downstream to verify successful eradication following the detection of several mosquitofish downstream of Sweetwater Dam. Ten collapsible minnow traps were set in Sweetwater Dam pond to verify that Western Mosquitofish were absent from the pond. No nonnative fish were captured or observed. Only Longfin Dace were captured, which were stocked on July 30th with funding from a different Department program.

Cave Creek was also surveyed from the fish barrier near the Apache Springs Ranch boundary (UTM 12R 523557/3509198) upstream through the unnamed tributary to Sweetwater Dam. Two crews walked the entire drainage with one crew walking upstream from the fish barrier and another pair walking downstream from Sweetwater Dam. A total of 26 dip net sweeps and 13 seine hauls were carried out within pools throughout the sampled reach and all surface water in the stream channel was visually searched. Only one Western Mosquitofish was detected and subsequently captured in the same pool where mosquitofish were captured on July 30th (519420/3508843). The mosquitofish captured in a single pool a few hundred meters downstream of Sweetwater Dam likely represent a few individuals that were able to persist in isolated pools in the stream channel following the pumping of Sweetwater Dam in June. Mosquitofish now appear to be restricted to a single pool and it is possible that all individuals have been removed from this pool during post-draw down monitoring efforts.

<u>Recommendations</u>: Minnow trap data and multiple visual observations of the pond since the draining effort in June all support the conclusion that Mosquitofish are eradicated from the pond. However, at least one intensive monitoring effort of Sweetwater Dam should occur in 2021 to verify eradication. In addition, the stream channel below Sweetwater Dam and Cave Creek should be surveyed again in 2021 to verify absence of Western Mosquitofish. This work will be completed using other Department funding sources.

Department staff are currently waiting for our partners to complete compliance in order to stock Cienega Creek lineage Gila Topminnow and Roundtail Chub¹ in Sweetwater Dam Pond in 2021. If Gila Topminnow are stocked, it will be reported under the Gila Topminnow Stockings project.

Forest Service staff installed a locked gate on the road to Sweetwater Dam on September 23, 2020, which should reduce the likelihood of nonnative fish being reintroduced to the pond.

Gila Topminnow stockings (Task AZ-2002-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - o Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - o 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.

Overall Background: 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

¹ Roundtail Chub from Cienega Creek were previously classified as Gila Chub

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 baited minnow trap sets per site, but dip nets or seines are sometimes used if habitat is amenable.

Fish Health Assessments During 2020:

Cold Spring. On February 18, 2020, Department staff collected 60 Gila Topminnow (Cottonwood Spring lineage) from Cold Spring (UTM 12S 602697/ 3652099) and transported them back to Department headquarters for a fish health assessment. Parasites or pathogens of concern were not detected in the assessment.

In addition, fish health assessments were carried out at three additional sites, however information cannot be included in this report consistent with ARS 17-495.

Sites Monitored or Stocked During 2020:

A table of mean catch per unit effort (CPUE) with standard error, and the proportion of young of year captured for each taxa by gear type at each location can be found in Appendix 2.

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 autumn 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. In August 2019, a total of 5,338 Gila Topminnow and 1,164 Desert Pupfish were captured.

Results: On July 21, 2020, Department staff monitored Gila Topminnow and Desert Pupfish in Black Canyon City Heritage Park Pond. The crew set 10 collapsible minnow traps and captured 2 Desert Pupfish and 12 Gila Topminnow. Department staff also performed three dip net sweeps and captured an additional 18 Gila Topminnow. The low capture rate was very surprising considering that several thousand topminnow were captured in 2019. Dissolved oxygen was low at the time of sampling (1.6 mg/L) so water quality could be an emerging issue for this location.

Recommendations: Because Gila Topminnow were last stocked in 2018, the population should be monitored until at least 2021, unless more are stocked. Desert Pupfish were last stocked into Black Canyon City Heritage Park Pond in 2017, but the status of the population is uncertain following the most recent monitoring results and should be monitored for at least one more year. Additional Desert Pupfish should eventually be stocked in the pond to increase the founding population size. Roundtail Chub¹ can be stocked in 2021 or later, after Desert Pupfish are established and water quality issues are resolved. Department staff will work with Black Canyon City Heritage Park to identify and address potential limiting factors in the pond (i.e., water quality) that may have caused crashes of the topminnow and pupfish populations.

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 to Charlebois Spring. In October 2018, Department staff captured a total of 983 Gila Topminnow in minnow traps and dip net sweeps. No fish were captured during monitoring in October 2019, but about 50 were observed in the original stocking pool. The low abundance of topminnow was likely related to severe flooding earlier in the year.

Results: On October 27, 2020, Department staff monitored Gila Topminnow at Charlebois Spring. A total of 12 collapsible minnow traps were set in the main spring and downstream pools and captured 38 Gila Topminnow (0 < 20 mm TL, $38 \ge 20 \text{ mm TL}$). The crew carried out five dip net

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

sweeps from the main spring downstream approximately 350 m to the confluence with La Barge Canyon and captured two additional Gila Topminnow. Several hundred more topminnow were visually observed in the original stocking pool so the monitoring data likely represents a substantial undercount of the population. Based on monitoring information, it appears that the topminnow population has recovered well from the decline in abundance last year following severe flooding. While relatively few fish were captured, topminnow can probably be considered established at this location based on the number of fish that were observed, but not captured in traps.

Recommendations: Although Gila Topminnow in Charlebois Spring appear to be established, additional stockings may be warranted to maintain genetic variability since the population drastically decreased in size between 2018 and 2019. The augmentation could be carried out by another Department program. Future monitoring can be transferred to another Department program or to Reclamation's long-term monitoring contract. We recommend that the USFS trim back the riparian vegetation 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 lands approximately 5 km upstream of the confluence with the San Pedro River. This perennial reach is at least 600 m long. 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. In April 2019, Department and Pima County staff stocked 564 Gila Topminnow (Redrock Canyon lineage) into Edgar Canyon (UTM 12S 543140/3590495). During the initial monitoring of the Edgar Canyon topminnow population in September 2019, Department staff captured a total of 802 Gila Topminnow with collapsible minnow traps, seines and dip nets.

Results: On September 16, 2020, Department staff set seven collapsible minnow traps in pool habitats near the stocking location (UTM 12S 543140/3590495) for a minimum soak time of 2 h and captured a total of 1,017 Gila Topminnow (416 < 20 mm TL, $601 \ge 20$ mm TL). The crew carried out 20 dip net sweeps and captured 96 more Gila Topminnow (66 < 20 mm TL, $30 \ge 20$ mm TL). Department staff also carried out three seine hauls and captured an additional 159 topminnow (87 < 20 mm TL, $72 \ge 20$ mm TL). Lowland Leopard Frogs and tadpoles were abundant throughout the surveyed reach. Despite unseasonable drought conditions and reduced extent of surface water, more Gila Topminnow were captured in 2020 than during the first monitoring in 2019.

At the time of monitoring, the perennial reach of Edgar Canyon did not appear to have been impacted by the Bighorn Fire. However, a substantial portion of the upper watershed was burned in the fire and impacts on stream habitat in Edgar Canyon in general, and topminnow specifically, will likely depend on precipitation patterns over the coming year.

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 aid population establishment if deemed necessary.

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 were absent in 2011, 2012, or 2013. Department staff began efforts to reestablish this population in 2016 by translocating 544 Gila Topminnow (Peck Canyon lineage). A total of 425 Gila Topminnow and 343 Longfin Dace were captured during the first monitoring effort in October 2017, but the captures declined to 312 Gila Topminnow and 58 Longfin Dace in 2018, and 24 Gila Topminnow and 144 Longfin Dace in 2019. Evidence of severe flooding preceding the survey in 2019 is the most likely explanation for the drastic reduction in topminnow abundance in 2019.

Results: On November 9, 2020, Department staff monitored Gila Topminnow in Hidden Water Spring. The crew set ten minnow traps for a minimum soak time of 2 h and captured a total of 455 Gila Topminnow (167 < 20 mm TL, $288 \ge 20 \text{ mm TL}$) and 290 Longfin Dace. The crew carried out five seine hauls and captured an additional 32 topminnow and 1 dace. An additional 11 dip net sweeps resulted in the capture of 8 topminnow and 14 dace. Topminnow were distributed throughout nearly all of the surveyed reach. The population and stream habitat seem to be recovering well following substantial flooding in 2019 when only 24 topminnow were captured. Part of the upper portion of the watershed is located within the burn footprint of the Bush Fire, however post-fire impacts were not observed within the surveyed portion of Hidden Water Spring.

Recommendations: The Gila Topminnow population in Hidden Water Spring rebounded well from severe flooding in 2019, with nearly 500 individuals captured in 2020. Gila Topminnow can be considered established in Hidden Water Spring and monitoring can be transferred to another Department program or agency. Additional stockings may still be beneficial to augment the population in the uppermost pool (UTM 12S 459353/3717249).

La Barge Spring

Background: La Barge Spring is located in La Barge Canyon near the confluence with Bluff Spring Canyon. Nonnative guppies were detected in the spring box and several small pools downstream

in 2017. The guppies were eradicated from the spring box in 2018 by diverting water from the spring box and manually pumping it dry. A total of 154 Gila Topminnow were stocked in La Barge Spring in April 2019. Nine topminnow were captured during the first monitoring effort in October 2019.

Results: On October 27, 2020, Department staff monitored the Gila Topminnow population in La Barge Spring. The crew completed eight dip net sweeps and captured 26 Gila Topminnow. The Sawtooth Fire burned over the spring box and several of the large riparian trees and almost all the shrubs in the vicinity of the spring were completely destroyed.

Recommendations: The spring box has already filled up about halfway with silt and sediment after the Sawtooth Fire. The slope upstream of the spring box has a substantial amount of loose soil and ash that is likely to run into the spring box with the next few major rain events. Consequently, it is very likely that the La Barge Spring population will be lost over the next year. The spring was stocked primarily for mosquito control and to inhibit the restocking of nonnative fishes. Nonetheless, the spring should be monitored until 2021 to determine if it has filled with sediment, if Gila Topminnow persist, or if more need to be stocked.

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 as part of a salvage effort. Only five topminnow were captured during monitoring in August 2018, but captures increased to 519 individuals in 2019.

Results: On August 3, 2020, Department staff monitored the Gila Topminnow population in Bill's Wildlife Pond. Ten minnow traps were set for a minimum soak time of 2 h and captured a total of 3,858 Gila Topminnow (2,066 <20 mm TL, 1,792 \ge 20 mm TL) and one Sonoran mud turtle in five of the traps. So many fish were captured that the crew ran out of daylight and was not able to count fish from the remaining five traps. Water quality appeared to be much improved, with over a foot of visibility and submersed aquatic vegetation present.

Recommendations: The Gila Topminnow population in Bill's Wildlife Pond appears to be well on the way toward being considered established, with several thousand fish captured in 2020. Because the population was last augmented in 2018, Bill's Wildlife Pond will be monitored one final time in 2021.

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. Desert Pupfish captures increased to 47 fish in 2018, and to 190 fish in 2019.

Results: On August 3, 2020, Department staff set ten minnow traps with floats overnight in Cottonwood Tank. The monitoring effort was initially planned to be a 2 h set but traps were unable to be pulled before dark. A total of 11 Desert Pupfish were captured in the traps. The results are similar to those of BLM monitoring efforts during 2020. Many more pupfish were visually observed, but capture probability continues to be highly variable at this location likely due to fluctuating water chemistry and aquatic vegetation density.

Recommendations: Despite a very similar trapping approach in 2020, Desert Pupfish catch declined substantially from 2019. It is unclear whether fluctuations in Desert Pupfish captures are the result of actual changes in abundance or whether capture probability is highly variable due to fluctuating water quality and vegetation density. Desert Pupfish are likely established in Cottonwood Pond since they have been detected in monitoring since 2014 despite variable captures. Monitoring of this location can be passed along to another Department program or agency in the future.

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. Topminnow were first stocked in Spring Water Wetland in 2013 and the population was determined to be established in 2016, with over 10,000 individuals captured during the final monitoring effort. 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. During the initial monitoring effort in August 2018, 1,161 Gila Topminnow and 71 Roundtail Chub¹ were captured. Roundtail Chub¹ catch during monitoring efforts in 2019 declined to 40 individuals.

Results: On August 3, 2020, Department staff set ten mini-hoop nets for a minimum soak time of two hours and captured one Roundtail Chub, two large Gila Topminnow and one Sonoran mud turtle. Several young of year chub were captured during Gila Topminnow collection efforts for

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

other Department projects at Spring Water Wetland earlier in 2020. The Roundtail Chub population in Spring Water Wetland was persisting at the time of monitoring, although apparently at a small size. BLM staff reported that Spring Water Wetland was nearly dry on October 16, with only a few inches of water remaining in some places. As of early December, there was very little additional rain and it is very possible the populations of both Roundtail Chub and Gila Topminnow could be lost from this location.

Recommendations: Monitoring of Roundtail Chub¹ should continue in 2021 because chub were last stocked in 2017, and the status of the population needs to be verified following reports of near drying by BLM staff. Additional stocking of Roundtail Chub into Spring Water Wetland is not recommended because of the apparent susceptibility of this location to extraordinary drought.

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 topminnow were captured each year from 2012 through 2017, and fewer than six pupfish were captured each year from 2013 through 2017. Desert Pupfish have not been captured since 2017. Captures of topminnow increased slightly in 2018 to 57 fish, but decreased to 30 topminnow in 2019. 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.

Results: On August 3, 2020, Department staff monitored Gila Topminnow in Murray Spring. A total of 20 collapsible minnow traps were set in Murray Spring for a minimum soak time of 2 h and captured a total of 70 Longfin Dace and 62 crayfish. The crew carried out five dip net sweeps above the barrier and failed to capture any Gila Topminnow.

Recommendations: Desert Pupfish are likely extirpated from Murray Spring since they have not been captured during three consecutive monitoring efforts since 2017. Gila Topminnow have been stocked four times since 2011 and no more than 57 topminnow have been captured in any annual monitoring efforts. This was the first monitoring effort since the last stocking in 2017 that Gila Topminnow were not captured. The vegetation is continuing to grow thicker which seems to be reducing the amount of open habitat and increasing shading on the stream, both of which probably negatively impact the ability of Gila Topminnow to establish at this location. Because Gila

Topminnow were not captured in 2020, this location should be monitored again in 2021 to verify their absence. Because this location was stocked with topminnow several times without evidence of establishment, additional stockings are not recommended unless habitat improvements are made.

Mattie Canyon.

Background: Mattie Canyon is a tributary to Cienega Creek. The Creek has a short perennial reach (~1,770 m in 2020) that historically supported Gila Topminnow, Roundtail Chub¹ and Longfin Dace. A flood in 2000 apparently displaced Gila Topminnow from Mattie Canyon. Roundtail Chub and Longfin Dace were able to move back upstream and recolonize habitat since the flood. Gila Topminnow apparently did recolonize, because during 2006, knowledgeable biologists observed abundant topminnow in the Mattie Canyon headwaters spring and in several pools downstream towards the tributary's confluence with Cienega Creek (Bodner et al. 2007). However, topminnow have not been detected in Mattie Canyon since.

Results: On August 27, 2020, Department staff assisted BLM and U.S. Fish and Wildlife Service staff in translocating topminnow into Mattie Canyon from Cienega Creek. Topminnow were collected from Cienega Creek in the vicinity of the confluence with Mattie Canyon with a combination of seines and dip nets. Fewer topminnow were captured than expected in this location, potentially due to the weak monsoon season and lack of typical summer flooding. A total of 116 Gila Topminnow were translocated to a series of two pools in Mattie Canyon (n = 28, 539918/3523504; n = 88, 540102/3523732) with no mortalities during capture or transport.

Recommendations: Because relatively few topminnow were able to be translocated to Mattie Canyon in 2020, more should be stocked to supplement the founding size of this population in 2021. Mattie Canyon is typically monitored by BLM staff as part of an annual monitoring program, and BLM staff can continue to monitor this population after augmentations occur.

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 located at 1,700 m elevation, has a surface area of about 255 m² and is about 2 m deep in the middle. Despite this relatively high elevation, winter water temperatures seem to be moderated by spring inputs and the south facing orientation of the pond. Vegetation (primarily sedges) line the perimeter of the pond 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 in August, 2018. During the initial monitoring in August, 2019 a total of 4,201 topminnow were captured.

¹ Roundtail Chub in Mattie Canyon were previously classified as Gila Chub.

Results: On August 4, 2020, Department staff monitored Gila Topminnow in Mud Spring. Ten minnow traps were set for a minimum soak time of 2 h and captured a total of 2,956 Gila Topminnow (2,137 <20 mm TL, 819 \ge 20 mm TL). In addition, 8 mud turtles and 1 Chiricahua leopard frog were captured.

Recommendations: Gila Topminnow should be monitored in Mud Spring until at least 2021 to confirm that topminnow are established at this location. The population has continued to persist in high numbers 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 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. During the initial monitoring in 2019, a total of 47 topminnow were captured despite topminnow visually being much more abundant.

Results: On August 4, 2020, Department staff monitored Gila Topminnow in Peterson Ranch Pond. Ten minnow traps were set for a minimum soak time of 2 h in Peterson Ranch Pond and captured a total of 302 Gila Topminnow (251 < 20 mm TL, $51 \ge 20 \text{ mm TL}$) and 24 Longfin Dace. Both size classes of topminnow were again visually more abundant than the capture 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.

Sabino Canyon

Background: Sabino Canyon is located northeast of Tucson, Arizona within the Coronado National Forest and Sabino Canyon Recreation Area. Sabino Canyon is a tributary to the Santa Cruz River and drains the Santa Catalina Mountains, flowing southwest to its confluence with Tanque Verde Wash in Tucson. Sabino Canyon was chemically treated in 1999 to remove nonnative Green Sunfish, and afterwards was stocked with salvaged Roundtail Chub¹ (Ehret and Dickens 2009a). Gila Topminnow were initially stocked in the Recreation Area near 'The Crack' in 2015 and augmented in 2016. These stockings resulted in the establishment of a population of topminnow mostly below Sabino Lake Dam. Stream habitat in a reach of Sabino Canyon located approximately 250 m upstream from the confluence with East Fork Sabino Canyon was evaluated

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

in 2017 and 2018 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 in June, 2018. The Gila Topminnow population in Sabino Canyon upstream of the East Fork was initially monitored in May, 2019. No topminnow were captured or observed. Immediately Following the monitoring effort, a total of 148 Roundtail Chub¹ (>100 mm TL) collected from downstream of Sabino Dam were stocked into a pool just downstream of the topminnow stocking location (UTM 12S 520836/3581045). In October, 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). A total of 350 Gila Topminnow were successfully stocked.

