

Gila River Basin Native Fishes Conservation Program:
Arizona Game and Fish Department's Native Fish Conservation Efforts During 2023

Cooperative Agreement R22AC00159
Between Bureau of Reclamation and Arizona Game and Fish Department
Annual Report
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Brian T. Hickerson, Crosby K. Hedden and Josh Walters
Arizona Game and Fish Department
5000 W. Carefree Highway
Phoenix, AZ 85086



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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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 Chub¹ *Gila intermedia* and Chiricahua leopard frog *Lithobates chiricahuensis* were added to the Program as species affected by operation of the CAP, and the Santa Cruz River drainage was added to its geographic scope.

The Program is funded by the U.S. Bureau of Reclamation (Reclamation), and is directed by the USFWS and Reclamation in cooperation with the New Mexico Department of Game and Fish (NMDGF) and Arizona Game and Fish Department (Department). Reclamation began 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 2007-2022 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*, Brook Stickleback *Culaea inconstans*, Brown Trout *Salmo trutta*, Channel Catfish *Ictalurus punctatus*, Common Carp *Cyprinus carpio*, Fathead Minnow *Pimephales promelas*, Flathead Catfish *Pylodictis olivaris*, 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 northern crayfish *Faxonius virilis*, and northern Mexican gartersnake *Thamnophis eques*.

This report summarizes Program work performed by the Department during 2023. For each priority action, work completed during 2023 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 2023 Department staff completed nine repatriation stockings into three waters: Spring Creek, Blue River and Sharp Spring (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 2023, Department staff 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 2023, 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 administered and managed Program projects identified in the agreement. Department staff finalized the 2022 annual report, began analyzing data and drafting the 2023 annual report, revised the FY24 workplan and drafted the FY25 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 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 regarding locations to stock and sources and lineages of fish appropriate for each translocation. 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 ($^{\circ}\text{C}$), 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

stocking site. 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 Chub², 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 (i.e., there are fifteen 100 m sub-reaches in the 14.6 km of the upper Blue River). A backpack electrofisher (Smith-Root; Model 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 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 Chub³ 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, UTM NAD83), 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.

² Including chub populations previously classified as Gila Chub.

³ Including chub populations previously classified as Gila Chub.

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.

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 Chub⁴ 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 should be monitored periodically after establishment by one or more of the cooperators for at least 10 years to determine population persistence and viability.

⁴ Including chub populations previously classified as Gila Chub.

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 goals are achieved. The catch of nonnatives across removal events is examined, and a decrease in abundance of the target nonnative species to low levels or to zero is 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 (/h or ind/h) of electrofishing effort, 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 change in mean relative abundance between years, and one-sided tests were used for taxa where mean relative abundance appeared to differ between 2022 to 2023. 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 Program R (R Development Core Team, 2023).

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 sub-project 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 Chub⁵, 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 been conducted annually since initiation. The number of sunfish removed from Reaches 1 and 2 has remained low, and far more sunfish are removed from Reach 3 each year since concerted efforts began there in 2014.

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

Results:

Nonnative Control. During April 25-26, 2023, Department staff removed Green Sunfish from Redfield Canyon. The crew backpack electrofished Reaches 1 and 2, from the confluence with Swamp Springs Canyon (563324/3588995) upstream to the waterfall barrier (563872/3589779; Figure 1). The crew electrofished for a total of 4,999 seconds and captured three Green Sunfish (Figure 2). The crew also set four mini-hoop nets in pools that have previously harbored the majority of the Green Sunfish in Reach 1 and captured a total of three Green Sunfish, five Roundtail Chub⁵, and three Sonora Sucker.

Department staff also completed three removal passes in Reach 3. The crew set 10 mini-hoop nets for three consecutive two hour sets in the pools near the wilderness boundary. A total of 20 Green Sunfish were captured (41-214 mm TL; Figure 3) with 17 captured during the first set and three captured during the second set, and none captured during the third set (Figure 2). Three additional Green Sunfish were captured by angling in Reach 3.

Overall, a total of 29 Green Sunfish were removed from Redfield Canyon in 2023. Total catch of Green Sunfish declined for the second consecutive year in Reach 3 (2021 n = 306, 2022 n = 109, 2023 n = 23) and was the lowest since concerted removal efforts began in this reach in 2014. Size structure of Green Sunfish in Reach 3 also shifted to predominantly larger individuals, with fish smaller than 70 mm TL absent from the catch (Figure 3). Given the relatively limited removal effort in a small portion of Reach 3, it is likely that environmental factors, particularly strong winter and spring runoff that may have flushed smaller fish downstream and delayed spawning activity, contributed to the relatively low number of Green Sunfish captured in Reach 3. Suppression efforts continue to prevent Green Sunfish from reestablishing in Reaches 1 and 2, but greater than average spring discharge in 2023 likely gave sunfish an extended opportunity to disperse upstream into Reaches 1 and 2 from Reach 3.

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 likely 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:

