Gila River Basin Native Fishes Conservation Program:
Arizona Game and Fish Department's Native Fish Conservation Efforts During 2022

Cooperative Agreement R22AC00159
Between Bureau of Reclamation and Arizona Game and Fish Department
Annual Report
January 20, 2023

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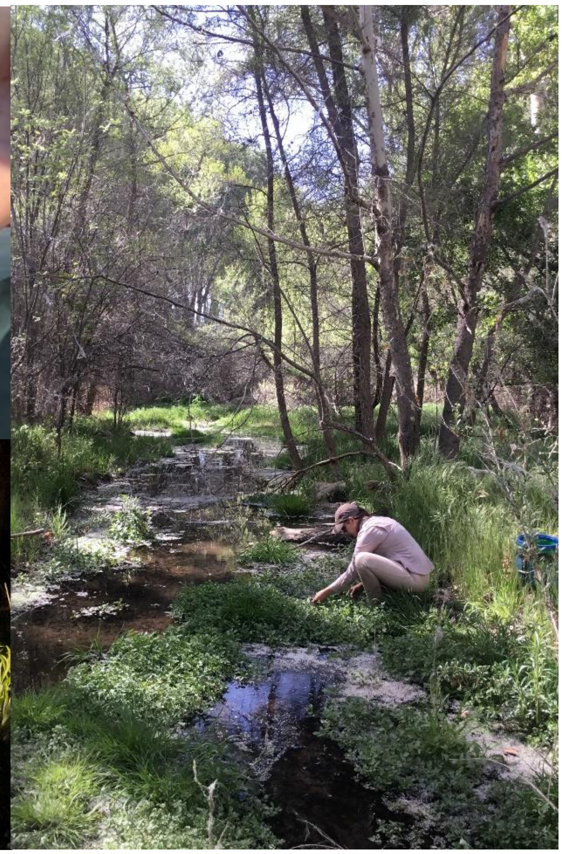

GILA RIVER BASIN NATIVE FISHES CONSERVATION PROGRAM

Program Cooperators:

Arizona Game and Fish Department Mission

To conserve, enhance, and restore Arizona's diverse wildlife resources and habitats through aggressive protection and management programs, and to provide wildlife resources and safe watercraft and off-highway vehicle recreation for the enjoyment, appreciation, and use by present and future generations.

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Acknowledgements

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Contents

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 administration of Program funding in 2015. The Department and Reclamation finalized an initial agreement (R16AC00077) in 2016, with the present agreement (R22AC00159) initiated in April, 2022. 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 finalized and approved

[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.

recovery plans for four of the five priority species, and a draft recovery plan for the Gila Chub (U.S. Fish and Wildlife Service 1983, 1991a, 1991b, 1998, 2002, 2015). Reports detailing work previously completed by the Department with Program funding from 2007-2021 are listed in Appendix 1.

In addition to the fish and amphibian species specified above, other species mentioned in this report include: Desert Pupfish *Cyprinodon macularius,* Desert Sucker *Catostomus clarkii*, Longfin Dace *Agosia chrysogaster,* Roundtail Chub *Gila robusta,* Sonora Sucker *Catostomus insignis*, Speckled Dace *Rhinichthys osculus*, Woundfin *Plagopterus argentissimus*, Black Bullhead *Ameiurus melas*, Black Crappie *Pomoxis nigromaculatus*, Bluegill *Lepomis macrochirus*, Brown Trout *Salmo trutta*, Common Carp *Cyprinus carpio*, Fathead Minnow *Pimephales promelas*, Green Sunfish *Lepomis cyanellus*, Largemouth Bass *Micropterus salmoides*, Red Shiner *Cyprinella lutrensis* Smallmouth Bass *Micropterus dolomieu*, Western Mosquitofish *Gambusia affinis,* and Yellow Bullhead *Ameiurus natalis*. Other aquatic species mentioned include lowland leopard frog *Lithobates yavapaiensis*, northern crayfish *Faxonius virilis*, and Northern Mexican gartersnake *Thamnophis eques*.

This report summarizes Program work performed by the Department during 2022. For each priority action, work completed during 2022 is presented, followed by recommendations.

PERFORMANCE MEASURES

Cooperative Agreement R22AC00159 between Reclamation and the Department specified the following performance measures.

1. Complete at least three repatriation stockings per calendar year under this agreement, with an overall goal to establish Gila Topminnow into a minimum of 10 surface waters, Loach Minnow into a minimum of 1 surface water, and Spikedace into a minimum of 1 surface water within the five-year performance period.

   Results: During 2022 Department staff completed repatriation stockings into five waters: Spring Creek (Spikedace), Blue River (Spikedace), Unnamed Drainage #68B (Gila Topminnow), Aravaipa Creek (Gila Topminnow), and Sharp Spring (Gila Topminnow, Appendix 2).

2. Control or eradicate non-native fish in at least one surface water per calendar year under this agreement, with an overall goal to eradicate or control non-native fish at a minimum of five unique surface waters within the five-year performance period.

   Results: During 2022, Department staff eradicated nonnative fish from Sharp Spring, and controlled nonnative fish at Harden Cienega Creek and Redfield Canyon.

3. Maintain and propagate populations of Loach Minnow, Spikedace and other rare species at the ARCC (formerly the Bubbling Ponds Native Fishes Conservation Facility) throughout the five-year performance period unless stakeholders determine that there is no longer a need for captive refuge and propagation at the facility. Also annually acquire Spikedace, Loach Minnow, and other rare species from the wild to maintain broodstocks of each lineage at ARCC.

   Results: During 2022, Department staff maintained and propagated three lineages of Spikedace (Aravaipa Creek, upper Gila River, and Gila Forks), five lineages of Loach Minnow (Blue River, Aravaipa Creek, San Francisco River, Bear Creek, and Gila Forks) one lineage of Roundtail Chub (Eagle Creek), and one putative lineage of Gila Topminnow (Parker Canyon).

GENERAL ACTIVITIES

Department staff coordinated with Reclamation and compiled information necessary to initiate a new cooperative agreement. Department staff administered and managed Program projects identified in the agreement. The program specialist promoted to the vacant program coordinator position and hired a replacement specialist and seasonal staff. Department staff finalized the 2021 annual report, began analyzing data and drafting the 2022 annual report, revised the FY23 workplan and drafted the FY24 work plan. Staff coordinated with intra-agency staff, other agencies, and private landowners to continue work on existing projects and to develop potential new projects. Staff revised and updated electronic data entry forms and corresponding formatting and summary scripts, entered data into survey and stocking datasets, and checked data for accuracy. Staff purchased and maintained vehicles and equipment as necessary to carry out fieldwork obligations. Staff also completed mandatory employee training.

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 translocations 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 select waters inhabited by target lineages using gear appropriate for the given water; typically 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 translocation site, 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 are 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 include: site name, date, time of arrival and stocking, participants, type of transport container, water quality in the tanks and site, counts of individuals stocked, condition of fish, fish behavior after release, and number of mortalities.

Fish Surveys: Backpack electrofishing was used at 100 m sub-reaches to survey translocated populations of Spikedace, Loach Minnow, and Roundtail Chub[2], and to assess habitats for fish translocations. The number of sub-reaches sampled was determined by length of target reach, with a minimum of three sub-reaches for short reaches and a goal of at least 10% of the reach length in longer streams (e.g., there are fifteen 100 m sub-reaches in the 14.6 km of the upper Blue River). A backpack electrofisher (Smith-Root; Model 12-B, LR24) was used to electrofish upstream through each sub-reach in a single pass. Three-pass depletion was carried out between two block nets at select fixed sub-reaches to estimate abundance and capture probability. 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 sub-reach, fish were processed and data were recorded. Captured fish were identified to species and counted. All Spikedace, Loach Minnow, and Roundtail Chub 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., *Catostomus sp.*). After processing, fish were released alive just downstream from where they

[2] Including chub populations previously classified as Gila Chub.

were captured. Fish were released a short distance below the downstream block net during depletion efforts. Data recorded for each sampling effort included: site name, site location (UTM NAD83), 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 Chub[3] 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 Chub monitoring. Typically, a minimum of 10 traps were set in each location 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 includes: 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, and counts of individuals within each species-size-class category.

For stock tank surveys, a bag seine was hauled across each tank for a minimum of three passes (unless the entire tank could be seined in one or two hauls, or the tank was too shallow to use a seine). Straight seine hauls or dip net sweeps were used in stock tanks too shallow for a bag seine.

Evaluation of Species Establishment: The goal of translocation efforts is to establish populations of Spikedace, Loach Minnow, Gila Topminnow, and Roundtail Chub to contribute to recovery of these species. A species is considered to have established (a successful translocation) 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 1991a) 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 translocated 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 translocated fish species and nonnative fish species;
2. evaluate trends in relative abundance (estimated as catch-per-unit effort) of the translocated species, sympatric native fishes, and nonnative fishes;
3. evaluate size-structure of each population of fish species to detect reproduction and recruitment to the population;
4. determine if translocated species have dispersed from the stocking area.

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[3] Including chub populations previously classified as Gila Chub.

Presence of individuals during post-stocking monitoring is evidence that the species has persisted. 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, sites are monitored for several years to determine whether or not the translocated species has established a population. The duration of monitoring varies by target species, and generally exceeds 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 are used for Gila Topminnow and Desert Pupfish. Spikedace and Loach Minnow have a longer potential lifespan (three to four years), and five years of post-stocking monitoring should be sufficient to determine if the species has established a population. Roundtail Chub[4] typically live about seven years. However, a yearly examination of size structure for five years after stocking is likely sufficient to determine if Roundtail Chub are established. Translocated 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. A variety of traps were used, depending on habitat size: mini-hoop nets (Promar® TR-502 collapsible traps; cylindrical, 0.85 m long x 0.3 m wide, with 9 mm mesh) and minnow traps (Promar® collapsible minnow traps; 0.46 m long x 0.3 m wide, with 2 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 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. Nonnative fish are typically measured to the nearest millimeter in total length (mm TL) to assess size structure of the target population.

Evaluation of Nonnative Fish Removal: There are two general goals for nonnative fish removals: suppression or eradication. For situations where barriers to nonnative fish invasion do not exist, the goal is to suppress nonnative populations until barriers can be installed. When barriers to nonnative fish invasion are in place, the goal is eradication. Multiple removals are conducted until

[4] Including chub populations previously classified as Gila Chub.

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 target nonnative fishes confirmed by eDNA sampling is evidence of eradication.

Statistical Analyses:

Relative abundance of select fish species in terms of catch per unit effort (CPUE) was calculated as fish per hour of electrofishing effort or soak time of passive gears (i.e., minnow trap, hoop net, gill nets), or fish per square meter sampled for active gears (i.e., dip net, straight seine). Abundance of all fish species encountered at sub-reaches where depletion sampling occurred was estimated via the Carle-Strub method using the "removal" function in the *FSA* package (Carle and Strub 1978, Ogle 2021). Differences in mean relative abundance of native fish between years were evaluated using multiple tests, depending on how data were distributed. The Shapiro-Wilk test of normality (*stats* package) was conducted to determine whether data were normally distributed. A two-sample t-test was used for normally distributed data (*stats* package). The non-parametric Wilcoxon rank-sum test was used for non-normal distributed data (*stats* package). Two-sided tests were used for taxa where there was not an obvious visual change in mean relative abundance between years, and one-sided tests were used for taxa where mean relative abundance visually differed between 2021 to 2022. Pearson's product-moment correlation coefficient was used to evaluate trends in mean relative abundance between species pairs across years (*stats* package). All statistical analyses were conducted within the statistical Program R (R Development Core Team, 2021).

Muleshoe ecosystem stream and spring repatriations (Task AZ-2003-1)
Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
  - Goal 3. Remove nonnative aquatic species threats.
  - Goal 4. Replicate populations and their associated native fish community into protected streams and other surface waters.
  - Goal 7. Monitor to quantitatively measure and evaluate project success in improving the status of target species and their habitats.

Recovery Objectives:

- Spikedace recovery objective 6.4. Monitor success/failure of reintroductions.
- Loach Minnow recovery objective 6.4. Monitor success/failure of reintroductions.
- 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.
- Gila Chub draft recovery plan objective 1.3.1. Eliminate or control problematic nonnative aquatic organisms
- Gila Chub draft recovery plan objective 2. Ensure representation, resiliency, and redundancy by expanding the size and number of populations within Gila Chub historical range via replication of remnant populations within each RU.
- Gila Chub draft recovery plan objective 7. Monitor remnant, repatriated, and refuge populations to inform adaptive management strategies.

Background: The purpose of this action is to establish Spikedace, Loach Minnow, Gila Topminnow, and Desert Pupfish into various waters on the Muleshoe Ranch Cooperative Management Area (CMA). The Muleshoe Ranch CMA is located on the western slopes of the Winchester and Galiuro mountains. The various waters and stream reaches previously included in this subproject are described in 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 Spikedace and Loach Minnow failed to establish in Redfield Canyon. However, Gila Topminnow have dispersed downstream from Swamp Springs Canyon and are now established in Redfield Canyon. In 2007, Roundtail Chub[5], Sonora Sucker, and Speckled Dace were translocated upstream of a waterfall in Redfield Canyon to expand their range in the system.

Green Sunfish control in Redfield Canyon started in 2007 and has continued every year since. The 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.

[5] Chub in Redfield Canyon were previously classified as Gila Chub.

Results:

Nonnative Control. During May 2-3, 2022, Department staff completed a Green Sunfish removal in Redfield Canyon. The crew backpack electrofished Reaches 1 and 2, from the confluence with Swamp Springs Canyon (UTM 12S 563324/3588995) upstream to the waterfall barrier (563872/3589779; Figure 1). The crew electrofished for a total of 4,782 seconds and captured four Green Sunfish (Figure 2). Native fish were not counted. Removal efforts occurred only during May because staff time was fully allocated to the Sharp Spring project in June. The crew also set five mini-hoop nets in pools that have previously harbored the majority of the Green Sunfish in Reach 1. A total of nine Roundtail Chub[5], six Sonora Sucker, and two Green Sunfish were captured in mini-hoop nets.

Department staff also completed two removal passes in Reach 3. The crew set 10 mini-hoop nets for two consecutive two hour sets in the pools near the wilderness boundary. A total of 109 Green Sunfish were captured (74-190 mm TL) with 50 captured during the first set and 59 captured during the second set (Figure 2, Figure 3).

Overall, a total of 115 Green Sunfish were removed from Redfield Canyon in 2022 (Figure 2). Green Sunfish reemerged in Reaches 1 and 2 after not being captured in 2021. It is unclear whether the absence of Green Sunfish last year can be attributed to the increased removal efforts in past years or the lack of sufficient flow for dispersal. Although the total catch in Reach 3 declined this year relative to both trips in 2021 (n=170, 136), mean mini-hoop net catch per unit effort (6.00 fish/h) increased relative to 2021 (5.17 fish/h). Although numbers appear to be marginally declining within this reach, mini-hoop net catch per unit effort has increased in past years which makes it difficult to assess the efficacy of removal efforts in this reach.

Recommendations: Department staff will continue to contact the downstream private landowners in Redfield Canyon and attempt to gain permission to access the property and remove sunfish from Reach 3. If permission is granted, the goal of Green Sunfish removal efforts should shift from suppression 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 Reaches 1 and 2 and should be continued until the status of the downstream population changes.

Tables and Figures:

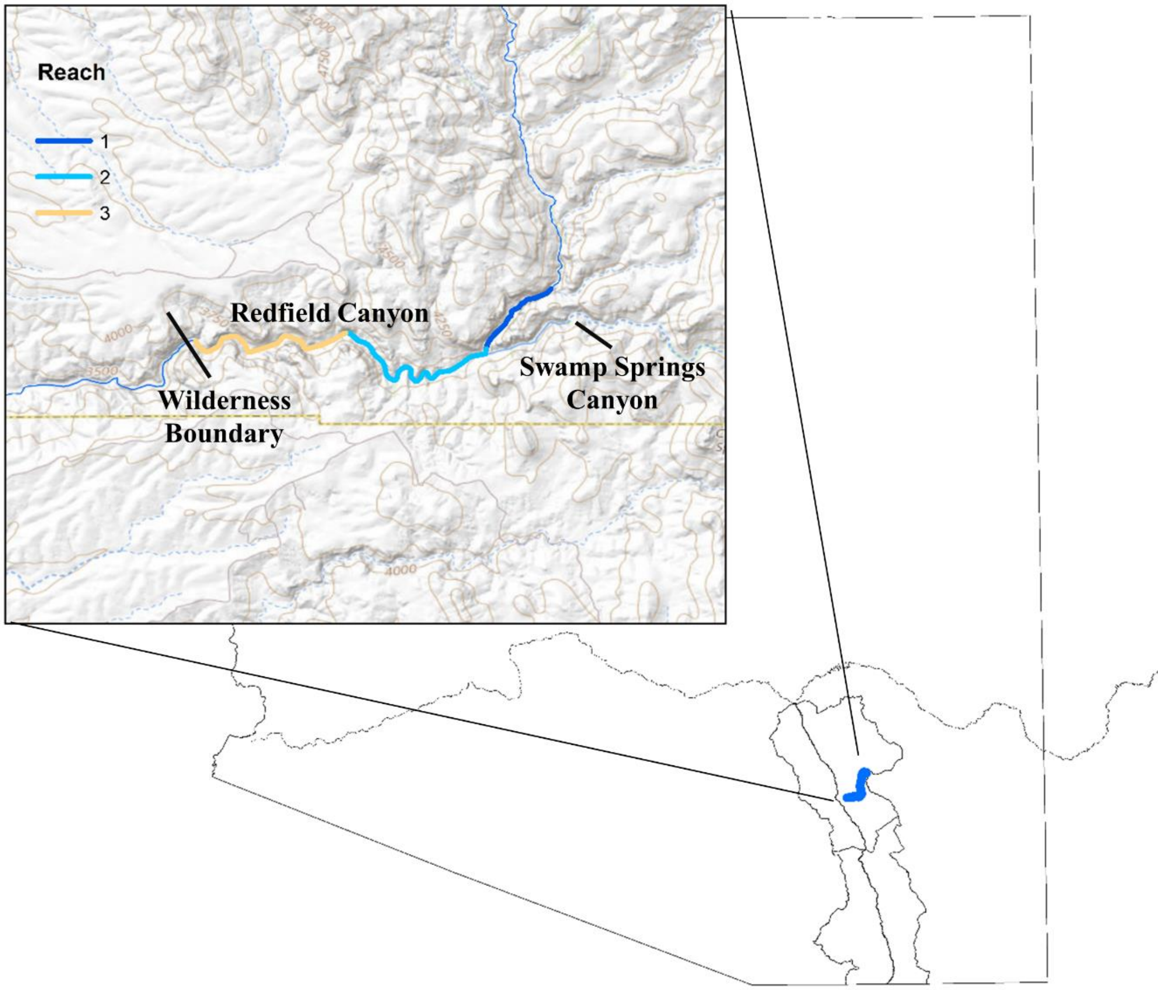

Figure 1.—Location of Redfield Canyon within the Gila River Basin and San Pedro River sub-basin. Inset map shows the location of sampling Reaches 1 (Swamp Springs Confluence upstream to Barrier), 2 (Rock House tributary upstream to Swamp Springs Confluence), and 3 (Wilderness Boundary upstream to Rock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

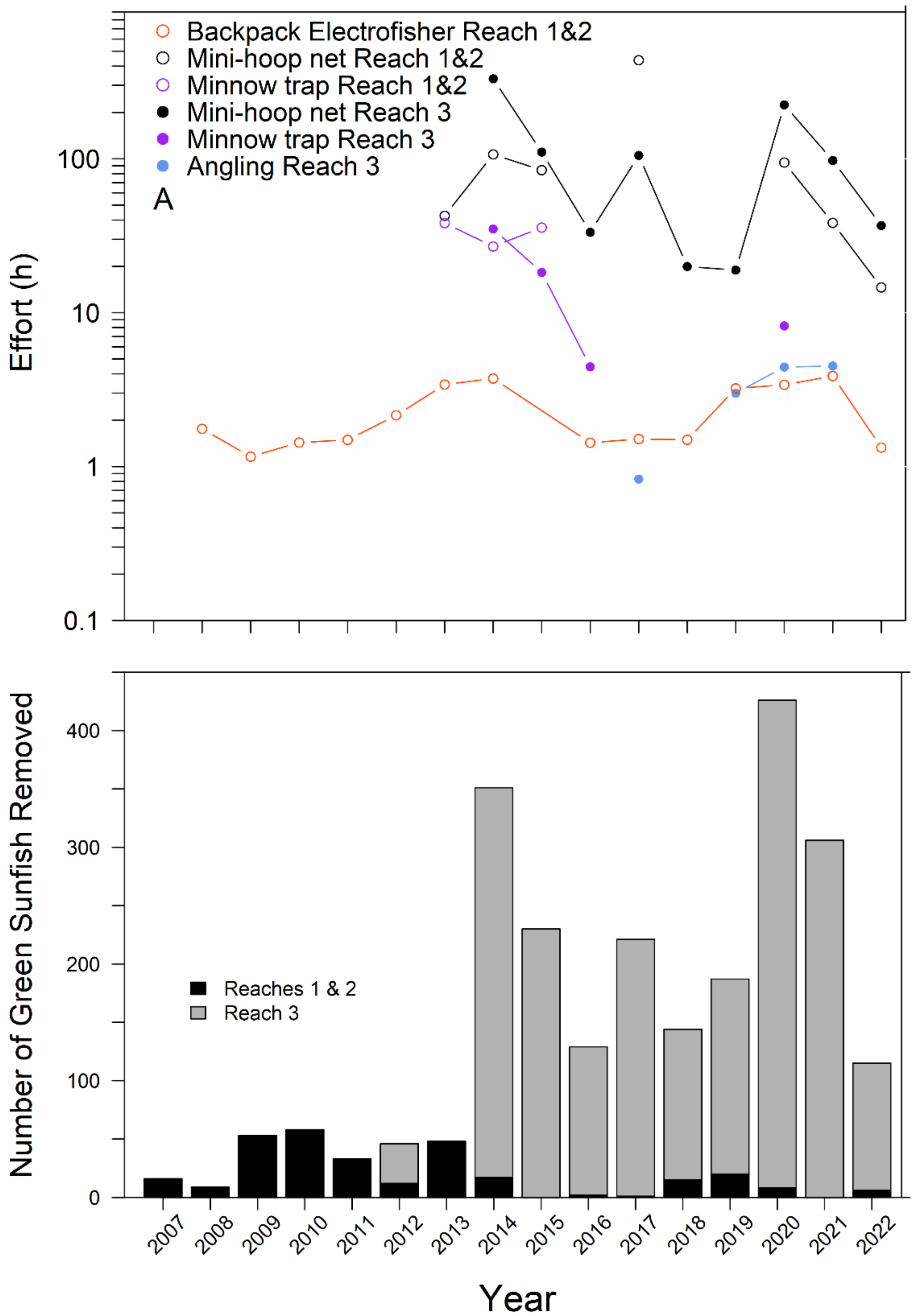

Figure 2.—Hours of removal effort by gear type and reach (A) and number of Green Sunfish removed during annual spring removal efforts and autumn monitoring from three reaches of Redfield Canyon, Arizona during 2007-2022 (B). Effort was not recorded for removals in 2007. Location and description of reaches within Redfield Canyon shown in Figure 1.

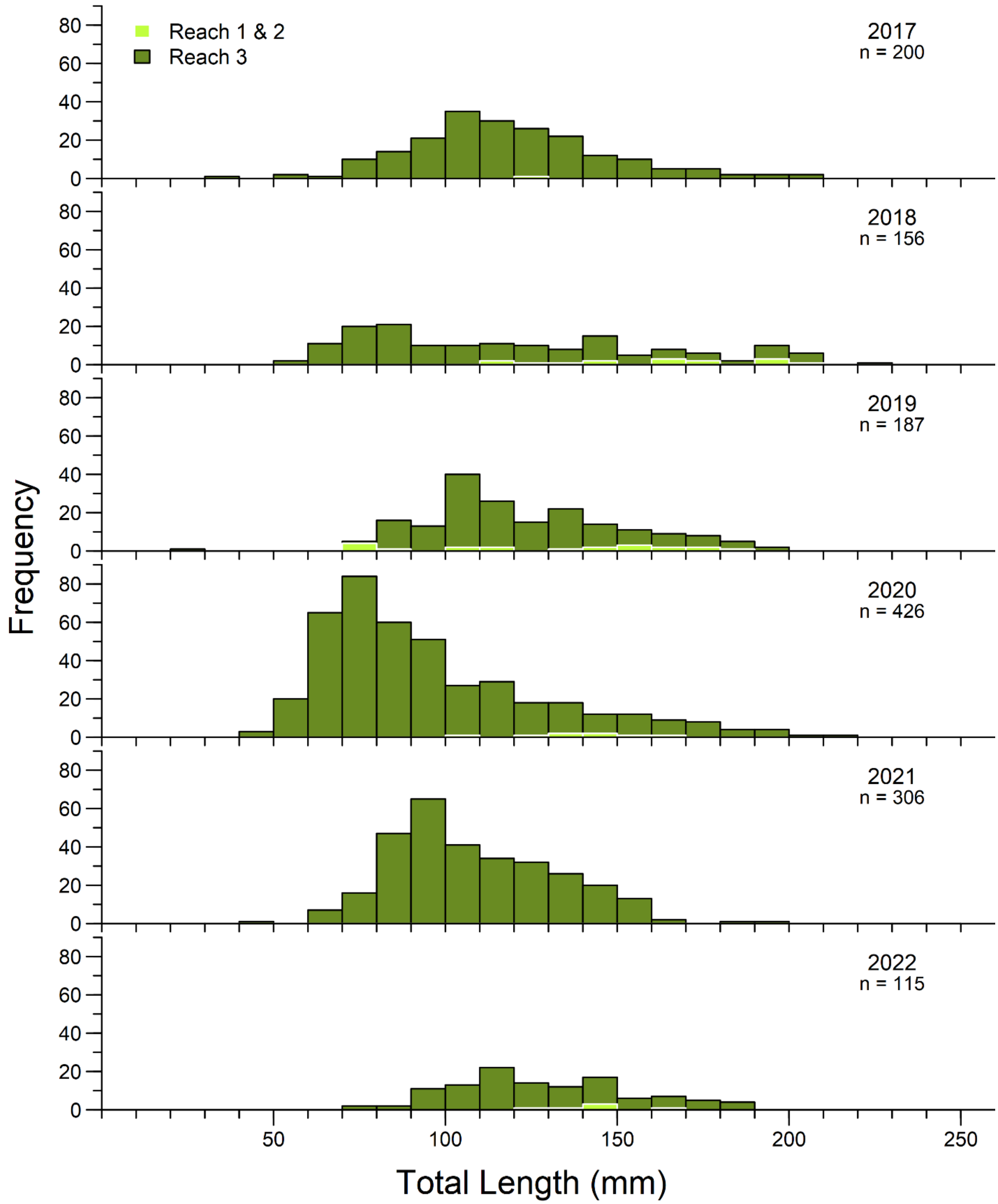

Figure 3.—Length frequency distribution of the number of Green Sunfish captured by reach during removal efforts and annual monitoring in Redfield Canyon, 2017 through 2022. Number of fish captured and measured each year is shown in the top right corner of each panel.

Gila Topminnow stockings (Task AZ-2002-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
    - Goal 4. Replicate populations and their associated native fish community into protected streams and other surface waters.
    - Goal 7. 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.
- 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.

Overall Background: The purpose of this action is to establish Gila Topminnow populations within the historic range of the species throughout the Gila River Basin in Arizona. Populations of Roundtail Chub[6] may also be established through this project. The Department coordinates with USFWS to determine stocking locations and appropriate donor locations and lineages. The strategy is to stock at least 500 Gila Topminnow initially or for any subsequent augmentations to establish a population. Populations are typically augmented if fewer than 100 fish are captured or observed during monitoring. After stocking, the populations are monitored at 6-months and then annually thereafter for three years after the last stocking event. If a population is considered established after the third post-stocking monitoring, the augmentation and monitoring responsibilities are passed on to other Department programs. Monitoring responsibilities may also be passed along to other agencies. Monitoring techniques are consistent from year to year for a given site, and usually involve setting a minimum of 10 baited minnow trap sets per site, often supplemented with dip netting or seining if habitat conditions allow. Background, results, and recommendations are presented separately for each site.