Results: On May 5, 2020, Department staff monitored Gila Topminnow and Roundtail Chub¹ populations in Sabino Canyon upstream of the East Fork of Sabino Canyon. The crew set 10 minnow traps in two pools where topminnow were stocked in October, 2019 and failed to capture or observe any Gila Topminnow. A temperature logger installed in October, 2019 was retrieved. The minimum overwinter temperature was 4.93°C, so winter water temperature is likely not the limiting habitat characteristic at this location (Figure 7). Flooding did not appear to be severe within the surveyed reach in 2020, so it is unclear which habitat attributes are currently limiting survival of topminnow at this site.

Department staff also set nine mini-hoop nets in the pool where Roundtail Chub¹ were stocked in June, 2019 and captured a total of 13 chub. A tenth mini-hoop net was not set because it was damaged. The crew carried out five seine hauls downstream of the original stocking pool and captured two additional Roundtail Chub¹. The chub have dispersed out of the original stocking pool and were observed at least 200 m downstream. Several juvenile chub (~ 30 mm TL) were observed, but evaded capture. The presence of juvenile chub suggests that some spawning may have already occurred since the initial stocking in 2019.

A substantial portion of the Sabino Canyon watershed is within the burn footprint of the Bighorn Fire, which burned most of the Santa Catalina Mountains in the summer of 2020. On October 3rd 2020, Department staff monitored native fish in Sabino Canyon from near the Forest Service boundary upstream to near 'The Crack'. The goal of the survey was to determine if any Roundtail Chub¹ or Gila Topminnow persisted after the first round of post-fire impacts earlier in the summer. The crew set 19 mini-hoop nets and four minnow traps for a minimum soak time of 2 h and captured a total of 217 Roundtail Chub, 769 Gila Topminnow and 24 Sonora Mud Turtles. Multiple age classes were present for both fish species. An additional 43 topminnow were captured in six dip net sweeps with some of the topminnow captured upstream of Sabino Dam. Fish were distributed in isolated pools from near the forest service boundary (517821/3574743) to just

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

upstream of 'The Crack' (520483/3578381). The catch data suggests that fish survived the first round of post-fire impacts, and are actually present in relatively high abundance despite the additional constraint of drought conditions restricting the amount of available stream habitat within the surveyed reach.

A thin layer of gray ash, typically less than an inch deep, was observed in and around the stream channel throughout the entire surveyed reach. However, there does not appear to have been much transport of larger material and sedimentation of pools appeared relatively minor at the time of the survey. The fish populations in Sabino Canyon will remain at risk of post-fire impacts until upland vegetation begins to reestablish.

Recommendations: Depending on the extent of post-fire impacts, it may be valuable to attempt to stock Gila Topminnow upstream of the confluence with East Fork Sabino Canyon one final time in 2021. If no additional topminnow are stocked in 2020, then monitoring of this population should occur until at least 2022. 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 Canyon. Chub were difficult to capture with the selected gears because of the depth of the pools where chub are present. Consequently, it may be worthwhile to consider adding snorkel surveys into the monitoring approach next year to better account for the number of fish present in these deeper habitats. The Department also recommends a hike-through survey from the 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.

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

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

upstream of the confluence with Mesquite Creek. A total of 829 Gila Topminnow were captured during the initial monitoring in November, 2017. During monitoring in 2018, a total of 2,020 Gila Topminnow and 65 Fathead Minnow were captured. In 2019, only 47 topminnow were captured during monitoring. 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 during monitoring in 2019, and likely contributed to the decline in catch at this location in 2019.

Results: On April 9, 2020, Department staff augmented the existing Gila Topminnow population in upper Tortilla Creek with Peck Canyon lineage fish collected on the previous day. A total of 374 fish were stocked near the original stocking location (UTM 12S 467239/3708608). There were 150 mortalities during transport, with the mortalities most likely attributable the rough hike in, which requires scrambling over boulders and obstacles and results in quite a bit of sloshing in the buckets.

On October 29, 2020, Department staff monitored Gila Topminnow in upper Tortilla Creek near the original stocking location. The crew set 12 minnow traps for a minimum soak time of two hours and captured a total of 305 Gila Topminnow (297 \geq 20 mm TL, 8 < 20 mm TL). Nine opportunistic dip net sweeps were carried out and resulted in the capture of an additional 37 topminnow (11 \geq 20 mm TL, 26 < 20 mm TL). Topminnow were present throughout the surveyed reach (466973/3708694 upstream to 467218/3708621) and visually appeared to be relatively abundant. The augmentation of this population in April appears to have helped the population rebound from the very low numbers observed following flooding last year. Interestingly, this is the second consecutive year that Fathead Minnow were not captured or observed at this location.

Recommendations: The Gila Topminnow population in upper Tortilla Creek should be monitored until at least 2022 to determine if they establish since the population was augmented in 2020.

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 which was inhabited by Longfin Dace. Downstream of the Miles Ranch Trailhead, there are several large plunge pools previously 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 ($238 \le 20 \text{ mm TL}$) during monitoring in October, 2017. A total of nine Gila Topminnow and 23 Longfin Dace were captured during monitoring in July, 2018. The Woodbury Fire began in June 2019 and burned 123,875 acres of the Superstition Mountains including part of the West Fork Pinto watershed. West Fork Pinto Creek was surveyed after the fire and a major flood event following the fire in October 2019. No fish were captured or observed near the topminnow stocking site or downstream of Miles Ranch. Sediment and debris were noted from the stocking site downstream to at least one kilometer below Miles Ranch and the stream channel near the original stocking location down to Miles Ranch was completely filled in with sand, with very little surface water.

Results: On November 16, 2020, Department staff monitored West Fork Pinto Creek. The stream was visually assessed near the original stocking location (UTM 12S 491033/3700110). A new stream channel has been cut through the sand and sediment which was completely filled during 2019. Young willows and cottonwoods are growing on the banks of the new channel and appear to be beginning to stabilize substrate on the channel margin. Compared to 2019, there was much more surface flow, likely due to recent rains but also due to the scouring of the channel that has occurred since monitoring in October 2019. Some pools have begun to reform in the vicinity of the stocking location.

Three minnow traps were set downstream of Miles Ranch below the fish barriers (494295/3700025) to see if any fish had tried to recolonize this reach from Pinto Creek or if any Gila Topminnow had dispersed downstream to this reach. No fish were captured or observed, but aquatic habitat in this reach appears to be improving following the fire, with pools beginning to scour out and increase in depth.

Recommendations: Monitoring of West Fork Pinto Creek should continue until 2021 to verify that fish are extirpated from the upper perennial reach, and to determine if conditions have improved enough to restock Gila Topminnow. Habitat was limited in upper West Fork Pinto Creek before the fire and it will take some time for the habitat is again suitable for fish. 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 reassessed in the future to determine if sufficient habitat is present for Roundtail Chub¹. There is still a substantial amount of mobile material in the stream channel, so recovery of stream habitat will depend heavily on the magnitude of flood events over the next few years. Because of this, it is uncertain when Gila Topminnow can be restocked into West Fork Pinto Creek.

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

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

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - o Goal 4. Remove nonnative aquatic species threats.
 - o Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - o 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.

Background: 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 invading upstream, 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 during the summer of 2014, after which the Department's CAMP staff assumed responsibility of the removal efforts above the diversion dam.

Reclamation finished construction of a fish barrier about 1.1 km upstream from Oak Creek in April 2015. Gila Topminnow were initially stocked in 2015 and were considered established above the barrier by 2019.

On May 11, 2015, 221 Spikedace (Aravaipa Creek lineage) were stocked above the barrier. Only three Spikedace were captured during the initial monitoring effort in 2015, and none were captured in 2016, so the population was augmented with 67 individuals in October, 2016. Spikedace

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

captures increased to 11 individuals in 2017 and the population was augmented in February 2018 with 1,076 Spikedace as part of an eDNA research study. Spikedace captures increased again to 20 during annual monitoring in 2018 and an additional 500 Spikedace were stocked in December, 2018. Spikedace captures increased to 36 fish during the first pass at each site in 2019. A three pass depletion estimate (Carle-Strub method) was carried out at the fixed site in 2019 with an estimate of 90 fish per 100 m sampled (95% CI = 0-183.29). The first evidence of natural reproduction was documented in 2018 with more reproduction occurring in 2019.

Results: On September 9, 2020, Department staff monitored 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 17 Spikedace were captured during the initial pass at each site, which is fewer than half the number of fish captured during first pass efforts in 2019 (Figure 8). Mean size of Spikedace captured was 66.8 mm TL (min = 50, max = 74; Figure 9). Both the decrease in catch and the increase in mean size compared to 2019 suggest that little reproduction occurred in Spring Creek in 2020. The absence of juvenile fish after two years consecutive years of spawning in this population is somewhat concerning. In addition to Spikedace, 295 Roundtail Chub¹, 4 Sonora Sucker, 119 Desert Sucker, 187 Longfin Dace and 681 Speckled Dace were captured during electrofishing (Table 2).

Three pass depletion electrofishing was carried out at the fixed site with block nets set at the downstream and upstream ends of the 100-m reach. A total of six Spikedace were captured during the two additional passes. Estimated abundance of Spikedace using a Carle-Strub method was 21 fish per hundred m with an estimated capture probability of 0.75 (Table 3; Carle and Strub 1978). Estimated capture probability of Spikedace was much higher in 2020 (0.75) than 2019 (0.21), which along with the lower estimated abundance (2019 n = 90) suggests that true abundance of Spikedace in Spring Creek likely declined since 2019.

Following the monitoring effort, ARCC staff and Kansas State Researchers released 101 PIT tagged fish into Spring Creek as part of an ongoing study on Spikedace and Loach Minnow survival and movement. Most of the Spikedace captured during monitoring were PIT tagged before being released.

<u>Recommendations</u>: Estimates of abundance and capture probability are providing greater insight than single pass electrofishing alone into the status and trends of the Spikedace population in Spring Creek, and should continue with future monitoring efforts. Since untagged Spikedace were last stocked in 2018, electrofishing monitoring should continue until at least 2023. Additional stockings may occur if deemed necessary.

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¹ Chub in Spring Creek were previously classified as Gila Chub

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

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - o Goal 4. Remove nonnative aquatic species threats.
 - o Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - o 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.

Background: 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 18 km of the Blue River, from Fritz Ranch to the confluence with the San Francisco River (Figure 10), additional efforts are taking place upstream (Figure 10). The Reclamation-funded 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. Spikedace, Loach Minnow and Roundtail Chub were stocked soon after the completion of the barrier. The populations in the lower Blue River were monitored annually from 2012-2019, with each augmented once between 2015 and 2016 and all three species were considered established as of 2019.

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. 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). Green Sunfish were found to be much more abundant than initially thought when 106 Green Sunfish were captured and removed throughout the lower Blue River during annual monitoring. 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.

Native fish conservations activities in the middle Blue River (McKittrick Creek confluence upstream to The Box; Figure 10) began in 2016 when 1,194 Roundtail Chub were stocked between The Box and Cole Flat. During the initial monitoring effort in 2017, a total of 57 Roundtail Chub were captured with hoop nets. Immediately following the chub monitoring, 448 Spikedace were collected from the Blue River at Juan Miller crossing and translocated 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. Following the monitoring, an additional 291 Spikedace were translocated from the Blue River near Juan Miller Crossing to the middle Blue River at Cole Flat. During annual monitoring in 2019 23 Spikedace, 9 Roundtail Chub, and 41 Loach Minnow were captured by electrofishing only. Capture efficiency during the monitoring was likely low due to the river being very turbid (~2-6 inches visibility) as a result of runoff from recent monsoon storms. Roundtail Chub catch declined each year from 2017 to 2019 despite additional electrofishing effort and juvenile Roundtail Chub were not captured within the monitoring reach. Following annual monitoring in 2019, a total of 100 Roundtail Chub were collected near Juan Miller Crossing and translocated to the middle Blue River near Cole Flat.

Results:

The Department completed native fish conservation actions in the lower Blue River and middle Blue River during 2020. Results for the lower Blue River are presented first.

During June 22-24, 2020, Department staff salvaged Spikedace, Loach Minnow and Roundtail Chub from the lower Blue River. The fish were salvaged due to concerns about post-fire impacts from the Brigham Fire impacting fish populations in the lower Blue River. Fish were collected by backpack electrofishing and seining both above and below Juan Miller crossing. A total of 226 Roundtail Chub of mixed size classes were transported to the upper Blue River and stocked in a beaver pond at Bobcat Flat near the New Mexico state line (UTM 12S 680688/3732342; Figure 10) with five mortalities during holding and transport. A little more than half of the Spikedace (n = 453) were stocked in the Blue River just downstream of upper Blue Campground (678665/3729743) with five mortalities and the remaining Spikedace (n = 373) were stocked just

downstream near the confluence with Jackson Canyon (678020/3728836) with 54 mortalities during transport and stocking. These stockings are the first stockings of Roundtail Chub and Spikedace in the upper Blue River. A total of 172 Loach Minnow were stocked in lower Campbell Blue Creek near the confluence with Turkey Creek (679251/3734439) with three mortalities during transport and stocking. An additional 313 Loach Minnow were transported to ARCC to augment the existing Blue River broodstock. There were 44 Loach Minnow mortalities during the extended holding and transport period, with a total of 269 live fish counted by ARCC staff.

Because fish salvage operations were deemed a higher priority, the annual piscivore removal effort was not completed in 2020. Department staff had also planned on conducting an eDNA survey of the lower Blue River to verify eradication of Green Sunfish in October. Unfortunately, sediment and ash from the Brigham Fire and Cow Canyon Fire increased the turbidity of the river to the point where eDNA sampling would not have been effective, so the effort was postponed until spring 2021.

During September 21-23, 2020, Department staff conducted the annual monitoring in the middle Blue River between The Box and McKittrick Creek. A total of 10 random and two fixed 100-meter transects were electrofished. Two randomly selected transects were located on private land and data is not presented consistent with ARS 17-495. Fish captured during the first pass in the other 10 transects, all on USFS property, totaled 180 Roundtail Chub, 117 Spikedace, 320 Loach Minnow, 902 Longfin Dace, 2,745 Desert Sucker, 587 Sonora Sucker, 2,586 Speckled Dace and one Brown Trout (Table 4). Roundtail Chub and Loach Minnow were captured at every site sampled and Spikedace were captured at all sites except the two most upstream sites located near The Box (Figure 11). The results of this year's monitoring effort represent a dramatic increase in the number of fish captured from 2019 and 2018 (Figure 12, 13). Mean electrofishing relative abundance of Spikedace experienced a significant increase from 2018 to 2019 and from 2019 to 2020 (Table 5). Mean electrofishing relative abundance of Roundtail Chub declined between 2018 and 2019, but significantly increased between 2019 and 2020 (Table 5).

Most of the increase in Roundtail Chub numbers can likely be attributed to a strong juvenile age class (20-65 mm TL; Figure 14). This is the first evidence of Roundtail Chub reproduction documented in this reach since the initial stocking in 2015. Similarly, most of the increase in Spikedace numbers can be attributed to a strong year class of juvenile fish (Figure 15). Loach Minnow also appeared to have a strong year class of juvenile fish this year. The lone Brown Trout captured was 304 mm TL.

Three-pass depletion electrofishing was performed at two fixed sites. Estimated abundance of Roundtail Chub per 100 m ranged from 28 to 78 individuals with an estimated capture probability of 0.47-0.64 (Table 6). Spikedace were not caught at the upper fixed site, and the estimate of

abundance at the lower fixed site was 30 individuals per 100 m with an estimated capture probability of 0.43 (Table 6). Estimated abundance of Loach Minnow per 100 m ranged from 133-199 individuals with an estimated capture probability of 0.17-0.39 (Table 6). The results of the depletion estimates suggest that capture probability was relatively low despite the low flow conditions. This is likely because there were so many fish in the stream that it was difficult to capture all of the fish each pass, which is unsurprising considering over 10,000 fish were captured and processed in three days of sampling.

Blue River discharge within the sampling reach was extremely low for this time of year, which is unsurprising considering the lack of monsoon precipitation and extended high temperatures. The two most downstream randomly selected sampling sites were dry upon arrival, and two more sites had to be reselected within the wetted portion of the reach. Partially as a result of the low water conditions, fish were highly consolidated and much easier to capture than during previous years of monitoring. In addition, long reaches of the Blue River upstream of the monitoring reach (approximately Marks Ranch to Grant Creek and Blue Cemetery to Jones Canyon) appeared to be completely dry. While the monitoring results suggest Roundtail Chub and Spikedace populations in the middle Blue River are on a promising trajectory, continued success is probably contingent on a normal level of precipitation over the next year.

A monitoring plan was drafted for the newly stocked Spikedace and Roundtail Chub in the upper Blue River. A manuscript detailing the success of the lower Blue River Native Fish Restoration Project was drafted and submitted to the North American Journal of Fisheries Management.

<u>Recommendations:</u> Green Sunfish have not been detected since 2016, and removals for this species should continue in 2021. Eradication of Green Sunfish from the lower Blue River should be confirmed by collecting eDNA samples throughout the surveyed reach in 2021 and utilizing the newly developed Green Sunfish marker.

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 2023 for Spikedace and 2024 for Roundtail Chub. Additional Spikedace and Roundtail Chub should be translocated from the lower Blue River as necessary to help establish populations.

Monitoring of the translocated populations of Spikedace and Roundtail Chub in the upper Blue River should begin in 2021. Populations of both species should be augmented with fish from the lower or middle Blue River as necessary to help establish 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.

<u>Background:</u> 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-m 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 to date through 2020 is provided in Table 11. Beginning in FY2021, this project will be incorporated into specific conservation projects (e.g., Gila Topminnow Stockings).

<u>Results:</u> A map showing locations of each of the streams assessed during 2019 is presented in Figure 16.

Agua Caliente Canyon. On May 4, 2020, Department staff assessed fish community and aquatic habitat in Agua Caliente Canyon. The intent of the survey was to assess habitat in the parallel Milagrosa Canyon, but Agua Caliente Canyon was accidentally surveyed instead. Agua Caliente was also on a list of sites to eventually assess so the data collected still has value. Surface water in Agua Caliente Canyon was intermittent from near where the trail joins the stream (UTM 12S 526671/3573385) upstream to where the survey ended near a large barrier falls (528493/3573991). It appears that surface water is probably restricted to a few bedrock pools during drier conditions. There were at least four natural fish-passage barrier falls throughout the sampled reach, however,

Green Sunfish were present in most deep pools throughout the surveyed reach including above the barriers. The crew set five minnow traps and five mini-hoop nets throughout the surveyed reach and captured of 10 Green Sunfish. In addition, five Green Sunfish were captured by angling and two Green Sunfish were captured in two dip net sweeps. Adult and juvenile size classes were present and several adults were observed guarding nests, so spawning appears to be occurring in Agua Caliente Canyon. There are several large stock tanks in the headwaters of the drainage which are likely the source of Green Sunfish to Agua Caliente Canyon.