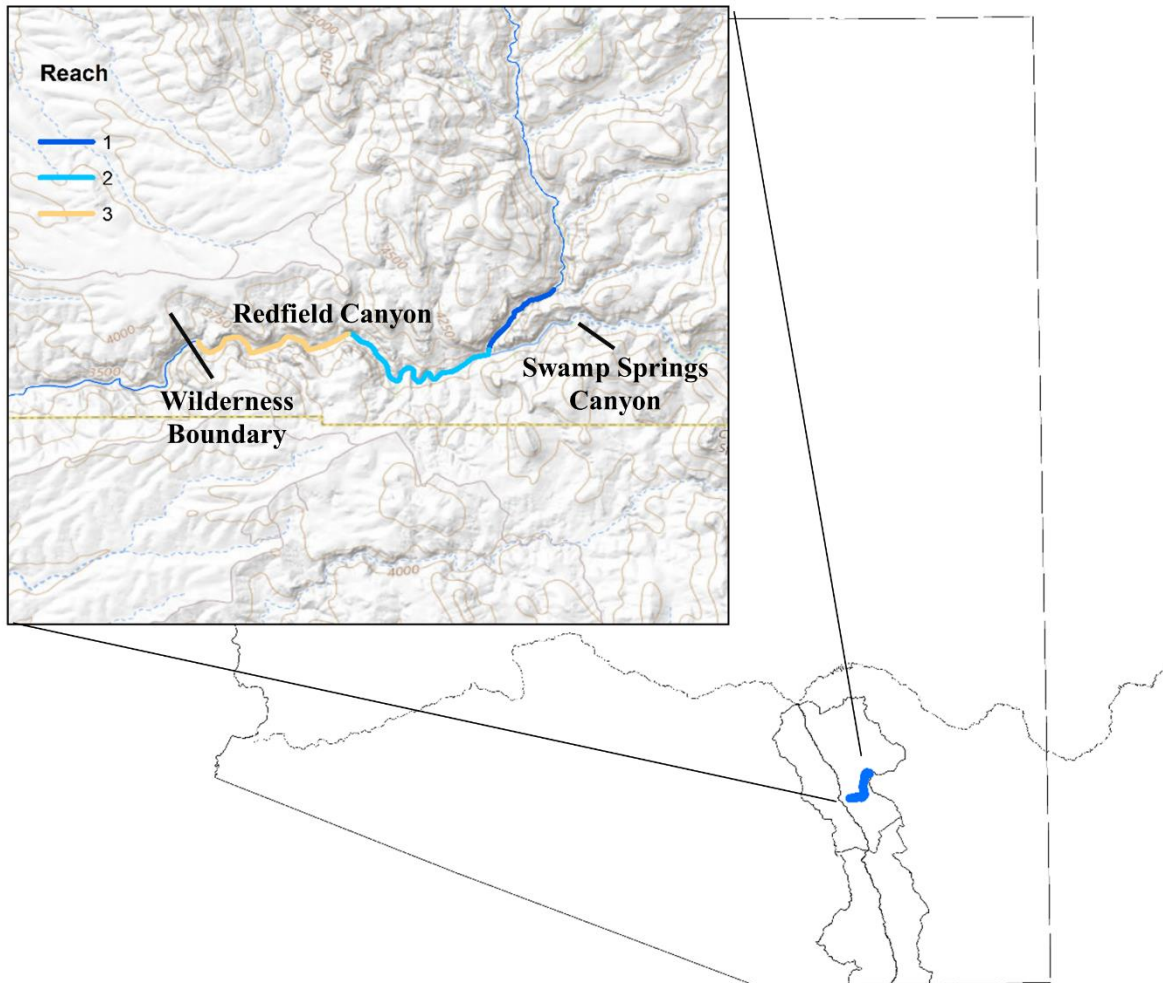


Figure 1.—Location of Redfield Canyon within the Gila River Basin and San Pedro River sub-basin. Inset map shows the location of sampling Reaches 1 (Swamp Springs Confluence upstream to Barrier), 2 (Rock House tributary upstream to Swamp Springs Confluence), and 3 (Wilderness Boundary upstream to Rock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

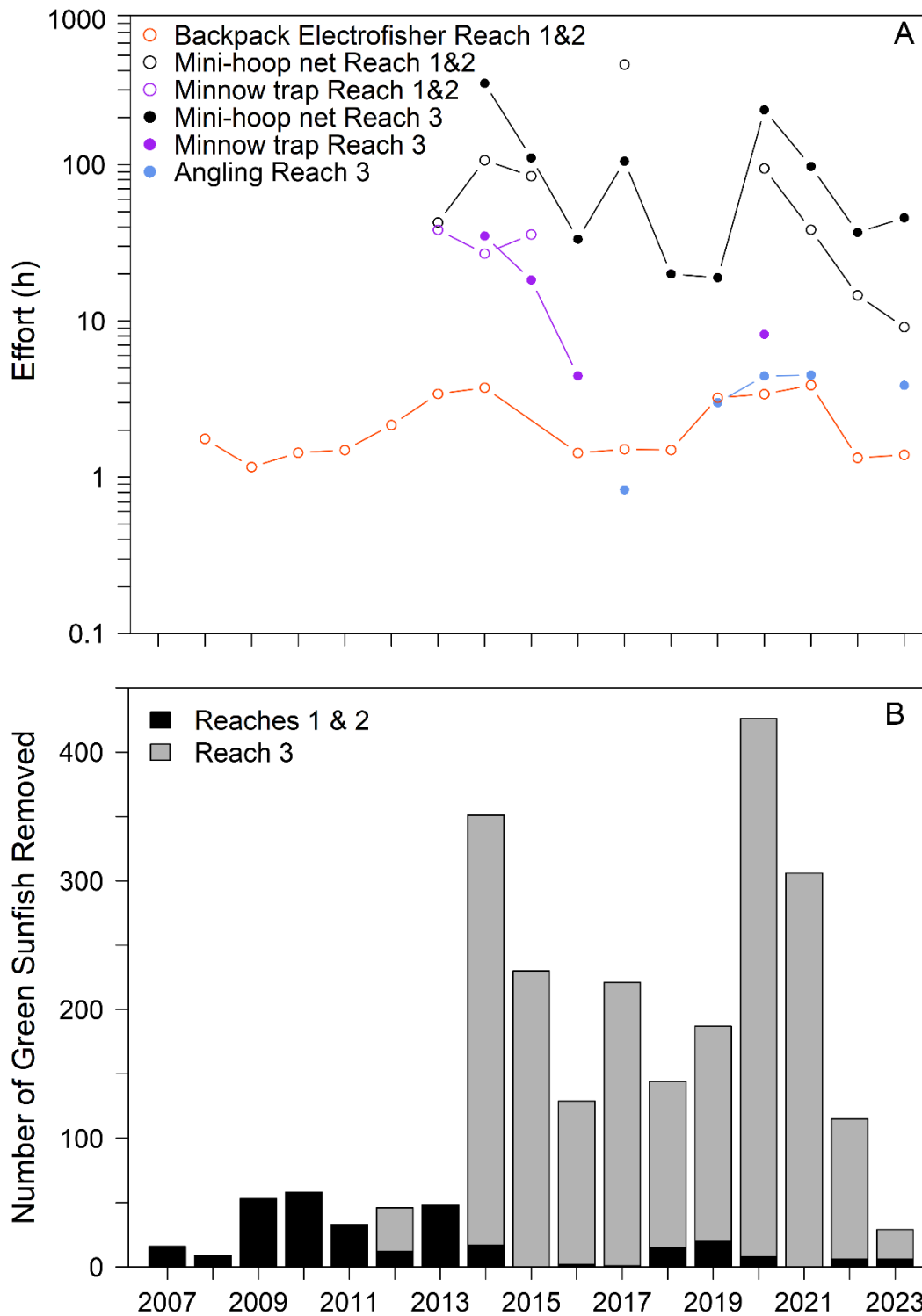


Figure 2.—Hours of removal effort by gear type and reach (A) and number of Green Sunfish removed during annual spring removal efforts and autumn monitoring from three reaches of Redfield Canyon, Arizona during 2007-2023 (B). Effort was not recorded for removals in 2007. Location and description of reaches within Redfield Canyon are included in Figure 1.