Fish Health Assessments During 2022:

[6] Populations of Roundtail Chub addressed by this project were formerly classified as Gila Chub.

Cold Spring. On March 14, 2022, Department staff collected 30 Gila Topminnow (Monkey Spring lineage) from Cold Spring for a fish health assessment. The fish were captured in 11 dip net sweeps. All fish were transported back to the fish health laboratory at Department headquarters. Subsequent analyses determined the fish were free of parasites or pathogens.

Walnut Spring #20. On February 28, 2022, Department staff collected 30 Gila Topminnow (Monkey Spring lineage) from Walnut Spring #20 for a fish health assessment. The fish were captured in three dip net sweeps. All fish were transported back to the fish health laboratory at Department headquarters. Subsequent analyses determined the fish were free of parasites or pathogens.

<u>Sites Monitored or Stocked During 2022</u>:
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 3.

Aravaipa Creek
Background: Aravaipa Creek is a tributary to the San Pedro River about 17 km south of the confluence of the Gila and San Pedro Rivers. It drains the east and north end of the Galuiro Mountains, the southwest portion of the Pinalenos, and the southern portion of the Santa Teresa Mountains. The creek becomes perennial at Aravaipa Spring near Stowe Gulch and flows west to the San Pedro River approximately 35 km. There are two constructed fish barriers (Reclamation funded) at the west end of the creek that prevent upstream movement of nonnative fishes. However, nonnative Green Sunfish, Yellow Bullhead, and Red Shiner were present in the creek before the barriers were constructed. Ongoing nonnative removals led by BLM have largely eliminated Green Sunfish, but Yellow Bullhead were still common as of April 2021. Topminnow did not previously exist in the stream, although there was an attempt to establish populations in 1969 and 1977 (Weedman 1999). In 2021, TNC staff recommended translocating Gila Topminnow into a spring-fed side channel of Aravaipa Creek that had not experienced flooding since 2006.

Results: On April 12, 2022, Department staff stocked 484 Bylas lineage Gila Topminnow into a spring-fed side channel of Aravaipa Creek on TNC property. A total of 503 topminnow were collected from the donor location and transported to Aravaipa Creek in aerated coolers, with 19 mortalities during transport. The majority of fish (n = 363) were stocked in the largest upstream pool while the remaining fish (n = 121) were stocked in another pool approximately 100 m downstream.

On September 30, 2022, Department staff completed the first post-stocking monitoring effort. A total of 10 minnow traps were set in pool habitats near the stocking location, and additional eight dip net sweeps and six seine hauls were conducted throughout the side channel while nets were fishing. A total of 98 Gila Topminnow (28 <20 mm TL, 70 ≥20 mm TL) were captured across all sampling techniques (63 captured seining, 23 with minnow traps, 12 with dip net sweeps).

Topminnow dispersed both upstream and downstream from the initial stocking location, now occupying the majority of the side-channel (Figure 4). Further, an individual topminnow was captured in the mainstem of Aravaipa Creek in the "Bunkhouse Reach" (~700 m downstream of the initial stocking location) during the University of Arizona annual sampling event concurrent to our monitoring.

Recommendations: Between the time of stocking and the initial monitoring, Aravaipa Creek experienced a large flooding event which impacted the side channel for the first time since 2006 (Figure 5). The persistence and recent reproduction of this new population suggests that establishment may occur even following an extraordinary flood event. Further, it appears this species has increased their distribution throughout the spring reach where it was initially stocked, and has attempted to make forays into the mainstem, albeit in low numbers. Further monitoring of this population will continue in future years and further augmentation efforts may occur if necessary.

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 furthest downstream perennial reach is located on Pima County lands approximately 5 km upstream of the confluence with the San Pedro River. This perennial reach was approximately 300 m long in September, 2019. 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). Previous monitoring efforts in September 2019 and September 2020 resulted in the capture of a total of 802 and 1,113 topminnow respectively. The Bighorn Fire burned a portion of the upper Edgar Canyon watershed in 2020. No topminnow were captured during annual monitoring in 2021 following substantial post-fire flooding within the watershed.

Results: On October 13, 2022, Department staff monitored Gila Topminnow in Edgar Canyon. The crew conducted a total of 10 dip net sweeps throughout the reach in available pool habitats. Minnow traps were not set as originally planned due to a lack of aquatic habitat with adequate depth for the traps to fish. No fish were captured or observed throughout the entirety of the reach. Several lowland leopard frogs were observed, indicating that some aquatic wildlife has been able to persist in this reach.

Substantial flooding occurred in Edgar Canyon during the 2021 monsoon season resulting in increased prevalence of fine sediments in the reach. It does not appear that flooding occurred to the same magnitude this year, although the stream appears to be actively incising through the newly deposited sediments and has become more channelized. Collectively, these changes have eliminated most pool habitat, which previously held topminnow. Although many of the pools have been inundated by sediment, at the bottom of the perennial reach a deep pool, as well as a headcut creating a small waterfall, has formed in the stream. This new pool was the deepest area of the stream observed and will be interesting to watch as it could lead to new potential habitat for fishes in the future.

Recommendations: This location possessed sufficient stream habitat conditions for interannual survival and reproduction of Gila Topminnow prior to the flooding. It may be valuable to attempt another stocking of Gila Topminnow at this location once the stream habitat is able to recover, which make take several years or longer. This location should be reevaluated within the next 3-5 years to determine whether aquatic habitat has sufficiently recovered to potentially support additional translocations of Gila Topminnow.

Las Cienegas National Conservation Area – Maternity Wildlife Pond

Background: Maternity Wildlife Pond is located in the Gardner Canyon drainage about 9.6 km upstream of the confluence with Cienega Creek. The pond was improved in 2020 which included dredging and installing a solar well to create a perennial water source for native fish and amphibians. In April 2021, Department staff translocated 248 Gila Topminnow from Empire Tank to Maternity Wildlife Pond. Initial monitoring following the stocking in August 2021 resulted in a total of 1,554 individuals being captured.

Results: On October 13, 2022, Department staff monitored the Gila Topminnow in Maternity Wildlife Pond. Ten minnow traps were set for a minimum soak time of two hours and a total of 1,591 Gila Topminnow (658 $<$20 mm TL, 933 $\geq$20 mm TL) were captured.

Recommendations: All wildlife ponds on Las Cienegas were either initially established or subsequently augmented with fish directly from Cienega Creek to ensure refuge populations are genetically representative of the relict lineage. The population should be augmented with fish directly from Cienega Creek in the future when the Cienega Creek population is sufficiently abundant to allow collection of at least 250 fish. Maternity Wildlife Pond should be monitored through at least 2023 to determine if the population will establish.

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 Chub[7] (Ehret and Dickens 2009). Gila Topminnow were initially stocked in the recreation area near 'The Crack' in 2015 and

[7] Chub stocked into Sabino Canyon were previously classified as Gila Chub.

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 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 Chub (>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 (520784/3581144). A total of 350 Gila Topminnow were successfully stocked. No Gila Topminnow were detected during monitoring in May 2020 or April 2021. A total of 15 and 10 Roundtail Chub were captured during monitoring in May 2020 and April 2021 respectively. The Bighorn Fire burned a substantial portion of the Sabino Canyon drainage in 2020.

Results: On July 12, 2022, Department staff monitored Gila Topminnow and Roundtail Chub in Sabino Canyon upstream of the East Fork of Sabino Canyon. The crew set 10 minnow traps in the vicinity of the topminnow stocking location and failed to capture or observe any topminnow. Topminnow have never been captured at this location following stockings in June 2018 and October 2019.

The crew also set 10 mini-hoop nets in the vicinity of the Roundtail Chub stocking location and captured a total of 24 chub. While the traps soaked the crew carried out six opportunistic seine hauls and captured 23 additional chub. Multiple size classes of fish were captured within the reach, which is additional evidence that fish are persisting and reproducing within the reach (Figure 6). Roundtail Chub were visually more abundant than the catch suggests. The crew visually observed an additional 88 chub while snorkeling the original stocking pool with multiple age classes present, including large adults.

Recommendations: Topminnow failed to persist at the stocking location following two translocation attempts despite a lack of a clear mechanism limiting persistence. A third and final translocation could be attempted in the absence of higher priority locations for Cienega Creek lineage Gila Topminnow. Chub populations should be monitored until at least 2024 and consideration should be given to translocating Roundtail Chub further upstream to Hutch’s Pool near West Fork Sabino Canyon to maximize the species range in the system and provide a genetic augmentation to the existing chub population which was founded with a relatively small number of individuals (<150). A hike-through survey from the pools near East Fork Sabino down to The

Crack would also be beneficial to determine if any chub or topminnow have dispersed downstream and occupied 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. 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 June 2017, Department staff stocked 548 Gila Topminnow (Peck Canyon lineage) into upper Tortilla Creek about 4.5 km upstream of the confluence with Mesquite Creek. More than 800 Gila Topminnow were captured during annual monitoring in 2017 and 2018. The Woodbury Fire began in June 2019 and burned 123,875 acres of the Superstition Mountains including the upper Tortilla Creek watershed, which caused substantial post-fire flooding. Subsequently, only 47 topminnow were captured during annual monitoring in 2019. The population was augmented with 374 topminnow in April 2020, with 322 and 2,245 topminnow captured during fall monitoring in 2020 and 2021 respectively.

Results: On October 25, 2022, Department staff monitored Gila Topminnow in Tortilla Creek near the original stocking location (UTM 12S 467239/3708608). The crew set 10 minnow traps for a minimum soak time of two hours and captured a total of 2,430 Gila Topminnow (265 <20 mm TL, 2,165 ≥ 20 mm). An additional 10 opportunistic dip net sweeps resulted in the capture of 84 Gila Topminnow. No Fathead Minnow were captured for the fourth consecutive year, suggesting the population is likely extirpated. Substantial flooding appears to have occurred within this reach within the last year, with the presence of flood debris up to 15 vertical feet above the current water level and evidence of channel reorganization throughout. Topminnow do not appear to have been negatively affected as a similar number of fish was captured in 2021 (n = 2,245).

Recommendations: The Gila Topminnow population seems to have recovered from post-fire impacts of the Woodbury Fire and demonstrated resilience to monsoon flooding in 2022. Evidence of reproduction, increasing relative abundance, and dispersal beyond the original stocking location has been consistently documented in Tortilla Creek, meeting criteria for population establishment.

Telegraph Canyon

Background: Telegraph Canyon is a tributary to Arnett Creek and drains from the north side of Picketpost Mountain. In 1992, the Department, Tonto National Forest, and USFWS identified an opportunity to reestablish a native fish community in Arnett Creek and its tributary Telegraph Canyon. In the late 1990s, a fish barrier was built, the stream was chemically treated to remove nonnative fishes, and native fish were stocked. Unfortunately, those fish did not establish populations, likely because too few were stocked and drought greatly reduced the amount of perennial water in the system. The partners re-evaluated the stream in 2007, and determined that the small amount of habitat was probably only suitable for Longfin Dace and Gila Topminnow. Longfin Dace were stocked in 2007 and established a population in Telegraph Canyon. In May 2017, a total of 522 Gila Topminnow were stocked into Arnett Creek just downstream of the Telegraph Canyon confluence. Few topminnow were captured during post-stocking monitoring from 2017-2019, and it is presumed the original population failed after the stream nearly entirely dried in 2018. Following completion of an invasive plant removal project in Telegraph Canyon by USFS staff in 2020, Department staff translocated 389 Redrock Canyon lineage Gila Topminnow from Walnut Spring #392 and one other location to Telegraph Canyon in May, 2021. Subsequent post stocking monitoring in October of 2021 resulted in the capture of 563 Gila Topminnow despite the Telegraph Fire burning a substantial portion of the upper watershed in June, 2021.

Results: On October 28, 2022, Department staff monitored Gila Topminnow in Telegraph Canyon. The crew set 10 collapsible minnow traps from the downstream end of surface water (UTM 12S 487203/3680205) up to the most upstream topminnow stocking location (486993/3679924) and captured a total of 117 Gila Topminnow (4 <20 mm TL, 113 ≥20 mm TL). The crew also carried out a total of 13 dipnet sweeps and captured an additional 48 Gila Topminnow (9 <20 mm TL, 39 ≥20 mm TL). The distribution of topminnow in Telegraph Canyon compressed relative to 2021, with fish apparently pushed out of the most upstream pools (Figure 7). Longfin Dace were not captured or observed during monitoring.

As minnow traps were soaking in Telegraph Canyon, the crew walked downstream to Arnett Creek near where topminnow were captured in 2018 (486392/3681070) in an attempt to identify if any fish remained in the reach. The crew carried out five dip net sweeps while walking the stream upstream to Telegraph Canyon and captured one Gila Topminnow and visually observed another within the stream (both ≥ 20 mm TL). Although only a few fish were present in the stream, this reach could eventually support an additional population of topminnow if surface water remains perennial and the watershed continues to recover from the Telegraph Fire.

It was apparent that substantial flooding had occurred within the last year in Telegraph Canyon and deposited large amounts of fine sediments within the reach. Deposition of these sediments may be the result of lagged effects from the Telegraph Fire that took place in 2021. Although evidence of flooding and potential fire effects appear to have disturbed the stream within the last year, topminnow have resisted the immediate effects of these disturbances and persisted within the reach.

Recommendations: Although numbers of Gila Topminnow decreased in Telegraph Canyon and Arnett Creek relative to 2021 (n=584), persistence of these fish following two consecutive years of post-fire effects exacerbated by above average monsoon seasons is promising for the prospects of this population establishing in the future. The Telegraph Canyon Gila Topminnow population should be monitored until 2024 to determine if the population is established.

Unnamed Drainage #68B

Background: Unnamed Drainage #68B is located on the Tonto National Forest and is a tributary to Mesquite Creek, which flows into Tortilla Creek, just upstream of Canyon Lake. Gila Topminnow were previously stocked in Mesquite Tank #2 (above Unnamed Drainage #68B) in 1982. 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 #68B and later dispersed into perennial pools in lower Mesquite Creek and lower Tortilla Creek. The original population occupying Unnamed Drainage 68B was founded with an unknown number of individuals and was never augmented. Despite these challenges, the population persisted until at least 2019, after which topminnow were not detected in three consecutive surveys from 2020-2021.

Results: During April 21-22, 2022, Department staff stocked 393 Cottonwood Spring lineage Gila Topminnow into Unnamed Drainage #68B. A total of 413 topminnow were collected from two donor locations, Walnut Spring #20 (n = 303) and Cold Spring (n = 110). Fish were transported to Unnamed Drainage #68B in aerated coolers, with 20 mortalities during transport. All fish were stocked into the largest pool at the confluence of the east and west prongs (464845/3711232).

On October 24, 2022, Department staff monitored the topminnow population in Unnamed Drainage #68B by setting 10 minnow traps in pools throughout the reach. A total of 854 Gila Topminnow (12 <20 mm TL, 783 $\geq$20 mm TL) were captured in minnow traps. An additional 109 topminnow (43 <20 mm TL, 66 $\geq$20 mm TL) were captured in four opportunistic seine hauls and 27 Topminnow (12 <20 mm TL, 15 $\geq$20 mm TL) in 12 dip net sweeps. Fish dispersed downstream of the original stocking location (Figure 8) into pools that later became isolated. A total of 22 fish were captured from an isolated pool that appeared to be at imminent risk of drying in the following days and translocated to a large pool upstream of the original stocking location (464691/3711295).

Recommendations: Gila Topminnow in Unnamed Drainage #68B are actively reproducing with more than twice as many fish captured in October than stocked in April. Monitoring of this population will continue for at least two more years unless additional establishment augmentations become necessary. If topminnow translocated upstream of the east and west prongs persist, it may be valuable to translocate additional fish upstream in 2023 to extend the distribution of the population in the system.

Rarick Canyon

Background: Rarick Canyon is a tributary to Red Tank Draw and consists of a series of perennial bedrock pools (Figure 9). A waterfall barrier (~10 meters high) in Rarick Canyon prevents upstream movement of nonnative fishes from the perennial reach of Red Tank Draw; however, a survey of isolated pools in the Rarick Canyon drainage from 2017 to 2018 detected Black Bullhead and Fathead Minnow upstream of the waterfall. Intensive mechanical removals efforts in 2019 resulted in the eradication of Black Bullhead from the Rarick Canyon drainage. Roundtail Chub[8] from Red Tank Draw were translocated above a natural barrier into three isolated pools in the Rarick Canyon drainage in 2019 and augmented in 2020 and 2021. Gila Topminnow (Redrock Canyon lineage) were also translocated to one of the same isolated pools above the barrier in April, 2020 (Figure 9). No topminnow were captured during annual monitoring in 2021.

Results: During October 4-5, 2022, Department staff monitored Gila Topminnow and Roundtail Chub in Rarick Canyon. The crew set a combination of 11 collapsible minnow traps and 10 mini-hoop nets in the four pools previously stocked with chub (F23, F20, F18, F17; Figure 9) for a soak time of nearly 22 hours. A total of 76 Roundtail Chub and 65 Fathead Minnow were captured. One additional Roundtail Chub was captured via angling. For the first time since monitoring began, two clear age-classes of Roundtail Chub were present (Figure 10). Gila Topminnow were not captured or observed for the second consecutive year. Similar to last year, flow was present throughout the entire surveyed reach, and most pools were at or near capacity. Although water was higher this year from rains the previous day, more than 15 times as many Roundtail Chub were captured relative to 2021 (n = 5). Roundtail Chub were also detected in every pool sampled, suggesting that chub continue to persist in each pool and/or individuals are dispersing downstream. The 2022 catch represents the most individuals captured during any monitoring event since the initial stocking of these fish in 2019. Further, this is the fewest Fathead Minnow captured since monitoring began, suggesting that chub might be utilizing Fathead Minnow as a prey item to the point where the Fathead Minnow population is decreasing.

Recommendations: The Rarick Canyon population of Roundtail Chub should be monitored for at least five years after the most recent augmentation (2026) to determine whether the population establishes. Translocating chub from the existing population upstream of the waterfall barrier to other potentially available pools upstream in Rarick Canyon could be considered in the future, after the population is established in the initial stocking locations. Additional translocations of Gila Topminnow are not recommended at this time due to low winter water temperatures likely limiting the potential for topminnow to persist overwinter at this location.

[8] Chub stocked into Rarick Canyon were previously classified as Gila Chub.

Tables and Figures:

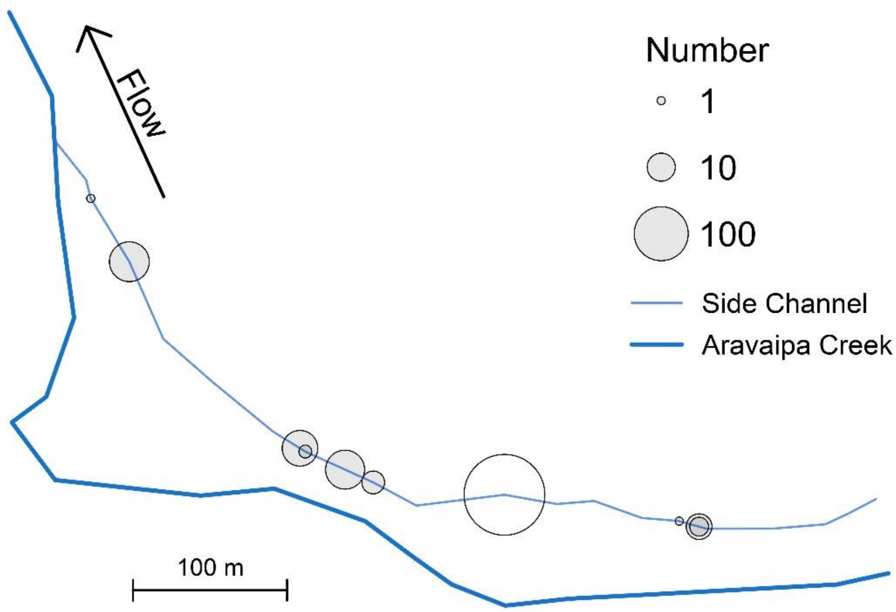

Figure 4.—Location and number of Gila Topminnow stocked and captured in Aravaipa Creek side-channel during 2022. The dark blue line represents the main channel of Aravaipa creek while the light blue line represents the spring fed side-channel. Open circles represent fish stockings while grey circles represent fish captured during monitoring.

Figure 5: Side by side comparison of the side-channel on Aravaipa Creek prior to and following flooding. Pre-flood pictures were taken during the initial stocking of Gila Topminnow in April 2022. Post-flood pictures were taken during post-stocking monitoring in September 2022. Photographs were taken from the same location.

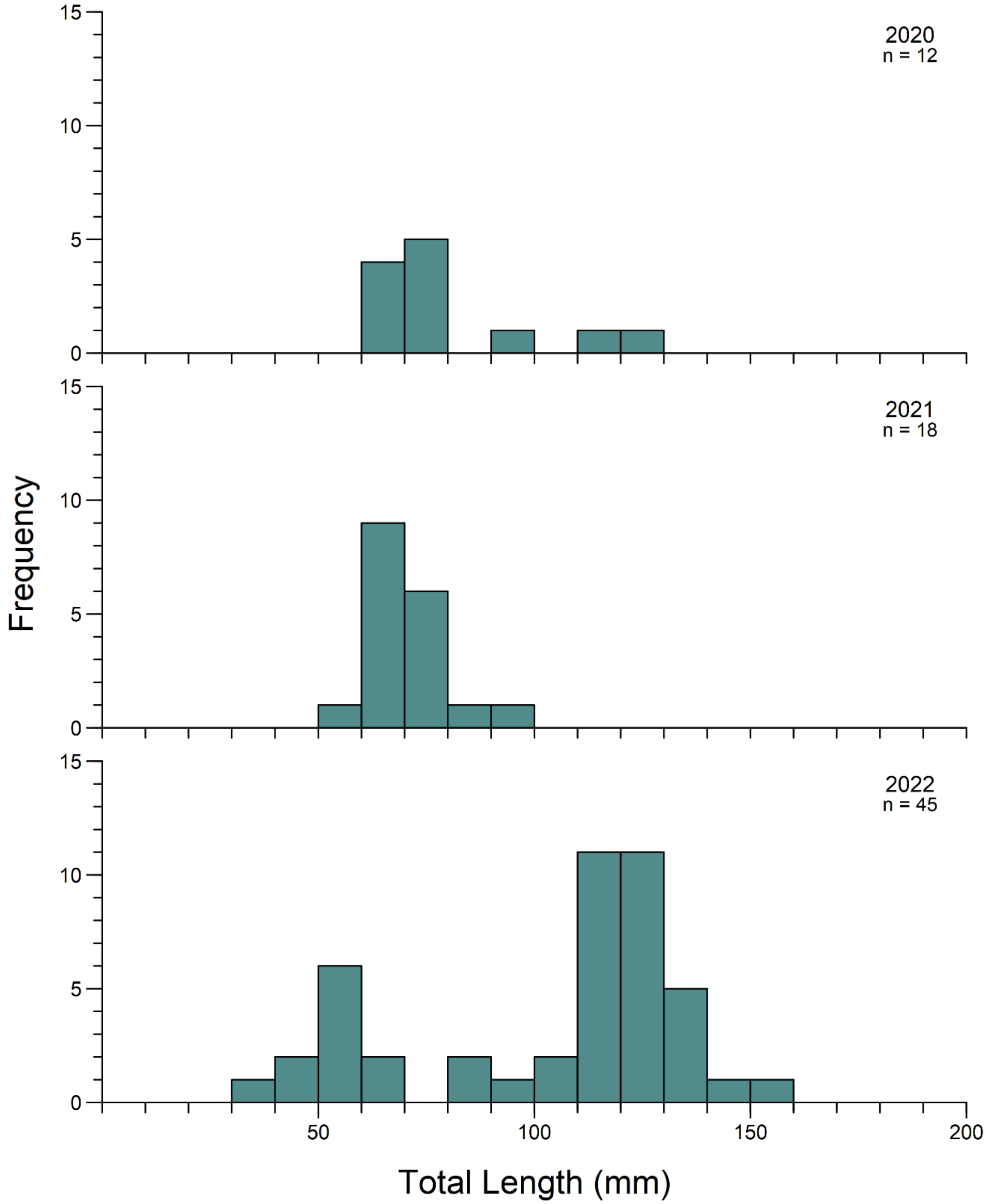

Figure 6.—Length frequency distribution of Roundtail Chub captured during annual monitoring in Sabino Canyon upstream of the confluence with East Fork Sabino Canyon, 2020 through 2022. Number of fish captured and measured each year is shown in the top right corner of each panel.

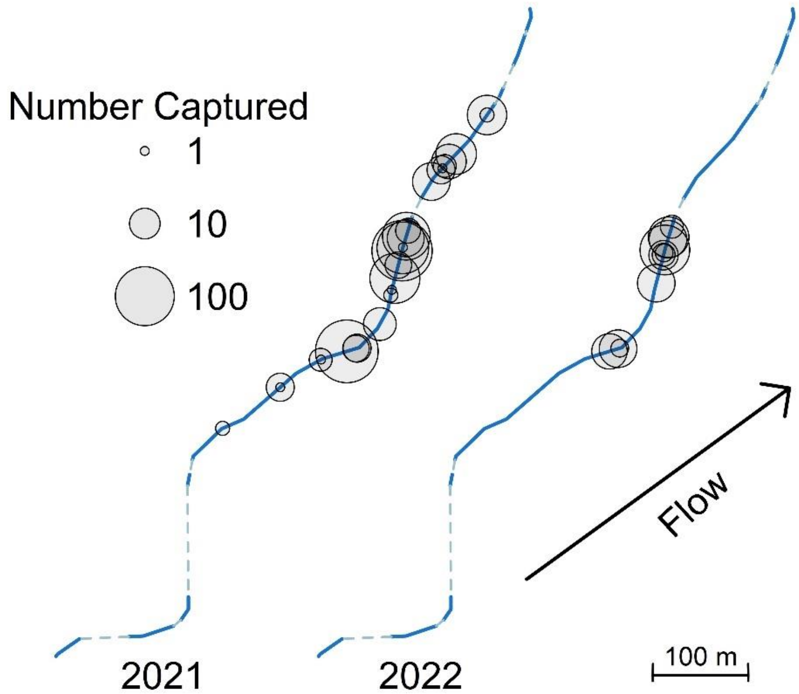

Figure 7.—Location and number of Gila Topminnow captured in Telegraph Canyon during annual monitoring in 2021 (left) and 2022 (right). The blue line represents the perennial portion of Telegraph Canyon while the dashed line represents intermittent portions.