Recommendations: Because there may be upstream sources of Green Sunfish and potentially limited surface water during drought conditions, Agua Caliente Canyon is not a high priority for native fish conservation projects at this time. Milagrosa Canyon will likely be surveyed in spring 2021.

Cañada Del Oro. During May 11-12, 2020, Department staff assessed fish community and aquatic habitat in Cañada del Oro, which drains the north side of the Santa Catalina Mountains. The first 3 km (most upstream) contained a few short dry sections, but mostly consisted of high gradient riffles and cascades interspersed with relatively small and shallow pools. Flow was continuous from about 1,067 m in elevation (UTM 12S 518690/3592110) downstream to where the survey ended near Biosphere 2 (515099/3603898). A majority of the stream consisted of high gradient riffles and shallow runs. Only six pools greater than 1 m in depth were documented, and nearly all of the pools were located within 1 km upstream and downstream of the junction of Charouleau Gap Road. One waterfall (516455/360338) was documented downstream of Irene Wash and likely is a fish barrier during most flows. Fish were not captured or observed during the survey. While surface flow was present for most of the surveyed extent, the stream in general was wide and shallow, and is likely much more intermittent following drier winters. Because of the lack of deep pools, any translocated fish populations would have limited resilience during drought years.

Recommendations: There may still be sufficient habitat in Cañada del Oro to support a small population of Roundtail Chub near the Charouleau Gap area. However, there does not appear to be sufficient habitat for Gila Topminnow within the surveyed reach. In addition, the Bighorn Fire burned nearly the entire Cañada Del Oro watershed in 2020, so there will likely be substantial post-fire impacts on stream habitat over the next several years. Cañada Del Oro should be reassessed in the future if there is a need and desire to replicate a population of Sabino Canyon lineage Roundtail Chub.

George Wise Spring. On March 31, 2020, Department staff assessed aquatic habitat and fish assemblage composition in George Wise Spring (UTM 12R 512372/3487732) and several tanks in the Ash Canyon drainage near Patagonia Lake.

Surface water was present in nearly the entire reach of Ash Canyon from George Wise Spring downstream to Patagonia Lake, but this reach is likely intermittent. Green Sunfish were observed in Ash Canyon immediately upstream of the lake. There were no apparent barriers to upstream movement of fish from Patagonia Lake to George Wise Spring, besides this reach likely being seasonally intermittent.

Department staff electrofished Ash Canyon within the newly constructed cattle exclosure (~300 m) and captured a total of 90 Green Sunfish. Most of the Green Sunfish were relatively small and one potential nest was observed, suggesting that spawning may be occurring within the George Wise Spring reach of Ash Canyon. The habitat within the exclosure consisted of a few long, slow pools with submergent aquatic vegetation and stable banks separated by short sections of shallow runs and riffles. The habitat at George Wise Spring appears to be of high quality for Gila Topminnow.

Following the sampling at George Wise Spring, an unnamed tank (512848/3488927) in the channel of Ash Canyon where Gila Topminnow were previously observed in 2006 was visited. Staff performed two bag seine hauls without capturing any fish, however two adult Green Sunfish were observed at the head of the tank. The tank is quite deep, potentially more than 4 m deep, and seining was likely not very effective. The presence of Green Sunfish in this tank suggests that sunfish can either travel upstream from Patagonia Lake to this location, that there is a source of Green Sunfish upstream in the watershed, or that they were stocked there.

To collect more information on potential sources of Green Sunfish in Ash Canyon, Department staff continued upstream from the unnamed tank to a location called Mata Siete Spring to determine whether any Green Sunfish were present. Pools in Ash Canyon were visually observed on the hike upstream but fish were not observed. Mata Siete Spring consisted of an off-channel masonry spring box covered in duck weed. No fish were observed in the spring. The spring appears to be a good location to introduce Gila Topminnow in the future, should Green Sunfish be removed from the drainage.

Finally, Department staff visited Henry Tank (515877/ 3492273) and an unnamed tank (516218/ 3492828) on USFS lands in the headwaters of the Ash Canyon drainage. Henry Tank is actually a series of two large and deep constructed dirt tanks, with the upstream tank spilling into the downstream tank. It appears that the downstream tank rarely, if ever spills. The unnamed tank was less than a vertical foot away from spilling and there was evidence that it had spilled recently, so this tank at least has the potential for fish to escape if they are present. The unnamed tank was quite large (\sim 80 m in length, and \sim 30 m wide) at nearly full pool. While fish were not observed in either of these tanks, it would be valuable to carry out bag seine hauls, set traps or set gill nets at some point in the future when water levels are lower to determine whether either of these locations could be a source of Green Sunfish to downstream reaches of Ash Canyon.

Recommendations: Stream habitat in George Wise Spring was suitable for both Gila Topminnow and Roundtail Chub. All five upstream tanks within the drainage should be surveyed to determine whether an upstream source of Green Sunfish exists within the drainage. Results of tank surveys will guide feasibility and removal strategies from this system. George Wise Spring and a few of the tanks are on Department property, which could potentially simplify the process for a chemical treatment, should one be necessary.

Houston Creek. On April 14, 2020, Department staff assessed fish assemblage and aquatic habitat in Houston Creek (tributary to the Verde River). The crew electrofished approximately 500 m of Houston Creek starting at the confluence with the Verde River upstream to near the confluence with Squaw Creek. They captured 61 Longfin Dace, 1 Fathead Minnow and 3 crayfish. Longfin Dace were present throughout much of the surveyed reach, but appeared to be less abundant in the most upstream kilometer of stream surveyed. No large-bodied fish were observed in the stream. No features that were likely to be fish passage barriers during all flows were located, but one waterfall (UTM 12S 436178/3794183) likely prevents upstream movement of fish during much of the year.

Flow was present throughout the entire surveyed reach. At least part of the reach is intermittent based on previous survey efforts and several years of satellite imagery. As a result of the higher than average flows during the spring of 2020, the conclusions that can be made about the amount of available habitat are limited. In general, the stream is relatively high gradient and is dominated by riffle habitats interspersed by step pools and shallow runs. Few pools greater than 1 m in depth were documented, and there are likely fewer deep pools during drier years.

Recommendations: The surveyed reach of Houston Creek does not appear to have much high-quality habitat for Gila Topminnow at this time, mostly due to the relatively high gradient of the stream and rarity of deep pools which might provide velocity refuge during flooding. Houston Creek may have sufficient habitat to support other native species like Roundtail Chub and Loach Minnow, but more fine-scaled surveys would need to be carried out during a dry period of a year with low precipitation to determine the minimum amount of habitat potentially available for these species.

Kayler Spring. On March 9, 2020, Department staff assessed aquatic habitat and fish community composition in Kayler Spring (UTM 12S 472079/3755886). There was little water sufficiently deep to soak minnow traps, however four traps were set with no fish captured. A total of 28 Longfin Dace (23 > 40 mm TL), 5 < 40 mm TL) were captured in six dip net sweeps from the downstream end of flow near the confluence with Tonto Creek (472068/3755550) upstream to the spring source (472101/3755890). A single Western Mosquitofish (> 20 mm TL) was captured near the

downstream end of flow. Gila Topminnow were not captured or observed. One Lowland Leopard frog was observed at the upstream end of perennial flow.

Aquatic habitat in Kayler Spring mostly consisted of shallow (< 5 cm depth) riffles flowing over sandy substrate. The channel split in several locations, was rarely more than 1 m in width and was heavily grown over with vegetation. Several of the larger riparian trees appeared to have died in recent years, suggesting that spring discharge may have declined. Barriers to upstream movement of fishes were not detected, and a previously identified barrier was either blown out or had been buried in sand since the last survey.

Recommendations: Gila Topminnow are likely extirpated from Kayler Spring and aquatic habitat does not appear to be of high enough quality to warrant further translocations of Gila Topminnow at this time. However, Kayler Spring currently provides about 300 m of aquatic habitat for Longfin Dace and lowland leopard frogs that is virtually free of nonnative species (including crayfish).

Reavis Creek. On April 7, 2020, Department staff reassessed habitat for Gila Topminnow in Reavis Creek. The burn severity of the 2019 Woodbury Fire in the upper Reavis Creek watershed was quite variable with some areas unburnt and others where virtually everything above ground level burned. Surface flow was present in Reavis Creek from Reavis Saddle Spring downstream to the most downstream pool documented during the 2019 assessment (UTM 12S 485505/3706286). Overall there appears to have been substantial flooding in Reavis Creek following the Woodbury Fire. Severe bank erosion, sediment deposition and scouring, and removal of riparian vegetation was observed throughout the surveyed reach. Most of the pools documented in 2019 were shallow gravel riffles in 2020. For example, the pool in which a temperature logger was installed was 1.22 m deep in 2019 and probably only about 0.1 m deep during the 2020 site visit. The temperature logger was buried under at least 0.5 m of sand and gravel, but was successfully retrieved after about 30 min of digging.

Recommendations: Aquatic habitat in Reavis Creek does not appear to be suitable for introduction of native fishes at this time. Habitat should be reassessed in 2-3 years after the uplands begin to revegetate and the stream channel begins to stabilize.

San Pedro River-Lower San Pedro River State Wildlife Area. On October 20, 2020, Department staff surveyed the San Pedro River within the Department's Lower San Pedro River State Wildlife Area. The property is made up of several parcels and the choice was made to survey towards the upstream end of the properties. The crew initially started at an unnamed road crossing (UTM 12S 529307/3629840) and traveled upstream for a few hundred meters without finding any water. The decision was made to turn around and continue downstream past the road crossing until water was finally located (528308/3630549) which was virtually continuous down to where the survey ended (528035/3630777). The extremely thick riparian area, especially in the dry reach, greatly slowed progress.

The crew carried out 10 dip net sweeps and 11 opportunistic seine hauls and captured a total of 111 Red Shiner, 2 Mosquitofish and 6 Longfin Dace. Several of the deeper pools were unable to be effectively sampled by seine or dip net, but at least one school of several hundred Red Shiner was observed in a deep pool. The presence of nonnative fish is concerning for establishment of native fish within this reach. Even with nonnative fish removal efforts, it would not be possible to maintain a nonnative-free reach due to lack of barriers and the presence of nonnative fish both upstream and downstream.

Recommendations: A majority of the San Pedro River within the Lower San Pedro River State Wildlife Area was not assessed in 2020. These remaining reaches should be assessed in 2021 and gears that can target large-bodied nonnative fishes should be deployed. If the San Pedro River is largely free of large bodied nonnative fishes within this reach, the deeper pools could be a potential location to replicate Aravaipa Creek lineage Roundtail Chub.

San Pedro River-Three Link Conservation Easement. On October 21, 2020, Department staff surveyed the San Pedro River with Reclamation staff on the Reclamation Three Link conservation easement from near where water begins to surface (UTM 12S 566014/ 3558225) downstream for about 1.6 km (566375/3559636). The crew carried out 12 opportunistic seine hauls and captured a total of 71 Longfin Dace. Reclamation's contractor also surveyed a 200-m long reach by electrofishing, and the only fish species captured was Longfin Dace. Stream habitat in this reach is much more complex and heterogeneous than lower San Pedro State Wildlife Area reach. The habitat mostly consisted of shallow glides and runs, with a few pools and riffles. Presence of surface water, including deep pools during this extremely dry year is very promising for the resilience of this site to drought. However the potential for occasional exposure to nonnative fishes is a possibility, as the only migration barriers are miles of typically dry streambed above and below this reach that become connected during large-scale floods.

Recommendations: There appears to be suitable habitat for Gila Topminnow, and probably suitable habitat for other species of native fish including suckers and Roundtail Chub in some of the deep pools that are >1 m in depth. Even with the risk of transport of nonnative fishes, this may be a worthy site for topminnow establishment that could potentially provide conservation value given the length of perennial water, and variety of suitable habitat. The presence of beavers in this portion of the river also suggests that deep pool habitat will continue to exist in this reach as habitat shifts and changes over time. Discussions with partner agencies and landowners are necessary before moving forward with any native fish conservation actions within this reach.

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.
 - o Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - o 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.

Background: Harden Cienega Creek is a tributary to the San Francisco River near the New Mexico state line. Roundtail Chub² 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. In April, 2015, a total of 102 Roundtail Chub¹ were translocated from lower Harden Cienega Creek to above the waterfall. Monitoring from 2017 to 2019 detected several hundred chub representing all size classes above the barrier. The population was augmented with five individuals from below the barrier in 2018 and 104 in 2019 in an effort to increase the genetic diversity above the barrier. In October 2019, Gila Topminnow (n = 631; Bylas Spring lineage) were first stocked in lower Harden Cienega Creek downstream of the waterfall barrier. A temperature logger was also installed at the stocking location at the time of stocking to track whether winter water temperatures were sufficient for topminnow establishment. Unfortunately, Green Sunfish were detected above the barrier during post-stocking monitoring, with one removed in 2017 and two in 2018. Four Green Sunfish were captured and removed downstream of the barrier in 2019, suggesting the population was increasing in abundance and distribution within Harden Cienega Creek. Because Green Sunfish were captured well upstream of the barrier, it was concluded that an upstream source of Green Sunfish exists in the Harden Cienega drainage.

Results:

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

² Roundtail Chub in Harden Cienega Creek were previously classified as Gila Chub

In February 2020, Department staff drafted a removal plan for the Harden Cienega Creek drainage, including upstream stock tanks in Arizona and New Mexico (Hickerson et al. 2020). 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.

During June 9-10, 2020, Department staff performed the first pass of nonnative removal efforts, monitored Gila Topminnow in lower Harden Cienega Creek, and monitored the Roundtail Chub¹ population above the barrier. All surface water was electrofished from the confluence with the San Francisco River upstream to near Prospect Canyon to target and remove Green Sunfish. All fish captured were counted and assigned to size classes from the start of perennial water upstream to the location where Gila Topminnow were stocked in October, 2019. Staff determined that the primary objective of electrofishing the entire perennial reach of Harden Cienega Creek could not be achieved if time was spent counting all fish captured. Consequently only sunfish were counted upstream of the topminnow stocking location. A total of 22 Green Sunfish, 872 Longfin Dace, 427 Speckled Dace, 407 Desert Sucker and 41 Sonora Sucker were captured by backpack electrofishing (Table 7).

In addition to backpack electrofishing, mini-hoop nets were set in pools where Green Sunfish had been captured during previous efforts or were too deep to effectively electrofish. An additional 16 Green Sunfish, 56 Roundtail Chub¹ and 4 Desert Sucker were captured, with 12 of the Green Sunfish captured in a single pool (UTM 12S 675647/3674186) where several sunfish were captured while electrofishing the previous day (Table 7).

Green Sunfish appear to have a patchy distribution within Harden Cienega Creek, with most of the fish concentrated within a few pools below the barrier falls (Figure 17). However, a single Green Sunfish was captured upstream of the barrier, which is continued evidence that the source of these fish is upstream of the falls. The smallest sunfish captured was only 70 mm TL, which suggests that spawning may have occurred in this reach, or that spawning occurred somewhere upstream and fish dispersed into the study reach within the last year (Figure 18). The mean length of Green Sunfish captured was 131.2 mm TL which could mean that Green Sunfish are already reproducing in this reach or that they are dispersing at smaller sizes than we have detected in the past.

The Roundtail Chub¹ population above the barrier falls was monitored by capturing and measuring the first 100 Roundtail Chub¹ encountered during electrofishing efforts above the waterfall barrier so that size structure of the chub population above the barrier could be compared to the previous several years of monitoring (Figure 19). Size structure of the population above the barrier has been relatively consistent since 2017 and is evidence that this population should be considered established.

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

Staff monitored the Gila Topminnow population by setting 16 minnow traps from the downstream end of flow (673702/3674794) upstream to the location where topminnow were initially stocked in 2019 (674768/3674598). A total of 79 Longfin Dace, 52 Speckled Dace and 14 Roundtail Chub¹ were captured, but Gila Topminnow were not captured or observed (Table 7).

Review of the temperature logger data suggests that winter water temperatures were more than sufficient for Gila Topminnow, with a minimum temperature of 6.8°C recorded over the winter (Figure 20). Similarly, there wasn't much evidence of large scale flooding in the stream over this past winter. Native fish and crayfish are very abundant in the lower reach and seem to occupy all available habitat. It is possible that there is little niche space available for topminnow to establish with so many other fish and crayfish already present. There is some support for this theory. A GoPro camera was placed in the stream to document the stocking in October, and upon review of the footage several chub could be seen eating topminnow during the stocking.

Tank Surveys. During July 6-9 and 13-16, 2020, Department staff surveyed for Green Sunfish in a total of 43 tanks in the Arizona portion of the Harden Cienega Creek drainage. No fish were detected in any stock tanks in Arizona. Of the tanks that were visited, 16 were dry (Figure 21). Of the remaining 27 tanks, two were sampled with a dip net, 10 were sampled with a straight seine and 15 were sampled with a bag seine.

One crew stopped at Ditch Tank in New Mexico to have lunch on July 7 and observed at least one Goldfish in the tank. On July 14 the crew stopped a Ditch Tank for lunch again to see if more fish could be observed. Green Sunfish were visually observed in the tank (Figure 21). Ditch Tank is fed by a ditch from Harden Cienega Creek and the dam is located within a few meters of the stream channel. There is evidence that this tank spills regularly and is likely at least one source of Green Sunfish to Harden Cienega Creek. There are relatively few tanks upstream of Ditch Tank in New Mexico, but there is also some flowing water on private property further upstream in Harden Cienega Creek which could also potentially support Green Sunfish.

Recommendations: Because topminnow were not captured during monitoring efforts in 2020, the population should be augmented again in the spring of 2021. Prior to stocking topminnow, we recommend capturing as many large chub (> 75 mm TL) as possible in the vicinity of the stocking site and relocating them a few hundred meters upstream or downstream to give topminnow more time to acclimate to local conditions and piscivorous fishes, and hopefully improve the chances for establishment success. If topminnow are stocked again in 2021, monitoring should continue until 2024.

More nonnative removal effort is warranted in Harden Cienega Creek in 2021 because more Green Sunfish were captured than expected in 2020 (n = 38), and the presence of small fish (70 mm TL) suggests that spawning may be occurring. Both backpack electrofishing and mini-hoop nets proved

effective at capturing and removing Green Sunfish, and this combination approach should continue. For future removal efforts, we recommend randomly selecting a few sub-reaches of the stream in which to count all native fish captured so that the response of native fish can still be tracked and crews can complete an entire removal pass.