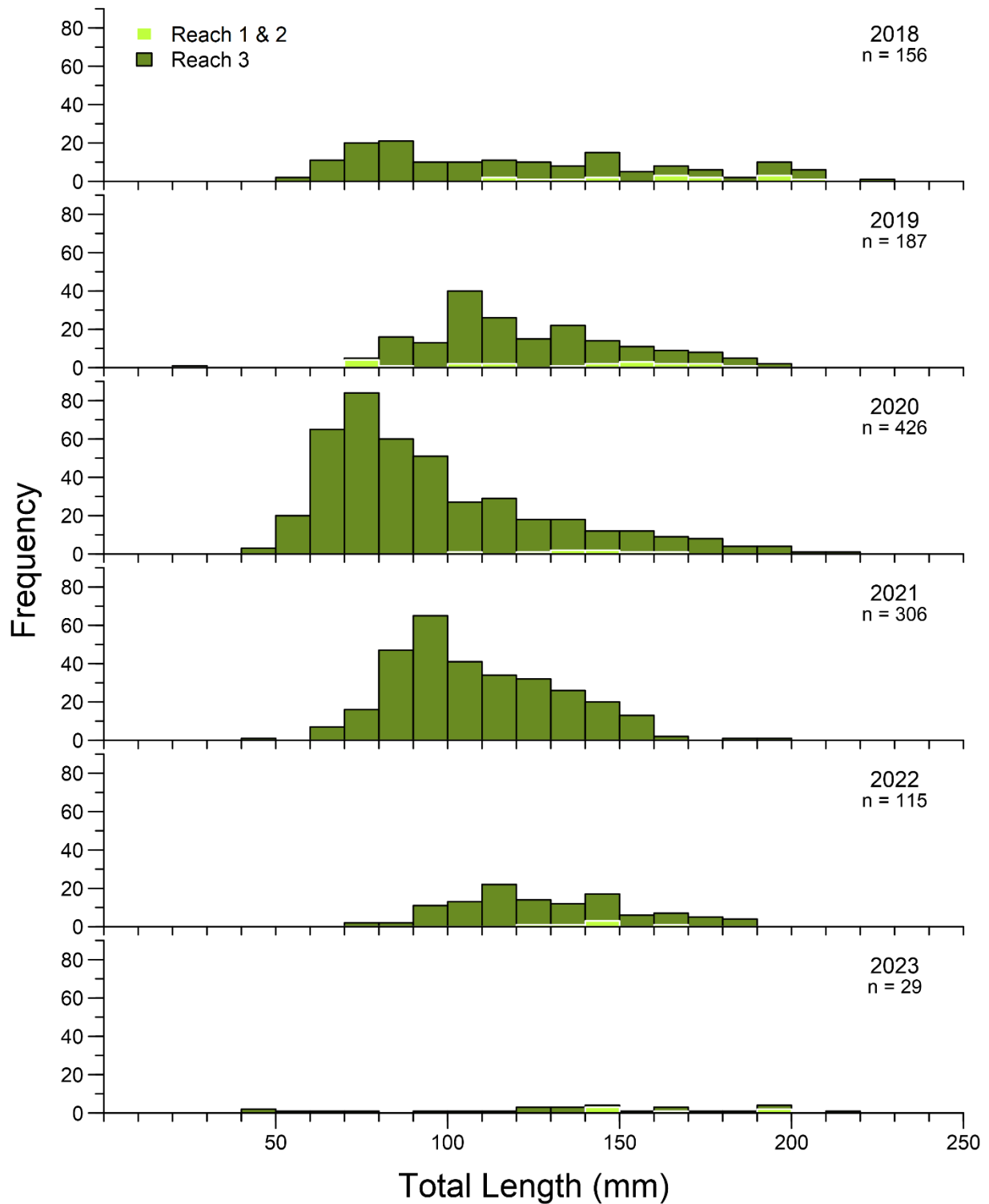


Figure 3.—Length frequency distribution of Green Sunfish captured by reach during removal efforts and annual monitoring in Redfield Canyon, 2018 through 2023. Number of fish captured and measured each year is shown in the top right corner of each panel.

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 Chub⁶ 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.

Sites Monitored or Stocked During 2023:

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

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 Galuero 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 April 2022, Department staff stocked 484 Bylas lineage Gila Topminnow into a spring-fed side channel near the upstream end of perennial flow. Major flooding from an above average monsoon season caused the side channel to flood for the first time since 2006. A total of 98 Gila Topminnow were captured during the initial post-stocking monitoring effort in September 2022, with an individual topminnow 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 monitoring.

Results: On October 2, 2023, Department staff monitored the Gila Topminnow population in the spring-fed side channel of Aravaipa Creek. A total of 10 minnow traps were set in pool habitats near the stocking location and captured 193 Gila Topminnow, 464 Longfin Dace, 114 Speckled Dace, 10 Sonora Sucker, and 3 Desert Sucker. An additional 14 dip net sweeps were carried out throughout the side channel while nets were fishing and captured an additional 36 Gila Topminnow, 7 Longfin Dace, 27 Speckled Dace, and 1 Sonora Sucker. Five straight seine hauls captured 58 Longfin Dace, 81 Speckled Dace, 3 Sonora Sucker, 1 Roundtail Chub, and 1 Loach Minnow, but failed to capture any Gila Topminnow.

The total number of Gila Topminnow captured across all sampling techniques ($n = 229$) was nearly double the number of topminnow captured in 2022 ($n = 98$). Mean relative abundance of Gila Topminnow captured in minnow traps significantly increased from 2022 to 2023 (2023 = 8.12/h, 2022 = 1.12/h; $W = 75.5$, $P = 0.022$). The proportion of young of year topminnow captured in 2023 (54 <20 mm TL, 175 \geq 20 mm TL; 23.6%) was similar to 2022 (28.6%), suggesting that reproduction has regularly occurred since the initial stocking.

Distribution of topminnow within the side channel continues to be relatively patchy (Figure 4), with most of the topminnow occurring in the few larger pools. Gila Topminnow were also

reportedly captured in the vicinity of Chimney Rock (approximately 3.6 km downstream of the original stocking location) during the University of Arizona fall annual monitoring (P. Reinthal, University of Arizona, personal communication) and near Horse Camp Canyon (approximately 18 km downstream of the original stocking location) during nonnative fish removal efforts by the Bureau of Land Management (H. Blasius, BLM, personal communication).

Recommendations:

The number of topminnow captured and relative abundance has increased since the initial monitoring, consistent with expectations following flooding in the summer of 2022. The number of topminnow captured is still below the ideal capture benchmark (i.e., 500 individuals) for topminnow monitoring. However, evidence of reproduction has been documented for two consecutive years, along with an increase in the relative abundance, and dispersal outside the immediate vicinity of the stocking site, which suggests this population is on a trajectory toward establishment barring any substantial changes in environmental conditions. Additional augmentations should be considered if captures or relative abundance substantially decline in 2024.

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. Post-stocking monitoring in August 2021 captured 1,554 topminnow with 1,591 topminnow captured in October 2022.

Results: On July 31, 2023, Department staff monitored Gila Topminnow in Maternity Wildlife Pond. Ten minnow traps were set for a minimum soak time of two hours and captured a total of 6,618 Gila Topminnow (2,240 <20 mm, 4,378 ≥20 mm).