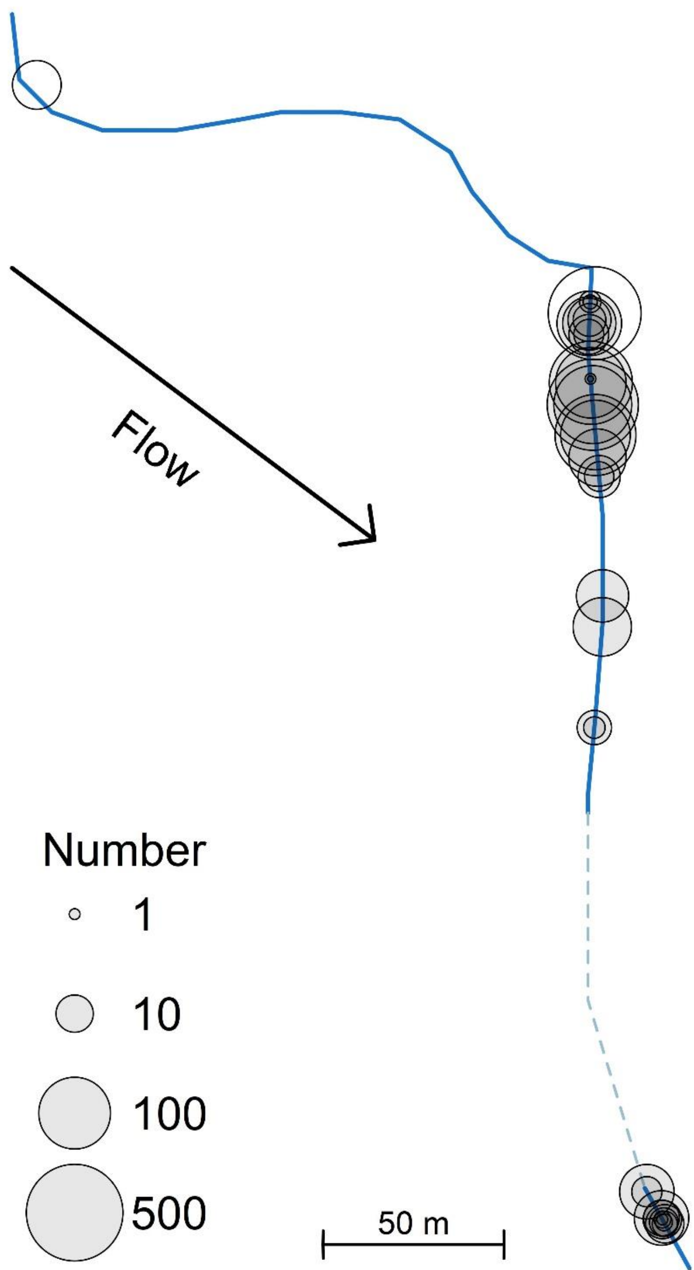

Figure 8.—Location and number of Gila Topminnow stocked and captured in Unnamed Drainage #68B in 2022. The solid blue line represents the perennial portion of Telegraph Canyon while the dashed line represents the intermittent portion. Open circles represent fish stockings while grey circles represent fish captured during monitoring.

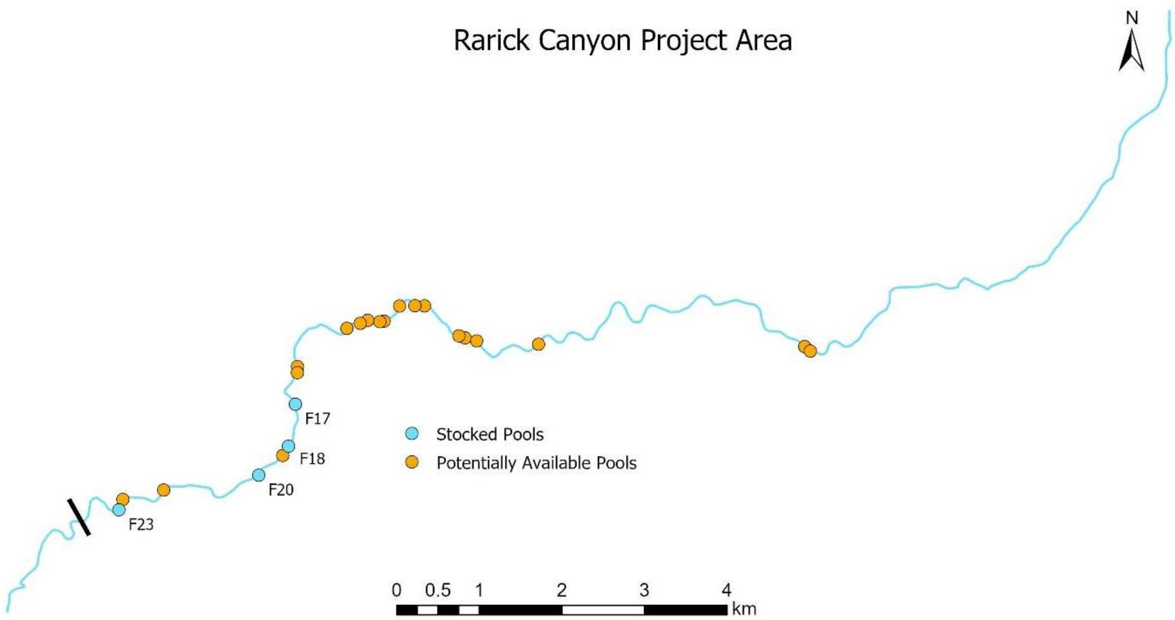

Figure 9.—Map of Rarick Canyon (blue line) including the location of the barrier falls (thick black line), names and locations of pools stocked with fish (blue dots), and the remaining potentially habitable pools in Rarick Canyon (orange dots).

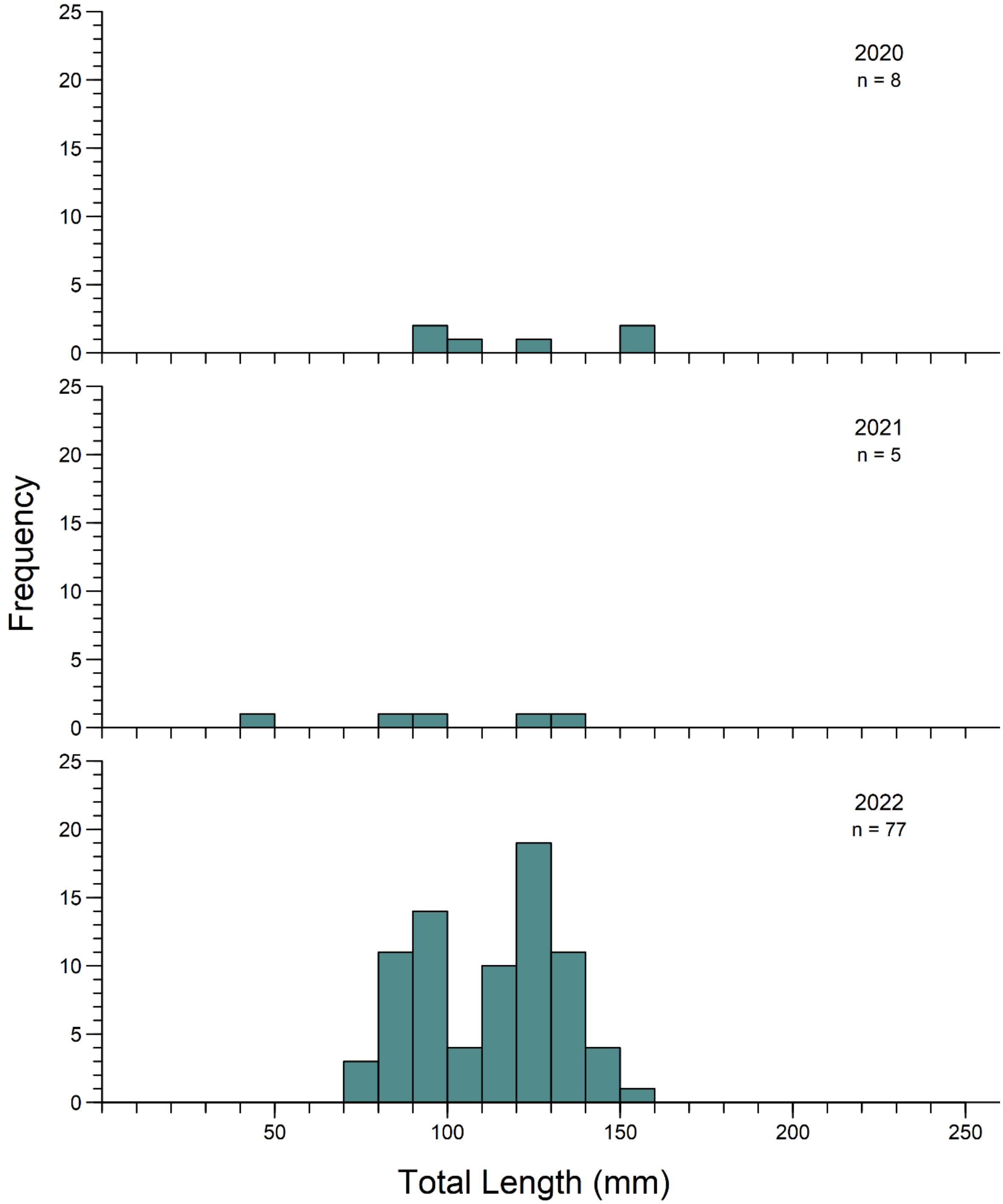

Figure 10.—Length frequency distribution of Roundtail Chub captured during annual monitoring in Rarick Canyon 2020 through 2022. Number of fish captured and measured each year is shown in the top right corner of each panel.

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

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
  - Goal 3. Remove nonnative aquatic species threats.
  - Goal 4. Replicate populations and their associated native fish community into protected streams and other surface waters.
  - Goal 7. 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.
- Gila Chub draft recovery plan objective 7. Monitor remnant, repatriated, and refuge populations to inform adaptive management strategies.

Background: Spring Creek is a tributary to Oak Creek in the Verde River drainage, and contains Roundtail Chub[9], Speckled Dace, Longfin Dace, Desert Sucker, and Northern Mexican gartersnake. A small diversion dam about 0.95 km upstream from the confluence with Oak Creek purportedly prevented most nonnative fishes from invading upstream, but there are historic records of Smallmouth Bass and Fathead Minnow in the system. 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 Conservation and Mitigation Program, and Gila River Basin Native Fish Conservation Program staff successfully eradicated Green Sunfish 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. However, topminnow were not detected during topminnow-specific monitoring by Reclamation's long-term monitoring contractor in 2022 (K. Shollenberger, Marsh and Associates, personal communication). Spikedace were initially stocked above the barrier in 2015, with

[9] Chub in Spring Creek were previously classified as Gila Chub.

additional stockings occurring in 2017, 2018, 2020, and 2021 (Figure 11). In total, 3,788 Spikedace have been translocated into Spring Creek upstream of the barrier. Beginning in 2020, some fish translocated from ARCC were PIT tagged as part of an ongoing study by Kansas State Researchers. Spikedace relative abundance was low during the first three years annual monitoring, and remained low until reproduction was first documented in 2018, with peak relative abundance documented in 2019 (Figure 11). Spikedace relative abundance declined through 2021, without evidence of reproduction since 2019.

Results: On March 10, 2022, Department staff translocated 1,717 Spikedace from ARCC to Spring Creek with three mortalities during transport (Appendix 2). A total of 192 of the translocated fish were PIT tagged on March 8 as part of an ongoing study by Kansas State University researchers.

On September 15, 2022, Department staff monitored Spikedace in Spring Creek. The crew targeted Spikedace by electrofishing one fixed 100 m reach and two randomly selected 100 m reaches. A total of 14 Spikedace were captured during the initial pass at each site, which is nearly three times the number of fish captured during first pass efforts in 2021 (Figure 11). In addition to Spikedace, 92 Roundtail Chub, 91 Desert Sucker, 94 Longfin Dace, and 311 Speckled Dace were captured during electrofishing (Table 1). Spikedace relative abundance also significantly increased relative to 2021 (2021 = 4.33/h, 2022 = 17.99/h; t = -547.58, P < 0.001), however, relative abundance remains low compared to 2019 (34.56/h, Figure 11). Mean size of Spikedace captured was 58.7 mm TL (min = 46, max = 80; Figure 12). The size structure of measured fish suggests that little to no reproduction has occurred in Spring Creek since 2019 (Figure 12).

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. One additional Spikedace was captured during the two additional passes. Estimated abundance of Spikedace using a Carle-Strub method was 10 fish per hundred m (±1.18) with an estimated capture probability of 0.91 (Table 2). The estimated abundance of Spikedace and the lower bound of the 95% confidence interval were greater than the abundance estimate and upper bound of the confidence interval in 2021 (N = 5±1.54; Figure 13), which suggests there was likely an increase in abundance. However, the increase in abundance was not as large as expected given the number of Spikedace stocked in March.

Of the 15 Spikedace captured, one was PIT tagged at ARCC and stocked in 2020, and another was tagged at ARCC and stocked in 2022. The tag data suggests that Spikedace have inter-annual survival in Spring Creek after stocking.

It was previously speculated that large Roundtail Chub could potentially be preying upon naïve hatchery Spikedace to the point that they were a primary ecological factor limiting survival and reproduction of translocated Spikedace (Hickerson et al. 2022). However, closer analysis of trends in relative abundance of the most frequently captured native fish species in Spring Creek from 2015-2022 reveals that relative abundance of Spikedace actually has a significant, positive correlation with Roundtail Chub (r = 0.834, t = 3.698, P = 0.010; Figure 14). Given these results, it is likely that other ecological factors (e.g., streamflow during the spawning season or density of nonnative crayfish) are potentially limiting reproduction, and consequently establishment, of Spikedace in Spring Creek.

Recommendations: Recaptures of PIT tagged fish suggests that environmental conditions in Spring Creek are sufficient for inter-annual survival of hatchery reared and translocated Spikedace. The lack of consistent reproduction and recruitment of translocated Spikedace in most years seems to be the limiting factor preventing this population from becoming established. Because Spikedace reproduction has been sporadic in Spring Creek to date, regular translocations may be necessary to maintain the population in years between successful spawns.

Additional Gila Topminnow should be stocked to restore the population that was either lost to flooding in 2021 or is existing at extremely low abundance.

Tables and Figures:

Table 1.—Summary of fish captured during the first pass at three 100 m electrofishing sub-reaches in Spring Creek during annual monitoring on September 15, 2022. Shown are the number of fish captured in each sub-reach (#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.

Sub-reach	Statistic	Roundtail Chub	Spikedace	Desert Sucker	Longfin Dace	Speckled Dace
Random-14	#Ind	12	2	40		56
	#Ind/h	111.06	16.00	320.00		435.63
Random-04	#Ind	63	3	20	12	62
	#Ind/h	224.16	9.60	69.40	20.90	187.84
Fixed-2	#Ind	17	9	31	82	193
	#Ind/h	49.09	24.91	83.59	272.94	523.50
Total	#Ind	92	14	91	94	311
	#Ind/h	150.17	17.99	148.84	71.02	354.27
	SE	(25.62)	(3.38)	(63.42)	(39.11)	(98.76)

Table 2.—Three-pass depletion estimates of abundance for all fish species captured per 100 m at the fixed sub-reach in Spring Creek during annual monitoring in 2022. 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, AGCH = Longfin Dace, and RHOS = Speckled Dace.

Stream	Site	Species	C1	C2	C3	N	N_LCI	N_UCI	*p*	*p*_LCI	*p*_UCI
Spring Creek	Fixed-02	MEFU	9	1	0	10	9.82	10.18	0.91	0.73	1.00
Spring Creek	Fixed-02	GIRO	17	13	6	43	29.52	56.48	0.44	0.19	0.68
Spring Creek	Fixed-02	AGCH	82	38	23	163	144.45	181.55	0.5	0.39	0.61
Spring Creek	Fixed-02	RHOS	193	107	71	467	413.69	520.31	0.41	0.33	0.49
Spring Creek	Fixed-02	CACL	31	9	5	46	42.5	49.5	0.67	0.52	0.83

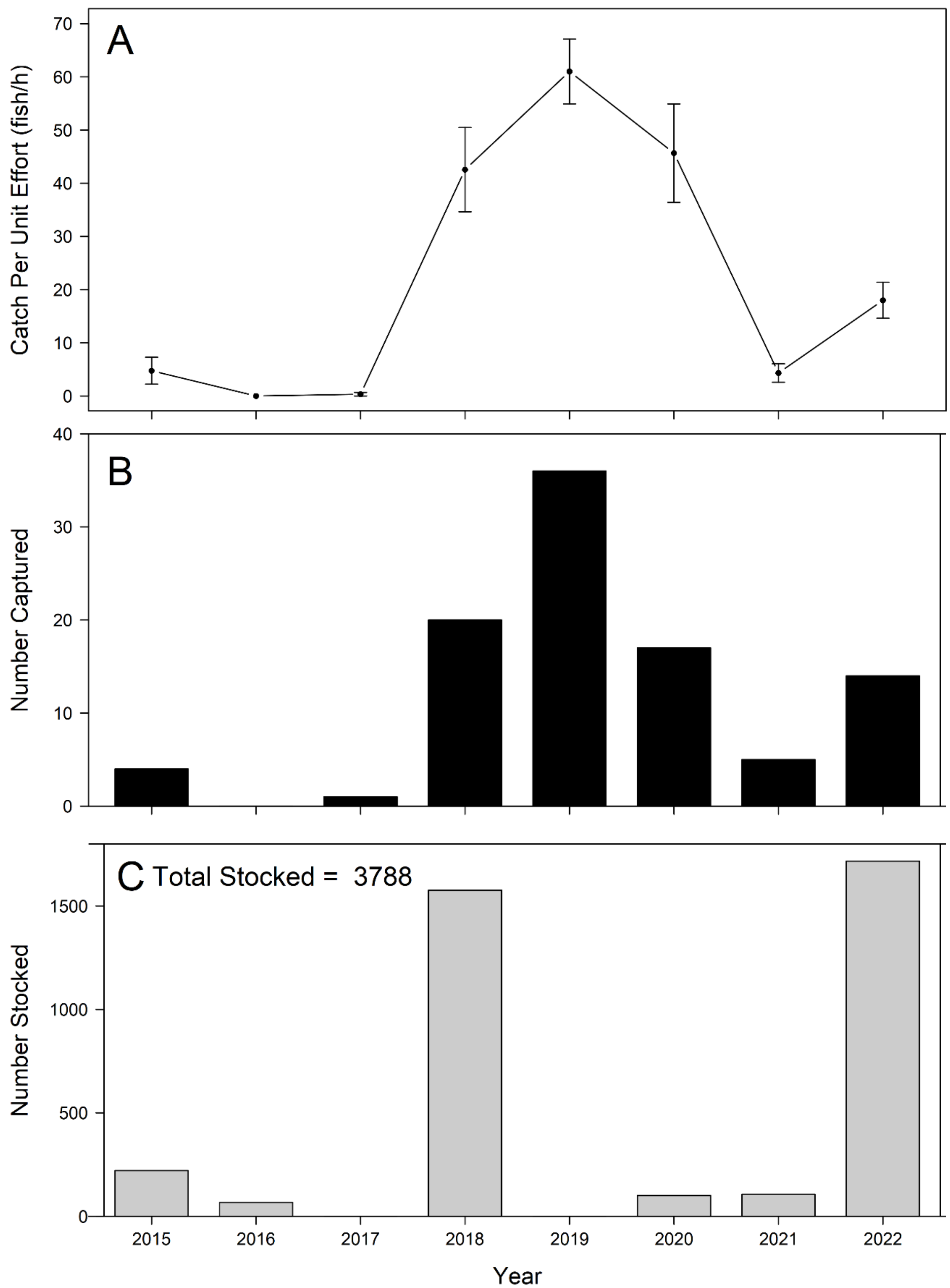

Figure 11.—Summary of Spikedace captured and stocked in Spring Creek, AZ, annually from 2015 to 2022 with (A) mean annual backpack electrofishing catch per unit effort (fish/h) with standard error bars, (B) total number of fish captured, and (C) total number of fish stocked.

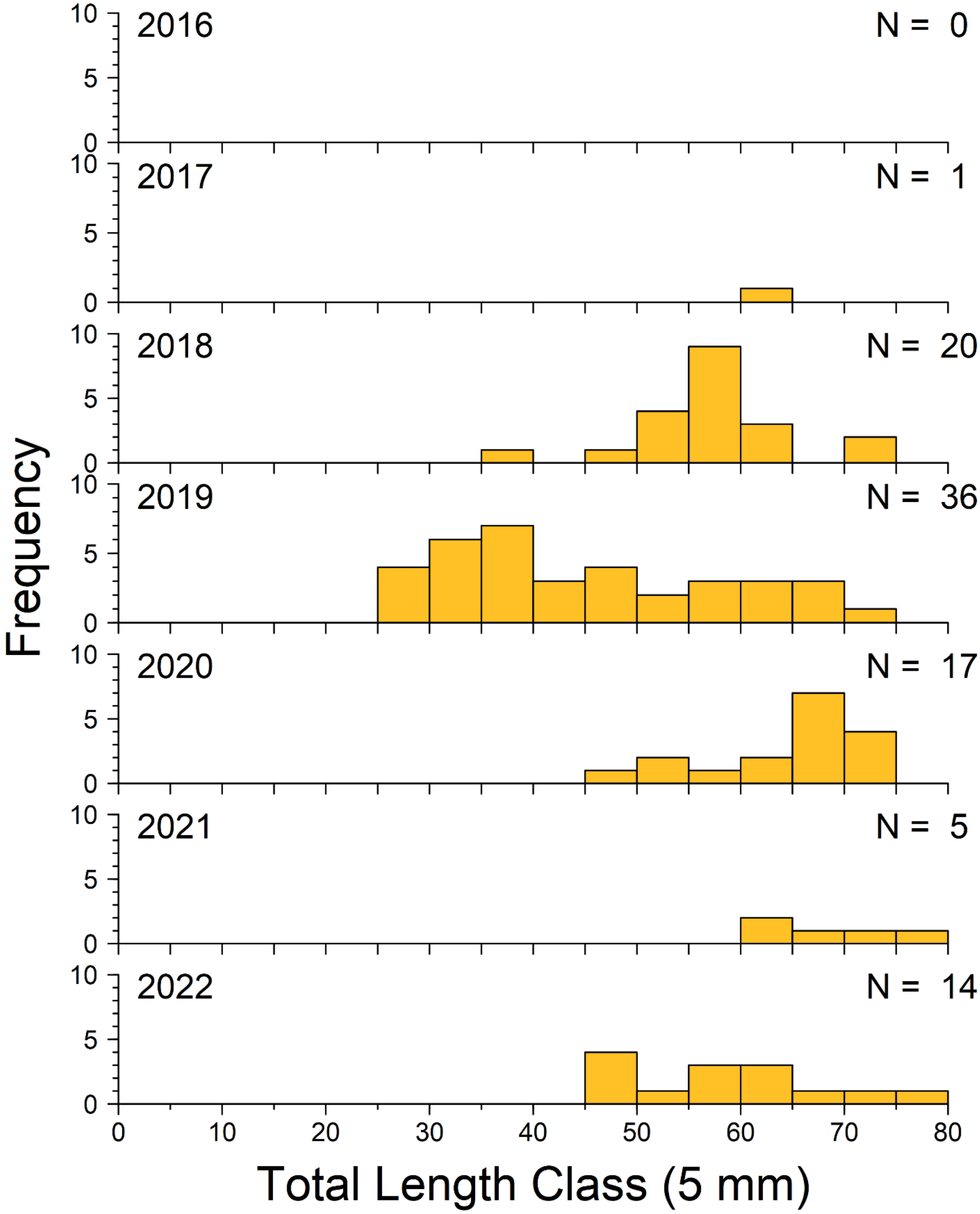

Figure 12.—Length frequency distribution of Spikedace captured during annual monitoring in Spring Creek, 2016 through 2022. Only fish captured on the first pass are included. Number of fish captured and measured each year is shown in the top right corner of each panel.

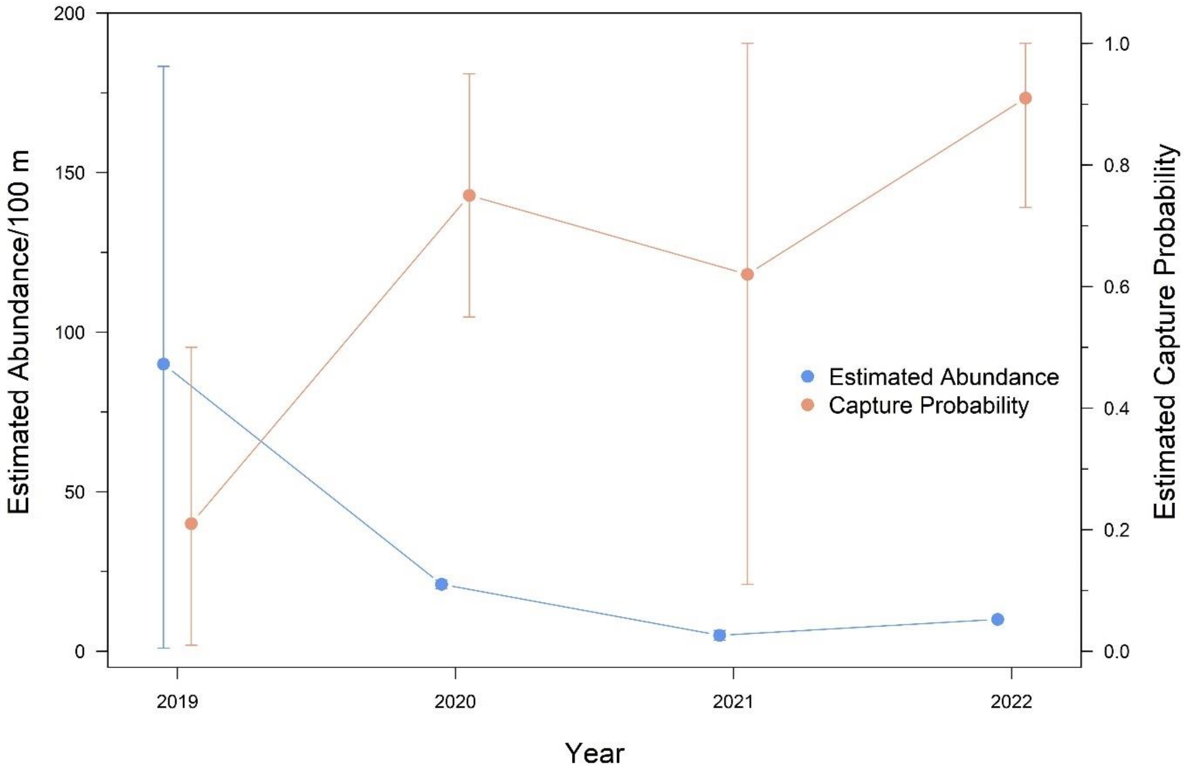

Figure 13.—Three-pass depletion estimates of Spikedace abundance per 100 m (blue points and lines) and capture probability (orange points and lines) at the fixed sub-reach in Spring Creek during annual monitoring from 2019-2022. Bars represent the lower and upper bounds of the 95% confidence interval of each estimate.

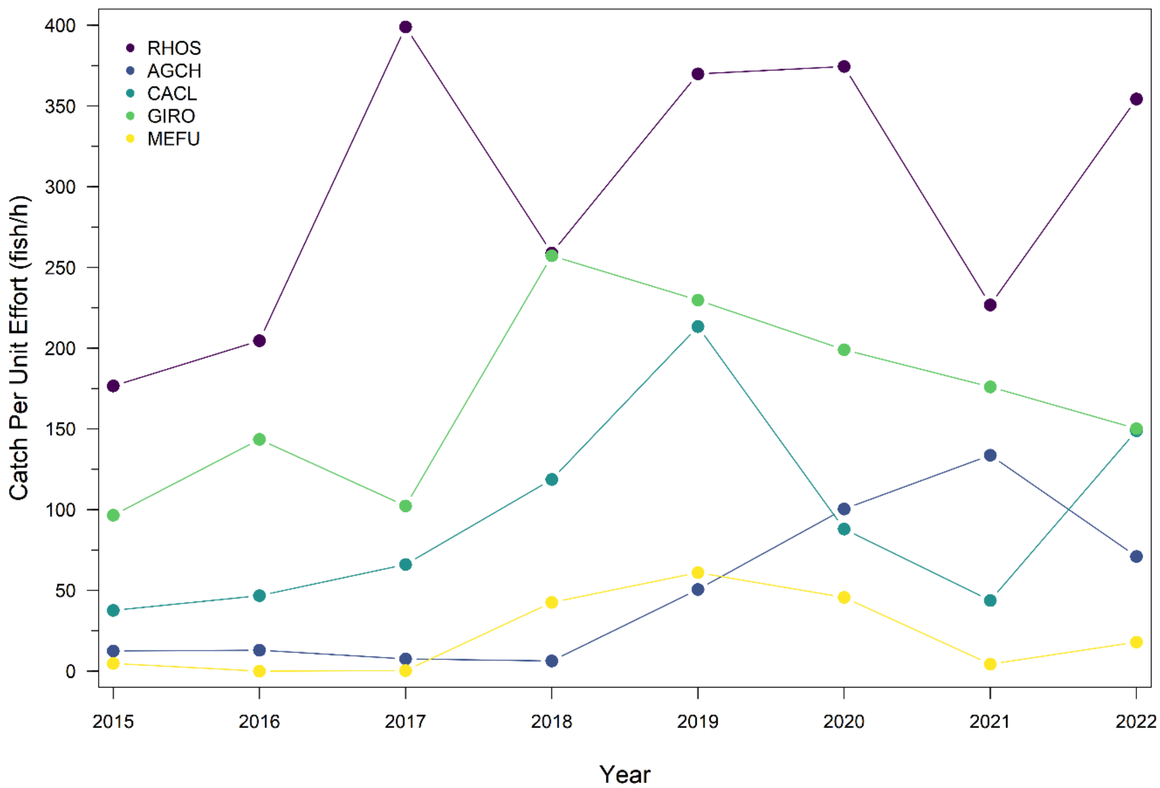

Figure 14.—Mean annual backpack electrofishing catch per unit effort (fish/h) of select native fish species (AGCH = Longfin Dace, CACL = Desert Sucker, GIRO = Roundtail Chub, MEFU = Spikedace, RHOS = Speckled Dace) captured in Spring Creek, AZ from 2015 to 2022. Standard error bars are not shown to improve clarity of mean catch per unit effort trends.