The source of Green Sunfish to upper Harden Cienega Creek does not appear to be any tanks in Arizona. However, Green Sunfish were present in one tank in New Mexico and there may be other potential source populations in New Mexico. Eradication of Green Sunfish from the Harden Cienega watershed will require working with partners in New Mexico to determine how to proceed with the identification and removal of Green Sunfish sources in the New Mexico portion of the drainage.

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.
 - o Goal 3. Protect native fish populations from nonnative fish invasions.
 - o Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - o 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 through Apache-Sitgreaves National Forest, San Carlos Apache, and private lands. Native fish documented in Eagle Creek include Spikedace, Loach Minnow, Roundtail Chub¹, Speckled Dace, Longfin Dace, Desert Sucker, Sonora Sucker, and Gila Trout. However, Spikedace and Loach Minnow were last recorded in 1989 and 1997 respectively. 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. 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. Neither Spikedace nor Loach Minnow were positively detected in any of the eDNA samples.

Results: During November 2-3, 2020, Department staff monitored Eagle Creek from the vicinity of Honeymoon campground upstream to the confluence with East Eagle Creek (monitoring reaches 3 and 4; Figure 22). The goal of the sampling was to collect some pre-barrier fish community data using a scaled-down version of the same protocol that will be used after completion of the barrier. Sampling consisted of backpack electrofishing within six randomly selected 100-m long transects. A three-pass depletion was carried out between two block nets at a single transect (5-4). A total of 74 Roundtail Chub², 199 Longfin Dace, 656 Speckled Dace, 1,618 Desert Sucker and 138 Sonora Sucker were captured during the first pass of electrofishing at each site (Table 8). An additional 36 Roundtail Chub, 13 Longfin Dace, 35 Speckled Dace, 404 Desert Sucker and 29 Sonora Sucker were captured during the second and third passes at the depletion site (Table 9). Roundtail Chub were captured at five of the six sites sampled and were only absent from the most downstream site (37-3; UTM 12S 641174/370458). Abundance of chub generally increased upstream and chub were most abundant in sites that had pools with woody cover or backwaters. Virtually all size classes of chub were present, with a mean size of 119.3 mm TL and a range from 43-278 mm TL.

A trip to collect eDNA samples from three remote forest service parcels of land in lower Eagle Creek was original planned for September, 2020. Unfortunately, because of Department COVID-

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

² Chub in the vicinity of Honeymoon Campground previously classified as Gila Chub.

19 restrictions on personnel traveling in the cockpit of aircraft the survey had to be postponed until 2021.

<u>Recommendations</u>: A complete pre-barrier fish survey of Eagle Creek should occur in 2021 if the barrier is likely to be built in 2022. In addition, collection of eDNA samples from USFS property on Eagle Creek downstream of Sheep Wash should occur in 2021; Department staff will backpack in if COVID-19 restrictions are still in place for helicopter transport.

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.
 - o Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - o 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.

<u>Background:</u> 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

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

Tank Draw is about 2.4 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 m 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. Intensive trapping efforts in 2019 resulted in the removal of 13 Black Bullhead from the isolated pools above the waterfall barrier. By August 2019, Black Bullhead were determined to be eradicated from Rarick Canyon. In October 2019, a total of 315 Roundtail Chub¹ were collected from Red Tank Draw and translocated to a series of three isolated pools above the barrier in Rarick Canyon. A removal plan for the Red Tank Draw drainage, including Rarick and Mullican Canyons, was drafted in March, 2019 (Hickerson and Robinson 2019).

<u>Results:</u> *Red Tank Draw.* During May 26-28, 2020, Department staff completed the first removal pass of the year and began the second removal pass. The crew used a combination of backpack electrofishing, mini-hoop nets and minnow traps and captured 811 Roundtail Chub¹, 17 Desert Sucker, 281 Green Sunfish and 69 Fathead Minnow during the first pass. All Green Sunfish and Fathead Minnow captured were removed from the stream.

During August 8-11, 2020, Department staff completed the second and third removal passes of the year in Red Tank Draw. The crew used a combination of backpack electrofishing, mini-hoop nets and minnow traps and captured 580 Roundtail Chub¹, 21 Desert Sucker, 269 Green Sunfish and 141 Fathead Minnow during the second pass. During the third pass a total of 719 Roundtail Chub¹, 321 Green Sunfish and 47 Fathead Minnow were captured. All Green Sunfish and Fathead Minnow captured were removed from the stream.

Juvenile Roundtail Chub are still relatively abundant in Red Tank Draw despite Green Sunfish still being present in high numbers (Figure 23). Mean total length of Green Sunfish has significantly changed between 2016 to 2020 (H = 429.28, df = 4, p < 0.001). This difference in mean size of

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

Green Sunfish is mostly attributable to significant decreases between 2017 and 2018 (W = 96652, p < 0.001) and 2018-2019 (W = 85344, p < 0.001). While length-frequency data suggests that Green Sunfish were able to spawn the last two years, a majority of the sunfish captured were less than 100 mm TL which means removal efforts may be on track to suppress spawning in this system in the coming years if at least the same amount of removal effort is applied and substantial numbers of sunfish do not disperse from upstream sources (Figure 24). Green Sunfish as small as 74 mm TL were observed to be ripe with eggs, which will likely make suppression of spawning much more difficult if sunfish are able to produce viable gametes at such a small size. Black Bullhead were not captured during removal efforts in 2020, which marks two consecutive years without any detections of this species within the removal reach.

Previous to this year's removal efforts, the most chub captured during a single pass within the removal reach was 550 individuals during the final pass of 2019 (Figure 25). More than 550 chub were captured during both the second and third passes this year, suggesting this population is continuing to do well, despite 300 individuals being translocated upstream to Rarick Canyon in October, 2019.

Rarick Canyon. On April 30, 2020, Department and Coconino National Forest staff stocked 649 Gila Topminnow into a single pool in Rarick Canyon (F18, UTM 12S 440122/3844798; Figure 26) upstream of the barrier falls. Roundtail Chub ¹ were stocked into this same pool in October, 2019. There was one mortality during transport and stocking, and the fish were in great condition at the time of release. Gila Topminnow (Redrock Canyon lineage) were collected on April 29, with two mortalities during the collection effort.

On October 13, 2020, Department staff monitored translocated populations of Gila Topminnow and Roundtail Chub in Rarick Canyon. Crews set five minnow traps and five mini-hoop nets in the most downstream pool (F23) and captured 4 Roundtail Chub and 213 Fathead Minnow (Figure 26). The entire streambed was dry between F23 and the next pool (F20) which was also stocked with chub last year. Pool F20 was greatly reduced in size to only about 3 m long by about 1 m wide. A single seine haul in F20 captured 17 Roundtail Chub and 323 Fathead Minnow. Unfortunately, the chub in this pool were not measured for total length due to a miscommunication. The crew set five mini-hoop nets and ten minnow traps in the next upstream pool (F18) that was previously stocked, and captured 447 Gila Topminnow, 2 Roundtail Chub and 498 Fathead Minnow. Approximately 43% of the topminnow captured were less than 20 mm TL, which suggests that topminnow were able to successfully reproduce since the initial stocking of 649 individuals in April. One crew walked upstream to the next pool that had water (F17; 440231/3845342; Figure 26) and carried out six seine hauls and captured 1,097 Fathead Minnow. All of the pools surveyed had substantially less water than has been observed during any previous

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

visit since 2018. The low water conditions are unsurprising considering the extraordinarily dry summer and fall of 2020.

Chub translocation. On October 15, 2020, Department staff translocated Roundtail Chub from Red Tank Draw to two isolated pools in Rarick Canyon. A total of 154 Roundtail Chub were collected from Red Tank Draw by backpack electrofishing and transported in aerated buckets to two pools above the waterfall barrier in Rarick Canyon. A total of 50 fish were translocated to the most downstream pool (F23; 438095/3844062; Figure 26). The remaining 104 fish were translocated to a pool (F17; 440231/3845342) upstream of 2019 stocking locations. There were no mortalities during collection, transport and stocking. Fish were in good condition at the time of stocking and behaved normally. Two juvenile Green Sunfish (< 50 mm TL) and one Fathead Minnow were captured and removed from Red Tank Draw during collection efforts.

Recommendations: The Gila Topminnow population in Rarick Canyon should be monitored for three years after the final stocking. If no additional topminnow are stocked, post-stocking monitoring of topminnow will be complete by 2023. 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, post-stocking monitoring will be completed by 2025. 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. The lack of water in Rarick Canyon upstream of the barrier during the severe drought conditions experienced in 2020 is somewhat concerning for the long-term viability of this population. However it is promising that chub persisted in all pools that were stocked in 2019, despite heavy flooding in the spring followed by severe drought conditions throughout the summer and fall.

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 will not be possible because the upstream source of nonnative fish would remain. In that situation, the goal of nonnative fish removals in Red Tank Draw should remain suppression of nonnative fish populations for the benefit the native fishes.

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.

- o Goal 4. Remove nonnative aquatic species threats.
- o Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
- o 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.

Background: 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 a nearby location 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 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

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

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.

Results: During 2020, Department staff engaged Arizona State Parks staff multiple times to identify a path forward for a chemical treatment of Sharp Spring. State Parks staff indicated that the Department needed to complete a Commercial Rental Permit for Research and Monitoring (CRPRM) in order to proceed with project planning. Department staff completed the CRPRM and will submit the permit to State Parks upon completion of internal Phase I Piscicide Project planning process. The Phase I planning document requires executive staff approval, and is one of three parts of the piscicide project planning process. Department staff drafted a Stage 1 Rotenone planning document for internal review, with submission anticipated in early 2021.

<u>Recommendations</u>: The GRB Technical Committee previously recommended dropping this project from the priority list. The nonnative fish removal planning portion of this project will be moved to the Topminnows Stocking project for FY2021. The Department proposes that this project be added to the FY2022 work plan so that the removal can be implemented that fiscal year.

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

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
 - o Goal 4. Remove nonnative aquatic species threats.
 - o Goal 5. Replicate populations and their associated native fish community into protected streams and other surface waters.
 - o 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 Ayer 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 (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 were 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 a treatment could be completed. 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 2020 Department staff communicated with a member of the Board of Directors for Boyce Thompson Arboretum State Park. The board member communicated that they had

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

purchased two large water storage tanks which would be connected to the wells. This new system would allow irrigation water to be drawn through the tanks, rather than the ponds, and the pond could be drawn down to eradicate the nonnative species. The drawdown could happen in late 2020 or early 2021. The park will work with the Department to salvage Desert Pupfish from the pond as it is being drawn down.

<u>Recommendations:</u> This project can be removed from the priority list and instead be incorporated into the Gila Topminnow Stockings Project. Department staff will continue to communicate with Boyce Thompson State Park to determine when the pond will be ready to commence with native fish restoration. Department staff will salvage Desert Pupfish and return them to the pond, after the pond is dried and then refilled. Gila Topminnow will also be restocked.

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
 - o Goal 4a. Eradicate nonnative aquatic species from a minimum of five surface waters to prepare them for repatriations of native fishes.
 - o Goal 5a. Replicate Gila topminnow stocks into a minimum of 10 surface waters.
 - o 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

Background: 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 2023. Nonnative control could commence afterwards. In 2019, Department staff assessed the feasibility of surveying stock tanks in the upper Verde River drainage for presence of nonnative fishes. Tanks most likely to support nonnative fish were identified using an automated approach developed in Program R to classify tanks as wet or dry using normalized difference water index (NDWI) values, and a scoring system based on perennial status, previous nonnative fish records, and distance to the Verde River. 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>Results:</u> Department staff participated in several monthly barrier meeting updates during the fiscal year. No fieldwork was completed, because the planning phase was extended.

Recommendations: Tank surveys in other drainages 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. We recommend that tank surveys be initiated in 2021, which is well before the treatment and before the barriers are constructed. The surveys will identify tanks with nonnative fishes, and provide information for planning nonnative removals in the tanks with fish. 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 camera or modified conductivity loggers in order to better understand the risk of dispersal

of nonnative fishes from stock tanks to the Verde River. Additionally, flow information for the Verde River including the discharge, travel times and the location of gaining and losing 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
 - o Goal 3. Improve propagation techniques for Spikedace and Loach Minnow
- Preventing Extinction and Managing Toward Recovery
 - O 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. 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 each year since 2007 is presented in Table 11. See Task AZ-2003-1 (Acquire Spikedace, Loach Minnow and rare populations of other native fish) for background information on each lineage. Number of fish brought into ARCC each year is presented in Table 1.

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. However, this topminnow broodstock was no longer needed, so all fish were stocked out. 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.

Results: The Department continued to operate ARCC in 2020. The ARCC maintains refuge populations of three lineages of Spikedace (Aravaipa Creek, upper Gila River, and Gila River Forks) and five lineages of Loach Minnow (Blue River, Aravaipa Creek, San Francisco River, and Gila River Forks). In 2020, ARCC produced 1,293 Aravaipa Creek Spikedace, 408 upper Gila River Spikedace, 833 Gila River Forks Spikedace, 16 Blue River Loach Minnow, 0 Aravaipa Creek Loach Minnow, 3 San Francisco River Loach Minnow, and 15 Gila River Forks Loach Minnow (Table 12). In addition, ARCC maintained a population of 146 Loach Minnow salvaged from Bear Creek, NM following the Tadpole Fire.

During 2018, ARCC staff started testing effects of fish density on propagation success of captive Spikedace and Loach Minnow. After a successful first year of trials staffed planned to conduct a second year of experiments using the exact same setup as 2018. Unfortunately, not enough wild Aravaipa lineage fish could be collected for the 2019 season to replace the brood stock lost during the previous year's testing. This resulted in all spawning raceways being setup identically to one another at the lowest most successful density identified during 2018 with no preference given to any one lineage. Due to COVID-19 and subsequent restrictions, ARCC staff continued with this raceway setup for the 2020 spawn season. 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 and placed in holding tanks without counting due to limited staff time from COVID restrictions. Algae were carefully removed as needed to minimize the potential effects of high algal biomass on spawning.

No new large scale physical improvements to ARCC were completed in 2020.

<u>Recommendations:</u> For 2021, ARCC staff will focus on running all raceways at the lowest density identified in 2018 with testing being conducted on Loach Minnow nest spacing using the highest population lineage. This will help verify that those densities do work to produce consistent larval counts as 2020 did not show the same success as 2019 and will help identify the ideal Loach Minnow nest spacing and resulting density.

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.

Fresno Canyon repatriations. Removed from priority list in 2019 because habitat was of insufficient size to support 500 Roundtail Chub¹.

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.

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¹ Chub planned to be stocked were previously classified as Gila Chub.

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FIGURES

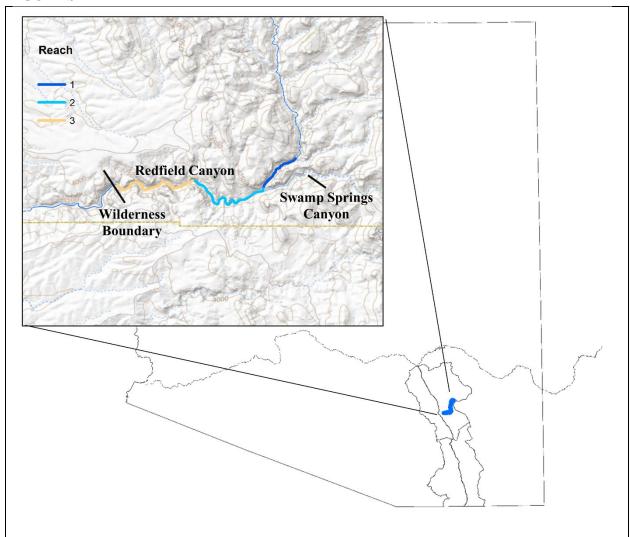


Figure 1.—Location of Redfield Canyon within the Gila River Basin and San Pedro River subbasin. Inset map shows the location of sampling Reaches 1 (Swamp Springs Confluence upstream to Barrier), 2 (Rock House tributary upstream to Swamp Springs Confluence), and 3 (Wilderness Boundary upstream to Rock House tributary).

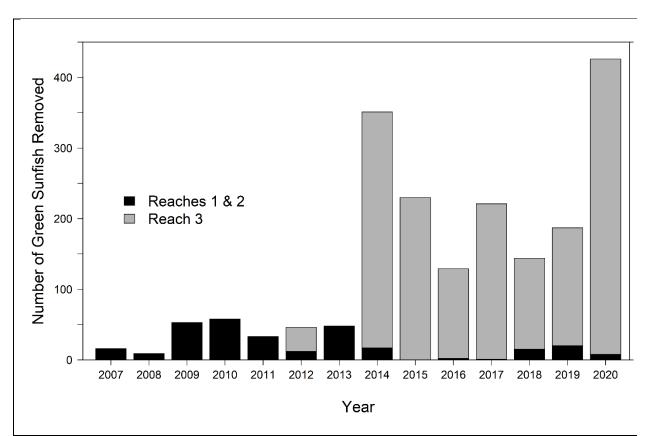


Figure 2.—Number of Green Sunfish removed during annual spring removal efforts and autumn monitoring from three reaches of Redfield Canyon, Arizona during 2007-2020. Location and description of reaches within Redfield Canyon shown in Figure 1.

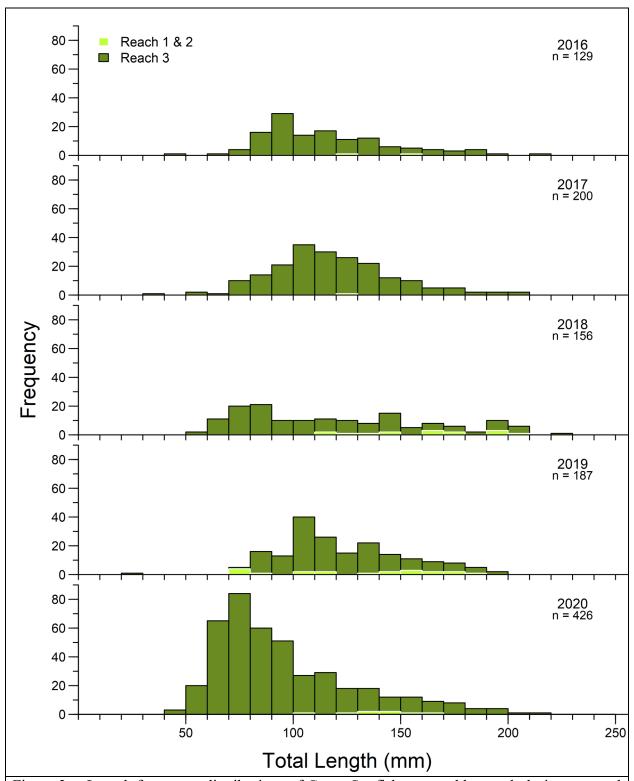


Figure 3.—Length frequency distributions of Green Sunfish captured by reach during removal efforts and annual monitoring in Redfield Canyon, 2016 through 2020.