The total number of Gila Topminnow captured in 2023 was more than four times greater than the number of topminnow captured in 2022 (n = 1,591). Mean relative abundance of Gila Topminnow captured in minnow traps significantly increased from 2022 to 2023 (2023 = 253.23/h, 2022 = 66.20/h; $W = 96$, $P = <0.001$). The proportion of young of year topminnow captured in 2023 (33.8%) was similar to 2022 (41.4%), suggesting that reproduction has been successful and consistent over the last year.

On August 1, 2023, Department staff collected 250 Gila Topminnow from Cienega Creek in the vicinity of the Mattie Canyon confluence and translocated them to Maternity Wildlife Pond with no mortalities during transport or stocking. The goal of the translocation was to contribute individuals directly from the Cienega Creek relict lineage to ensure that the population in the pond is an adequate genetic replicate.

Recommendations: Maternity Wildlife Pond is currently meeting all criteria for an established population, with the final post stocking monitoring planned for 2024. The draft Gila Topminnow Genetic Management Plan calls for the establishment of a few ‘primary’ donor sites for each Gila Topminnow lineage. We recommend considering Maternity Wildlife Pond as a primary donor site for the Cienega Creek lineage because it can produce a large number of individuals, is relatively easy to access, and has recently been augmented with individuals translocated directly from Cienega Creek. In addition, semi-natural ponds like Maternity Wildlife Pond tend to develop water quality issues with age, so this pond will likely have optimal environmental conditions for topminnow for several more years, given its relatively young age.

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 Chub⁷ (Ehret and Dickens 2009). Gila Topminnow were initially stocked in the recreation area near ‘The Crack’ in 2015 and augmented in 2016. These stockings resulted in the establishment of a population of topminnow mostly below Sabino Lake Dam.

Stream habitat in a reach of Sabino Canyon located approximately 250 m upstream from the confluence with East Fork Sabino Canyon was evaluated in 2017 and 2018 and identified as suitable for Gila Topminnow. A total of 557 Gila Topminnow were translocated from the large pools immediately below Sabino Dam to Sabino Canyon upstream of the confluence with East Fork Sabino Canyon in June 2018. The Gila Topminnow population in Sabino Canyon upstream of the East Fork was initially monitored in May, 2019. No topminnow were captured or observed. Immediately following the monitoring effort, a total of 148 Roundtail Chub¹ (>100 mm TL) collected from downstream of Sabino Dam were stocked into a pool just downstream of the topminnow stocking location (520836/3581045). In October 2019, Department staff collected an additional 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 but a total of 15, 10, and 47 Roundtail Chub were captured during monitoring in 2020, 2021, and 2022 respectively. The Bighorn Fire burned a substantial portion of the Sabino Canyon drainage in 2020.

Results: On May 5, 2023, Department staff monitored Gila Topminnow and Roundtail Chub in Sabino Canyon upstream of the confluence with 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

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

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 chub stocking location and captured three Roundtail Chub. Relative abundance of Roundtail Chub captured in hoop nets has been too variable to make valuable statistical comparisons between years, and tends to substantially underrepresent the true number of individuals at the stocking site (Hickerson et al. 2022). While nets were soaking, the crew walked approximately 200 meters upstream into the East Fork of Sabino Canyon and chub were present in the two most downstream pools (Figure 5). The crew snorkeled in the two pools in the East Fork of Sabino Canyon and observed 35 Roundtail Chub. Several pairs of chub were observed actively spawning under mats of tree roots. A small waterfall (~3 m high) currently appears to be acting as a fish barrier, preventing natural dispersal of chub into a few additional pools located in the lower few hundred meters of the East Fork Sabino Canyon. Young of year chub continue to be captured within this reach, suggesting that reproduction has regularly occurred since the initial translocation (Figure 6).

Following monitoring in the vicinity of the stocking sites, the crew walked downstream from the confluence with East Fork Sabino Canyon to Sabino Canyon Trail #23 to investigate dispersal of chub downstream from the original stocking location (Figure 5). The crew conducted three seine hauls and two dip net sweeps and captured a total of six Roundtail Chub. The crew visually observed hundreds of chub for approximately 1.4 km downstream of the East Fork Sabino Canyon confluence (Figure 5). The crew failed to observe fish in a high gradient reach referred to as the 'boulder field' in archived Region V maps. As the name implies, this reach consists of a series of plunge pools, large boulders, and bedrock waterfalls with several probable fish barriers.

Recommendations: Topminnow failed to persist at the stocking location following two translocation attempts despite a lack of a clear mechanism limiting persistence. The chub population has persisted for at least four years, has likely increased in abundance, and has substantially expanded in distribution since the initial stocking, meeting most criteria for considering a population established. The chub population should be monitored in 2024 to confirm that the population is established following five years of monitoring. Consideration should be given to translocating Roundtail Chub to the upstream extent of designated critical habitat in Sabino Canyon at the confluence with West Fork Sabino Canyon, as well as into suitable pools in the East Fork of 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).

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 on Arnett Creek, 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 and 2022 resulted in the capture of 563 and 165 Gila Topminnow respectively despite the Telegraph Fire burning a substantial portion of the upper watershed in June, 2021.

Results: On October 26, 2023, Department staff monitored Gila Topminnow in Telegraph Canyon. The crew set 10 collapsible minnow traps from the downstream end of surface water (487131/3680068) up to the most upstream topminnow stocking location (486999/3679922) and captured a total of 38 Gila Topminnow. The crew also carried out 16 dip net sweeps in Telegraph Canyon and captured an additional 109 Gila Topminnow (11 <20 mm TL, 98 ≥20 mm TL). No Longfin Dace were captured or observed for the second consecutive year. The number of topminnow captured in minnow traps has substantially declined with each year of monitoring (2021 n = 398, 2022 n = 117, 2023 n = 38) but mean relative abundance did not significantly decline compared to 2022 (2023 = 1.85/h, 2022 = 4.57/h; $W = 41$, $P = 0.241$).

Topminnow distribution in Telegraph Canyon has steadily decreased since 2021, with topminnow only captured or observed in two pools in 2023 (Figure 7). Sediment, sand and gravel displaced from the uplands during the Telegraph Fire in 2021 continues to make its way through the system with some pools scouring some years and filling in others. The crew observed a pool approximately 325 m upstream of the 2021 stocking location (486942/3679653) that had substantially scoured out and was of similar size and depth to the two pools still occupied by topminnow. The crew transported 100 of the Gila Topminnow captured upstream to the recently scoured pool in an attempt to expand the distribution of topminnow in the system and provide an opportunity to recolonize smaller pools that they may have no longer been able to access (Figure 7).