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

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
  - Goal 3. Remove nonnative aquatic species threats.
  - Goal 4. Replicate populations and their associated native fish community into protected streams and other surface waters.
  - Goal 7. 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.

Overall 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 included construction of a fish barrier, mechanical removal of non-native fishes, and translocation 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 (lower Blue River; Figure 15). A synthesis of conservation efforts leading to the eradication of nonnative fishes and establishment of Spikedace and Roundtail Chub populations in the lower Blue River through 2019 can be found in Hickerson et al. (2021a). The entire native fish community in the lower Blue River has experienced substantial decline following impacts from the Bringham and Cow Canyon fires in 2020 (Shollenberger et al. 2021, Shollenberger et al. 2022). Additional efforts to establish populations of Spikedace and Roundtail Chub are now taking place in the middle and upper Blue River (Figure 15).

Middle Blue River

The middle Blue River project area includes 5.5 km of the Blue River from the confluence with McKittrick Creek upstream to The Box (Figure 15). Roundtail Chub were initially stocked in the middle Blue River in 2016 (n = 1,194), with a subsequent augmentation in 2019 (n = 100; Figure 16). Spikedace were initially stocked in 2017 (n = 448), with a subsequent augmentation in 2018 (n = 291; Figure 17). Monitoring of the Roundtail Chub population began in 2017 when only hoop nets were set. In September, 2018, a monitoring approach was implemented that involved electrofishing ten randomly selected and two fixed 100 m sub-reaches within three river reaches:

McKittrick Creek to KP Creek (n = 5), KP Creek to Cole Flat (n = 4), and Cole Flat to the natural waterfall barrier at The Box (n = 3). Hoop netting was dropped from the monitoring protocol following the 2018 monitoring effort.

Upper Blue River
The upper Blue River project area includes 14.8 km of the Blue River from Blue Crossing upstream to New Mexico state line (Figure 15). Spikedace and Roundtail Chub were initially stocked in 2020 when 826 Spikedace and 226 Roundtail Chub were translocated from the lower Blue River due to concerns over post-fire impacts from the Brigham Fire, and stocked at Bobcat Flat and near Upper Blue Campground respectively. In September, 2021, a monitoring approach was implemented that involved electrofishing 12 randomly selected and three fixed 100 m sub-reaches within three river reaches: Blue Crossing to Swafford Canyon (n = 5), Swafford Canyon to Upper Blue Campground (n = 5), and Upper Blue Campground to the New Mexico state line (n = 5).

Results:
Lower Blue River
On March 15, 2022, Department staff translocated 705 Gila River lineage Spikedace into the lower Blue River near Juan Miller Crossing from ARCC (Appendix 2). The purpose of the translocation was to augment the population following post-fire impacts from the Bringham and Cow Canyon Fires. There were no mortalities during stocking and transport.

Middle Blue River
During September 26 to 28, 2022, Department staff electrofished a total of ten randomly selected and two fixed 100 m long sub-reaches. A total of 14 Spikedace, 17 Roundtail Chub, 8 Loach Minnow, 19 Longfin Dace, 142 Speckled Dace, 171 Desert Sucker, 68 Sonora Sucker, and 1 Brown Trout were captured during the first pass (Table 3). The number of Spikedace captured is three times fewer fish captured during first pass efforts in 2021 (n = 42; Figure 17). Similarly, Roundtail Chub captures declined sharply relative to 2021 (n = 80; Figure 16). Despite a decrease in the number of fish captured for all species, the percent composition of Spikedace (3.12%) and Roundtail Chub (3.86%) relative to all fish captured was similar to percent composition in 2021 for both species (Spikedace = 2.49%, Roundtail Chub = 4.74%).

Mean relative abundance of all native fish, except Spikedace and Sonora Sucker, significantly declined from 2021 to 2022 (Table 4). Importantly, relative abundance of most native fish species was the lowest on record since monitoring began in 2018 (Figure 18), with the exception of the two translocated species, where Roundtail Chub relative abundance was only lower in 2019 (6.91/h), and Spikedace relative abundance was only lower in 2018 (3.20/h).

Young-of-year Roundtail Chub and Spikedace were absent from the catch, which marks two consecutive years without apparent spawning for both species (Figure 19, Figure 20). The mean size of Spikedace captured has continued to grow since the last time young of year fish were captured in 2020 (2020 = 38.35, 2021 = 54.66, 2022 = 65.21; Figure 20). Most of the Spikedace captured in 2022 were likely at least 2.5 years old, and are approaching the end of their typical lifespan in the wild of about 3 years. There are two primary year classes of fish that have been consistently captured during monitoring: the chub initially stocked in 2016 which are still present and relatively large (~320-370 mm TL), and the strong year class of chub spawned in 2020, which are growing (~90-140 mm TL), but likely need additional time to reach reproductive maturity (Figure 19). The lack of spawning since 2020 could be attributed to the lack of sufficient flows for successful spawning for a second consecutive spring.

Spikedace were captured at six sub-reaches and Roundtail Chub at four sub-reaches, which is a decline in distribution relative to 2021 when Spikedace were captured at nine sub-reaches and Roundtail Chub at all twelve sub-reaches (Figure 21). Spikedace still seem to have difficulty accessing the most upstream 400–500 m of the monitoring reach, probably because of the steeper gradient in this area, and have not yet been captured upstream of sub-reach 4.

Depletion sampling at the lower fixed site (KP Creek confluence) was not successful due to the presence of elevated flows and small debris (i.e., leaves) from rains the previous evening, which caused both block nets to fail during the first pass. Estimates of Roundtail Chub ($N = 14 \pm 1.60$) abundance at the upper fixed site slightly declined in comparison to 2021 ($N = 29 \pm 9.61$, Table 5). Spikedace were not captured at the upper fixed site, although absence from this location is not surprising as this species has not been captured at or upstream of the fixed sub-reach in the past. Despite greater discharge and more turbid flows this year, capture probability for most taxa was similar to last year, which suggests that environmental conditions probably did not contribute much to our lower catch this year (Table 5).

The decrease in fish numbers in the middle Blue River is not surprising considering this reach was subject to two years of poor winter precipitation, followed by two intense monsoon seasons which brought with it flooding and post-fire debris from the Cow Canyon Fire. Further, evidence of effects from the Cow Canyon Fire are still present within the reach, with increased levels of turbidity and the proportion of fine sediments visually appearing higher than pre-fire conditions. Despite the difficult environmental conditions for fish populations, both Spikedace and Roundtail Chub have demonstrated some resilience within the system, which will likely allow both species to take advantage of better conditions in the future.

Upper Blue River

During September 19-27, 2022, Department staff electrofished a total of 12 randomly selected and three fixed 100 meter long sub-reaches starting at Blue Crossing Campground upstream to the New Mexico state line. A total of 2 Spikedace, 17 Roundtail Chub, 76 Loach Minnow, 582 Longfin Dace, 1,489 Speckled Dace, 694 Desert Sucker, 220 Sonora Sucker and 42 Brown Trout were captured during the first pass (Table 6). The number and mean relative abundance of Spikedace is similar to results from first pass efforts in 2021 ($n = 4$; Table 7; Figure 22). Roundtail Chub captures substantially increased relative to 2021 (n = 1; Figure 23), but mean relative abundance did not significantly change (Table 7). Relative abundance of all other native fish taxa, except Loach Minnow, significantly declined from 2021 to 2022 (Table 7; Figure 24).

Young of year Spikedace (Figure 25) and Roundtail Chub (Figure 26) were not detected, for the second consecutive year. Similar to the middle Blue River, all Spikedace captured were large adults (~70 mm TL) that are approaching the end of their typical lifespan in the wild of about 3 years. The number of young of year fish of all taxa was noticeably reduced relative to sampling in 2021. Continued persistence of translocated Roundtail Chub and Spikedace in this reach suggests that environmental conditions are sufficient to support these taxa, however neither species has likely experienced conditions conducive to spawning since the initial translocation in 2020.

Spatial distribution of both Spikedace and Roundtail Chub continues to be patchy within the Upper Blue River. Spikedace were captured at one sub-reach (2R43) during the first pass with an additional individual captured during the second pass at a depletion site (2F45). All Spikedace captured in 2021 and 2022 were in the same vicinity as the initial stocking locations (Figure 27). While Spikedace have shown site fidelity, dispersal from the initial stocking location has not been documented. Roundtail Chub were captured at four sub-reaches, with fish captured near both the bottom and top of the monitoring reach, which suggests that chub have dispersed and persisted in suitable habitats throughout the monitoring reach (Figure 27).

Three-pass depletions were carried out at one fixed sub-reach in each of the three monitoring reaches. Roundtail Chub were only captured at the most downstream fixed site (1F46), however estimated capture probability (0.15) was quite low, which suggests that chub may be more abundant within the upper Blue River than the first pass data alone suggests (Table 8). Only one Spikedace was captured within the depletion sites, so estimates of abundance and capture probability for Spikedace are still of limited utility at this time (Table 8).

The mean relative abundance of Brown Trout captured in 2022 was not significantly different than 2021 (Table 7). However mean size of Brown Trout captured was significantly greater than in 2021 (2021 = 102.34 mm TL, 2022 = 159.55 mm TL, $W = 805.5$, $P = 0.011$). Length-frequency data suggests that the strong year class of juvenile fish captured in 2021 survived through the summer and recruited into the population (Figure 28). However, the relatively small number of Brown Trout that reach large adult sizes (i.e., >300 mm TL) in the Upper Blue River suggests that predation from Brown Trout is not likely to be a primary factor limiting native fish populations at this time.

Relative abundance of all native fish taxa in the upper Blue River other than Spikedace, Roundtail Chub, and Loach Minnow declined relative to 2021 despite this reach of river being spared from the post-fire impacts affecting the middle and lower Blue River reaches. These declines suggest that other factors, likely an atypical flow regime, are also negatively influencing native fish populations throughout the Blue River. The watershed has experienced two consecutive years of below-average winter precipitation and spring runoff, which likely limits spawning potential for most native fish taxa, followed by two summers of above average monsoon flooding, which may limit survival or persistence of any juvenile fishes produced. Despite the difficult environmental conditions for fish populations, both Spikedace and Roundtail Chub have demonstrated the ability to persist within the upper Blue River, which may mean that both species can take advantage of better hydrologic conditions in the future to successfully establish populations.

Recommendations:

The lack of hydrologic conditions sufficient for spawning and survival of juvenile fish is likely a major factor limiting establishment of Spikedace and Roundtail Chub populations in both the middle and upper Blue River. Nearly all Spikedace captured in both reaches in 2022 were large, old fish that likely only have one more opportunity to spawn before the end of their typical lifespan in the wild. Additional Spikedace need to be translocated into both reaches, to increase the potential for reproductive output if hydrologic conditions in the Blue River improve in 2023. Without stocking, there is little opportunity for either Spikedace population to become established in the near term. Relative abundance of Roundtail Chub in the middle Blue River has consistently declined since the most recent spawning event in 2020, and has remained low in the upper Blue River through two years of monitoring efforts. Translocation of additional Roundtail Chub is likely necessary to ensure continued persistence of chub in both reaches and increase the potential for reproductive output when sufficient hydrologic conditions are present.

Preliminary reports from monitoring in the lower Blue River in 2022 suggests that some Spikedace from the stocking in March 2022 survived, but reproduction of these fish was not documented, and no Roundtail Chub were captured (K. Shollenberger, Marsh and Associates, personal communication). Additional Spikedace and Roundtail Chub should be stocked into the lower Blue River in 2023 to help reestablish this population that showed remarkable potential prior to 2020.

If the middle and upper Blue River populations are stocked with Spikedace and Roundtail Chub in 2023, monitoring should occur through 2028 to determine whether populations establish. Multiple stockings and eight years of monitoring were required to determine population establishment in the lower Blue River, so more stocking and additional years of monitoring may be required when environmental conditions cause population setbacks.

Tables and Figures:

Table 3.—Summary of fish captured during the first pass of backpack electrofishing within each monitoring reach in the middle Blue River during annual monitoring from September 26-28, 2022. Shown for each reach is the number of sub-reaches sampled (N), number of fish captured (#Ind), the mean relative abundance (number of fish captured per hour of electrofishing effort; #Ind/h) and standard error of mean relative abundance (SE).

Reach	N	Statistic	Loach Minnow	Roundtail Chub	Spikedace	Desert Sucker	Longfin Dace	Sonora Sucker	Speckled Dace	Brown Trout
1	5	#Ind	2	0	5	36	13	16	25	0
		#Ind/h	1.90	0	4.00	56.68	10.19	18.66	22.32	0
		SE	(1.47)	(0)	(1.60)	(23.30)	(5.59)	(5.33)	(5.49)	(0)
2	3	#Ind	1	1	9	75	4	29	41	0
		#Ind/h	0.73	1.85	16.86	66.98	4.64	26.04	31.17	0
		SE	(0.73)	(1.85)	(4.75)	(6.05)	(2.20)	(3.35)	(7.76)	(0)
3	4	#Ind	5	16	0	60	2	23	76	1
		#Ind/h	13.35	22.62	0	67.09	1.71	23.07	92.32	0.70
		SE	(5.69)	(4.05)	(0)	(8.20)	(1.17)	(6.41)	(26.85)	(0.70)
Total	12	#Ind	8	17	14	171	19	68	142	1
		#Ind/h	6.26	11.46	7.26	63.41	5.73	22.52	51.28	0.25
		SE	(2.50)	(2.55)	(2.02)	(8.72)	(2.29)	(3.00)	(11.56)	(0.25)

Table 4.—Pairwise Wilcoxon signed rank test statistics evaluating changes in mean relative abundance of all fish taxa captured in the middle Blue River between 2021 and 2022. Two-sided tests were used for taxa where there was not an obvious visual change in mean relative abundance, and one-sided tests were used for taxa where mean relative abundance visually declined from 2021 to 2022. Significant values are indicated with an asterisk (*).

Taxa	Test Type	*W*	*P*
Spikedace	Two-sided	47	0.146
Roundtail Chub	One-sided	16	<0.001*
Loach Minnow	One-sided	11	<0.001*
Longfin Dace	One-sided	21.5	0.002*
Speckled Dace	One-sided	7	<0.001*
Desert Sucker	One-sided	9	<0.001*
Sonora Sucker	Two-sided	82	0.583

Table 5.—Three-pass depletion estimates of abundance for all fish species captured per 100 m at each fixed sub-reach in the middle Blue River during annual monitoring in 2022. 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, RHCO = Loach Minnow, CACL = Desert Sucker, CAIN = Sonora Sucker, AGCH = Longfin Dace, and RHOS = Speckled Dace.

Sub-reach	Species	C1	C2	C3	N	N_LCI	N_UCI	*p*	*p*_LCI	*p*_UCI
Fixed-04	RHCO	5	2	2	9	6.59	11.41	0.60	0.20	1.00
Fixed-04	GIRO	9	4	1	14	12.40	15.60	0.70	0.43	0.97
Fixed-04	RHOS	48	16	16	91	77.03	104.97	0.50	0.35	0.65
Fixed-04	CACL	34	20	19	107	59.94	154.06	0.31	0.11	0.51
Fixed-04	CAIN	12	2	8	29	11.10	46.90	0.36	0.01	0.71
Fixed-04	AGCH	2	3	3	11	0.00	26.69	0.31	0.00	0.94

Table 6.—Summary of fish captured during the first pass of backpack electrofishing within each monitoring reach in the upper Blue River during annual monitoring from September 19-27, 2022. Shown for each reach is the number of sub-reaches sampled (N), number of fish captured (#Ind), the mean relative abundance (number of fish captured per hour of electrofishing effort; #Ind/h) and standard error of mean relative abundance (SE).

Reach	N	Statistic	Loach Minnow	Roundtail Chub	Spikedace	Desert Sucker	Longfin Dace	Sonora Sucker	Speckled Dace	Brown Trout
1	5	#Ind	28	12	0	236	214	74	482	8
		#Ind/h	50.64	14.30	0	140.08	149.25	58.92	234.44	5.45
		SE	(6.94)	(3.56)	(0)	(17.61)	(27.87)	(13.97)	(41.28)	(1.60)
2	5	#Ind	24	0	2	214	223	48	461	24
		#Ind/h	30.46	0	0.98	134.05	165.86	36.74	296.65	29.87
		SE	(4.65)	(0)	(0.68)	(15.61)	(37.31)	(6.98)	(27.65)	(4.84)
3	5	#Ind	24	5	0	244	145	98	546	10
		#Ind/h	31.56	11.37	0	208.79	132.52	114.55	410.54	10.74
		SE	(5.04)	(4.71)	(0)	(26.68)	(22.85)	(19.06)	(38.41)	(3.02)
Total	15	#Ind	76	17	2	694	582	220	1489	42
		#Ind/h	37.82	8.99	0.35	163.96	148.71	73.94	325.24	17.46
		SE	(3.37)	(2.20)	(0.24)	(12.73)	(17.14)	(9.44)	(21.84)	(2.55)

Table 7.—Pairwise Wilcoxon signed rank test statistics evaluating changes in mean relative abundance of all fish taxa captured in the upper Blue River between 2021 and 2022. Two-sided tests were used for taxa where there was not an obvious visual change in mean relative abundance, and one-sided tests were used for taxa where mean relative abundance visually declined from 2021 to 2022. Significant values are indicated with an asterisk (*).

Taxa	Test Type	*W*	*P*
Spikedace	Two-sided	97	0.292
Roundtail Chub	Two-sided	137	0.125
Loach Minnow	Two-sided	82	0.213
Longfin Dace	One-sided	45	0.002*
Speckled Dace	One-sided	23	<0.001*
Desert Sucker	One-sided	44	0.002*
Sonora Sucker	One-sided	55	0.008*
Brown Trout	Two-sided	117.5	0.851

Table 8.—Three-pass depletion estimates of abundance for all fish species captured per 100 m at each fixed sub-reach in the upper Blue River during annual monitoring in 2022. 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, RHCO = Loach Minnow, CACL = Desert Sucker, CAIN = Sonora Sucker, AGCH = Longfin Dace, RHOS = Speckled Dace, and SATR = Brown Trout.

Sub-reach	Species	C1	C2	C3	N	N_LCI	N_UCI	*p*	*p*_LCI	*p*_UCI
1-46F	GIRO	5	5	9	46	0.00	166.66	0.15	0	0.63
1-46F	AGCH	33	10	14	68	51.67	84.33	0.45	0.25	0.64
1-46F	RHOS	23	21	10	72	44.11	99.89	0.36	0.14	0.58
1-46F	CACL	19	3	8	34	25.21	42.79	0.49	0.24	0.74
1-46F	CAIN	41	13	11	71	61.98	80.02	0.55	0.39	0.71
1-46F	SATR	3	0	0	3	3.00	3.00	1.00	NA	NA
2-47F	RHCO	1	1	1	3	0.51	5.49	0.50	0.00	1.00
2-47F	MEFU	0	1	0	1	0.00	1.00	0.50	0.00	1.00
2-47F	AGCH	25	11	9	52	39.91	64.09	0.47	0.26	0.68
2-47F	RHOS	88	58	50	315	209.34	420.66	0.28	0.15	0.40
2-47F	CACL	22	12	7	47	36.08	57.92	0.48	0.27	0.70
2-47F	CAIN	10	19	6	55	11.53	98.47	0.28	0.00	0.58
2-47F	SATR	2	1	0	3	2.48	3.52	0.75	0.23	1.00
3-45F	RHCO	3	3	1	7	4.66	9.34	0.58	0.11	1.00
3-45F	AGCH	21	10	10	52	32.58	71.42	0.39	0.15	0.64
3-45F	RHOS	56	32	28	163	113.25	212.75	0.34	0.18	0.49
3-45F	CACL	6	5	3	16	8.37	23.63	0.45	0.06	0.84
3-45F	CAIN	4	1	1	6	4.70	7.30	0.67	0.23	1.00
3-45F	SATR	0	1	0	1	0.00	2.44	0.5	0.00	1.00

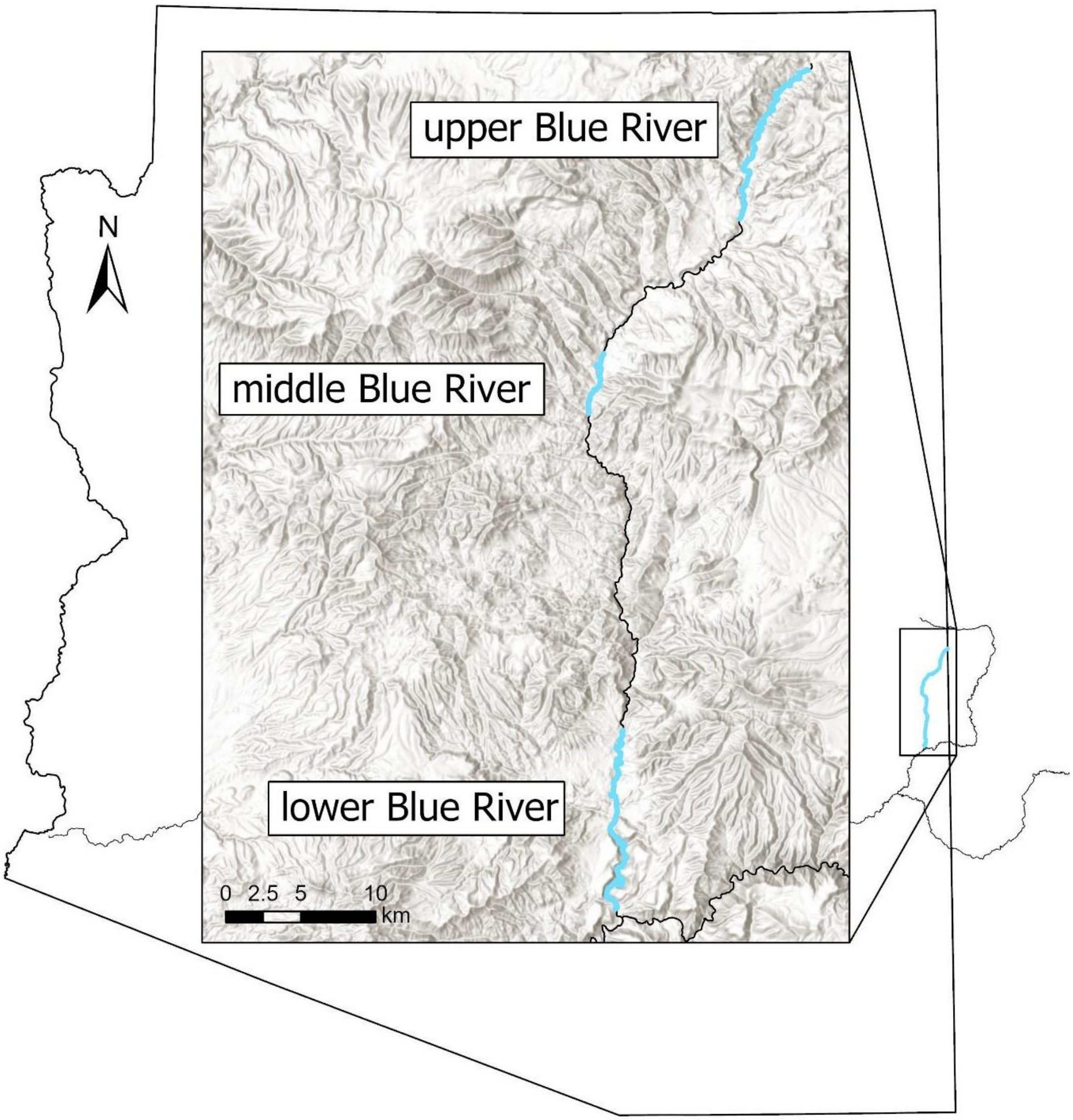

Figure 15.—Map showing the upper (New Mexico border downstream to Blue Crossing Campground), middle (The Box downstream to Fritz Ranch), and lower (Fritz Ranch downstream to the barrier) project areas of the Blue River.

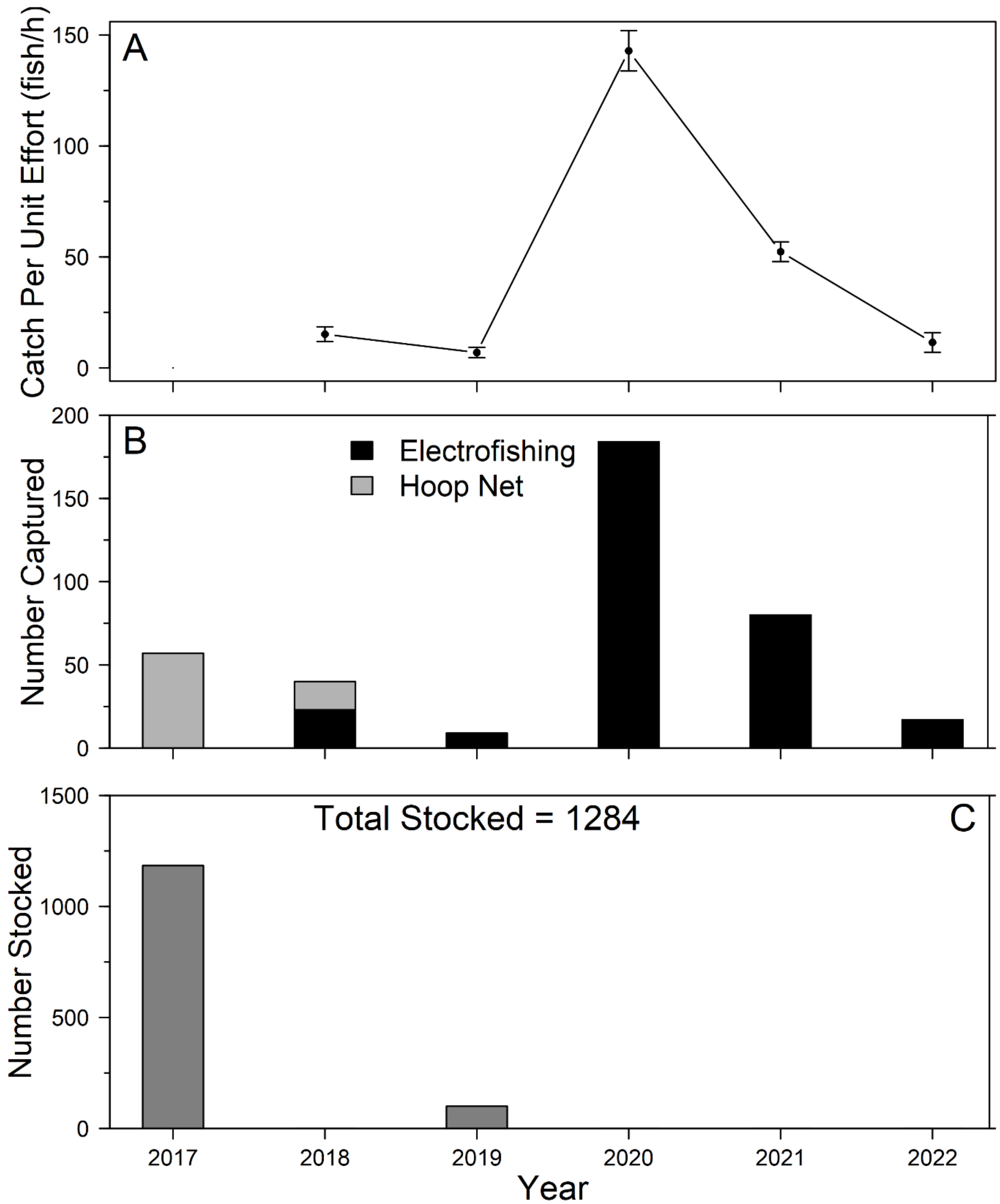

Figure 16.—Summary of Roundtail Chub captured and stocked in the middle Blue River, annually from 2017 to 2022 with (A) mean catch per unit effort (fish/h) for backpack electrofishing with standard error bars, (B) total number of fish captured by gear type (hoop nets in gray, backpack electrofishing 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.