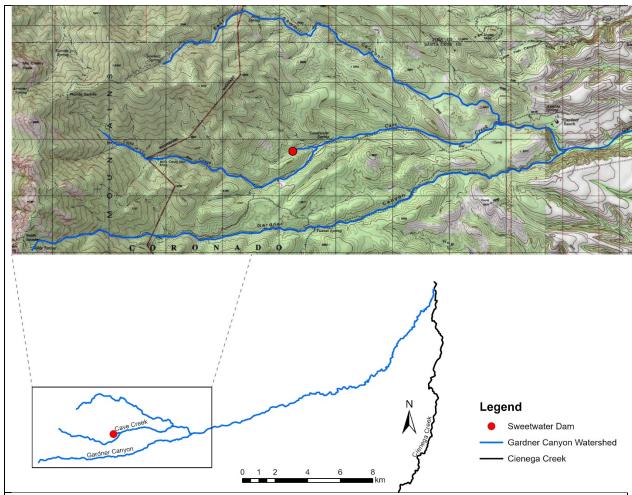


Figure 4.—Location of Sweetwater Dam in relation to the Cienega Creek watershed. Inset map displays the location of Sweetwater Dam in the upper Gardner Canyon watershed.



Figure 5.—Photo of Sweetwater Dam Pond on June 16, 2020 at the onset of draw down efforts.



Figure 6.—Photo of Sweetwater Dam Pond on June 17, 2020 at the completion of draw down efforts.

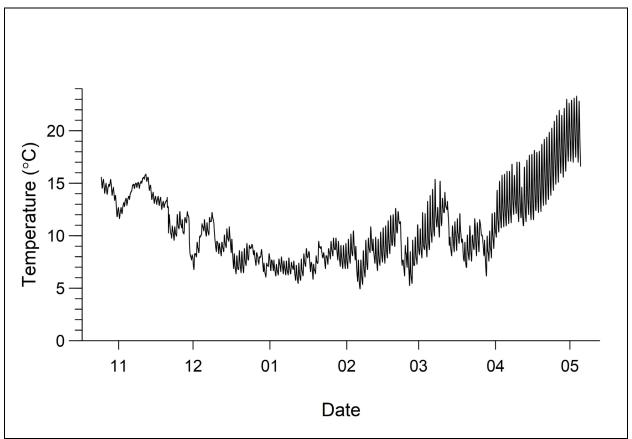


Figure 7.—Plot of stream temperature in Sabino Canyon at Gila Topminnow stocking location upstream of the confluence with East Fork Sabino Canyon from October 25, 2019 to May 5, 2020.

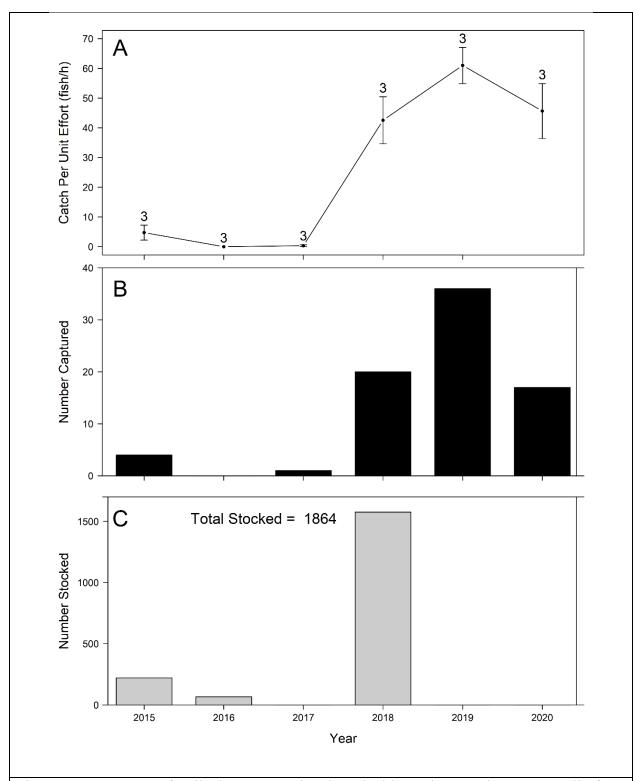


Figure 8.—Summary of Spikedace captured and stocked in Spring Creek, AZ, annually from 2015 to 2020 with (A) mean annual backpack electrofishing catch per unit effort (fish/h), (B) total number of fish captured, and (C) total number of fish stocked.

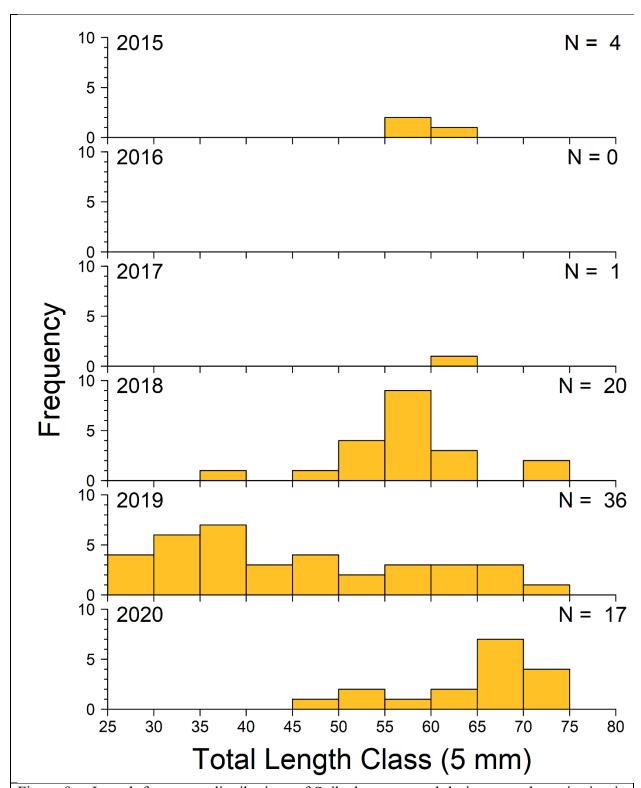


Figure 9.—Length frequency distributions of Spikedace captured during annual monitoring in Spring Creek, 2015 through 2020. Included are first pass fish only.

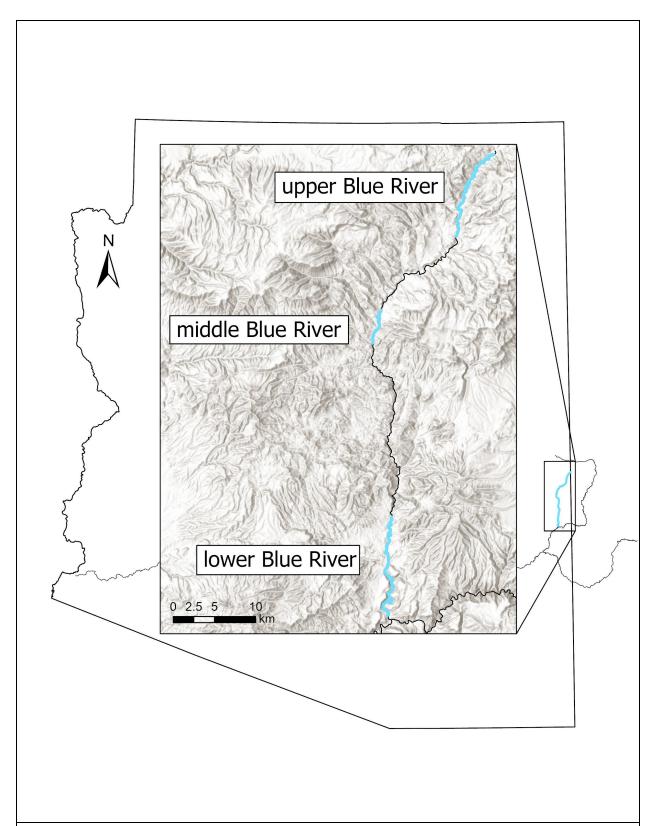


Figure 10.—Map showing the upper (New Mexico border downstream to Blue Crossing Campground), middle (The Box downstream to Fritz Ranch), and lower (Fritz Ranch downstream to the barrier) project areas of the Blue River.

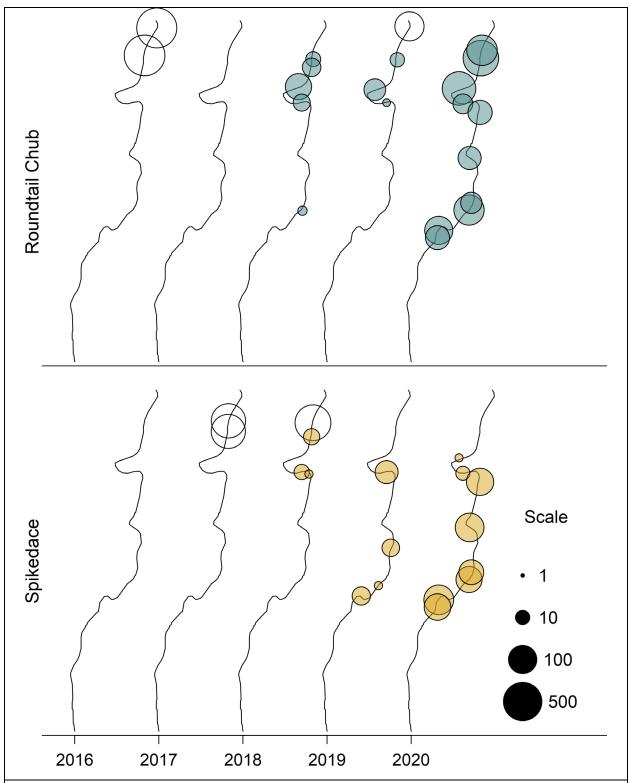


Figure 11.—Roundtail Chub (top row) and Spikedace (bottom row) stocking locations (open circles) and mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the middle Blue River from 2016-2020. Size of points indicates either the number of fish stocked or the relative abundance during monitoring at a particular location.

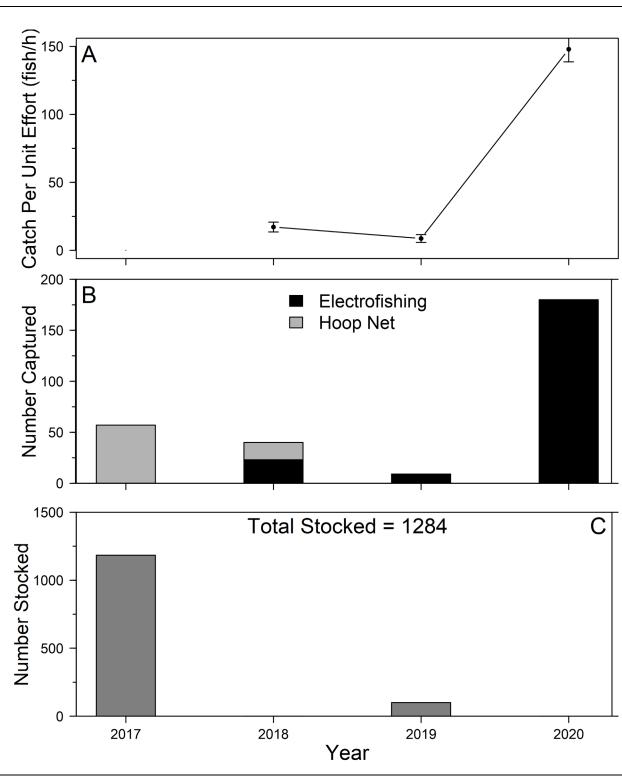


Figure 12.—Summary of Roundtail Chub captured and stocked in the middle Blue River, annually from 2017 to 2020 with (A) mean catch per unit effort (fish/h) for backpack electrofishing with standard error bars, (B) total number of fish captured by gear type (hoop nets in gray, backpack electrofishing in black), and (C) total number of fish stocked. Catch per unit effort is not displayed for hoop nets in panel A because it was less than one fish per hour.

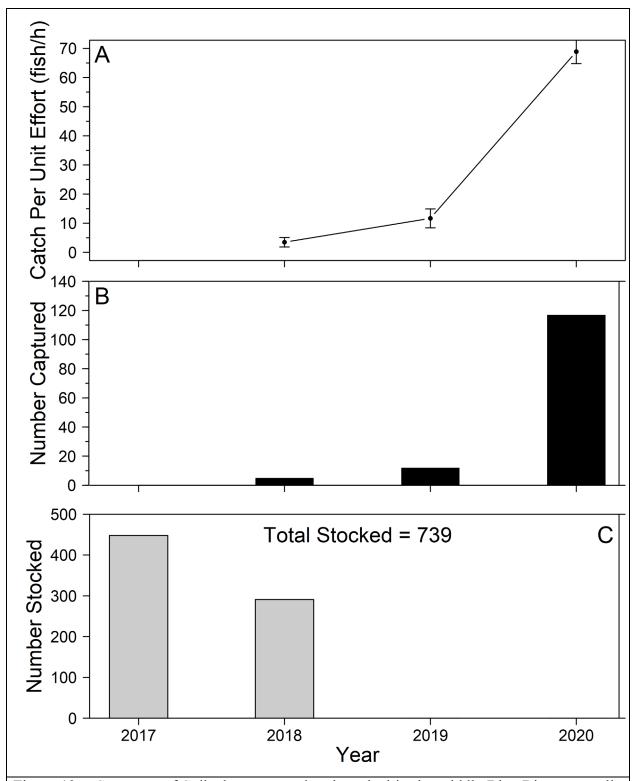


Figure 13.—Summary of Spikedace captured and stocked in the middle Blue River, annually from 2017 to 2020 with (A) mean catch per unit effort (fish/h) with standard error bars, (B) total number of fish captured, and (C) total number of fish stocked.

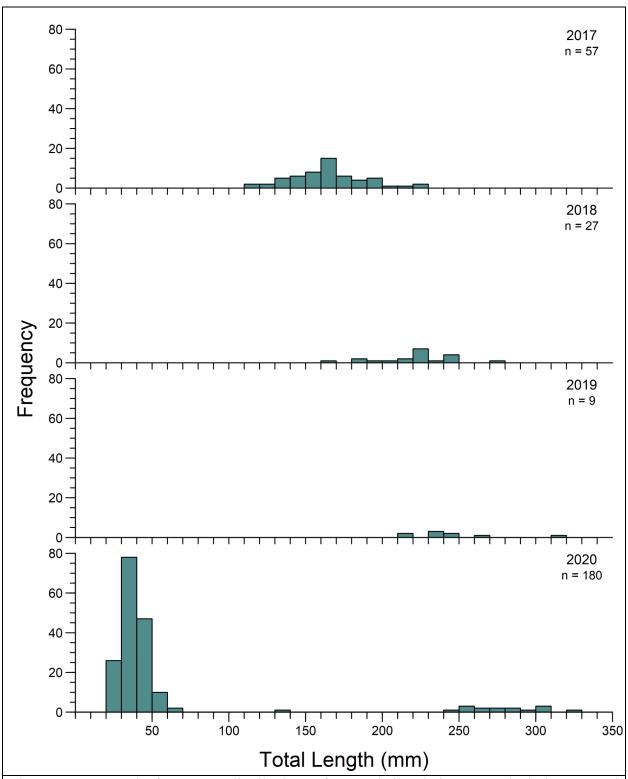


Figure 14.—Length frequency distribution of Roundtail Chub captured during annual monitoring in the middle Blue River, from 2017 to 2020.

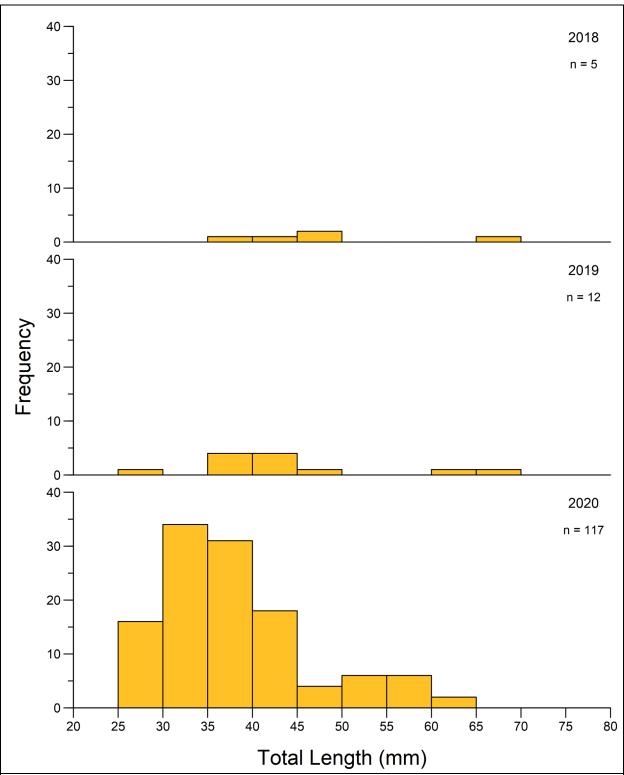


Figure 15.—Length frequency distribution of Spikedace captured during annual monitoring in the middle Blue River, from 2018 to 2020.

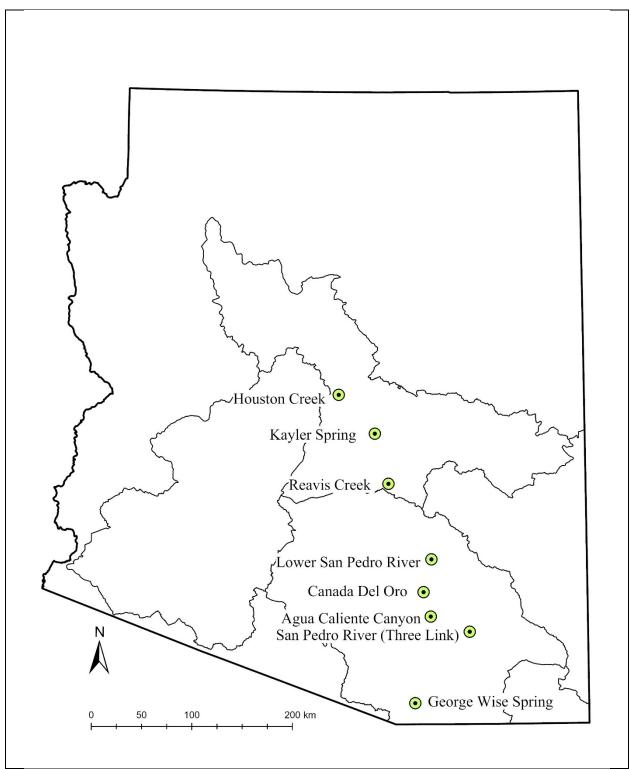


Figure 16.—Map showing locations of streams and ponds assessed for suitable fish habitat in the Gila River basin in 2020. The finer lines delineate the four digit hydrologic unit codes (HUCs) within the Gila River basin.