As minnow traps were soaking in Telegraph Canyon, the crew walked downstream to Arnett Creek near the 2017 topminnow stocking location to confirm persistence of topminnow observed in 2021 and 2022. The crew set two minnow traps in the 2017 stocking location (just downstream of the confluence of Arnett Creek and Telegraph Canyon) for an average soak time of 1.8 hours and captured a total of 212 topminnow (40 <20 mm TL, 172 ≥20 mm TL). This is by far the most topminnow that have been captured since the initial stocking in 2017. Gila Topminnow have now

been documented in Arnett Creek for three consecutive years, which represents a substantial, but unexpected, benefit from the stocking of Telegraph Canyon.

Recommendations: Monitoring of Telegraph Canyon and Arnett Creek should continue in 2024. Currently the topminnow population in Telegraph Canyon is falling short of meeting criteria for establishment, as relative abundance and distribution have declined since the initial monitoring effort. If relative abundance and distribution do not substantially increase in 2024, additional augmentations should be considered to help the population establish. Environmental conditions necessary for the population to thrive are more likely to occur with time since the Telegraph Fire.

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 to 2021. In April 2022, Department staff stocked 393 Cottonwood Spring lineage Gila Topminnow into Unnamed Drainage #68B and during post-stocking monitoring in October a total of 990 topminnow were captured. A total of 22 Gila Topminnow were captured in a small isolated pool that appeared to be at immediate risk of drying, and were translocated to a large pool upstream of the stocking location and a natural fish barrier (464691/3711295) in an effort to expand the distribution of the topminnow population (Figure 8).

Results: On October 25, 2023, Department staff monitored Gila Topminnow in Unnamed Drainage 68B near the original stocking location (464845/3711232). The crew set ten minnow traps for a minimum soak time of two hours and captured a total of 1,422 Gila Topminnow (83 <20 mm TL, 1,339 ≥20 mm TL) with five mortalities during capture and handling (Figure 8). Total catch increased relative to 2022 but mean relative abundance did not significantly differ from 2022 (2023 = 37.97/h, 2022 = 36.32/h; $W = 69.5$, $P = 0.781$). The proportion of young of year topminnow captured in 2023 (5.8%) was very similar to 2022 (7.9%).

The crew also set five minnow traps and carried out one dip net sweep in several of the pools upstream of the original stocking location and a natural fish barrier to determine whether the topminnow translocated upstream in 2022 were able to persist. A total of 13 Gila Topminnow were captured (1 <20 mm TL, 12 ≥20 mm TL), providing evidence that topminnow have persisted and reproduced upstream of the original stocking location (Figure 9).

Occupied habitat at the original stocking location was restricted to just three pools at the time of monitoring, so the decision was made to move 500 of the Gila Topminnow captured in the vicinity of the original stocking location upstream to the pool that salvaged fish were translocated to in 2022 (Figure 9). If topminnow continue to persist and thrive in the pools upstream of the original stocking location, it will increase topminnow distribution by an additional 150 meters and add several more occupied pools (Figure 9)

While traps were soaking, the crew walked upstream to Mesquite Tank #2. The tank visually appeared to be a promising habitat for Gila Topminnow and was nearly full despite a relatively poor monsoon season in 2023. The drain at the bottom of the dam that impounds the tank is currently capped, but a slight trickle from the pipe supports several more pools downstream, many of which seemed as large or larger than those immediately upstream of the original topminnow stocking location.

Recommendations: The Gila Topminnow population in Unnamed Drainage #68B is currently meeting all criteria for population establishment, with reproduction documented each year, an increase in the catch along with stable relative abundance, and persistence within the extended upstream distribution. Monitoring of Unnamed Drainage #68B should continue for at least one more year, with at least one final genetic augmentation after the final post-stocking monitoring effort. Department staff should coordinate with Tonto National Forest staff to determine whether topminnow can be translocated to Mesquite Tank #2 to expand the distribution of topminnow in the system, and potentially increase the population's resilience to disturbance in the long term.

Rarick Canyon

Background: Rarick Canyon is a tributary to Red Tank Draw and consists of a series of perennial bedrock pools (Figure 10). A waterfall barrier (~10 m high) in Rarick Canyon prevents upstream movement of nonnative fishes from the perennial reach of Red Tank Draw. A survey of isolated pools in the Rarick Canyon drainage from 2017 to 2018 detected Black Bullhead. Intensive mechanical removals efforts in 2019 resulted in the eradication of Black Bullhead from the Rarick Canyon drainage. Roundtail Chub from Red Tank Draw were translocated above a natural barrier into four isolated pools in Rarick Canyon 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 (F18, Figure 10). No topminnow were captured during annual monitoring in 2021 or 2022.

Results: During October 11-12, 2023, Department staff monitored Gila Topminnow and Roundtail Chub in Rarick Canyon. The crew set a combination of 7 collapsible minnow traps and 16 mini-hoop nets in the four pools previously stocked with chub (F23, F20, F18, F17) for a soak time of nearly 24 hours and captured a total of 113 Roundtail Chub and 25 Fathead Minnow (Figure 11). One additional minnow trap was set in a pool that was not previously stocked with chub (F22) and captured an additional 17 Roundtail Chub and 8 Fathead Minnow. Angling throughout the

monitoring reach resulted in the capture of a total of 10 Roundtail Chub. Gila Topminnow were not captured or observed for the third consecutive year.

Despite capturing nearly twice as many chub in 2023 ($n = 140$) relative to 2022 ($n = 77$), mean relative abundance of chub captured in minnow traps and mini-hoop nets was not significantly greater (2023 = 0.22/h, 2022 = 0.15/h, $W = 310$, $P = 0.132$; Figure 12). The relative abundance of chub has generally increased through time though, consistent with the increase in number of fish captured. Interestingly, both the number and relative abundance of Fathead Minnow has substantially declined since 2020 (Figure 12). The decline of Fathead Minnow relative to the increase in Roundtail Chub could be evidence that Fathead Minnow are being utilized as a prey item to the point where population numbers are substantially declining, or that environmental conditions favorable for Roundtail Chub are detrimental to the Fathead Minnow population, or possibly both.

Roundtail Chub were also detected in three additional pools that were never directly stocked with chub, suggesting that chub have the ability to disperse throughout the system (at least downstream of F17 to the waterfall barrier) when flows are present (Figure 11).

The length frequency histogram shows a structured population, with four fairly distinct age classes of fish (Figure 13). Young of year chub have proven difficult to capture in Rarick Canyon in the past, with only one individual ~50 mm TL captured in 2021. However, the presence of multiple age classes captured in 2023 suggests spawning has regularly occurred in Rarick Canyon, and individuals spawned in Rarick Canyon have already or may soon be close to recruiting into the population (Figure 13).

Recommendations: The Rarick Canyon Roundtail Chub population has shown evidence of consistent reproduction, is increasing in relative abundance, expanding in distribution, and individuals are possibly recruiting into the population, meeting all criteria for population establishment. Monitoring should continue for five years after the most recent establishment translocation (2026) to confirm that the population continues to meet criteria for establishment. 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.