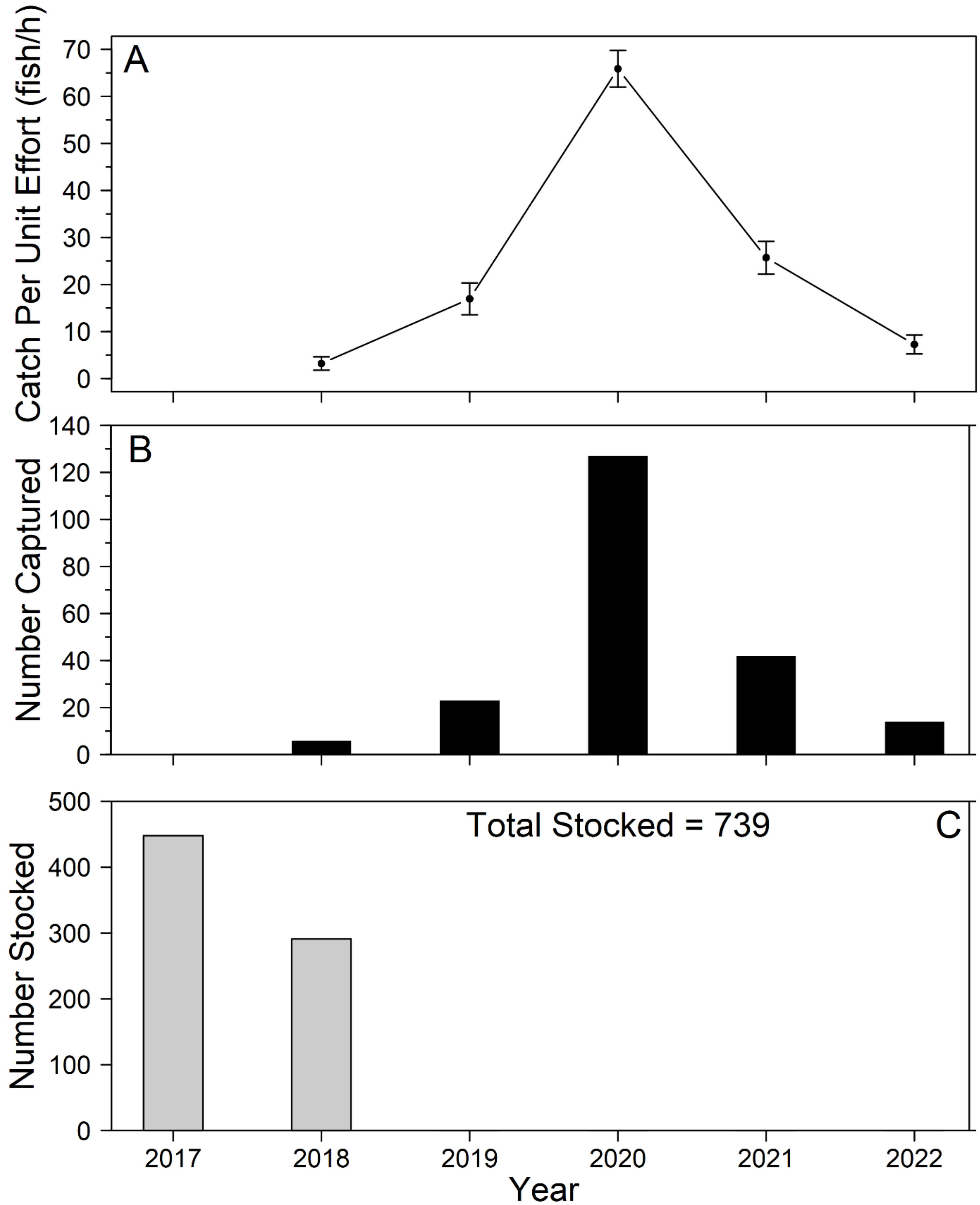

Figure 17.—Summary of Spikedace captured and stocked in the middle Blue River, annually from 2017 to 2022 with (A) mean catch per unit effort (fish/h) with standard error bars, (B) total number of fish captured, and (C) total number of fish stocked.

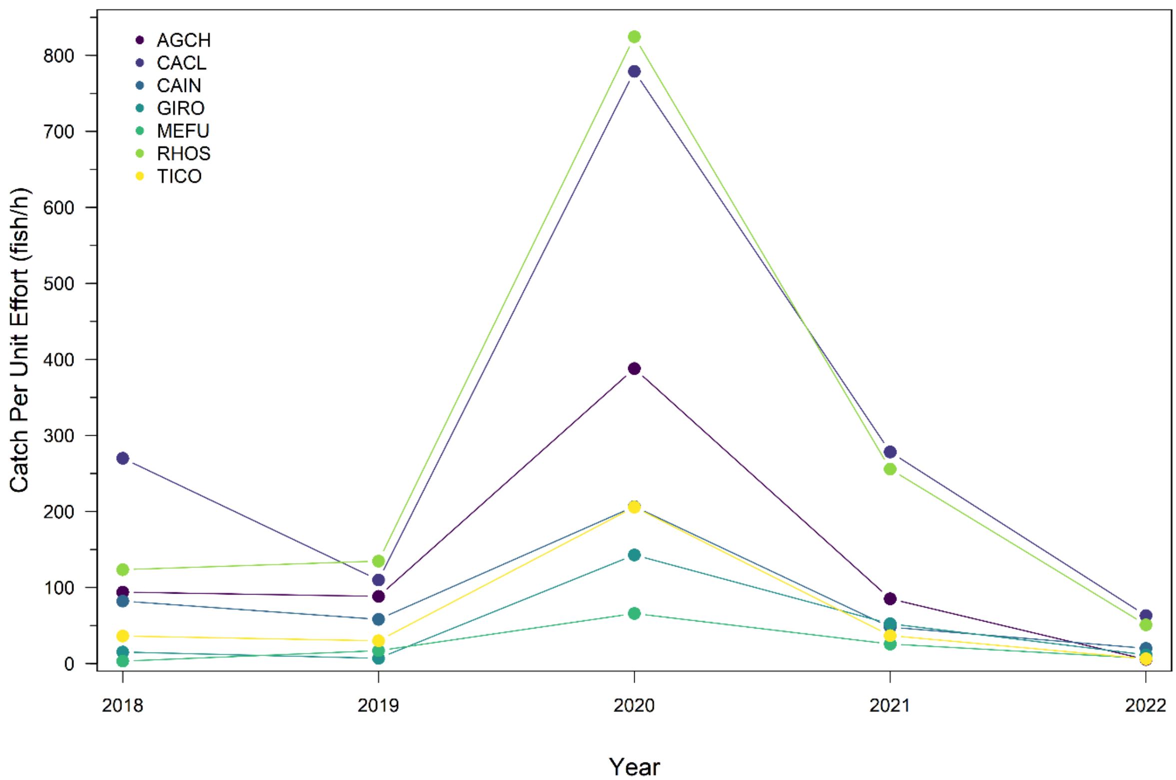

Figure 18.—Mean annual backpack electrofishing catch per unit effort (fish/h) of all native fish species (AGCH = Longfin Dace, CACL = Desert Sucker, CAIN = Sonora Sucker, GIRO = Roundtail Chub, MEFU = Spikedace, RHOS = Speckled Dace, TICO = Loach Minnow) captured in the Middle Blue River, AZ from 2018 to 2022. Standard error bars are not shown to improve clarity of mean catch per unit effort trends.

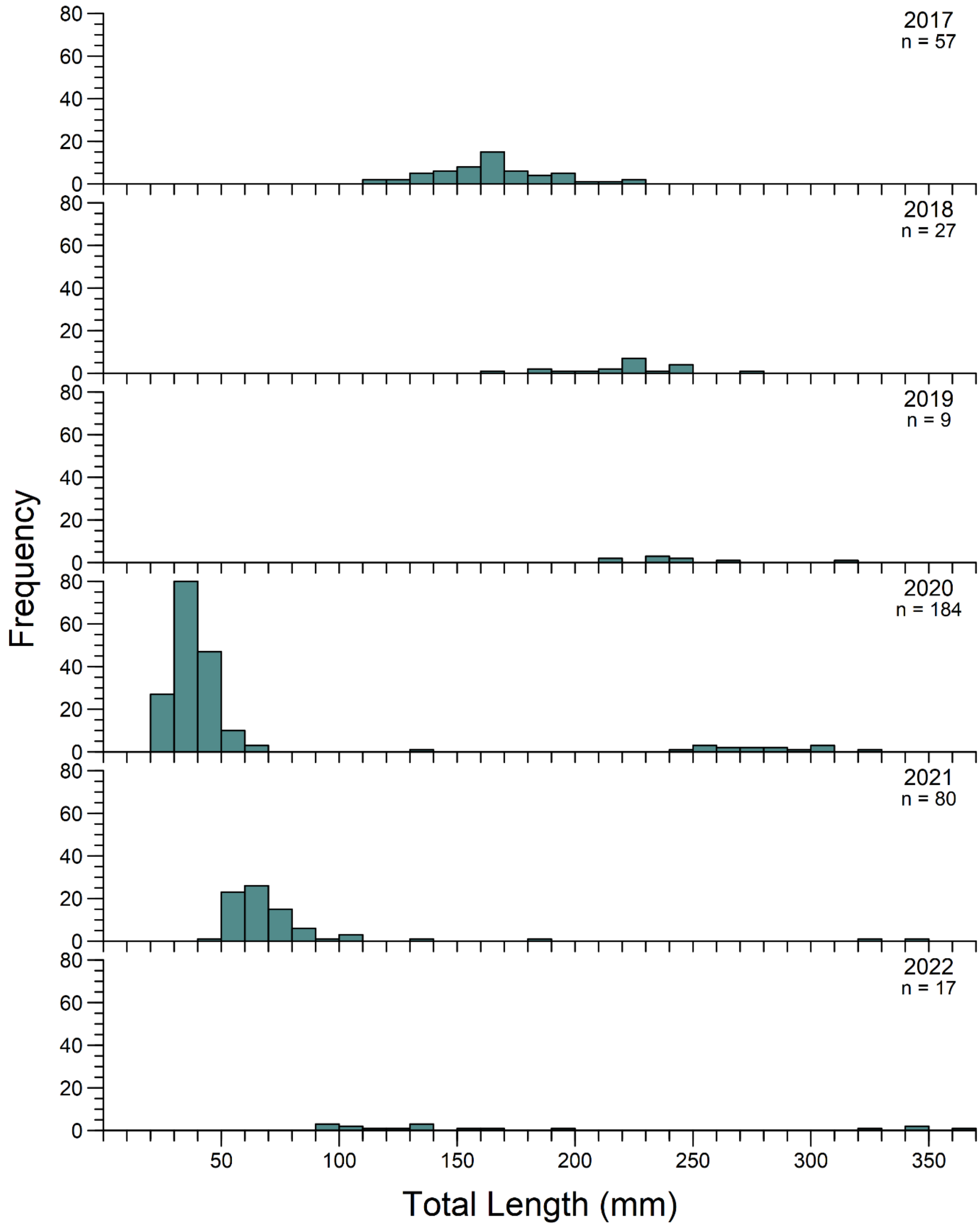

Figure 19.— Length frequency distribution of Roundtail Chub captured during annual monitoring in the middle Blue River, from 2017 to 2022. Only fish captured on the first pass are included. Number of fish captured and measured each year is shown in the top right corner of each panel.

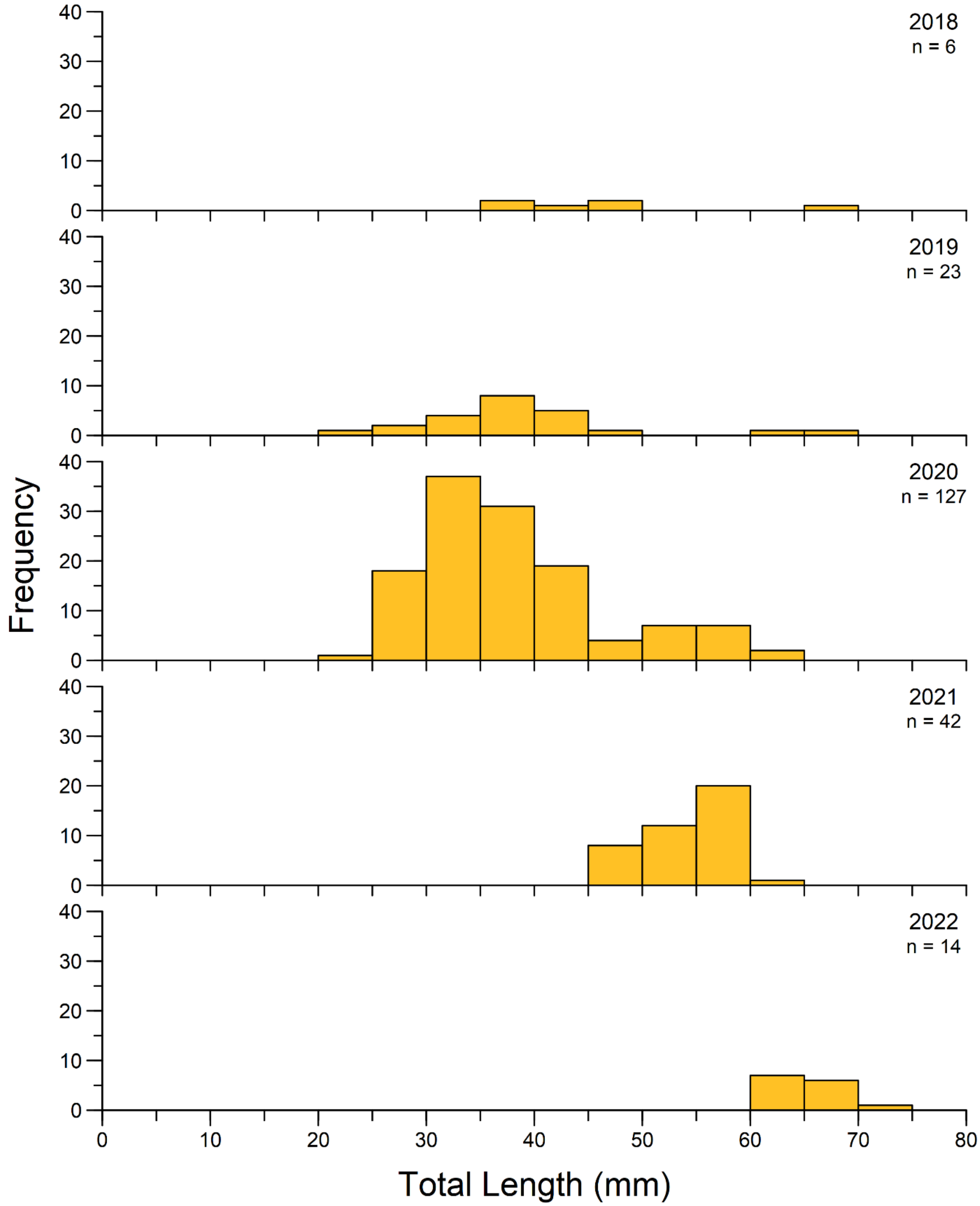

Figure 20.— Length frequency distribution of Spikedace captured during annual monitoring in the middle Blue River, from 2018 to 2022. Only fish captured on the first pass are included. Number of fish captured and measured each year is shown in the top right corner of each panel.

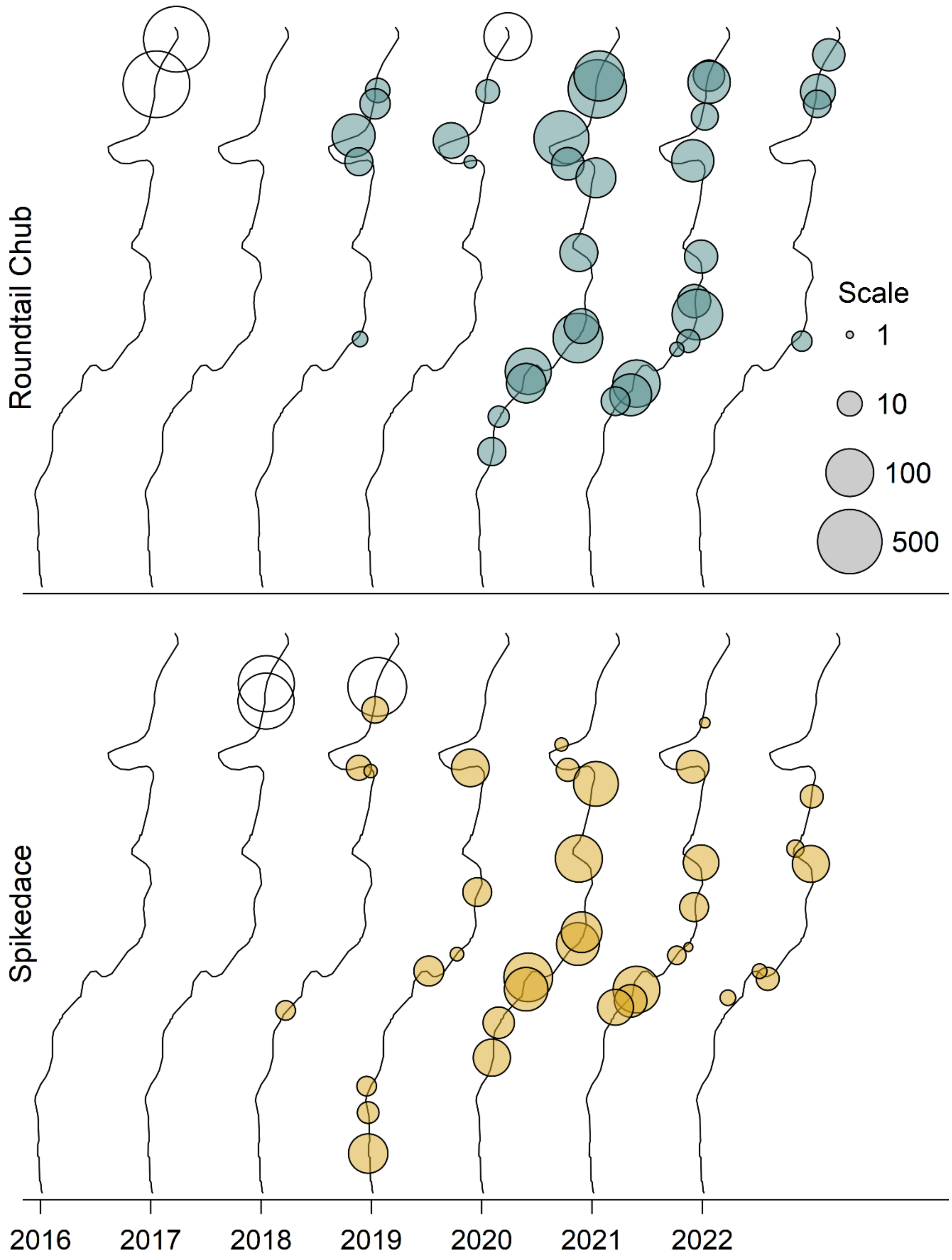

Figure 21.—Roundtail Chub (top row) and Spikedace (bottom row) stocking locations (open circles) and mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the middle Blue River from 2016-2022. Size of points indicates either the number of fish stocked or the relative abundance during monitoring at a particular location.

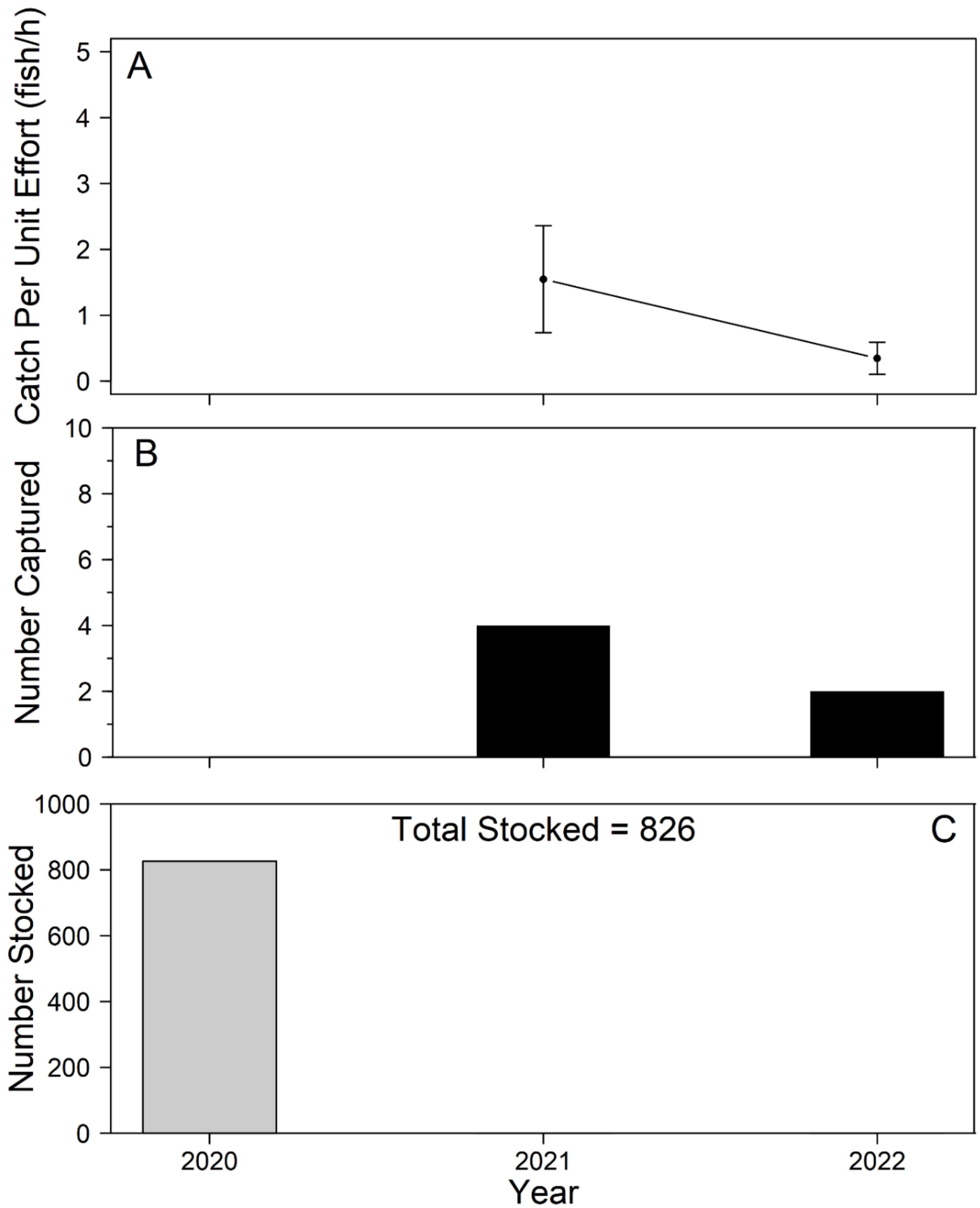

Figure 22.—Summary of Spikedace captured and stocked in the upper Blue River, annually from 2020 to 2022 with (A) mean catch per unit effort (fish/h) with standard error bars, (B) total number of fish captured, and (C) total number of fish stocked.

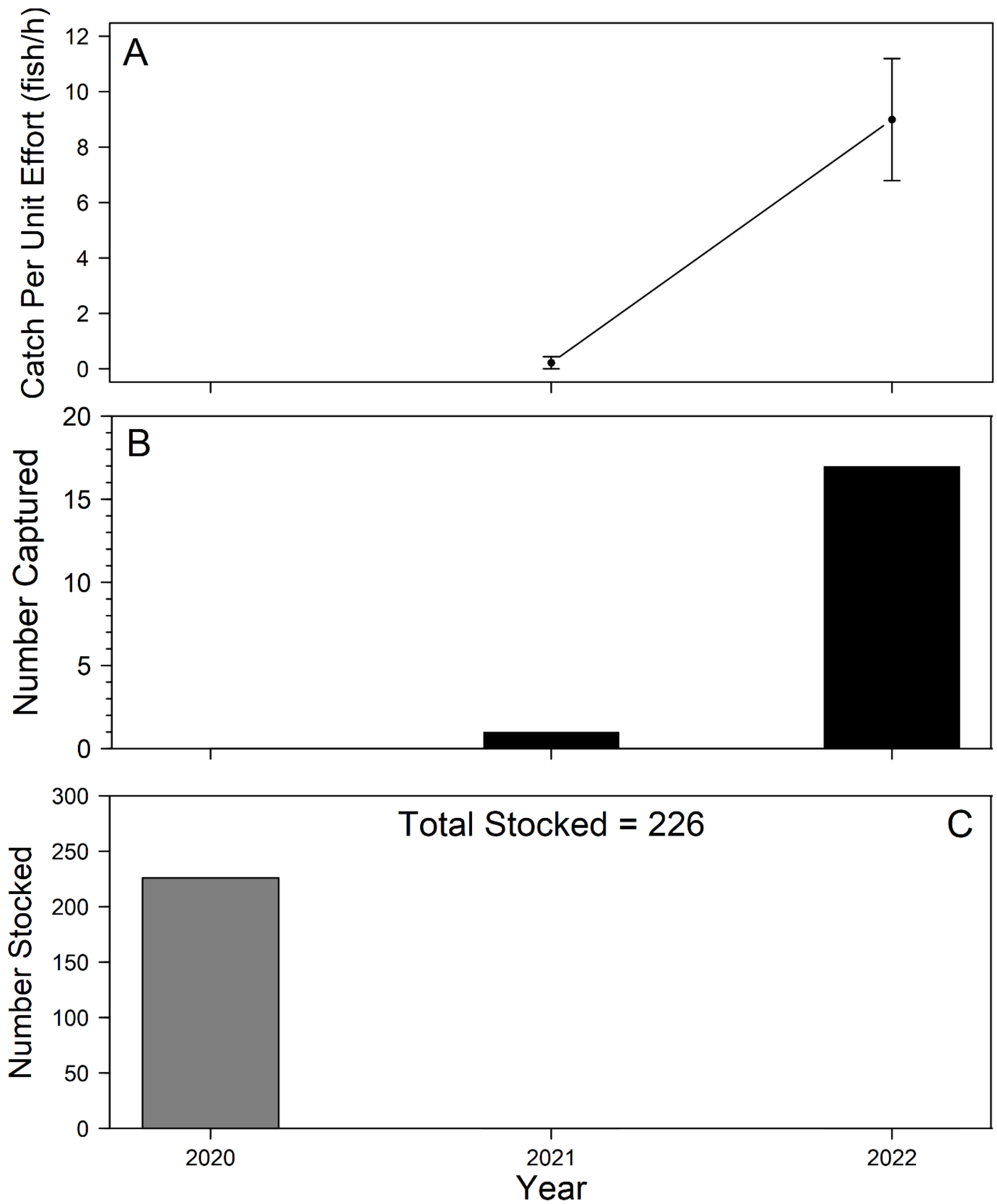

Figure 23.—Summary of Roundtail Chub captured and stocked in the upper Blue River, annually from 2020 to 2022 with (A) mean catch per unit effort (fish/h) with standard error bars, (B) total number of fish captured, and (C) total number of fish stocked.