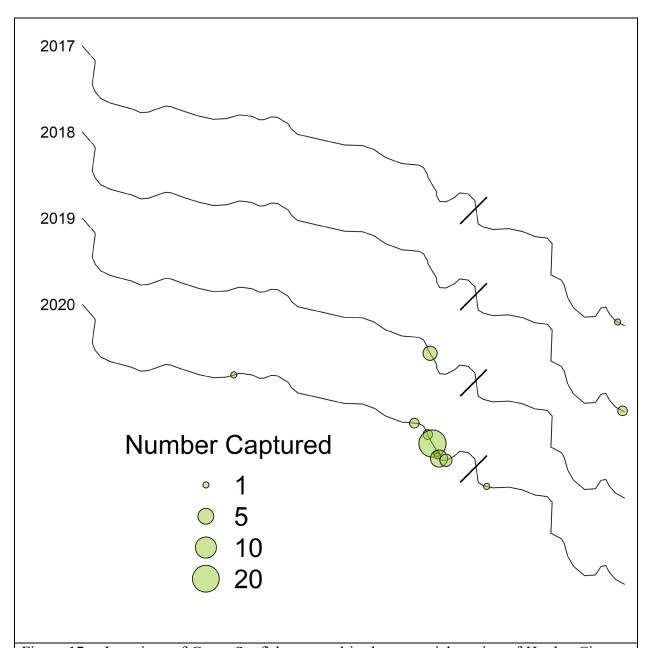


Figure 17.—Locations of Green Sunfish captured in the perennial portion of Harden Cienega Creek from 2017-2020. The barrier location is indicated by a diagonal line. Size of points indicates number of fish captured at a particular location during nonnative removal or monitoring efforts. Monitoring during 2017-2019 was only above the barrier.

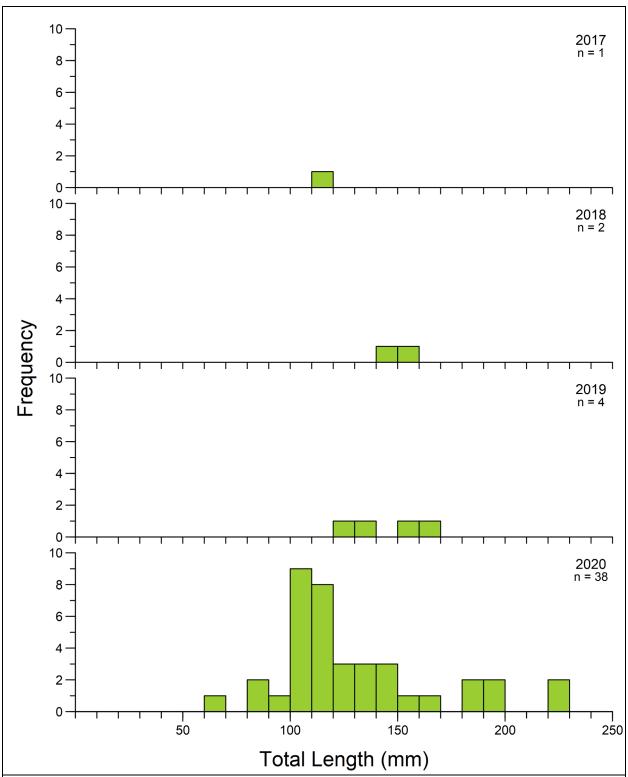


Figure 18.—Length frequency distribution of Green Sunfish captured and removed during annual monitoring and nonnative removal efforts in Harden Cienega Creek, from 2017 to 2020.

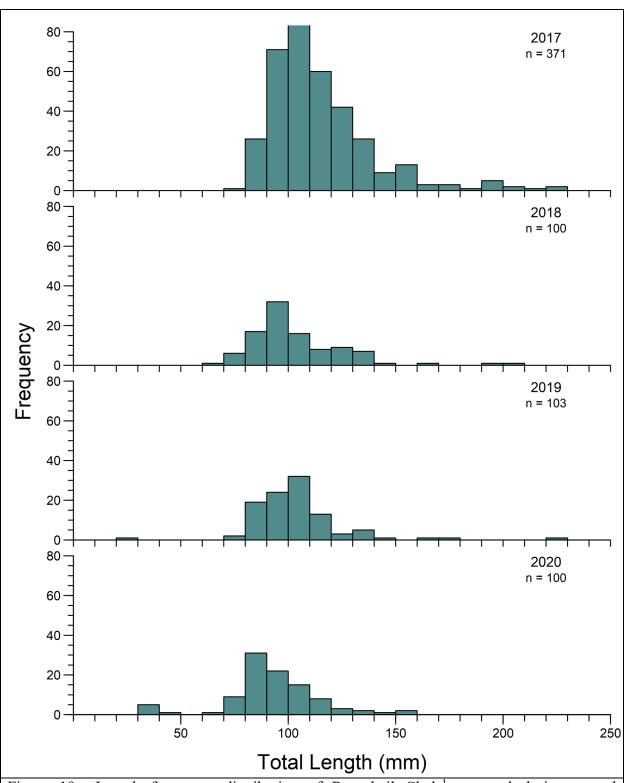


Figure 19.—Length frequency distribution of Roundtail Chub¹ captured during annual monitoring above the waterfall barrier in Harden Cienega Creek, from 2017 to 2020. Only the first 100 Roundtail Chub¹ captured were measured in 2018 and 2020.

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

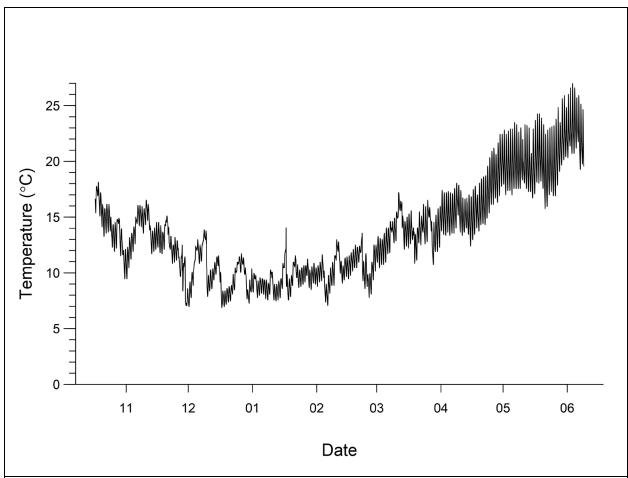


Figure 20.—Plot of stream temperature in Harden Cienega Creek at Gila Topminnow stocking location from October 17th, 2019 to June 8th, 2020.

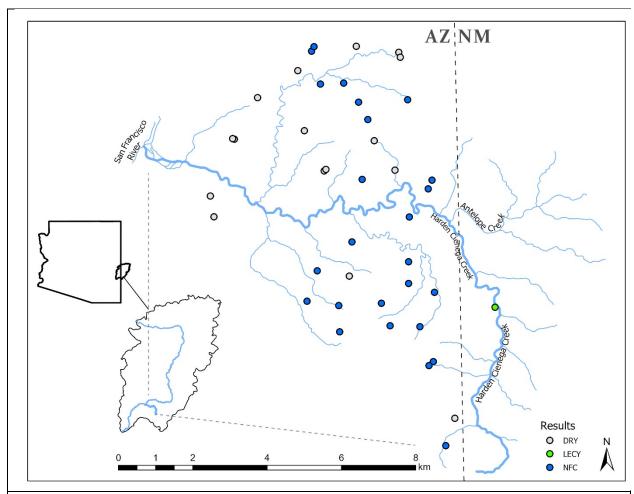


Figure 21.—Map of all 43 tanks surveyed in the Arizona portion of the Harden Cienega Creek watershed during 2020. Shown are the 16 dry tanks (grey points) and 27 wet tanks (blue points), the latter of which were sampled by bag seine, straight seine or dip net. Also shown is Ditch Tank (green point) in New Mexico where Common Goldfish and Green Sunfish were detected.

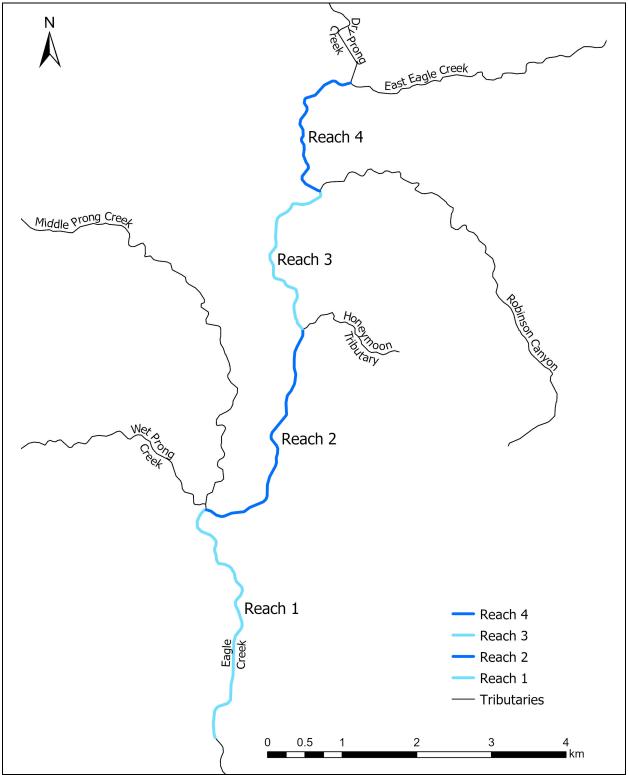


Figure 22.—Map of the four sampling reaches that comprise the Eagle Creek study area above the tentative barrier location. Preliminary sampling in 2020 was conducted exclusively in Reaches 3 and 4.

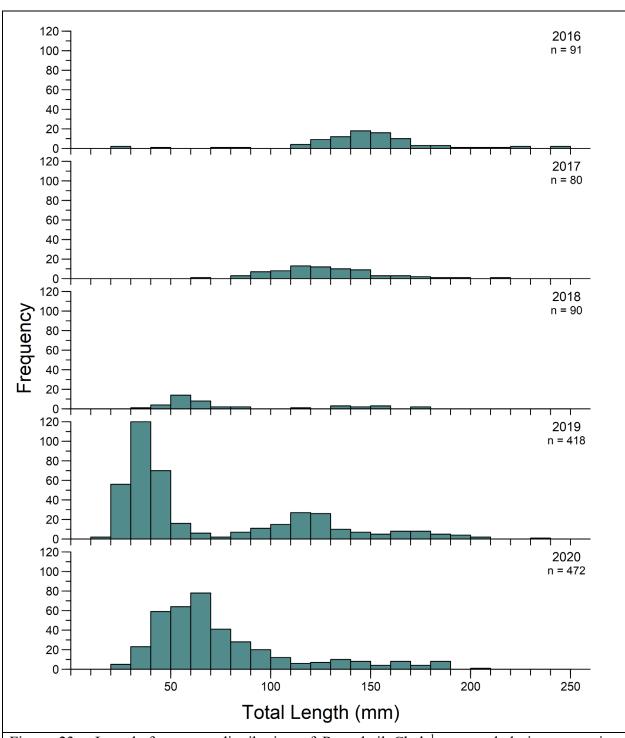


Figure 23.—Length frequency distribution of Roundtail Chub¹ captured during nonnative removal efforts in Red Tank Draw, from 2016 to 2020. In general, only the first 100 Roundtail Chub¹ captured per pass were measured between 2018 and 2020.

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

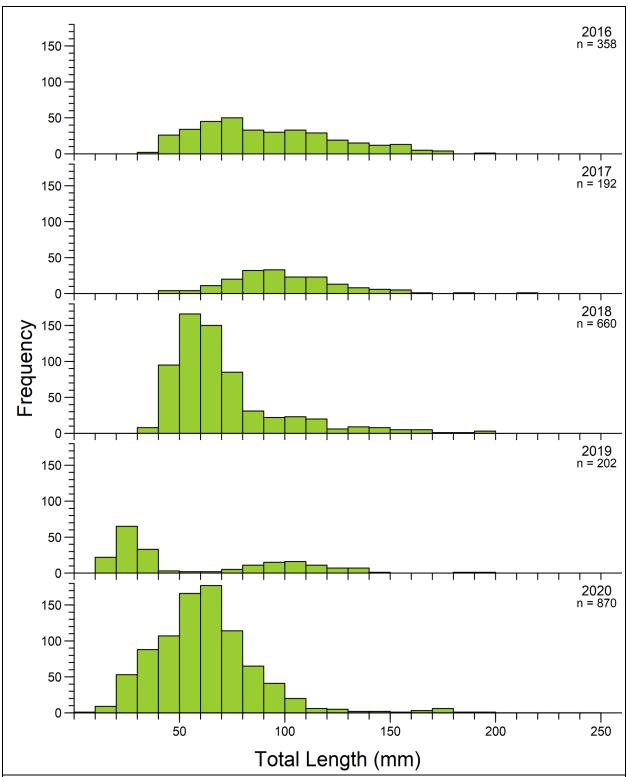


Figure 24.—Length frequency distribution of Green Sunfish captured during nonnative removal efforts in Red Tank Draw, from 2016 to 2020.

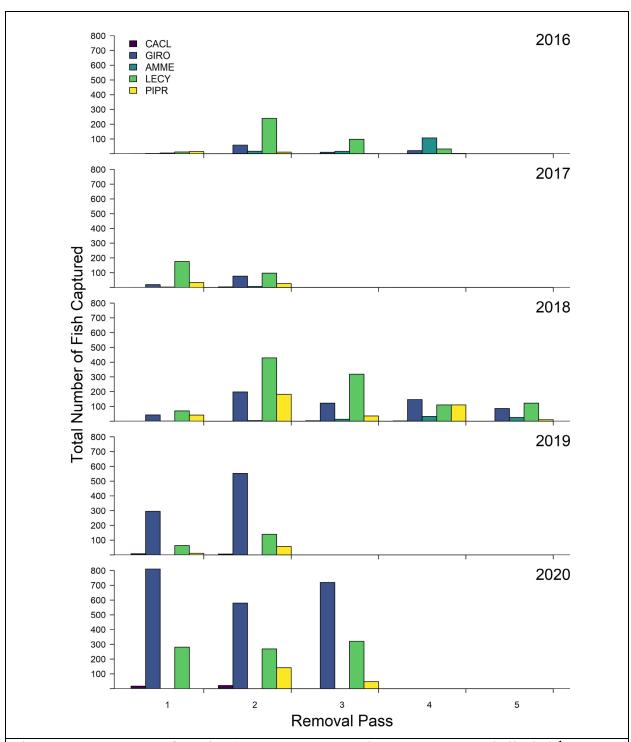


Figure 25.—Summary of species (CACL = Desert Sucker, GIRO = Roundtail Chub¹, AMME = Black Bullhead, LECY = Green Sunfish, PIPR = Fathead Minnow) captured in each removal pass in Red Tank Draw from 2016 to 2020. Total number of fish captured includes fish captured by backpack electrofishing, mini-hoop nets, minnow traps and angling.

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

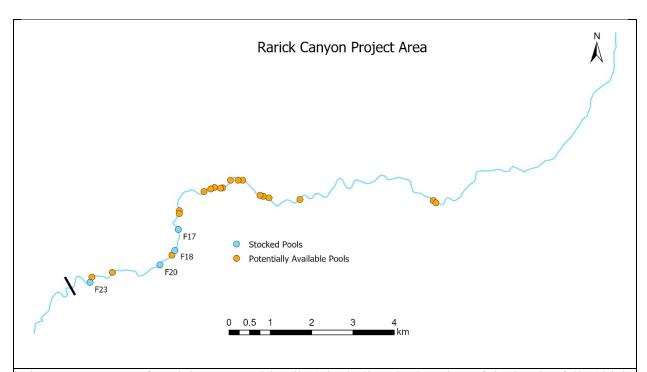


Figure 26.—Map of Rarick Canyon (blue line) including the location of the barrier falls (thick black line), names and locations of pools stocked with fish (blue dots), and the remaining potentially habitable pools in Rarick Canyon (orange dots).

TABLESTable 1.—Summary of number of Spikedace (MEFU) and Loach Minnow (RHCO), of each lineage, brought into the Aquatic Research and Conservation Center from 2010 to 2020.

Taxa	Extant Lineage/Stream	2010^{1}	2011 ²	2012	2013 ³	2014 ⁴	2015 ⁵	2016 ⁶	20177	2018	2019	2020
MEFU	Upper Gila River, NM											
	Gila River Forks, NM	250	148			XX				1		
	Aravaipa Creek (& tribs)		XX		XX	26	150	80	160		322	49
RHCO	Upper Gila River, NM											
	Gila River Forks, NM	100	434			61			110	145		
	San Francisco R., NM (& tribs)				41							
	Blue River (& tribs)		27					12		223	80	269
	Aravaipa Creek (& tribs)		XX		XX	48	50	200	100		57	82

^{1.} Robinson 2010

^{2.} Robinson 2011

^{3.} Robinson 2014

^{4.} Crowder and Robinson 2015

Robinson 2016

^{6.} Robinson et al. 2017

^{7.} Robinson and Mosher 2018

Table 2.—Summary of fish captured during the first pass at three 100-m electrofishing transects in Spring Creek during annual monitoring during September 9, 2020. Shown are the number of fish captured in each transect (#Ind), the mean number of fish captured per hour of electrofishing effort (#Ind/h), and the overall mean and standard error of the catch rate.