Sites Assessed During 2023:

Willow Creek

On May 31, 2023, Department staff assessed stream habitat for native fish in Willow Creek. Willow Creek is a tributary to Saguaro Lake originating in the Goldfield Mountains on the Tonto National Forest. The crew walked 5.6 km of stream and identified 12 isolated bedrock pools (Figure 14). At least two natural fish barriers, including a large waterfall barrier (> 15 m) near the

confluence with Canyon Lake were also identified (Figure 14). Pool dimensions varied among pools, but were generally relatively small (Table 1). Previous surveys by regional staff in May and November, 2022 surveyed only the lower grouping of pools and documented four pools, so it is likely that at least two of the pools are not perennial through periods of drought.

The six most downstream pools in Willow Creek (pools 1-6) could potentially support populations of Gila Topminnow. The recovery value of a topminnow population in Willow Creek could be limited given the isolated nature of the pools and the relatively small amount of habitat. However, similar habitat (isolated bedrock pools in a canyon) has been occupied for years in nearby La Barge Canyon and Unnamed Drainage #68B. Conversations should take place with partners at Tonto National Forest and U.S. Fish and Wildlife Service to discuss whether further conversation and investigation for potentially translocating topminnow into Willow Creek are warranted.

Fish Creek

On June 1, 2023, Department staff assessed stream habitat for native fish in Fish Creek. Fish Creek is a tributary to Apache Lake that was previously occupied by a population of Roundtail Chub and Longfin Dace (Weedman et al. 1996). The stream was subsequently invaded by Green Sunfish, and Roundtail Chub were extirpated sometime shortly thereafter (Weedman et al. 1996). Green Sunfish persisted until at least 2006 (Gill and Carveth 2006). The Woodbury Fire burned most of the upper watershed of fish creek in 2019. An upstream reach of Fish Creek was previously assessed for native fish habitat by Department staff in 2019, weeks before the Woodbury Fire began (Hickerson et al. 2020b.). The purpose of this assessment was to determine whether Green Sunfish remained in lower Fish Creek following post-fire impacts from the Woodbury Fire, identify potential fish barriers, and document the extent of perennial flow.

Two crews walked approximately 10.9 km of the stream channel, with one crew hiking from the Apache Trail bridge down, and the other crew hiking upstream from the bridge to near the confluence with Lost Dutch Canyon (Figure 15). A total of about 7.7 km of surface water were present, with a majority of the water occurring upstream of the bridge (Figure 15). At least 10 pools with a maximum depth greater than 1 m were identified, with all but one located upstream of the bridge (Figure 15). Two traps were set upstream of the Apache trail bridge for approximately 2 hours. No fish were captured or observed throughout the stream.

Much of the stream habitat contained substantial deposits of silt, sand, and gravel likely from the Woodbury Fire (Figure 16). In the reach upstream of the bridge, most locations that would normally facilitate the creation of pools were mostly filled in with substrate. Downstream of the bridge, most of the stream habitat consisted of a shallow (0.1 – 0.2 m deep), narrow channel that has carved through deposited sediment. The stream likely needs more time for habitat to recover from post-fire effects from the Woodbury Fire as silt, sand, and gravel continues to flush through the system and more pool habitat is able to scour out. The stream should be reassessed in the future to evaluate stream habitat recovery and determine whether environmental conditions have sufficiently improved to pursue Roundtail Chub reintroduction attempts.

Sycamore Creek

On June 27, 2023, Department and Prescott National Forest staff completed wet/dry mapping, visual surveys, and snorkel surveys in a 4.1 km reach of Sycamore Creek from the Middle Box to Double T Falls (Figure 17). A Roundtail Chub population in Sycamore Creek exists from Middle Box upstream to a natural waterfall barrier at Double T Falls. Prescott National Forest Staff previously recommended evaluating the potential to translocate Roundtail Chub upstream of Double T Falls to extend their distribution within the system. The purpose of this survey was to confirm the extent of surface water during the driest time of year, and conduct a census of the Roundtail Chub population to determine whether the population could support collections for translocation efforts.

A total of a little more than 2 km of surface water was present within the surveyed reach (Figure 17). The crew counted fish in all pools greater than 1 m in depth, while all pools shallower than 1 m were visually surveyed. Nine pools were snorkeled and visual surveys occurred at 29 locations within the sampled reach. A total of 2,475 Roundtail Chub (1,981 \leq 50mm, 19 51-100mm, and 478 >100mm) and nine Rainbow Trout (all >130mm) were observed. Adult Roundtail Chub were observed downstream at Middle Box, and in the larger pools near Double T Falls, with almost exclusively juveniles observed in the reach between. The chub population is sufficiently large to support collections for translocations upstream of Double T Falls, barring any drastic changes in environmental conditions. Translocations should occur as soon as Prescott National Forest staff complete remaining compliance requirements.

Tables and Figures:

Table 1.—Name, location (easting, northing), and maximum length, width, and depth (m) of each of the 12 pools identified in Willow Creek on May 31, 2023.

Pool Number	Easting	Northing	Length	Width	Depth
Pool 1	457301	3711361	16.5	10.5	3.70
Pool 2	457278	3711339	7.4	3.8	0.74
Pool 3	457254	3711292	10.0	8.4	3.10
Pool 4	457216	3711296	4.8	2.6	2.60
Pool 5	457262	3711263	4.4	2.7	1.85
Pool 6	457403	3711102	2.2	1.7	0.50
Pool 7	457666	3709259	4.0	2.8	0.70
Pool 8	457689	3709346	7.0	4.0	1.00
Pool 9	457689	3709360	10.0	5.0	5.00
Pool 10	457687	3709362	8.0	6.0	1.50
Pool 11	457635	3709535	8.0	6.0	1.20
Pool 12	457643	3709429	12.0	8.0	0.50