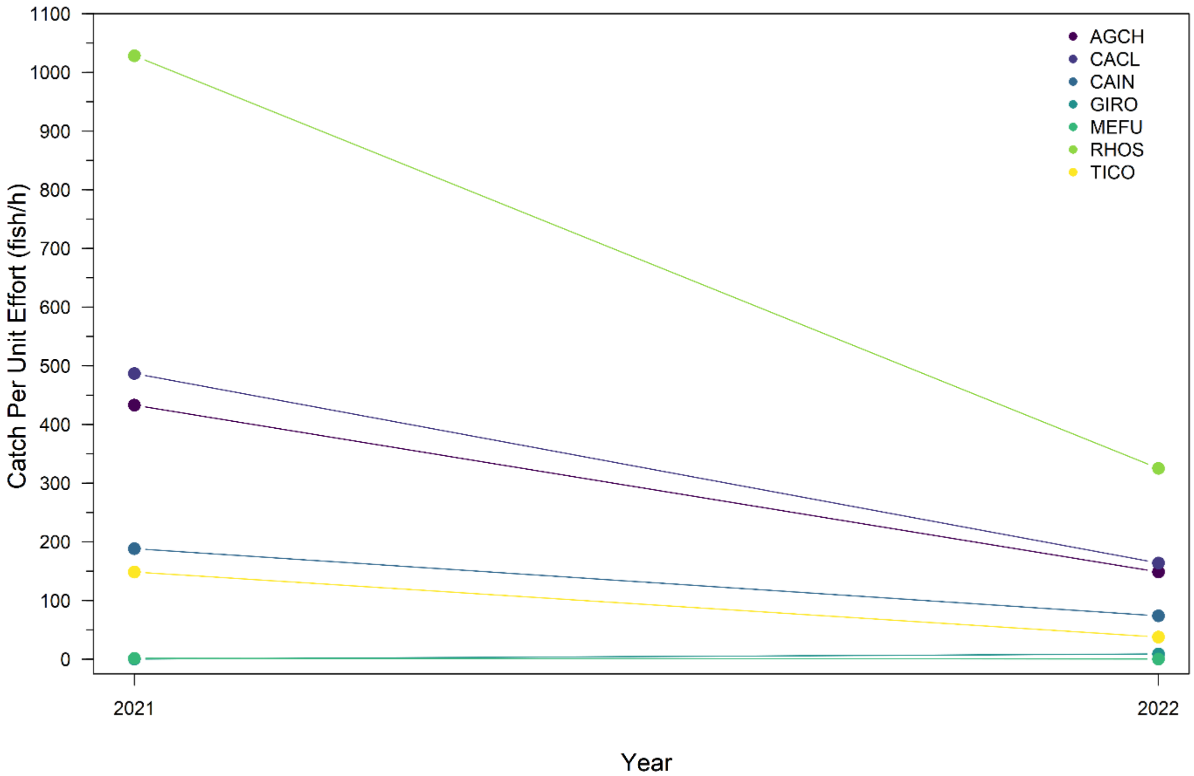

Figure 24.—Mean annual backpack electrofishing catch per unit effort (fish/h) of all native fish species (AGCH = Longfin Dace, CACL = Desert Sucker, CAIN = Sonora Sucker, GIRO = Roundtail Chub, MEFU = Spikedace, RHOS = Speckled Dace, TICO = Loach Minnow) captured in the Upper Blue River, AZ from 2021 to 2022. Standard error bars are not shown to improve clarity of mean catch per unit effort trends.

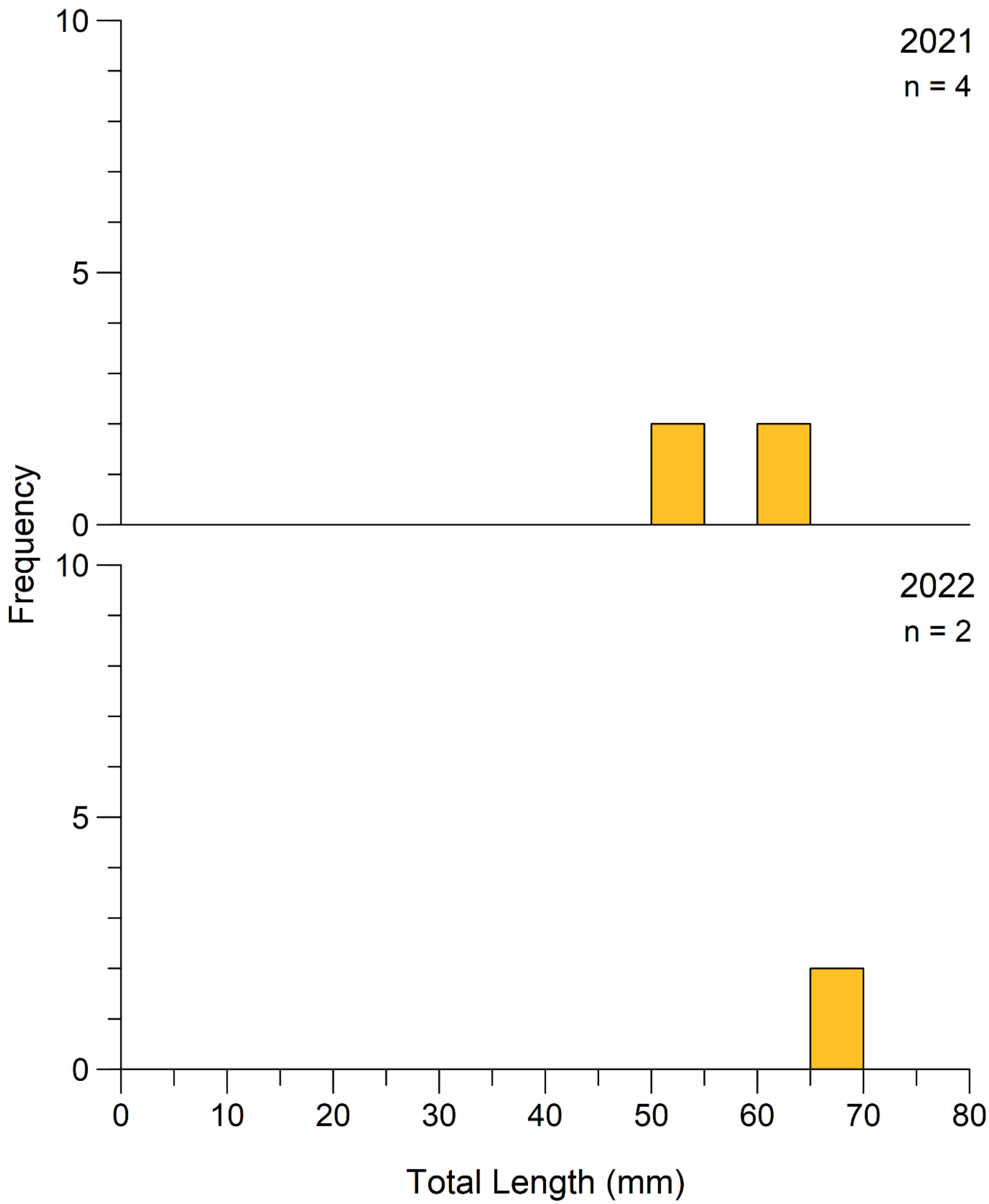

Figure 25.— Length frequency distribution of Spikedace captured during annual monitoring in the upper Blue River, from 2021 to 2022. Number of fish captured and measured each year is shown in the top right corner of each panel.

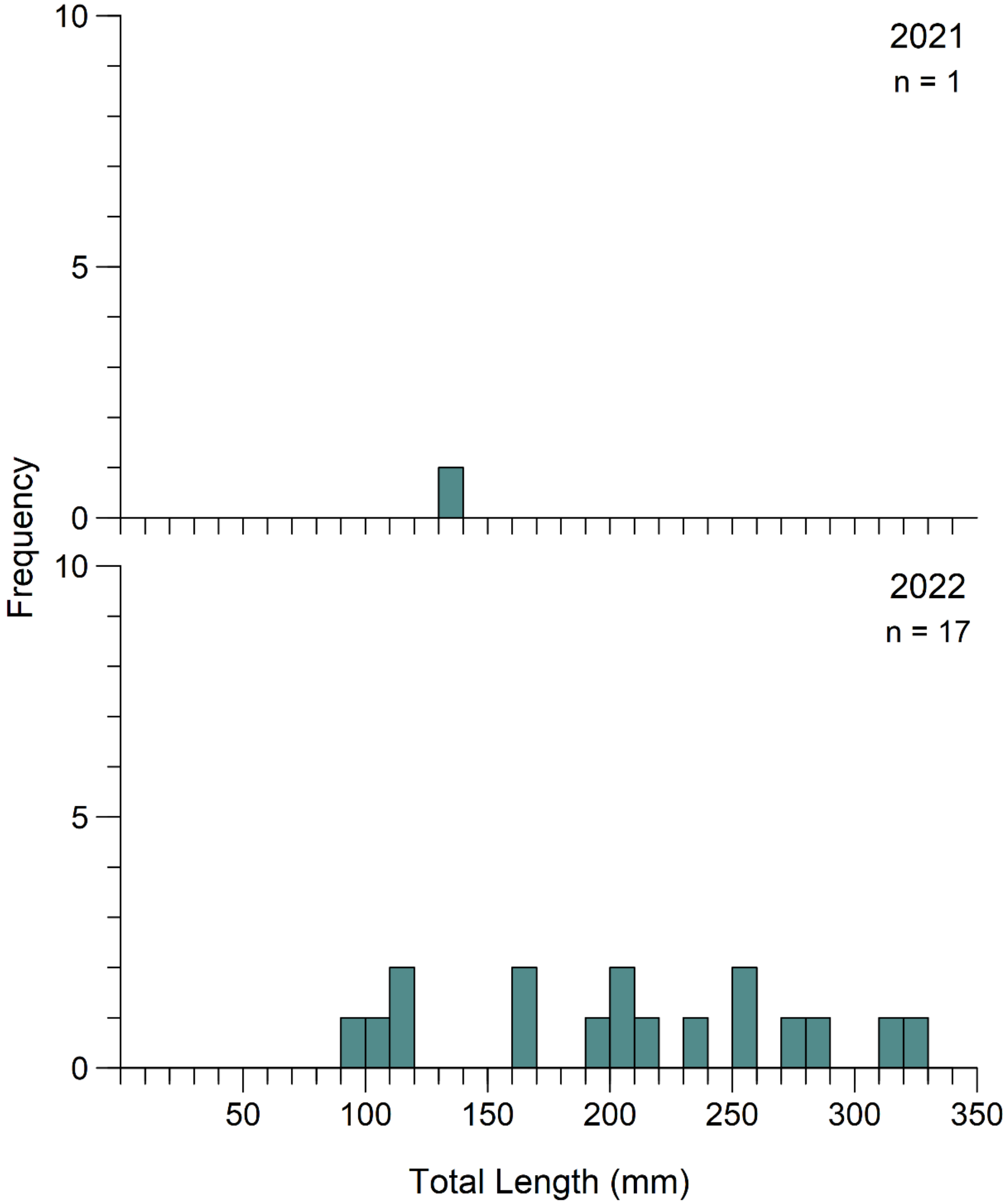

Figure 26.— Length frequency distribution of Roundtail Chub captured during annual monitoring in the upper Blue River, from 2021 to 2022. Number of fish captured and measured each year is shown in the top right corner of each panel.

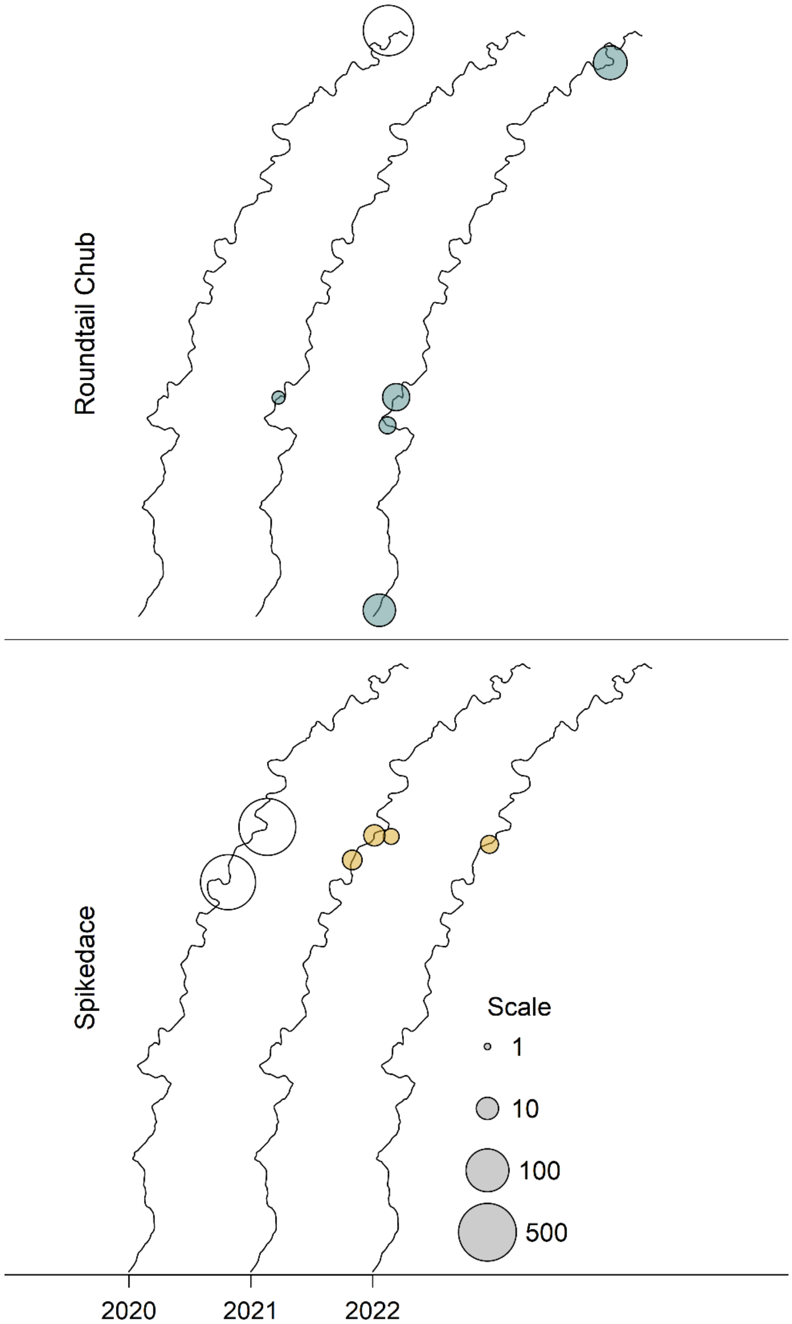

Figure 27.—Roundtail Chub (top row) and Spikedace (bottom row) stocking locations (open circles) and mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the middle Blue River from 2020-2022. Size of points indicates either the number of fish stocked or the relative abundance during monitoring at a particular location.

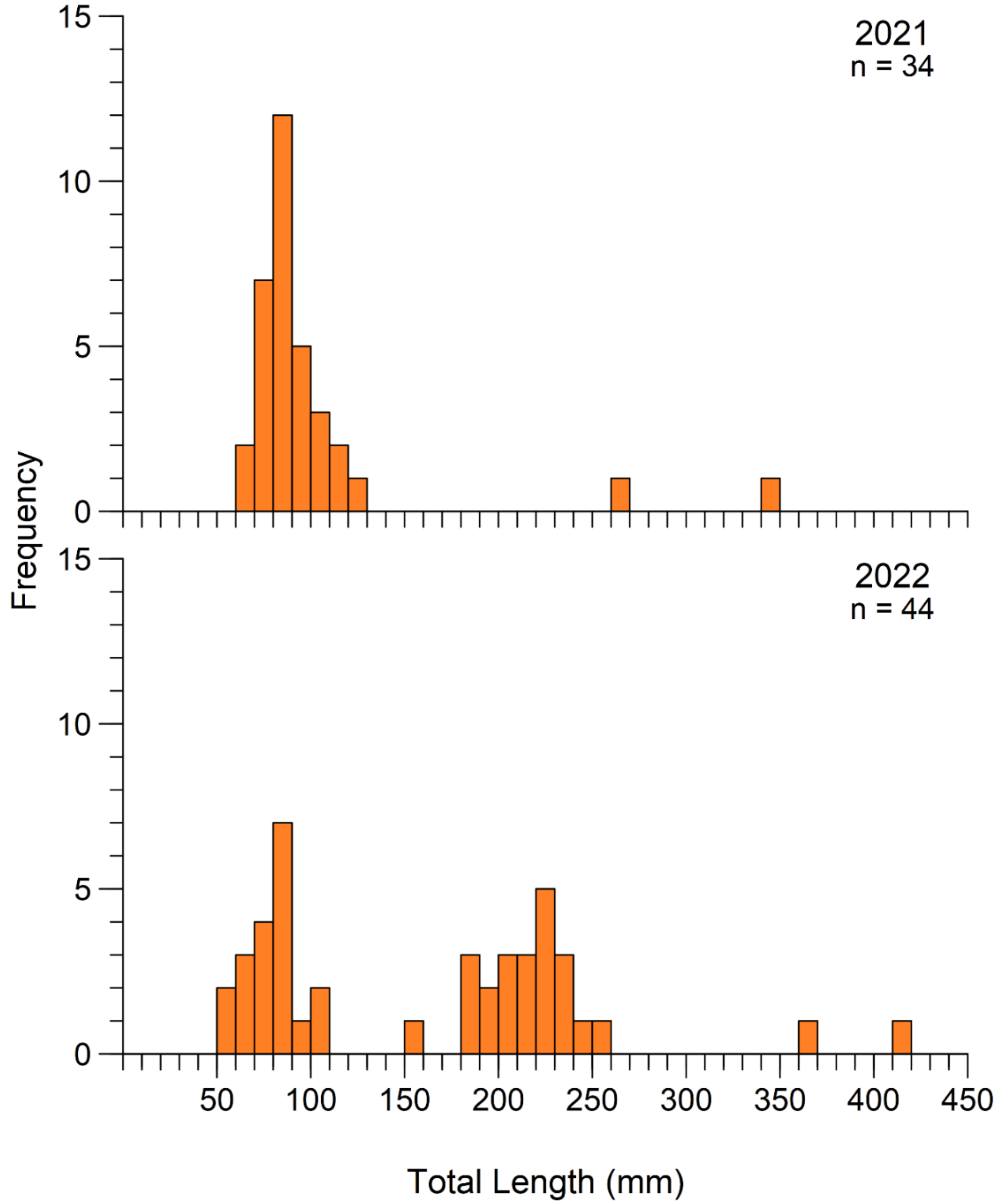

Figure 28.— Length frequency distribution of Brown Trout captured during annual monitoring in the upper Blue River, from 2021 to 2022. Number of fish captured and measured each year is shown in the top right corner of each panel.

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

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
  - Goal 3. Remove nonnative aquatic species threats.
  - Goal 4. Replicate populations and their associated native fish community into protected streams and other surface waters.
  - Goal 7. 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 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.
- 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.

Background: Harden Cienega Creek is a tributary to the San Francisco River near the New Mexico state line. Roundtail Chub[10] 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 Chub. Chub were initially translocated from lower Harden Cienega Creek to the previously unoccupied reach upstream of the waterfall in 2015, with genetic augmentations in 2018 and 2019. Monitoring from 2017 to 2020 detected several hundred chub representing all size classes above the barrier. Gila Topminnow (n = 631; Bylas Spring lineage) were first stocked in lower Harden Cienega Creek downstream of the waterfall barrier in 2019 and augmented in 2021. 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 more abundant and broadly distributed within Harden Cienega Creek. A removal plan was drafted (Hickerson et al. 2020) and Green Sunfish removal efforts were initiated in 2020. Because Green Sunfish were captured well upstream of the barrier on multiple occasions, it was concluded that an upstream source of Green Sunfish exists in the Harden Cienega drainage. Surveys of all 43 stock tanks in the Arizona portion

[10] Chub in Harden Cienega Creek were previously classified as Gila Chub.

of the Harden Cienega Creek watershed failed to detect any fish. However, Green Sunfish were detected in at least three tanks in New Mexico during surveys in 2021 (Figure 29).

Results:

Gila Topminnow Monitoring

On June 7, 2022, Department staff monitored Gila Topminnow by setting ten minnow traps in the vicinity of the stocking location for a soak time of approximately six hours. A total of 18 Longfin Dace, 2 Speckled Dace, and 2 Roundtail Chub were captured. No Gila Topminnow were captured or observed during the monitoring or removal efforts.

Green Sunfish Removal

On May 10, 2022, Department staff completed the first Green Sunfish removal pass of the year in Harden Cienega Creek. The stream was electrofished for 12,746 seconds from the start of flow (UTM 12S 673650/3674847; approximately 200 m upstream from the confluence with the San Francisco River) upstream to the terminus of perennial water near Prospect Canyon with no sunfish captured. Two juvenile Common Carp (170, 192 mm TL) were captured in the most downstream extent of flow near the confluence with the San Francisco River. The crew also set seven mini-hoop nets in pools too deep to sample effectively with the backpack electrofishing equipment and captured a total of three Green Sunfish, four Desert Sucker, and 21 Roundtail Chub. All Green Sunfish captured were of adult size, consistent with trends from the past few years (Figure 30).

On June 7, 2022, Department staff completed the second Green Sunfish removal pass of the year in Harden Cienega Creek. The stream was electrofished for 9,257 seconds from the start of flow upstream to the terminus of perennial water near Prospect Canyon with no sunfish captured. A total of 21 Red Shiner were captured and removed in the vicinity of the San Francisco River confluence. The crew also set eight mini-hoop nets in pools too deep to sample effectively with the backpack electrofishing equipment and captured one Green Sunfish (136 mm TL), one Sonora Sucker, and 25 Roundtail Chub. The lone Green Sunfish was captured in a pool where the majority of Green Sunfish have been captured during previous removal efforts (Figure 31).

Green Sunfish captures have consistently declined with each full removal pass (2020 pass 1 = 38, 2021 pass 1 = 16, 2021 pass 2 = 7, 2022 pass 1 = 3, 2022 pass 2 = 1; Figure 31). This decline has probably been aided by the drought conditions which have likely prohibited movement of sunfish into Harden Cienega Creek from upstream sources. In addition, Green Sunfish still do not appear to be spawning in Harden Cienega Creek, as only adult fish have been captured during removal passes (Figure 30). Current removal efforts seem to be on track to eradicate Green Sunfish from Harden Cienega Creek in the near future, if dispersal from upstream sources of Green Sunfish can be prevented in the near future.

Tank Surveys

During July 19-20, 2022, Department staff assisted New Mexico Department of Fish and Game (NMDFG) staff with surveys of stock tanks in the Harden Cienega Creek drainage in New Mexico.

The crew seined Ditch Tank because Green Sunfish were previously detected in 2020 (alive) and 2021 (dead). Two seine hauls were carried out and no fish were captured, but tiger salamanders were relatively abundant. The crew assisted NMDFG staff with measurements of the length, width and depth of Ditch Tank, California Tank, and Distill Tank. Presence of Green Sunfish in California tank and Distill Tank was confirmed with dip net sweeps at each tank.

Recommendations: Topminnow have not been captured following the initial stocking in 2019 and subsequent augmentation in 2021. It is not clear what is currently limiting survival of Gila Topminnow in Harden Cienega Creek, but predation pressure from the existing chub population is one potential limiting factor. Translocation of additional Gila Topminnow into Harden Cienega Creek is not recommended at this time.

Continued nonnative removal effort is warranted in Harden Cienega Creek in 2023 because Green Sunfish are still present, and it is possible that additional fish may have dispersed into this reach with monsoon rains. Both backpack electrofishing and mini-hoop nets proved effective at capturing and removing Green Sunfish, and this combination approach should continue. Eradication seems achievable if the upstream sources can be eradicated in the near future.

Multiple stock tanks in New Mexico continue to support populations of Green Sunfish in 2021, which may be the sources of sunfish to downstream reaches. Should NMGFD wish to pursue eradication efforts, the Department will assist however possible. The remaining three tanks on private property should also be sampled if permission can be obtained from the property owners.

Tables and Figures:

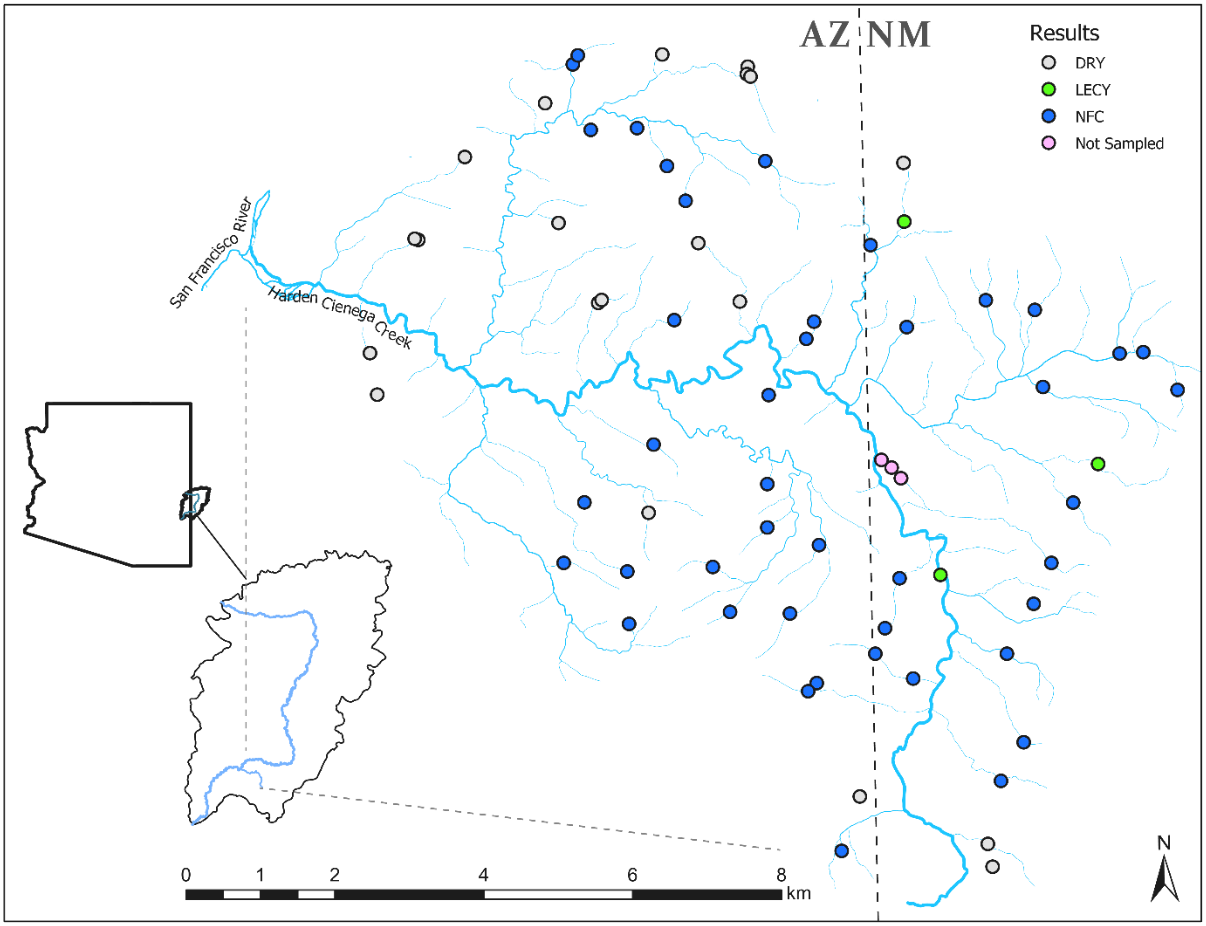

Figure 29.—Map of all tanks surveyed in the Arizona and New Mexico portions of the Harden Cienega Creek drainage during 2020 and 2021. Show are tanks that were dry upon arrival (grey points), and tanks that contained water and were sampled by bag seine, straight seine or dip net where no fish were captured (blue points). Also shown are Ditch Tank, Distill Tank and California Tank where Green Sunfish were detected (green points). Three tanks on private property remain in New Mexico remain to be sampled (pink points).