	Roundtail		Desert	Sonora	Longfin	
Statistic	Chub ¹	Spikedace	Sucker	Sucker	Dace	Speckled Dace
#Ind	73	1	33			43
#Ind/h	179.20	4.32	83.55			139.85
#Ind	42	1	8	4	3	81
#Ind/h	122.82	0.93	33.09	3.70	10.23	183.93
#Ind #Ind/h	98 302.82	15 86.60	48 166.13		102 329.62	241 772.60
#Ind #Ind/h	213 199.04	17 45.66	89 87.99	4 1.61	105 100.40 (57.62)	365 374.42 (55.58)
	#Ind #Ind/h #Ind/h #Ind/h #Ind/h	Statistic Chub¹ #Ind 73 #Ind/h 179.20 #Ind 42 #Ind/h 122.82 #Ind 98 #Ind/h 302.82 #Ind 213 #Ind/h 199.04	Statistic Chub¹ Spikedace #Ind 73 1 #Ind/h 179.20 4.32 #Ind 42 1 #Ind/h 122.82 0.93 #Ind 98 15 #Ind/h 302.82 86.60 #Ind 213 17 #Ind/h 199.04 45.66	Statistic Chub¹ Spikedace Sucker #Ind 73 1 33 #Ind/h 179.20 4.32 83.55 #Ind 42 1 8 #Ind/h 122.82 0.93 33.09 #Ind 98 15 48 #Ind/h 302.82 86.60 166.13 #Ind 213 17 89 #Ind/h 199.04 45.66 87.99	Statistic Chub¹ Spikedace Sucker Sucker #Ind 73 1 33 #Ind/h 179.20 4.32 83.55 #Ind 42 1 8 4 #Ind/h 122.82 0.93 33.09 3.70 #Ind 98 15 48 #Ind/h 302.82 86.60 166.13 #Ind 213 17 89 4 #Ind/h 199.04 45.66 87.99 1.61	Statistic Chub¹ Spikedace Sucker Sucker Dace #Ind 73 1 33 1 33 1 33 1 33 1 34

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

Table 3.—Three-pass depletion estimates of abundance for all fish species captured per 100 m at the fixed site in Spring Creek during annual monitoring in 2020. Included is the number of fish caught in each pass (C1, C2, C3), Carle-Strub three pass abundance estimate (N), lower (N_LCI) and upper (N_UCI) 95% confidence interval of the abundance estimate, estimated capture probability (p), and the lower (p_LCI) and upper (p_UCI) 95% confidence interval of the estimate of capture probability. Species codes are MEFU = Spikedace, GIRO = Roundtail Chub, CACL = Desert Sucker, AGCH = Longfin Dace, and RHOS = Speckled Dace.

Stream	Site	Species	C1	C2	C3	N	N_LCI	N_UCI	p	p_LCI	<i>p</i> _UCI
Spring Creek	Fixed-02	MEFU	15	5	1	21	19.62	22.38	0.75	0.55	0.95
Spring Creek	Fixed-02	GIRO	98	57	25	207	184.96	229.04	0.49	0.39	0.59
Spring Creek	Fixed-02	CACL	48	10	20	92	74.46	109.54	0.46	0.30	0.62
Spring Creek	Fixed-02	AGCH	102	60	22	207	188.08	225.92	0.52	0.42	0.61
Spring Creek	Fixed-02	RHOS	241	207	109	827	693.92	960.08	0.31	0.24	0.38

Table 4.—Summary of fish captured within each survey reach at 10, 100-m electrofishing transects in the middle Blue River during annual monitoring during September 21-23, 2020. 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	Brown
Reach	N	Statistic	Minnow	Chub	Spikedace	Sucker	Dace	Sucker	Dace	Trout
1	3	#Ind	55	28	51	481	311	143	807	
		#Ind/h	160.48	59.84	93.39	670.21	420.33	195.43	877.18	
		SE	(14.46)	(7.65)	(6.61)	(82.25)	(44.93)	(31.14)	(116.69)	
2	4	#Ind	189	30	65	1611	484	242	1000	1
		#Ind/h	219.08	67.73	67.25	1000.97	333.83	191.44	659.64	0.98
		SE	(7.56)	(9.75)	(4.58)	(66.98)	(33.79)	(255.27)	(69.18)	(0.98)
3	3	#Ind	76	122	1	653	107	202	779	
		#Ind/h	130.67	203.12	0.87	653.36	123.08	237.5	786.17	
		SE	(8.92)	(12.56)	(0.87)	(39.68)	(22.21)	(30.37)	(93.25)	
Total	10	#Ind	320	180	117	2745	902	587	2586	1
		#Ind/h	187.91	147.93	68.89	829.20	292.62	209.80	747.79	0.46
		SE	(5.90)	(9.28)	(4.08)	(40.92)	(23.32)	(16.99)	(50.84)	(0.46)

Table 5.—Pairwise Wilcoxon signed rank test results (test statistic W and p-value) evaluating increases in mean relative abundance of Spikedace and Roundtail Chub between years during annual monitoring in the middle Blue River. Significant values are indicated with an asterisks (*).

	Spil	redace	Roundtail Chub			
Years	\overline{W}	P	W	P		
2018-2019	38	0.038*	7	0.898		
2019-2020	58	0.015*	76	<0.001*		

Table 6.—Three-pass depletion estimates of abundance for all fish species captured per 100 m at each fixed site in the Middle Blue River during annual monitoring in 2020. Included is the number of fish caught in each pass (C1, C2, C3), Carle-Strub three pass abundance estimate (N), lower (N_LCI) and upper (N_UCI) 95% confidence interval of the abundance estimate, estimated capture probability (p), and the lower (p_LCI) and upper (p_UCI) 95% confidence interval of the estimate of capture probability. Species codes are MEFU = Spikedace, GIRO = Roundtail Chub, TICO = Loach Minnow, CACL = Desert Sucker, CAIN = Sonora Sucker, AGCH = Longfin Dace, and RHOS = Speckled Dace.

Site	Species	C1	C2	C3	N	N_LCI	N_UCI	p	p_LCI	p_UCI
Fixed-04	MEFU	0	0	0	NA	NA	NA	NA	NA	NA
Fixed-04	GIRO	34	24	9	78	63.04	92.96	0.47	0.30	0.64
Fixed-04	TICO	33	19	29	190	0	403.38	0.18	0	0.39
Fixed-04	CACL	172	79	91	504	402.40	605.60	0.31	0.22	0.41
Fixed-04	CAIN	64	10	19	101	91.01	110.99	0.56	0.44	0.69
Fixed-04	AGCH	47	25	20	118	88.24	147.76	0.39	0.23	0.55
Fixed-04	RHOS	345	139	96	660	623.88	696.12	0.50	0.45	0.56
Fixed-28	MEFU	11	10	4	30	18.19	41.81	0.43	0.13	0.73
Fixed-28	GIRO	16	10	1	28	24.73	31.27	0.64	0.43	0.85
Fixed-28	TICO	51	31	21	133	110.56	165.43	0.39	0.23	0.54
Fixed-28	CACL	442	297	163	1174	1076.48	1271.52	0.39	0.33	0.44
Fixed-28	CAIN	112	82	20	242	220.83	263.17	0.51	0.42	0.60
Fixed-28	AGCH	149	92	68	431	353.22	508.78	0.34	0.25	0.44
Fixed-28	RHOS	272	162	148	918	748.59	1087.41	0.28	0.21	0.36

Table 7.—Summary of fish captured by gear type across all nonnative fish removals, Gila Topminnow monitoring and Roundtail Chub monitoring efforts in Harden Cienega Creek in 2020. Shown for each gear type are the total number of fish captured (#Ind), the mean relative abundance (number of fish captured per hour of electrofishing effort, or per hour of trap effort; #Ind/h) and standard error of mean relative abundance (SE).

		Roundtail	Desert	Longfin	Sonora	Speckled	Green
Gear	Statistic	Chub	Sucker	Dace	Sucker	Dace	Sunfish
Backpack Electrofisher	#Ind	142	407	872	41	427	22
_	#Ind/h	76.71	275.39	545.55	27.74	267.40	0.07
	SE	(1.04)	(61.74)	(243.10)	(6.10)	(106.89)	(0.07)
Mini-hoop Net	#Ind	56	4				16
•	#Ind/h	1.21	0.05				0.81
	SE	(0.26)	(0.04)				(0.16)
Collapsible Minnow							
Trap	#Ind	14		79		52	
-	#Ind/h	0.35		1.09		0.78	
	SE	(0.14)		(0.27)		(0.21)	

Table 8.—Summary of fish captured within each survey reach at six, 100-m electrofishing transects in the Eagle Creek during monitoring during November 2-3, 2020. 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).

			Roundtail	Desert	Longfin	Sonora	Speckled
Reach	N	Statistic	Chub	Sucker	Dace	Sucker	Dace
3	3	#Ind	10	850	93	62	375
		#Ind/h	19.26	976.66	105.22	87.55	508.54
		SE	(4.67)	(99.06)	(27.74)	(18.87)	(95.57)
4	3	#Ind	64	768	106	76	281
		#Ind/h	128.05	749.38	161.21	72.27	497.97
		SE	(8.58)	(122.26)	(33.35)	(17.29)	(84.99)
Total	6	#Ind	74	1618	199	138	656
		#Ind/h	107.04	857.61	137.46	79.51	502.91
		SE	(8.36)	(80.21)	(22.73)	(12.64)	(62.86)

Table 9.—Three-pass depletion estimates of abundance for all fish species captured per 100 m at the fixed site in Eagle Creek during November 2-3, 2020. Included is the number of fish caught in each pass (C1, C2, C3), Carle-Strub three pass abundance estimate (N), lower (N_LCI) and upper (N_UCI) 95% confidence interval of the abundance estimate, estimated capture probability (p), and the lower (p_LCI) and upper (p_UCI) 95% confidence interval of the estimate of capture probability. Species codes are: Species codes are GIRO = Roundtail Chub, CACL = Desert Sucker, CAIN = Sonora Sucker, AGCH = Longfin Dace, and RHOS = Speckled Dace.

Stream	Site	Species	C1	C2	C3	N	N_LCI	N_UCI	p	p_LCI	<i>p</i> _UCI
Eagle Creek	4-5	GIRO	39	19	17	95	69.50	120.50	0.40	0.22	0.58
Eagle Creek	4-5	CACL	346	248	156	1078	941.87	1214.13	0.33	0.27	0.38
Eagle Creek	4-5	CAIN	42	19	10	78	68.04	87.96	0.54	0.39	0.69
Eagle Creek	4-5	AGCH	23	10	3	37	33.50	40.50	0.65	0.48	0.83
Eagle Creek	4-5	RHOS	70	20	15	112	130.34	120.66	0.60	0.48	0.71

Table 10.—Summary of fish captured by gear type during each year of nonnative fish removal efforts in Red Tank Draw from 2016-2020. Shown for each year and gear type is the total number of fish captured (#Ind), the mean relative abundance (number of fish captured per hour of electrofishing effort, or per hour of trap effort; #Ind/h) and standard error of mean relative abundance (SE).

	():		Roundtail	Desert	Green	Black	Fathead
Year	Gear	Statistic	Chub	Sucker	Sunfish	Bullhead	Minnow
2016	Backpack Electrofisher	#Ind	78		205	122	23
		#Ind/h	109.16		472.22	55.22	16.53
		SE	(11.34)		(26.12)	(9.70)	(2.95)
							_
2016	Mini-hoop Net	#Ind	12		72	23	3
		#Ind/h	0.11		0.46	0.23	0.20
		SE	(0.01)		(0.02)	(0.03)	(0.14)
2017	Backpack Electrofisher	#Ind	96	4	185	10	54
2017	Dackpack Electronisher	#Ind/h	37.02	3.48	45.03	3.63	19.25
		#IIId/II SE	(1.62)	(3.48)	(1.58)	(0.57)	(2.56)
		SL	(1.02)	(3.40)	(1.50)	(0.57)	(2.30)
2017	Mini-hoop Net	#Ind			27		2
	1	#Ind/h			1.29		0.11
		SE			(0.14)		(0.01)
							` /
2018	Backpack Electrofisher	#Ind	570	5	894	26	371
		#Ind/h	152.73	3.82	242.09	15.14	66.07
		SE	(13.95)	(0.82)	(5.14)	(1.21)	(15.92)
2010	M:: 1 NI.4	μт 1	20		1.40	42	7
2018	Mini-hoop Net	#Ind #Ind/h	20 0.18		148 1.28	43 2.90	7 0.31
		#IIId/II SE	(0.03)		(0.13)	(0.33)	(0.13)
		SE	(0.03)		(0.13)	(0.33)	(0.13)
	Collapsible Minnow						
2018	Trap	#Ind	1		4	6	
	1	#Ind/h	0.05		1.07	1.16	
		SE	(0.05)		(1.07)	(0.18)	
			` ,		, ,	, ,	
2019	Backpack Electrofisher	#Ind	845	14	188		67
		#Ind/h	399.96	7.37	203.93		53.87
		SE	(11.10)	(0.45)	(15.49)		(17.35)
					_		
2019	Mini-hoop Net	#Ind	1		6		
		#Ind/h	0.33		0.44		
		SE	(0.33)		(0.08)		
	Collapsible Minnow						
2019	Trap	#Ind	1		8		1
2017	11ap	n mu	1		O		1

		#Ind/h	0.21		1.71	0.21
		SE	(0.21)		(1.71)	(0.21)
2020	Backpack Electrofisher	#Ind	2096	38	749	232
		#Ind/h	254.17	15.49	254.50	89.39
		SE	(10.75)	(1.67)	(8.44)	(27.42)
2020	Mini-hoop Net	#Ind	10		101	14
		#Ind/h	0.35		4.31	0.69
		SE	(0.13)		(0.29)	(0.34)
	Collapsible Minnow					
2020	Trap	#Ind	4		21	11
		#Ind/h	0.16		0.864	0.42
		SE	(0.03)		(0.17)	(0.30)

Table 11.—Waters assessed during 2014 through 2020 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. Species codes are MEFU = Spikedace, TICO = Loach Minnow, GIRO = Roundtail Chub, CACL = Desert Sucker, CAIN = Sonora Sucker, AGCH = Longfin Dace, RHOS = Speckled Dace, and POOC = Gila Topminnow.

			Upst	tream	Down	stream		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
5/06/2019	Reavis Creek	Salt	483927	3701853	485505	3706286	1487	>6400	POOC
5/07/2019	Fish Canyon	Salt	480065	3705586	478984	3705377	1036	>1500	GIRO
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
3/9/2020	Kayler Spring	Salt	472097	3755880	472096	3755497	740	450	None
3/31/2020	George Wise Spring	Santa Cruz	512395	3487781	512258	3487515	1197	300	POOC, GIRO
4/7/2020	Reavis Creek	Salt	483927	3701853	485505	3706286	1487	>6400	None
4/14/2020	Houston Creek	Verde	434181	3797131	436953	3793613	747	<4000	GIRO
5/4/2020	Agua Caliente Canyon	Santa Cruz	528493	3573991	526671	3573385	889	>1300	GIRO
5/11/2020	Canada Del Oro	Santa Cruz	518690	3592110	515099	3603898	1384	>20000	GIRO

10/20/2020	San Pedro River (lower San Pedro State Wildlife Area)	San Pedro	529307	3629840	528035	3630777	672	>500	POOC, GIRO, CACL, CAIN
10/21/2020	San Pedro River (Three Link Conservation Easement)	San Pedro	566014	3558225	566375	3559636	1000	>1600	POOC, GIRO, CACL, CAIN

Table 12.—Summary of number of broodstock (#B), number of offspring produced (#P), number of offspring stocked (#S) for each species and lineage held at the Aquatic Research and Conservation Center, from 2008 through 2020. Numbers stocked do not include fish transferred to New Mexico.

Taxa	Extant Lineage/Stream		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Spikedace	upper Gila River, NM	$\#\mathbf{B}$							380	392	531	267	159	254	219
		#P	740	165	2555	539	1300		1000	296	0	384	352	2404	408
		#S	448	165	545		539			296		327	0		
	Gila River Forks	$\#\mathbf{B}$		17	267				250	204	138	122	83	71	76
		# P	NA	0	379	0	800	700	300		0	1183	195	1132	833
		#S										1000	0		
	Aravaipa Creek	#B							480	412	262	382	331	523	529
		#P	1650	410	5993	4663	3471		221	35	120	1347	3214	4250	2182
		#S	1600	386	2954	4663	3471			221	67		2234		2897
Loach Minnow	upper Gila River, NM	#B							NA	NA	NA	NA	NA	NA	
		#P													
		#S													
	Gila River Forks	#B							57	81	96	128	97	169	121
		#P	NA	0	0	0			250		220	7	1207	665	15
		#S										159	0		
	San Francisco R., NM	#B							27	119	215	314	318	231	208
		#P	NA	NA					500		26	177	1627	601	3
		#S										243	0		
	Blue River	#B	XX	XX	XX	150		XX	180	245	214	156	117	290	266
		#P	670	22	164	722		1500	288		426	47	6	713	16
		#S	678		156		217	310	288		390		0		500
	Aravaipa Creek	#B	XX	XX	XX	XX		XX	340	316	297	490	439	354	337
	-	#P	3250	274	1623	1035		951	0		265	305	1848	1398	57
		#S	4003	156	1561	527		951					0		300
Roundtail Chub	Eagle Creek	#B				XX	-		85	85	101	99	99	99	98
		#P				149		0	1500	2000	0	57	0	0	0

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APPENDICES

Appendix 1.—Summary of native fish stocked in Arizona during 2020 by the Department under the Gila River Basin Native Fishes Conservation Program. Easting and Northing are in UTMs (NAD 83; zone 12S).

							#	#
Taxa	Water Name	Site Name		Northing	Date	Lineage	Stocked	Mortalities
Gila Topminnow	Tortilla Creek	4.6 km above Mesquite Wash	467237	3708616	4/9/2020	Peck Canyon	374	150
Gila Topminnow	Rarick Canyon	F18	525269	3490499	4/30/2020	Redrock Canyon	650	2
Loach Minnow	Bonita Creek	Midnight Canyon	635275	3653300	5/7/2020	Aravaipa Creek	473	1
Loach Minnow	Hot Springs Canyon	Wildcat Canyon	569200	3580090	5/22/2020	Aravaipa Creek	299	1
Spikedace	Hot Springs Canyon	Wildcat Canyon	569185	3580087	5/22/2020	Aravaipa Creek	332	1
Loach Minnow	Campbell Blue Creek	DS Turkey Creek	679251	3734439	6/23/2020	Blue River	172	3
Spikedace	Blue River	Upper Blue Campground	678665	3729743	6/23/2020	Gila Forks	453	5
Spikedace	Blue River	Jackson Canyon	678020	3728836	6/23/2020	Gila Forks	373	54
Roundtail Chub	Blue River	Bobcat Flat	678721	3729802	6/23/2020	Eagle Creek	226	5
Gila Topminnow	Mattie Canyon	Mattie Canyon	540102	3523732	8/27/2020	Cienega Creek	116	0
Roundtail Chub ¹	Rarick Canyon	F23	438094	3844065	10/15/2020	Red Tank Draw	50	0
Roundtail Chub ¹	Rarick Canyon	F17	436290	3841769	10/15/2020	Red Tank Draw	105	0

¹ Chub translocated from Red Tank Draw previously classified as Gila Chub.