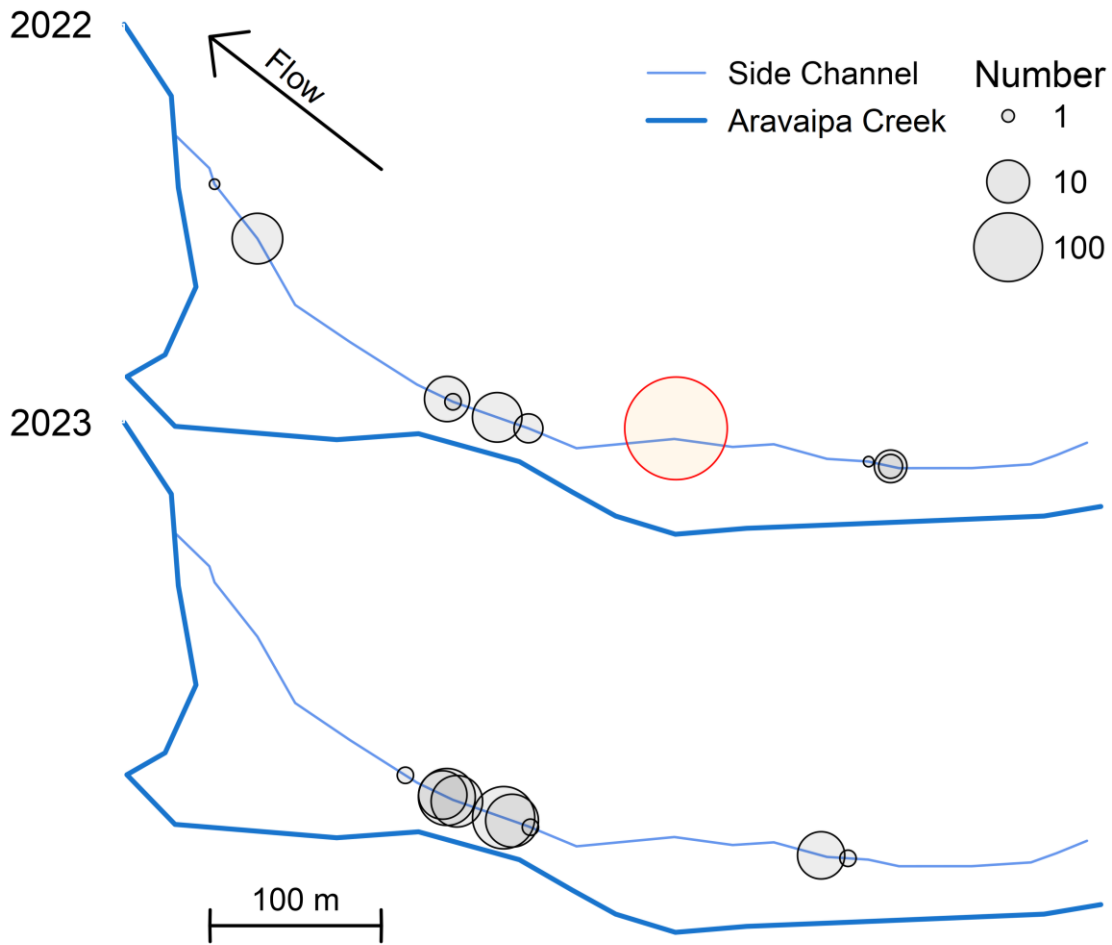


Figure 4.—Location and number of Gila Topminnow stocked and captured in the Aravaipa Creek side-channel from 2022 to 2023 (from top to bottom). The dark blue line represents the main channel of Aravaipa creek while the light blue line represents the spring fed side-channel. Orange circles represent the location and number of fish stocked, while grey circles represent the location and number of fish captured during monitoring.



Figure 5.—Map showing the 2019 Roundtail Chub stocking location (pink point), the locations where Roundtail Chub were captured outside of the vicinity of the original stocking location in 2023 (grey points), the stream reaches where Roundtail Chub were visually observed (light blue lines), the stream reaches where Roundtail Chub appeared to be absent (dark blue lines), and the location where Roundtail Chub were restocked following the Aspen Fire in 2005 (orange dot).

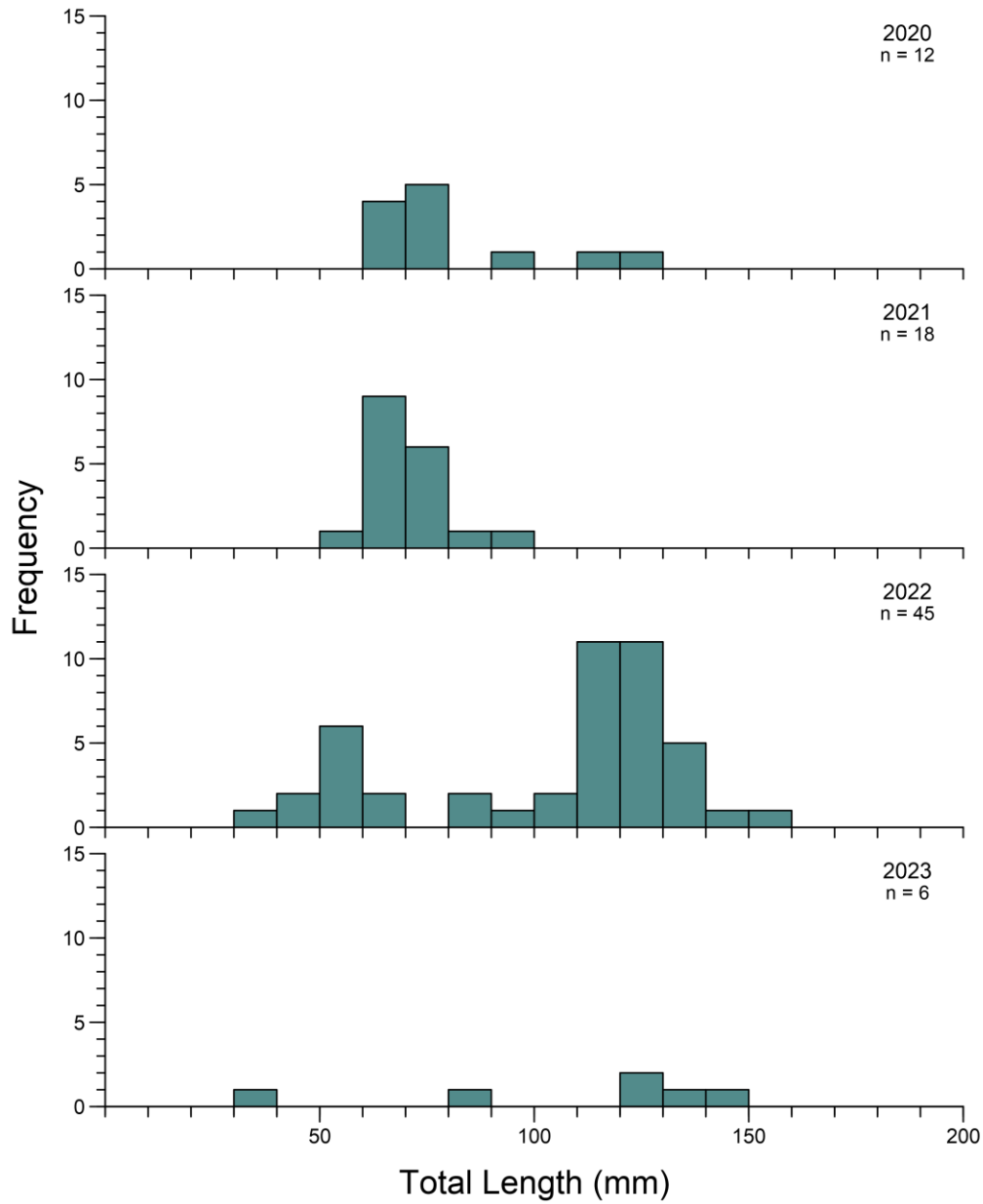


Figure 6.—Length frequency distribution of Roundtail Chub captured during annual monitoring in Sabino Canyon in the vicinity of the confluence with East Fork Sabino Canyon from 2020 to 2023. Number of fish captured and measured each year is shown in the top right corner of each panel.