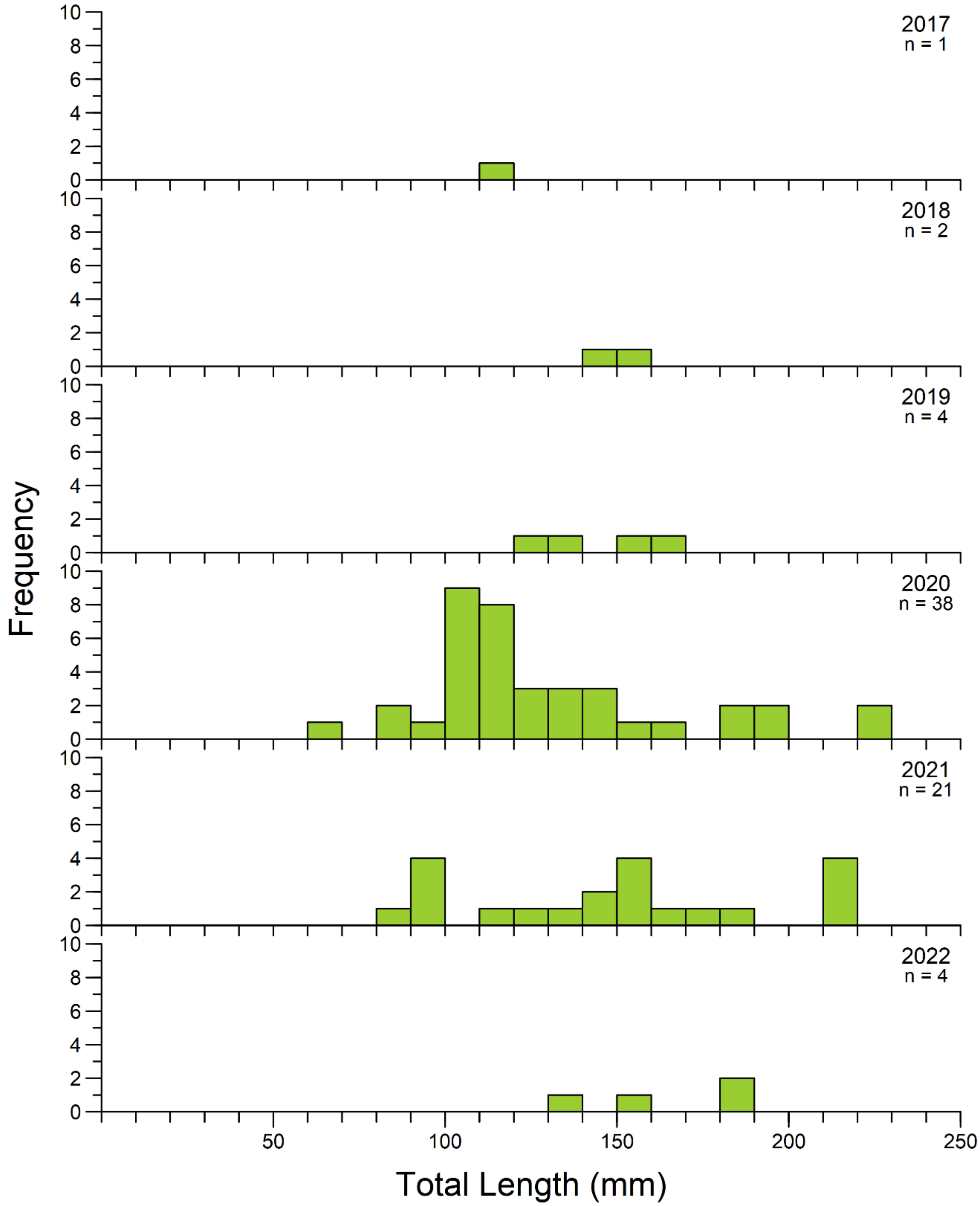

Figure 30.—Length frequency distribution of the number of Green Sunfish captured and removed during annual monitoring and nonnative removal efforts in Harden Cienega Creek, from 2017 to 2022. Number of fish captured and measured each year is shown in the top right corner of each panel.

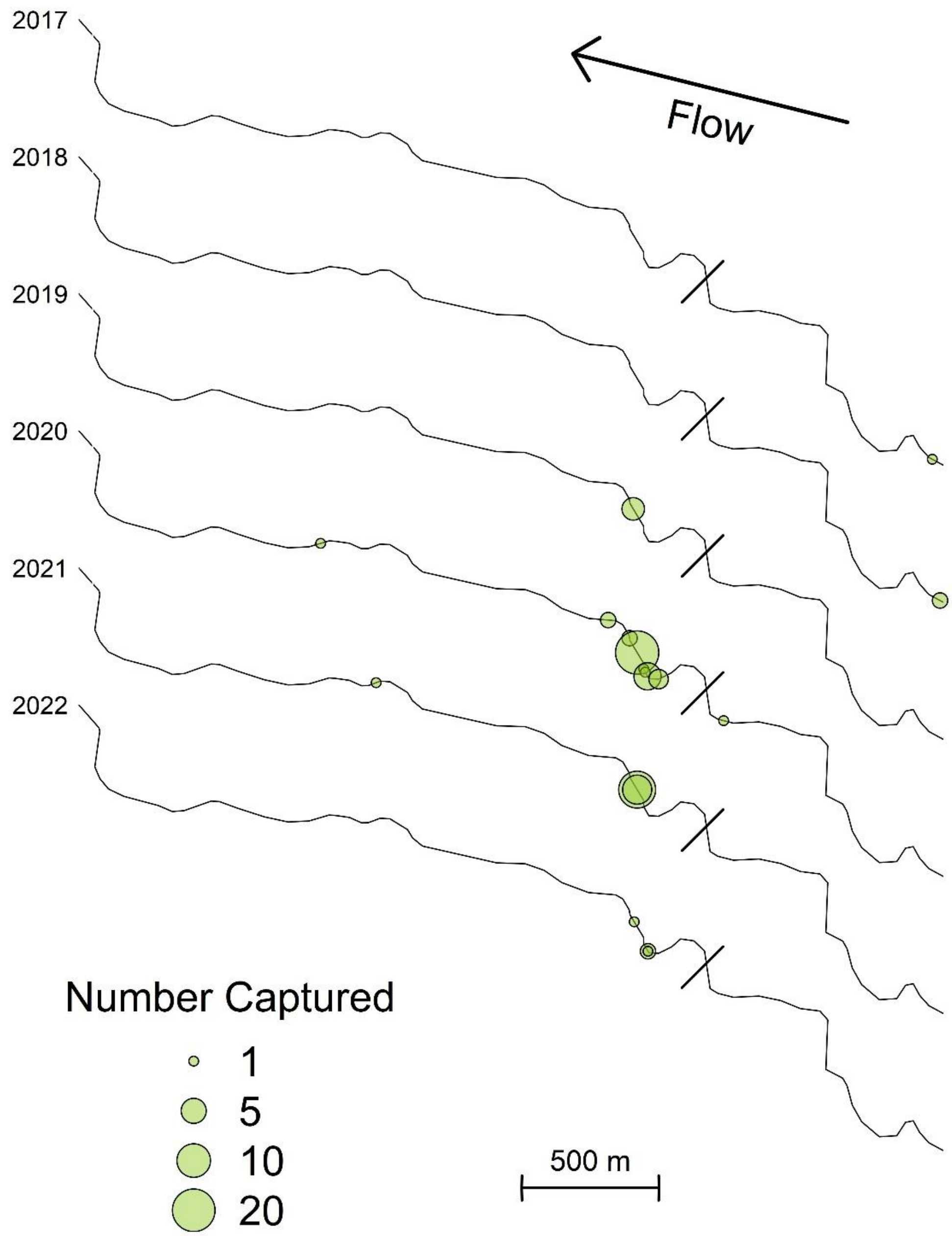

Figure 31.—Locations of Green Sunfish captured in the perennial portion of Harden Cienega Creek from 2017-2022. The barrier location is indicated by a diagonal line. Size of points indicates number of fish captured at a particular location during nonnative removal or monitoring efforts. Monitoring during 2017-2019 was only upstream of the barrier.

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

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
    - Goal 3. Remove nonnative aquatic species threats.
    - Goal 4. Replicate populations and their associated native fish community into protected streams and other surface waters.
    - Goal 7. 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 series of perennial cienega pools located on San Rafael State Natural Area, which is owned by Arizona State Parks and Trails (AZSP). The drainage is tributary to the Santa Cruz River, about 2 km from the international border with Mexico. Sharp Springs was historically occupied by a relict population of Gila Topminnow. Nonnative Western Mosquitofish were first detected in Sharp Springs in 1979. Monitoring by the Department and partners documented the gradual decline and eventual disappearance of Gila Topminnow, which was last detected in 2001. Extirpation of topminnow has primarily been attributed to predation and competition with nonnative mosquitofish. The purpose of this project is to eradicate Western Mosquitofish from Sharp Spring and reintroduce Sharp Spring lineage Gila Topminnow. Eradication of Western Mosquitofish would also create an opportunity to potentially replicate a population of upper Santa Cruz River Roundtail Chub[11] in Sharp Spring.

[11] Roundtail Chub in the upper Santa Cruz River previously classified as Gila Chub.

In June 2013, Department staff attempted to eradicate Western Mosquitofish by draining the pools in Sharp Spring with gasoline powered pumps. The two most upstream pools were pumped down, but not entirely drained due to the unexpected depth of fine sediment in the bottom of the pools, which retained water (and fish) and fouled the pumps. The pools partially refilled overnight, and live mosquitofish were observed the following morning. The pumping effort was abandoned because the pools could not be completely dried. It was determined that a rotenone treatment was the only management option likely to achieve successful eradication of mosquitofish.

Conversations with AZSP about a potential rotenone treatment of Sharp Springs began in 2017 and gained momentum in early 2020. In 2021, Department staff coordinated with AZSP staff and obtained a permit necessary to proceed with the Department's Piscicide Treatment Planning and Procedures Manual (PTPPM). Stages 1 and 2 were completed in 2021, and stage 3 was initiated, which included hosting a public meeting and completing internal environmental compliance. Commission approval for the treatment was received in early March, 2022.

Results:

On March 22, 2022, Department staff collected 30 Gila Topminnow (Sharp Spring lineage) from AD Wash for a fish health assessment in support of translocations to Sharp Spring later in the year. No parasites or pathogens of concern were detected.

On March 22, 2022, Department staff collected 30 Gila Topminnow (Sharp Spring lineage) from the SRP Pond at Page Springs hatchery for a fish health assessment in support of translocations to Sharp Spring later in the year. Parasitic yellow grub were detected in most of the fish.

On March 28, 2022, Department staff collected 30 Gila Topminnow (Sharp Spring lineage) from Mud Spring for a fish health assessment in support of translocations to Sharp Spring later in the year. No parasites or pathogens of concern were detected.

On April 6, 2022, Department staff completed a bioassay at Sharp Spring. The crew collected mosquitofish from Sharp Spring with a dip net and placed ten fish (5 <20 mm TL, 5 ≥20 mm TL) into an insulated bucket containing 10 L of site water for each of the six treatments (Control, 12.5, 25, 50, 100, 200 ppb active rotenone) recommended in the Rotenone SOP (Finlayson et al. 2018). A stock solution was made by adding 1 mL CFT Legumine to 1 L of fresh filtered water, and the correct amount of stock solution (0, 2.5, 5, 10, 20, 40 mL) was then added to each of the treatment buckets using a pipette. Contact time was recorded from the time stock solution was added to each of the treatments. Fish were observed at 0.5, 1, 2, 4, 6, and 8 h contact time to determine how many fish were alive or dead. An individual was determined to be dead when it had already lost equilibrium and there was no movement or respiration for 30+ seconds. All fish died within 8 h contact time in all treatments except the 12.5 ppb treatment and the control (Figure 32). The two fish in the control treatment that died were small (<20 mm TL) males, with the other control fish showing no signs of distress. Several other small males that were kept in the original collection bucket with a lid and an aerator also died, so these small fish just might not have handled capture and holding as well as larger fish. In general, smaller fish died well before larger fish and males died before females. Large females were the last fish to die in all treatments.

During May 24-26, 2022, Department staff measured pool volumes of all 15 pools at Sharp Springs (Figure 33) in preparation for rotenone treatments. The crew also prepared and organized gear for the upcoming treatment.

During May 31-June 2, 2022, Department staff applied rotenone to all 15 pools in Sharp Springs in an attempt to eradicate Western Mosquitofish. Preparations for the treatment occurred on May 31. Rotenone was applied to all pools on June 1, and all pools were deactivated following at least 8 hours of contact time. Water samples were collected on June 2 until rotenone concentrations were below the threshold required to remove placarding and leave the treatment area.

During June 6-7, 2022, Department and Reclamation staff set a total of 28 minnow traps in each of the 15 pools within the treatment area of Sharp Springs for a total soak time of 611 hours. One trap was set for every 10 m of pool length. The crew checked and reset each trap five times with a minimum soak time of at least two hours between checks, including one overnight soak. Two pools (6 and 9) dried during the survey to the point where they were too shallow to effectively fish a minnow trap, so traps were only set three times in these pools. A total of 202 Western Mosquitofish were captured in only four pools: SP02, SP03, SP13, SP14

During June 13-15, 2022, Department staff applied rotenone to all 15 pools in Sharp Springs in an attempt to eradicate Western Mosquitofish. Preparations for the treatment occurred on June 13. Rotenone was applied to all pools on June 14, and all pools were deactivated following at least 8 hours of contact time. Water samples were collected on June 15 until rotenone concentrations were non-detectable.

During June 20-21, 2022, Department staff set a total of 28 minnow traps in 13 of the 15 pools within the treatment area for a total soak time of 669 hours. One trap was set for every 10 m of pool length. The crew checked and reset each trap five times with a minimum soak time of at least two hours between checks, including one overnight soak. Two pools (6 and 9) were too shallow to effectively fish a minnow trap, so the entirety of each pool was sampled with two dip nets sweeps. No mosquitofish were captured or observed and the treatment was determined to be a success.

During June 27-28, 2022, Department staff and a volunteer translocated Sharp Spring lineage Gila Topminnow to Sharp Spring. The crew collected 253 Gila Topminnow from AD Wash using dip nets on the morning of June 27. A total of 132 fish were stocked in pool 10 and 130 in pool 2 at Sharp Spring with three mortalities during transport and stocking. More fish were stocked than were collected at AD Wash because 12 Gila Topminnow were born in the transport tank in transit to Sharp Spring. The crew collected 406 Gila Topminnow from Mud Spring on the morning of June 28 using a combination of minnow traps and dip nets. A total of 200 fish were stocked in pool 10 and 206 fish in pool 2 with no mortalities during transport and stocking. The fish were in great condition at the time of release and behaved normally.

On September 9, 2022, Department staff collected 271 female fish from the SRP Pond at Page Springs hatchery and held them in aquaria at Department Headquarters in an effort to produce offspring free of yellow grub. Only 12 offspring were produced, with three individuals failing to survive in captivity. The remaining adult fish were returned to SRP Pond on October 18, 2022, with a total of 108 mortalities during captivity.

On October 12, 2022, Department staff monitored translocated populations of Gila Topminnow in Sharp Springs. The crew set 5 minnow traps in each of the pools stocked in June (SP02, SP10) and conducted opportunistic dip net sweeps in additional pools downstream that were not stocked to see if this species dispersed throughout the reach. The crew captured a total of 135 Gila Topminnow (35 <20 mm TL, 100 ≥20 mm TL) between both stocked pools sampled with minnow traps. No fish were captured in downstream pools utilizing dip net sweeps. Further, no mosquitofish were captured or observed in any of the pools at Sharp Spring, providing further confirmation this species was eradicated during the rotenone treatments. Immediately after monitoring, an additional nine Gila Topminnow were stocked between Pool 2 and Pool 10.

<u>Recommendations</u>: Additional Gila Topminnow from other Sharp Spring lineage donor populations should be translocated in 2023 to recreate as much of the original genetic diversity as possible in Sharp Spring. If the number of topminnow captured in Pools 2 and 10 increases in 2023, some of the captured fish should be moved to other pools within Sharp Spring. Efforts should be made to discuss the potential to translocate upper Santa Cruz Roundtail Chub into some of the pools within Sharp Spring.

Tables and Figures:

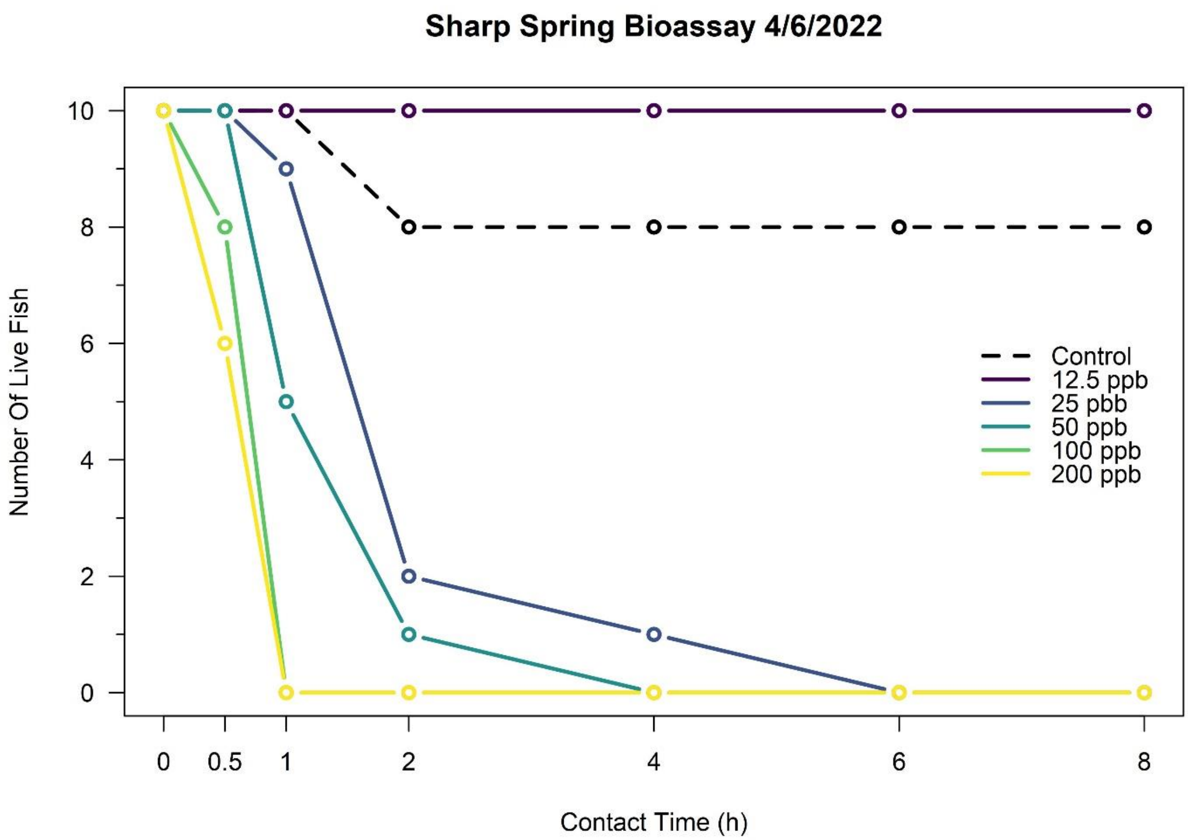

Figure 32.—Number of live Western Mosquitofish by contact time for each of the six active rotenone concentrations tested during the bioassay effort at Sharp Spring on April 6, 2022.

Figure 33.—Detail of Sharp Spring treatment area indicated by the blue line. Included is the approximate location of each of the 15 pools (white dots) from upstream (P1) to downstream (P15).

Upper Verde River native fish restoration (Task AZ-2020-1)

Strategic Plan Goals:

- Preventing Extinction and Managing Toward Recovery
  - Goal 3. Remove nonnative aquatic species threats.
  - Goal 4. Replicate populations and their associated native fish community into protected streams and other surface waters.
  - Goal 7. 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.
- 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 is currently focused on assessing the feasibility of nonnative fish control efforts should Reclamation construct fish barriers on the Verde River. 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 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. The goal of tank surveys is to understand the species composition and distribution of nonnative fish in the upper Verde River drainage and the potential for nonnative fish to disperse to the Verde River. Of the 146 tanks identified as having the greatest risk to the Verde River, a total of 52 were surveyed by Department

Gila River Basin Native Fish Program staff in 2021. Of the tanks surveyed, a total of 13 contained nonnative fish, including nine with just Green Sunfish. Of the other four tanks that possessed fish, they contained a combination of Bluegill, Yellow Bullhead, Black Crappie, and Largemouth Bass; although each species was not found in every tank.

Results: Of the 146 tanks identified as having the greatest risk to the Verde River, a total of 35 were surveyed by Department Gila River Basin Native Fish Program staff in 2022. All tanks with water present were sampled with three hauls of a bag seine (see general methods) unless noted otherwise. A combination of gill nets and angling were used to sample Hell Canyon Tank due to the size of the tank. The total number of fish captured at each location, mean relative abundance (CPUE), coordinates, and water presence for each tank surveyed in 2022 can be found in Table 9. A map of the location and species composition of tanks sampled in 2022 can be found in Figure 34.

During July 13-14, 2022, Department staff conducted tank surveys in the upper Verde River drainage. A total of two tanks were visited on the Kaibab National Forest. Green Sunfish, Black Bullhead and Yellow Bullhead were detected in Hell Canyon Tank, while Barrata Tank contained Green Sunfish and Fathead Minnow.

During July 26-27, 2022, Department staff conducted tank surveys in the upper Verde River drainage. A total of 11 tanks were visited on the private property owned by Northern Arizona University (Hat Ranch), as well as the Kaibab National Forest. Three tanks were documented as dry, two on the Kaibab National Forest (Evans Tank #1 and Evans Tank #2) and one on the Hat Ranch property (EK Tank). Largemouth Bass, Bluegill, Green Sunfish, and Black Crappie were detected in Bill Williams Loop tank on Hat Ranch. Further, Mosquitofish, Bluegill, and Largemouth Bass were found in Heifer Tank #1 on Hat Ranch. The remaining tanks were fishless.

During August 1-3, 2022, Department staff conducted tank surveys in the upper Verde River basin. A total of 21 tanks were visited on private property owned by Northern Arizona University (Hat Ranch), as well as the Kaibab National Forest. Three tanks were documented as dry, two on the Kaibab National Forest (Susan Tank and Winter Camp Tank) and one on Hat Ranch (Hell Canyon Tank #1). Green Sunfish were detected in Turkey Tank. The remaining tanks were fishless.

During August 9, 2022, Department staff conducted tank surveys in the upper Verde River basin. One tank was visited on private property. Green Sunfish was the only fish species detected in Lockett Tank.

Overall, nonnative fishes were detected in six of the 35 tanks sampled in 2022 (Table 9; Figure 34). Of the tanks where nonnative fish were detected, Green Sunfish were the most common (n = 5), followed by Largemouth Bass and Bluegill (n = 2), with Fathead Minnow, Yellow Bullhead, Black Bullhead, Black Crappie, and Western Mosquitofish each occurring in only one tank. Multiple size classes of Green Sunfish were detected in many of the tanks which suggests that these populations are self-sustaining.

Department staff also participated in several monthly barrier meeting updates in 2022.

Recommendations: Although surveys of tanks within 30 km of the Verde River where access has been granted are complete (Figure 35), stream channels downstream of stock tanks containing nonnative fishes should be surveyed to determine the community composition, persistence, and distribution of nonnative fish in these drainages. It would also be valuable to survey Big Chino Wash and Williamson Valley Wash at their confluence during a period of declining flows to better evaluate the risk of dispersal of nonnative fish from these drainages during periods of flow, since access was denied to sample most tanks in these large watersheds. It may also be valuable to survey additional tanks farther than 30 km (straight line distance) away from the Verde River if there are concerns about additional tanks potentially serving as sources of nonnative fish dispersal to the Verde River. Information about the frequency and duration of hydrologic connections between tributary streams (Hell Canyon, Chino Valley Wash, etc.) and the Verde River could also be evaluated with trail camera or modified conductivity loggers to better understand the risk of dispersal of nonnative fishes from stock tanks to the Verde River. Department staff will continue to participate in Verde River barrier calls and will begin internal discussions about how to manage nonnative fish in stock tanks and tributaries to the upper Verde River watershed.

Tables and Figures:

Table 9.—Summary of stock tanks and stream sites surveyed in the upper Verde River drainage in 2022. Included for each location is the site easting and northing (UTM NAD83 12S), whether water was present (status), gear type, species captured, total number of individuals captured, and the catch per unit effort (CPUE; fish/m^2 for bag seine and straight seine, fish/h for gill nets and angling) for each species at each location.