Appendix 2.—Summary of monitoring results during 2020 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	2020
Desert Pupfish	Black Canyon City Heritage Pond	7/21/2020	Minnow Trap	10	#Ind	2
					%YOY	0
					Mean CPUE	0.11
					SE	0.07
Desert Pupfish	Black Canyon City Heritage Pond	7/21/2020	Dip Net	3	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Desert Pupfish	Las Cienegas NCA – Cottonwood Tank	8/3/2020	Minnow Trap	10	#Ind	11
					%YOY	0
					Mean CPUE	0.11
					SE	0.05
Gila Topminnow	Bass Canyon	9/15/2020	Minnow Trap	13	#Ind	2
					%YOY	50
					Mean CPUE	0.03
					SE	0.03
Gila Topminnow	Bass Canyon	9/15/2020	Seine	3	#Ind	51
					%YOY	86
					Mean CPUE	5.67
					SE	3.27
Gila Topminnow	Black Canyon City Heritage Pond	7/21/2020	Minnow Trap	10	#Ind	12
					%YOY	50
					Mean CPUE	0.63
					SE	0.24
Gila Topminnow	Black Canyon City Heritage Pond	7/21/2020	Dip Net	3	#Ind	18
					%YOY	100

					Mean CPUE SE	32.43 11.25
Gila Topminnow	Charlebois Spring	10/27/2020	Minnow Trap	12	#Ind %YOY Mean CPUE SE	38 0 1.85 1.16
Gila Topminnow	Charlebois Spring	10/27/2020	Dip Net	5	#Ind %YOY Mean CPUE SE	2 0 0.9 0.57
Gila Topminnow	Double R Canyon	9/15/2020	Minnow Trap	10	#Ind %YOY Mean CPUE SE	10 20 0.68 0.43
Gila Topminnow	Double R Canyon	9/15/2020	Dip Net	3	#Ind %YOY Mean CPUE SE	15 93 14.59 4.08
Gila Topminnow	Double R Canyon	9/15/2020	Seine	2	#Ind %YOY Mean CPUE SE	193 74 14.69 0.4
Gila Topminnow	Edgar Canyon	9/16/2020	Minnow Trap	7	#Ind %YOY Mean CPUE SE	1017 41 80.72 16.48
Gila Topminnow	Edgar Canyon	9/16/2020	Dip Net	20	#Ind %YOY Mean CPUE SE	96 69 17.08 5.25
Gila Topminnow	Edgar Canyon	9/16/2020	Seine	3	#Ind	159

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					%YOY Mean CPUE SE	55 7.57 4.37
Gila Topminnow	Harden Cienega Creek	6/9/2020	Minnow Trap	16	#Ind %YOY Mean CPUE SE	0 0 0
Gila Topminnow	Harden Cienega Creek	6/9/2020	Backpack Electrofisher	1	#Ind %YOY Mean CPUE SE	0 0 0
Gila Topminnow	Hidden Water Spring	11/9/2020	Minnow Trap	10	#Ind %YOY Mean CPUE SE	455 37 23.05 4.13
Gila Topminnow	Hidden Water Spring	11/9/2020	Dip Net	11	#Ind %YOY Mean CPUE SE	8 88 2.46 1.07
Gila Topminnow	Hidden Water Spring	11/9/2020	Seine	5	#Ind %YOY Mean CPUE SE	32 75 3.56 2.25
Gila Topminnow	La Barge Spring	10/27/2020	Dip Net	8	#Ind %YOY Mean CPUE SE	26 35 15.92 2.28
Gila Topminnow	Las Cienegas – Bill's Wildlife Pond	8/3/2020	Minnow Trap	5	#Ind %YOY Mean CPUE SE	3858 54 314.14 49.59

Gila Topminnow	Mud Spring - Coronado National Forest	8/4/2020	Minnow Trap	10	#Ind %YOY Mean CPUE SE	2956 72 123.87 15.7
Gila Topminnow	Peterson Ranch Pond	8/4/2020	Minnow Trap	10	#Ind %YOY Mean CPUE SE	302 83 17.85 8.25
Gila Topminnow	Rarick Canyon	10/13/2020	Minnow Trap	15	#Ind %YOY Mean CPUE SE	447 43 18.14 7.26
Gila Topminnow	Sabino Canyon	5/5/2020	Minnow Trap	10	#Ind %YOY Mean CPUE SE	0 0 0 0
Gila Topminnow	Tortilla Creek	10/29/2020	Minnow Trap	12	#Ind %YOY Mean CPUE SE	305 3 14.38 3.85
Gila Topminnow	Tortilla Creek	10/29/2020	Dip Net	9	#Ind %YOY Mean CPUE SE	37 70 18.76 4.49
Loach Minnow	Blue River	9/21/2020	Backpack Electrofisher	10	#Ind %YOY Mean CPUE SE	320 44 187.91 5.90
Roundtail Chub	Blue River	9/21/2020	Backpack Electrofisher	10	#Ind %YOY Mean CPUE	180 82 147.93

					SE	9.28
Roundtail Chub	Eagle Creek	11/2/2020	Backpack Electrofisher	6	#Ind	74
					%YOY	0
					Mean CPUE	107.04
					SE	8.36
Roundtail Chub	Harden Cienega Creek	6/9/2020	Minnow Trap	16	#Ind	14
					%YOY	0
					Mean CPUE	0.35
					SE	0.14
Roundtail Chub	Harden Cienega Creek	6/10/2020	Mini-Hoop Net	12	#Ind	56
	-		•		%YOY	0
					Mean CPUE	1.21
					SE	0.26
Roundtail Chub	Rarick Canyon	10/13/2020	Minnow Trap	15	#Ind	2
	·		·		%YOY	0
					Mean CPUE	0.05
					SE	0.04
Roundtail Chub	Rarick Canyon	10/13/2020	Mini-Hoop Net	10	#Ind	4
	•		•		%YOY	0
					Mean CPUE	0.17
					SE	0.07
Roundtail Chub	Rarick Canyon	10/13/2020	Seine	6	#Ind	17
					%YOY	0
					Mean CPUE	0.57
					SE	0.37
Roundtail Chub	Sabino Canyon	5/5/2020	Mini-Hoop Net	9	#Ind	13
	,		1	-	%YOY	0
					Mean CPUE	1.22
					SE	0.24
Roundtail Chub	Sabino Canyon	5/5/2020	Seine	5	#Ind	2
220 Marwii Ciido	zaeme ean, en	0,0,2020	S VIII V	ŭ	%YOY	100
					, 01 01	100

					Mean CPUE SE	0.03 0.03
Roundtail Chub	Las Cienegas – Spring Water Wetland	8/3/2020	Mini-Hoop Net	10	#Ind %YOY Mean CPUE SE	1 0 0.05 0.05
Spikedace	Blue River	9/21/2020	Backpack Electrofisher	10	#Ind %YOY Mean CPUE SE	117 67 68.89 4.08
Spikedace	Spring Creek	9/9/2020	Backpack Electrofisher	3	#Ind %YOY Mean CPUE SE	17 0 45.66 9.25

Appendix 3.—Populations of Threatened and Endangered species repatriated under the Gila River Basin Native Fishes Conservation Program from 2007 through 2020. 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. Populations on private property are not listed consistent with ARS 17-495.

C	M-4 1-4:	Linner	Deutinstad Leasting	Year	Population
Species Gila Topminnow	Metapopulation Bylas Springs	Lineage Bylas Springs	Replicated Locations Bass Canyon (Muleshoe Ranch CMA)	Replicated 2014-2018	Status/Size TBD
Ona Tophiliniow	Dylas Springs	Dylas Springs	• • •		
			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	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
			Wildcat Canyon (Muleshoe Ranch CMA)	2014	500-999
	Upper Santa Cruz	Sharp Spring	Buckhorn Spring	2011	500-999
			Black Canyon City Heritage Pond	2018	TBD
			Chalky Spring	2009	Failed
			Fossil Creek	2007-2010	500-999
			Morgan City Wash	2009	500-999
			Mud Spring (Coronado NF)	2018	TBD

Species	Metapopulation	Lineage	Replicated Locations	Year Replicated	Population Status/Size
1	1 1		Page Springs Hatchery SRP Topminnow Pond	2009	100-499
			Robbins Butte Stop Sign Tank	2015	1000-4999
			Robbins Butte Swimming Pool Tank	2015	1000-4999
			Peterson Ranch Pond	2018	TBD
			West Fork Pinto Creek	2017	TBD
	Lower Santa Cruz	Peck Canyon	Hidden Water Spring	2016	500-999
			Phoenix Zoo Ranarium	2012	1000-4999
			Rock Spring	2013-2014	Failed
			Sheepshead Canyon	2014-2016	1000-4999
			Spring Creek	2015-2016	1000-4999
			Tortilla Creek (upper)	2017-2020	TBD
		Redrock Canyon	Arnett Creek	2017	TBD
			Edgar Canyon	2019	TBD
			Rarick Canyon	2020	TBD
			Walnut Spring (#392)	2012-2013	500-1000
	Monkey&Cottonwood	Cottonwood Spr	Ben Spring (San Pedro Riparian NCA)	2011	Failed
			Horse Thief Draw (San Pedro Riparian NCA)	2011	Failed
			Pemberton Pond (McDowell Mountain Reg. Park)	2009	Failed
			Usery Mountain Regional Park pond	2011	TBD
		Monkey Spring	Cottonwood Spring (Goldfield Mountains)	2008, 2019	TBD
			Mud Spring (#18) augmentation	2008	100-499
			Spur Cross Ranch Cons. Area Solar Oasis Pond	2009	Failed
			Willow Spring (White Tank Mountain Reg. Park)	2009	Failed
	Cienega Creek	Cienega Creek	Bill's Wildlife Pond (Las Cienegas NCA)	2016-2018	TBD
			Clyne Tank (Las Cienegas NCA)	2015-2016	500-999
			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

Species	Metapopulation	Lineage	Replicated Locations	Year Replicated	Population Status/Size
•	1 1		Little Nogales Spring (Las Cienegas NCA)	2012	Failed
			Mattie Canyon	2020	TBD
			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	500-999
	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
			Larry & Charlie Tank (Muleshoe Ranch CMA)	2009	100-499
			Little Joe Spring (San Pedro Riparian NCA)	2013	500-999
			Mint Spring (Muleshoe Ranch CMA)	2015-2016	100-499
			Mud Spring (#18)	2007-2009	100-499
			Murray Spring (San Pedro Riparian NCA)	2011-2014	Failed
			Nursery Tank (McDowell Mnt. Regional Park)	2010, 2019	TBD
			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 Tule Creek 2007-2009 Failed Tule Creek 2007-2009 Failed Walnut Spring (#20) 2008 Failed Canyon Hassayampa Arnett Creek 2007 2098 1000-4999 River Hassayampa Arnett Creek 2007 0-99 River Hassayampa 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 Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Fossil Creek Bue River Bonita Creek (lower) 2008 Failed Bue River Bonita Creek (lower) 2008 Failed Bonita Creek (upper) 2009-2020 TBD Campbell Blue Creek Spring Creek 2007-2020 1000-4999 Spikedace Aravaipa Creek Fossil Creek 2007-2010 TBD Spikedace Aravaipa Creek Fossil Creek 2007-2020 1000-4999 Spikedace	Species	Metapopulation	Lineage	Replicated Locations	Year Replicated	Population Status/Size
Robbins Butte Wildlife Area Twin Tanks 2009 1000-4999 1000	1	1 1	<u> </u>			500-999
Secret Spring (Muleshoe Ranch CMA)				Robbins Butte Wildlife Area Cottonwood Tank	2010	1000-4999
Spur Cross Ranch Cons. Area Solar Oasis pond 2009 500-999				Robbins Butte Wildlife Area Twin Tanks	2009	1000-4999
Swamp Spring (Muleshoe Ranch CMA) 2007 Failed Tule Creek 2007-2009 Failed Swalnut Spring (#20) 2008 1000-4999 10000-4999 10000-4999 1000000000000000000000000000000				Secret Spring (Muleshoe Ranch CMA)	2007-2011	100-499
Tule Creek 2007-2009 Failed Valnut Spring (#20) 2008 Failed Valnut Spring (#20) 2008 Failed Valnut Spring (#20) 2008 7000-4999				Spur Cross Ranch Cons. Area Solar Oasis pond	2009	500-999
Mainut Spring (#20) 2008 Failed Canyon 2008 1000-4999 10000-4999 10000-4999 100000-4999 1000000000000000000000000000000000				Swamp Spring (Muleshoe Ranch CMA)	2007	Failed
Coal Mine Fresno Canyon 2008 1000-4999 10000-4999 10000-4999 10000-4999 10000-4999 10000-4999				Tule Creek	2007-2009	Failed
Canyon				Walnut Spring (#20)	2008	Failed
River Hassayampa Telegraph Canyon 2007 100-499 River 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 Coach Minnow Aravaipa Creek Hot Springs Canyon (Muleshoe Ranch CMA) 2007-2020 100-499 Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Fossil Creek 2007-2013 Failed Fossil Creek 2007-2013 Failed Fossil Creek (lower) 2008 Failed Bonita Creek (lupper) 2009-2020 TBD Campbell Blue Creek 2007-2020 1000-4999 Spikedace Aravaipa Creek Fossil Creek 2007-2020 1000-4999 Spring Creek 2015-2020 TBD Hot Springs Canyon (Muleshoe Ranch CMA) 2007-2020 0-99 Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Hot Springs Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Upper Gila River Blue River (lower) 2012, 2015 5000-9999 Blue River (middle) 2017-2018 TBD	Longfin Dace			Fresno Canyon	2008	1000-4999
River Hidden Water Spr Rock Creek 2016 Failed Seven Sprs Wash Spur Cross Ranch Cons. Area Solar Oasis pond 2008 0-99 1000-4999 1			• •	Arnett Creek	2007	0-99
Seven Sprs Wash Spur Cross Ranch Cons. Area Solar Oasis pond 2008 0-99			• 1	Telegraph Canyon	2007	100-499
Tangle Creek Fossil Creek Springs Canyon (Muleshoe Ranch CMA) 2007-2020 1000-4999			Hidden Water Spr	Rock Creek	2016	Failed
Aravaipa Creek Hot Springs Canyon (Muleshoe Ranch CMA) 2007-2020 100-499 Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Fossil Creek 2007-2013 Failed Fossil Creek Bonita Creek (lower) 2008 Failed Bonita Creek (upper) 2009-2020 TBD Campbell Blue Creek 2020 1000-4999 Spikedace Aravaipa Creek Fossil Creek 2007-2020 1000-4999 Spring Creek 2015-2020 TBD Hot Springs Canyon (Muleshoe Ranch CMA) 2007-2020 0-99 Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Upper Gila River Blue River (lower) 2012, 2015 5000-9999 Blue River (middle) 2017-2018 TBD			Seven Sprs Wash	Spur Cross Ranch Cons. Area Solar Oasis pond	2008	0-99
Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Fossil Creek 2007-2013 Failed Blue River Bonita Creek (lower) 2008 Failed Bonita Creek (upper) 2009-2020 TBD Campbell Blue Creek 2020 1000-4999			Tangle Creek	Fossil Creek	2008-2009	1000-4999
Fossil Creek 2007-2013 Failed Bonita Creek (lower) 2008 Failed Bonita Creek (upper) 2009-2020 TBD Campbell Blue Creek 2020 1000-4999	Loach Minnow		Aravaipa Creek	Hot Springs Canyon (Muleshoe Ranch CMA)	2007-2020	100-499
Blue River Bonita Creek (lower) 2008 Failed				Redfield Canyon (Muleshoe Ranch CMA)	2007-2010	Failed
Bonita Creek (upper) 2009-2020 TBD				Fossil Creek	2007-2013	Failed
Campbell Blue Creek 2020 1000-4999 Spikedace			Blue River	Bonita Creek (lower)	2008	Failed
Aravaipa Creek Fossil Creek 2007-2020 1000-4999 Spring Creek 2015-2020 TBD Hot Springs Canyon (Muleshoe Ranch CMA) 2007-2020 0-99 Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Upper Gila River Blue River (lower) 2012, 2015 5000-9999 Blue River (middle) 2017-2018 TBD				Bonita Creek (upper)	2009-2020	TBD
Spring Creek 2015-2020 TBD Hot Springs Canyon (Muleshoe Ranch CMA) 2007-2020 0-99 Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Upper Gila River Blue River (lower) 2012, 2015 5000-9999 Blue River (middle) 2017-2018 TBD				Campbell Blue Creek	2020	1000-4999
Hot Springs Canyon (Muleshoe Ranch CMA) 2007-2020 0-99 Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Upper Gila River Blue River (lower) 2012, 2015 5000-9999 Blue River (middle) 2017-2018 TBD	Spikedace		Aravaipa Creek	Fossil Creek	2007-2020	1000-4999
Redfield Canyon (Muleshoe Ranch CMA) 2007-2010 Failed Upper Gila River Blue River (lower) 2012, 2015 5000-9999 Blue River (middle) 2017-2018 TBD				Spring Creek	2015-2020	TBD
Upper Gila River Blue River (lower) 2012, 2015 5000-9999 Blue River (middle) 2017-2018 TBD				Hot Springs Canyon (Muleshoe Ranch CMA)	2007-2020	0-99
Blue River (middle) 2017-2018 TBD				Redfield Canyon (Muleshoe Ranch CMA)	2007-2010	Failed
			Upper Gila River	Blue River (lower)	2012, 2015	5000-9999
Blue River (upper) 2020 TBD				Blue River (middle)	2017-2018	TBD
				Blue River (upper)	2020	TBD

Species	Metapopulation	Lineage	Replicated Locations	Year Replicated	Population Status/Size
1	1 1	<u> </u>	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
		Sabino Canyon ¹	Sabino Canyon (upper)	2019	TBD
		Sabino Canyon ¹	Romero Canyon ³	2019	100-499
		Dix Creek ¹	Redrock Wildlife Area, NM	2010-2011	Failed
		Eagle Creek	Blue River (lower)	2012-2016	1000-4999
		Eagle Creek	Blue River (middle)	2017, 2019	TBD
		Eagle Creek	Blue River (upper)	2020	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	2019-2020	TBD
Razorback Sucker		Lake Mohave	Fossil Creek	2008-2014	Failed

¹ Chub in these locations were previously classified as Gila Chub.
² An augmentation stocking because Loach Minnow already existed in Campbell Blue Creek.
³ An augmentation stocking because chub already existed in Romero Canyon.