Water Name	Easting	Northing	Status	Gear	Taxa	Catch	CPUE
Baratta Tank	371812	3878964	Wet	Bag Seine	Fathead Minnow	164	1.64
Baratta Tank	371812	3878964	Wet	Bag Seine	Green Sunfish	2	0.02
Bill Williams Loop Tank	383655	3893027	Wet	Bag Seine	Green Sunfish	1	0.01
Bill Williams Loop Tank	383655	3893027	Wet	Bag Seine	Bluegill	664	0.13
Bill Williams Loop Tank	383655	3893027	Wet	Bag Seine	Largemouth Bass	1	0.01
Bill Williams Loop Tank	383655	3893027	Wet	Bag Seine	Black Crappie	190	0.02
Coleman Lake	392666	3890156	Wet	Bag Seine	No Fish	0	0
Devil Dog Tank #2	380393	3890738	Wet	Bag Seine	No Fish	0	0
EK Tank	384642	3893574	Dry	Bag Seine	No Fish	0	0
Evans Tank #1	384123	3894486	Dry	Bag Seine	No Fish	0	0
Evans Tank #2	384140	3894547	Dry	Bag Seine	No Fish	0	0
Floe Tank	373765	3887393	Wet	Bag Seine	No Fish	0	0
Green Tank	369753	3890610	Wet	Bag Seine	No Fish	0	0
Guijada Tank	384074	3894127	Wet	Bag Seine	No Fish	0	0
Heifer Tank #1	384762	3893323	Wet	Bag Seine	Bluegill	1942	1.07
Heifer Tank #1	384762	3893323	Wet	Bag Seine	Largemouth Bass	116	0.06
Heifer Tank #1	384762	3893323	Wet	Bag Seine	Western Mosquitofish	2	0.01
Heifer Tank #2	385028	3893157	Wet	Bag Seine	No Fish	0	0
Hell Canyon Tank	372015	3883020	Wet	Angling/Gill Net	Black Bullhead	3	0.19
Hell Canyon Tank	372015	3883020	Wet	Angling/Gill Net	Yellow Bullhead	14	3.7
Hell Canyon Tank	372015	3883020	Wet	Angling/Gill Net	Green Sunfish	71	1.07
Hells Canyon Tank #1	385387	3892333	Dry	Bag Seine	No Fish	0	0
Island Tank	392528	3890069	Wet	Bag Seine/Straight Seine	No Fish	0	0
JC Tank	385874	3892009	Wet	Bag Seine/Straight Seine	No Fish	0	0
Jesus Tank	381498	3894248	Wet	Bag Seine	No Fish	0	0

Kessler Tank	379347	3890242	Wet	Bag Seine	No Fish	0	0
Kunde Tank	394660	3889146	Wet	Bag Seine	No Fish	0	0
Last Tank	394382	3882781	Wet	Bag Seine	No Fish	0	0
Lockett Tank	395052	3890286	Wet	Bag Seine	Green Sunfish	948	1.73
Lower JC Tank	385479	3892123	Wet	Bag Seine	No Fish	0	0
Mayes Tank	372003	3887758	Wet	Bag Seine	No Fish	0	0
Mud Seep Tank	397138	3886215	Wet	Bag Seine	No Fish	0	0
Nagiller Tank	379224	3891835	Wet	Bag Seine	No Fish	0	0
North Tank	369193	3890940	Wet	Bag Seine	No Fish	0	0
Pot Tank	391434	3880328	Wet	Bag Seine	No Fish	0	0
Rabbit Bill Tank	379154	3892928	Wet	Bag Seine	No Fish	0	0
Secret Tank	393155	3875169	Wet	Bag Seine	No Fish	0	0
Summit Tank	394978	3886980	Wet	Bag Seine	No Fish	0	0
Susan Tank	393594	3876228	Dry	Bag Seine	No Fish	0	0
Turkey Tank	395800	3886004	Wet	Bag Seine	Green Sunfish	528	0.47
Wash Tub Tank	375143	3892066	Wet	Bag Seine	No Fish	0	0
Winter Camp Tank	391443	3876033	Dry	Bag Seine	No Fish	0	0
XA Tank	384737	3892939	Wet	Bag Seine	No Fish	0	0

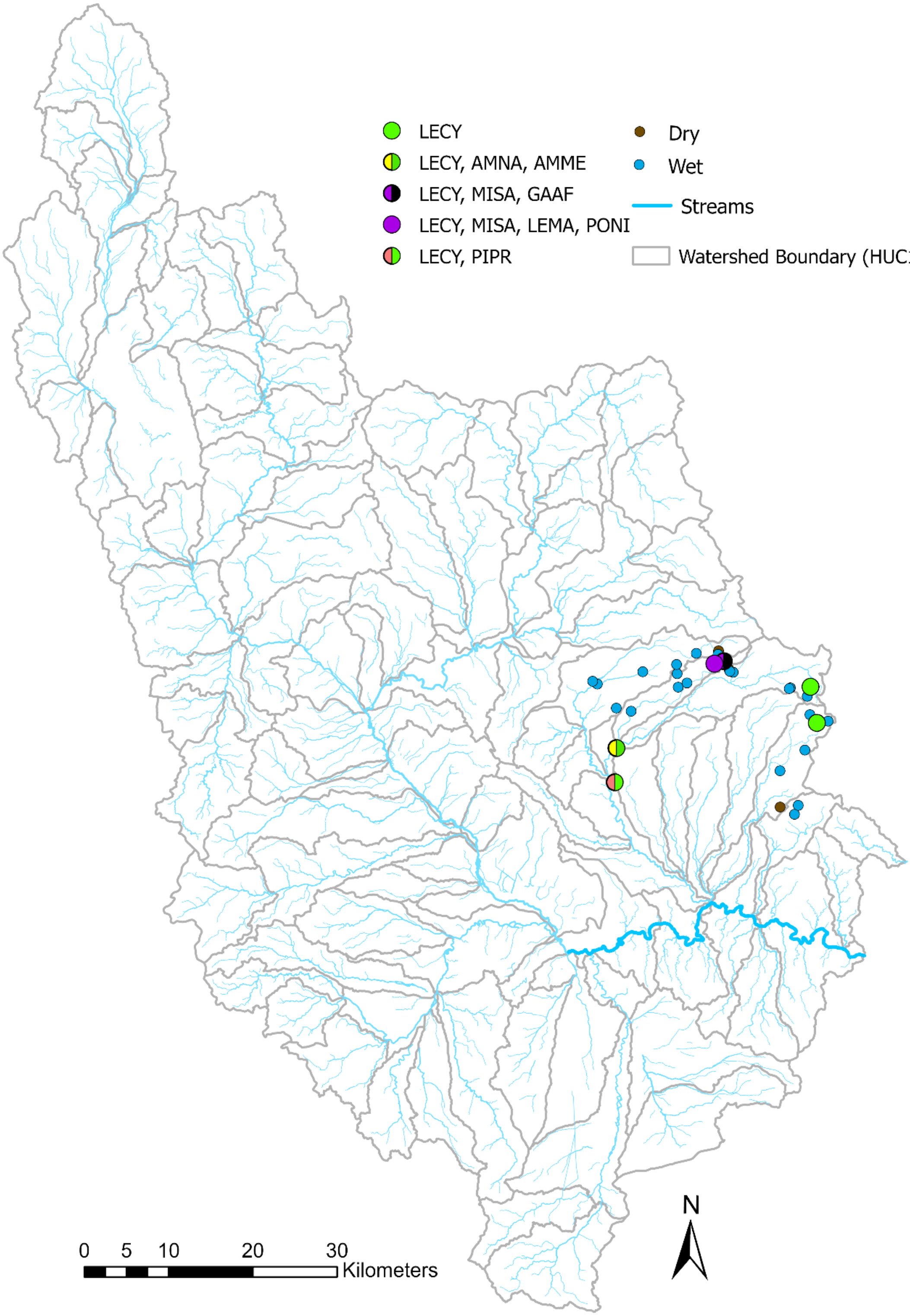

Figure 34.—Map of tanks sampled within the upper Verde River watershed and the assemblage of fish species detected (AMNA = Yellow Bullhead, LECY = Green Sunfish, MISA = Largemouth Bass, LEMA = Bluegill, PONI = Black Crappie, PIPR = Fathead Minnow) during the 2022 field season.

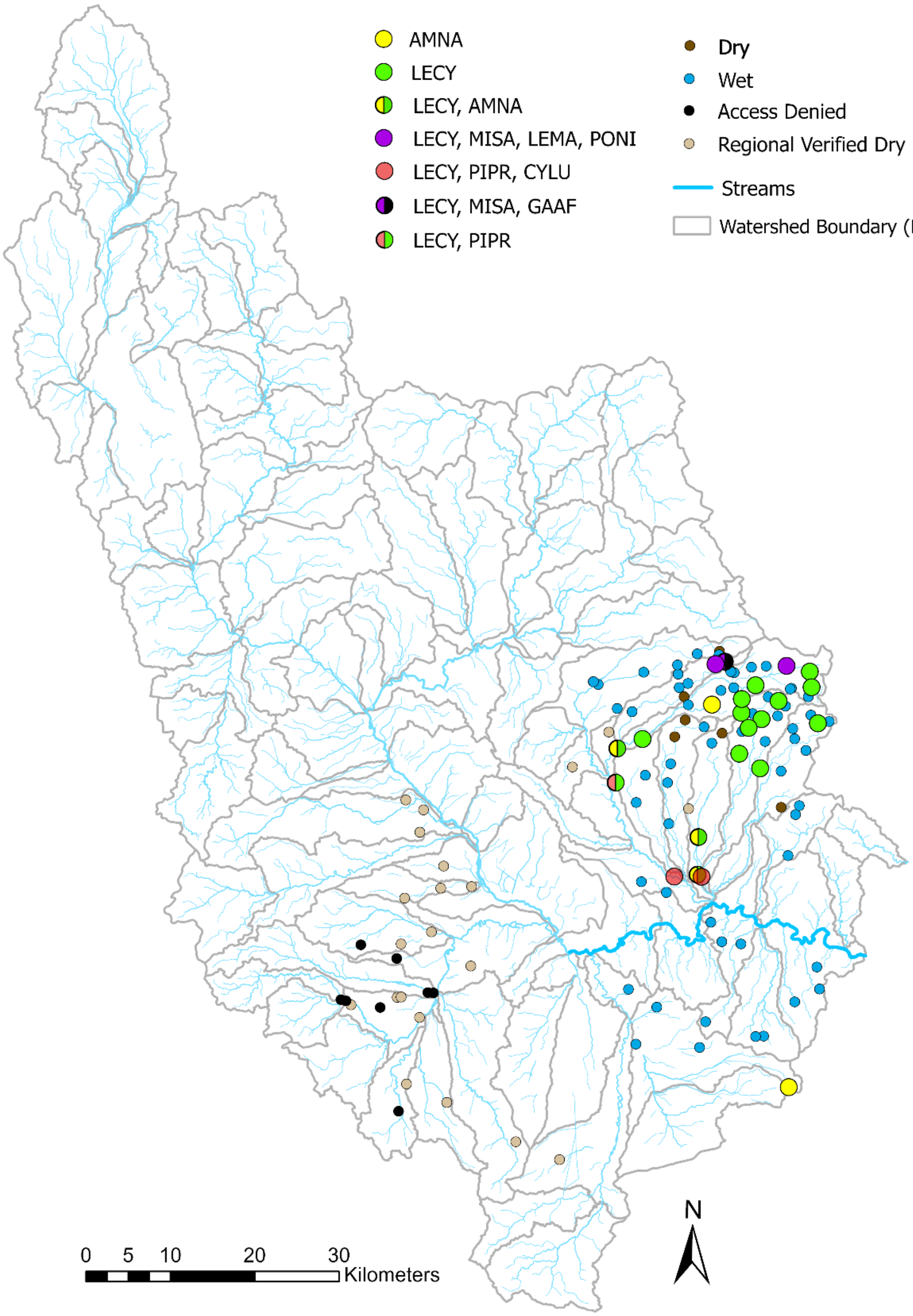

Figure 35.—Map of tanks sampled within the upper Verde River watershed and the assemblage of fish species detected (AMNA = Yellow Bullhead, LECY = Green Sunfish, MISA = Largemouth Bass, LEMA = Bluegill, PONI = Black Crappie, PIPR = Fathead Minnow) during the 2021 and 2022 field season.

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

Strategic Plan Goals**:**

- Scientific Foundation
  - Goal 3. Improve propagation techniques for Spikedace and Loach Minnow
- Preventing Extinction and Managing Toward Recovery
  - Goal 1. Maintain the Aquatic Research and Conservation Center (ARCC) and explore alternative locations for establishment of hatchery stocks of upper Gila and San Francisco River lineages of Spikedace and Loach minnow.

Recovery Objectives:

- Spikedace recovery objective 8. Plan and conduct investigations on captive holding, propagation and rearing.
- 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. Plan and conduct investigations on captive holding, propagation and rearing.
- 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 Topminnow 1999 draft revised 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.

Background: Reclamation funded construction of the Aquatic Research and Conservation Center (ARCC) 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 translocations. A number of improvements were made to the facility between 2000 and 2007. Beginning in 2014, Reclamation began providing funds (through USFWS) for a variety of improvements to ARCC, including new spawning raceways between existing structures, a new quarantine building, and new ponds.

Spikedace and Loach Minnow from all extant lineages were previously acquired under a separate sub-project (Task AZ-2003-1: Acquire Spikedace, Loach Minnow and rare populations of other native fish) and brought to ARCC, to establish refuge populations and support propagation efforts. The goal is to have each lineage represented by 500 adults. There are few natural populations left, and removing too many fish at a time could have negative impacts. The number of fish to collect 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. If necessary, new individuals are brought into ARCC every year to maintain the population size and genetic diversity with wild stock. Counts of Spikedace and Loach Minnow brought into ARCC, brood stock, fish produced, and fish stocked each year since 2015 are presented in Table 10.

At various times Woundfin, Gila Topminnow, and Desert Pupfish were also brought to the facility to propagate fish in support of translocation efforts. Eagle Creek Roundtail Chub were brought to the facility in 2010 to establish a refuge population and support propagation efforts for the Blue River project. The putative Parker Canyon lineage of Gila Topminnow was brought to the facility to establish a refuge population in 2022, pending further genetic investigations. The facility also holds various other species for research or educational purposes.

In 2018, ARCC staff began testing effects of fish density on propagation success of captive Spikedace and Loach Minnow. After a successful first year of trials, staff planned to conduct a second year of experiments using the exact same design as 2018. Unfortunately, not enough wild Aravaipa Creek 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 identical to one another at the lowest 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 and 2021 spawning seasons. The number of raceways used for each lineage was dependent on the overall brood stock size and need for larval fish, 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 and counted once per week and placed in holding tanks. Algae were carefully removed as needed to minimize the potential effects of high algal biomass on spawning.

Results:

ARCC O&M

The Department continued to operate ARCC in 2022. The ARCC propagates and 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, Gila River Forks, and Bear Creek). In addition, a stock of lower Eagle Creek lineage Roundtail Chub was propagated to support conservation efforts in the Blue River. The putative Parker Canyon lineage of topminnow also continued to persist at the facility as of the end of 2022.

In 2022, ARCC produced 393 Aravaipa Creek Spikedace, 466 upper Gila River Spikedace, 1252 Gila River Forks Spikedace, 278 Blue River Loach Minnow, 168 Aravaipa Creek Loach Minnow, 310 San Francisco River Loach Minnow, 65 Bear Creek Loach Minnow, and 475 Gila River Forks Loach Minnow (Table 10).

During the 2022 spawn season, ARCC staff began the first year of a Loach Minnow nest spacing study. Building on the success of previous years using the 38 cm spacing established in 2018, ARCC staff also evaluated 25 cm and 50 cm nest spacing. Blue River Loach Minnow were used in the first year of trials, but due to an abundance of aquatic vegetation larval fish removal efforts were complicated.

No new large-scale physical improvements to ARCC were completed in 2022.

Acquire Spikedace, Loach Minnow, and rare populations of other native fish
On July 18, 2022, Department staff collected 35 Longfin Dace from Aravaipa Creek on TNC property located at the West end of the canyon. Longfin Dace were collected as a surrogate for Spikedace and Loach Minnow. The fish were transported to the fish health laboratory at Department Headquarters for a fish health inspection. The crew carried out three seine hauls to capture the required number of Longfin Dace for the assessment and also captured and released 12 juvenile Loach Minnow.

On August 15, 2022, Department staff collected Speckled Dace from the Blue River near Blue River Crossing Campground for a fish health assessment. Speckled Dace were collected as a surrogate for Loach Minnow. The crew utilized a combination of electrofishing, straight seining, and kick seining to collect fish. A total of 40 Speckled Dace were transported to the fish health laboratory at Department Headquarters in an aerated cooler. An additional 64 Specked Dace, 22 Longfin Dace, 15 Desert Sucker, and 4 Loach Minnow were captured and released.

On November 29, 2022, Department staff collected Loach Minnow from the Blue River between Blue Crossing Campground upstream to the New Mexico state line. The crew electrofished three locations in the reach for a total of 4,069 seconds in an attempt to acquire fish to supplement the broodstock at ARCC. A total of four Loach Minnow were collected and transported to the hatchery. An additional five juvenile Loach Minnow (<40 mm TL) were captured but released.

On December 19, 2022, Department staff collected Loach Minnow and Spikedace from Nature Conservancy property on the east side of Aravaipa Creek. The crew collected a total of 27 Spikedace and 23 Loach Minnow in 14 seine hauls. All fish were transported to ARCC in aerated coolers with no mortalities during collection or transport.

Recommendations: For 2023, ARCC staff will focus on running all raceways at the lowest density identified in 2018 with a second year of testing conducted on Loach Minnow nest spacing using the most abundant lineage. This research will help identify the ideal Loach Minnow nest spacing in hopes of increasing the number of spawning individuals and larvae produced without a need for more spawning raceways.

Additional years of research are needed to determine if 38 cm is the optimal spacing for Loach Minnow nests or if it is possible to reduce the distance between nests and increase the number of nests and brood stock fish per spawning raceway.

Recommendations for acquiring wild fish in 2023 include continuing to collect Spikedace and Loach Minnow from remnant populations, with goals to minimize impacts on remnant population while also acquiring the number of fish necessary to maintain a refuge population of at least 500 adults. Aquatic Research and Conservation Center staff should coordinate with NMDGF regarding acquiring more stock of the New Mexico lineages.

Tables and Figures:

Table 10.—Summary of number of broodstock (#B), number of offspring produced (#P), number of offspring stocked (#S), and number of wild fish brought in to augment existing broodstock (#A), for each species and lineage held at the Aquatic Research and Conservation Center, from 2015 through 2022. Data for years prior to 2015 can be located in Hickerson et al. (2021b; Table 1, Table 12). Numbers stocked do not include fish transferred to New Mexico.

Taxa	Extant Lineage/Stream		2015	2016	2017	2018	2019	2020	2021	2022
Spikedace	upper Gila River	#B	392	531	267	159	254	219	176	131
		#P	296	0	384	352	2404	408	914	466
		#S	296	0	327	0	0	0	0	0
		#A	0	0	0	0	0	0	0	0
Spikedace	Gila River Forks	#B	204	138	122	83	71	76	151	120
		#P	0	0	1183	195	1132	833	203	1252
		#S	0	0	1000	0	0	0	0	705
		#A	0	0	0	1	0	0	52	0
Spikedace	Aravaipa Creek	#B	412	262	382	331	523	529	379	158
		#P	35	120	1347	3214	4250	2182	1032	393
		#S	221	67	0	2234	0	2897	106	1707
		#A	150	80	160	0	322	49	0	27
Loach Minnow	Bear Creek	#B							112	66
		#P							196	65
		#S							0	0
		#A							0	0
Loach Minnow	Gila River Forks	#B	81	96	128	97	169	121	0	58
		#P	0	220	7	1207	665	15	0	475
		#S	0	0	159	0	0	0	0	0
		#A	0	0	110	145	0	0	102	0
Loach Minnow	San Francisco R.	#B	119	215	314	318	231	208	173	92
		#P	0	26	177	1627	601	3	541	310
		#S	0	0	243	0	0	0	0	0
		#A	0	0	0	0	0	0	0	0
Loach Minnow	Blue River	#B	245	214	156	117	290	266	364	244
		#P	0	426	47	6	713	16	919	278
		#S	0	390	0	0	0	500	400	0
		#A	0	12	0	223	80	269	130	4
Loach Minnow	Aravaipa Creek	#B	316	297	490	439	354	337	261	200
		#P	0	265	305	1848	1398	57	504	168
		#S	0	0	0	0	0	300	0	0
		#A	50	200	100	0	57	82	0	23
Roundtail Chub	Eagle Creek	#B	85	101	99	99	99	98	84	81
		#P	2000	0	57	0	0	0	0	7405
		#S	876	1194	0	0	0	0	0	0
		#A	0	0	0	0	0	0	0	0

Projects Removed from Priority List in 2022

Red Tank Draw native fish restoration (Task AZ-2016-2). Dropped from priority list due to replication of Red Tank Draw lineage Roundtail Chub in Rarick Canyon, and the inability to control upstream source of nonnative fish on private lands.

West Fork Black River Nonnative Fish Removal (Task AZ-2021-1). Dropped from priority list due to anticipated rotenone treatment.

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APPENDICIES

Appendix 1.—Reports detailing work completed by the Arizona Game and Fish Department under the Gila River Basin Native Fishes Conservation Program from 2007-2022.

Year	Citation
2007	C. Carter. 2007. Three Forks Loach Minnow Survey, August 28-30, 2007. *Arizona Game and Fish Department,* Phoenix, AZ.
2008	Robinson, A.T. 2008. Arnett Creek and Telegraph Canyon 1-Year Post-Stocking Monitoring, July 23, 2008. *Arizona Game and Fish Department*, Phoenix, AZ.
2008	Robinson, A.T., J. Bahm, and C. Carter. Loach Minnow Survey in the Three Forks Area, East Fork Black River, July - August, 2008. *Arizona Game and Fish Department*, Phoenix, AZ.
2008	Robinson, A.T. 2008. Mineral Creek Fish Survey, April 21-22, 2008. *Arizona Game and Fish Department*, Phoenix, AZ.
2008	Robinson, A.T. 2008. Mud Springs #18: Gila Topminnow and Desert Pupfish Monitoring on November 6, 2008. *Arizona Game and Fish Department*, Phoenix, AZ.
2009	Robinson, A.T. 2009. Muleshoe Cooperative Management Area Native Fish Repatriations, One-Year Post-Stocking Monitoring and First Augmentation Stocking September 15-17, 2008. *Arizona Game and Fish Department*, Phoenix, AZ.
2009	Robinson, A.T., D. Orabutt, and C. Crowder. 2009. Loach Minnow Survey of East Fork Black River and Tributaries during July 2009. *Arizona Game and Fish Department*, Phoenix, AZ.
2009	Robinson, A.T. 2009. Repatriation of Native Fishes to Fossil Creek: Summary of Monitoring and Stocking During 2008. *Arizona Game and Fish Department*, Phoenix, AZ.

2010	Robinson, A.T. 2010. Arizona Native Fish Recovery and Nonnative Fish Control. *Arizona Game and Fish Department*, Phoenix, AZ.
2010	Robinson, A.T., C. Crowder, and D. Boyarski. 2010. Mechanical removal of Nonnative Fishes from the Blue River During June 1-3, 2009. *Arizona Game and Fish Department*, Phoenix, AZ.
2010	Robinson, A.T., R. Timmons, D. Boyarski, and C. Crowder. Muleshoe Cooperative Management Area Native Fish Restoration: Monitoring and Stocking During 2009. *Arizona Game and Fish Department*, Phoenix, AZ.
2010	Boyarski, D.E., A.T. Robinson, and C.D. Crowder. 2010. Repatriation of Native Fishes to Fossil Creek: Summary of Monitoring and Stocking During 2009. *Arizona Game and Fish Department*, Phoenix, AZ.
2010	Robinson, A.T., D. Orabutt, and C. Crowder. 2010. Devils Canyon and Mineral Creek Fish Surveys During 2009. *Arizona Game and Fish Department*, Phoenix, AZ.
2010	Crowder, C.D., D.E. Orabutt, and A.T. Robinson. 2010. Gila Topminnow and Desert Pupfish Repatriations to Morgan City Wash and Chalky Spring, Monitoring and Stocking During 2010. *Arizona Game and Fish Department*, Phoenix, AZ.
2010	Robinson, A.T. 2010. Mud Springs #18: Gila Topminnow and Desert Pupfish Monitoring on August 26, 2009. *Arizona Game and Fish Department*, Phoenix, AZ.
2011	Robinson, A.T., R. Timmons, and C. Crowder. 2011. Muleshoe Cooperative Management Area Native Fish Restoration: Monitoring and Stocking During 2010. *Arizona Game and Fish Department*, Phoenix, AZ.

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2011	Robinson, A.T. 2011. Mud Springs #18: Gila Topminnow and Desert Pupfish Monitoring on July 26, 2010. *Arizona Game and Fish Department*, Phoenix, AZ.
2011	Mosher, K.R., C.D. Crowder, and A.T. Robinson. 2011. O'Donnell Canyon and Turkey Creek Fish Surveys During 2010. *Arizona Game and Fish Department*, Phoenix, AZ.
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2011	Yarush, K., A.T. Robinson, and C.D. Crowder. 2011. Attempted Establishment of Desert Pupfish in Walnut Springs, Mazatzal Mountains, Arizona, 2008-2011. *Arizona Game and Fish Department*, Phoenix, AZ.
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Appendix 2.—Summary of native fish stocked in Arizona during 2022 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	Easting	Northing	Date	Lineage	# Stocked	# Mortalities
Spikedace	Spring Creek	416130	3847280	3/10/2022	Aravaipa Creek	1,717	3
Spikedace	Blue River	667959	3685054	3/15/2022	Upper Gila River	705	0
Gila Topminnow	Aravaipa Creek	557163	3637751	4/12/2022	Bylas Spring Complex	484	17
Gila Topminnow	Unnamed Drainage #68B	464845	3711232	4/20-21/2022	Cottonwood Spring	393	20
Gila Topminnow	Sharp Springs	540487	3468810	6/27-28/2022	Sharp Springs	668	3
Gila Topminnow	Sharp Springs	540487	3468810	10/12/2022	Sharp Springs	9	0

Appendix 3.—Summary of monitoring results during 2022 for the five priority species and other target native fish species that were previously stocked into various waters in the Gila River Basin Arizona. Included is the number of sites sampled, number of individuals captured at a particular location (#Ind), the proportion of young of year individuals captured (%YOY), Mean relative abundance (CPUE) and standard error of the mean relative abundance (SE).

Taxa	Location	Date	Gear Type	Sample Size	Statistics	2022
Gila Topminnow	Aravaipa Creek	9/30/2022	Minnow Trap	10	#Ind	23
					%YOY	17
					Mean CPUE	1.91
					SE	1.17
Gila Topminnow	Aravaipa Creek	9/30/2022	Dip Net	8	#Ind	12
					%YOY	92
					Mean CPUE	13.51
					SE	7
Gila Topminnow	Aravaipa Creek	9/30/2022	Seine	6	#Ind	63
					%YOY	21
					Mean CPUE	0.54
					SE	0.17
Gila Topminnow	Arnett Creek	10/27/2022	Dip Net	5	#Ind	1
					%YOY	0
					Mean CPUE	0.36
					SE	0.36
Gila Topminnow	Maternity Wildlife Pond	10/13/2022	Minnow Trap	10	#Ind	1591
					%YOY	41
					Mean CPUE	74.26
					SE	12.45
Gila Topminnow	Sharp Spring	10/12/2022	Minnow Trap	10	#Ind	135
					%YOY	26
					Mean CPUE	8.09
					SE	2.15
Gila Topminnow	Telegraph Canyon	10/27/2022	Minnow Trap	10	#Ind	117

					%YOY	3
					Mean CPUE	6.01
					SE	2.01
Gila Topminnow	Telegraph Canyon	10/27/2022	Dip Net	13	#Ind	48
					%YOY	19
					Mean CPUE	8.11
					SE	2.77
Gila Topminnow	Tortilla Creek	10/25/2022	Minnow Trap	10	#Ind	2430
					%YOY	11
					Mean CPUE	103.42
					SE	11.28
Gila Topminnow	Tortilla Creek	10/25/2022	Dip Net	10	#Ind	95
					%YOY	37
					Mean CPUE	24.81
					SE	9.72
Gila Topminnow	Unnamed Drainage 68b	10/24/2022	Minnow Trap	10	#Ind	854
					%YOY	8
					Mean CPUE	58.01
					SE	13.9
Gila Topminnow	Unnamed Drainage 68b	10/24/2022	Dip Net	12	#Ind	27
					%YOY	44
					Mean CPUE	10.81
					SE	3.34
Gila Topminnow	Unnamed Drainage 68b	10/24/2022	Seine	4	#Ind	109
					%YOY	39
					Mean CPUE	3.57
					SE	1.04
Loach Minnow	Upper Blue River	9/19/2022	Backpack Electrofisher	15	#Ind	76
					%YOY	8
					Mean CPUE	37.82
					SE	3.37

Loach Minnow	Middle Blue River	9/26/2022	Backpack Electrofisher	12	#Ind	8
					%YOY	0
					Mean CPUE	6.26
					SE	2.50
Roundtail Chub	Upper Blue River	9/19/2022	Backpack Electrofisher	15	#Ind	17
					%YOY	0
					Mean CPUE	8.99
					SE	2.20
Roundtail Chub	Middle Blue River	9/26/2022	Backpack Electrofisher	12	#Ind	17
					%YOY	0
					Mean CPUE	11.46
					SE	2.55
Roundtail Chub	Rarick Canyon	10/4/2022	Angling	1	#Ind	1
					%YOY	0
					Mean CPUE	6
					SE	0
Roundtail Chub	Rarick Canyon	10/4/2022	Minnow Trap	11	#Ind	24
					%YOY	0
					Mean CPUE	0.59
					SE	0.07
Roundtail Chub	Rarick Canyon	10/4/2022	Mini-Hoop Net	10	#Ind	52
					%YOY	0
					Mean CPUE	0.92
					SE	0.08
Roundtail Chub	Sabino Canyon	7/12/2022	Mini-Hoop Net	10	#Ind	24
					%YOY	0
					Mean CPUE	7.1
					SE	0.84
Roundtail Chub	Sabino Canyon	7/12/2022	Snorkel Survey	2	#Ind	88
					%YOY	NA
					Mean CPUE	264

					SE	102
Roundtail Chub	Sabino Canyon	7/12/2022	Seine	6	#Ind	23
					%YOY	13
					Mean CPUE	0.3
					SE	0.03
Spikedace	Upper Blue River	9/19/2022	Backpack Electrofisher	15	#Ind	2
					%YOY	0
					Mean CPUE	0.35
					SE	0.24
Spikedace	Middle Blue River	9/26/2022	Backpack Electrofisher	12	#Ind	14
					%YOY	0
					Mean CPUE	7.26
					SE	2.02
Spikedace	Spring Creek	9/15/2022	Backpack Electrofisher	3	#Ind	14
					%YOY	0
					Mean CPUE	17.99
					SE	3.38