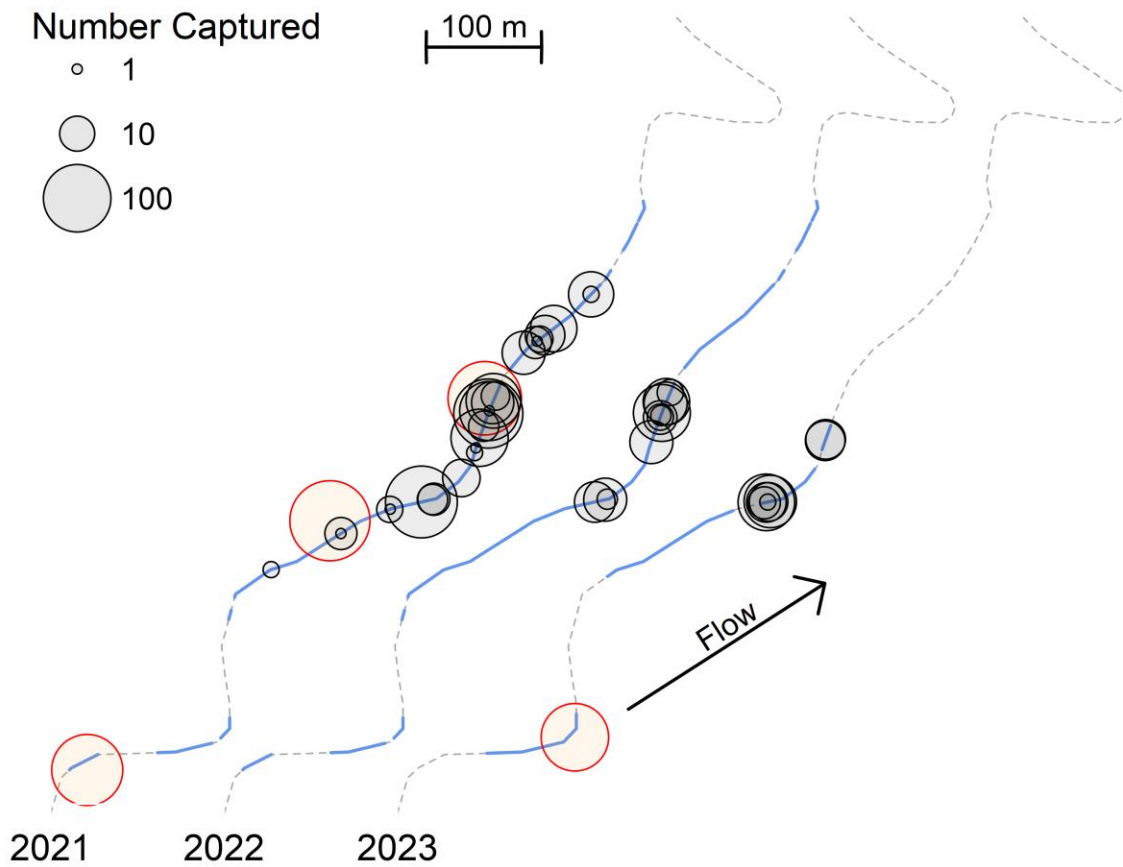


Figure 7.—Location and number of Gila Topminnow captured in Telegraph Canyon during annual monitoring from 2021 to 2023 (from left to right). The blue line represents the perennial portion of Telegraph Canyon (based on the most recent wet-dry mapping) while the dashed line represents intermittent portions. Orange circles represent the location and number of fish released, while grey circles represent the location and number of fish captured during monitoring.



Figure 8.—Photo of pool upstream of the original stocking location and natural fish barrier in Unnamed Drainage #68B where salvaged topminnow were translocated in 2022 and augmented in 2023.

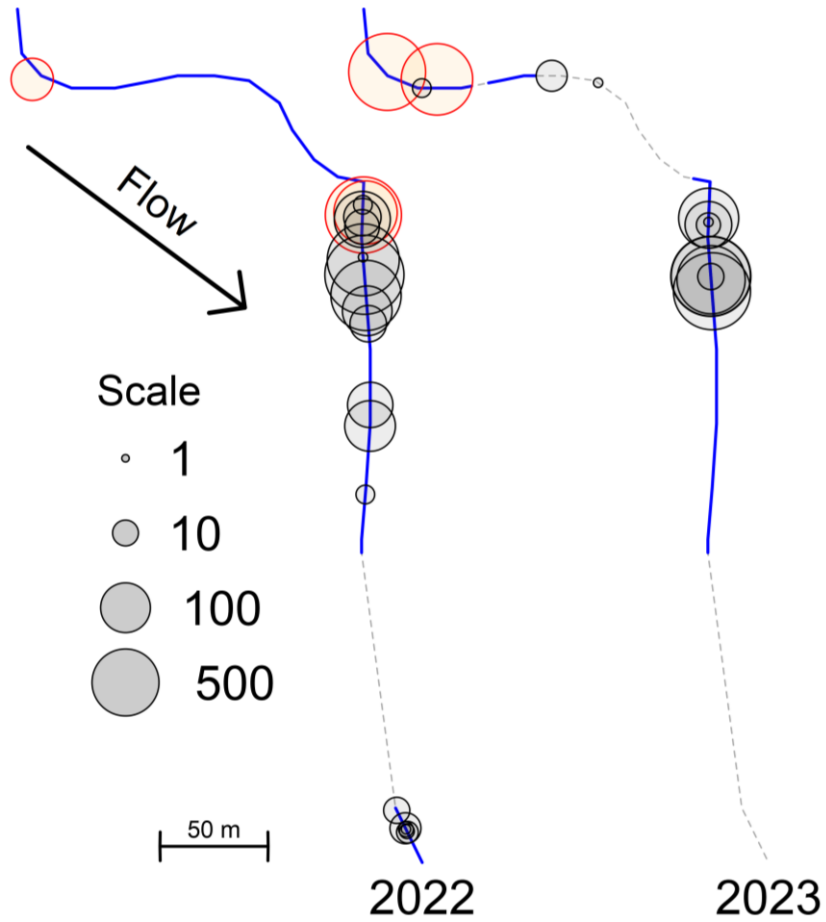


Figure 9.— Location and number of Gila Topminnow captured in Unnamed Drainage #68B during annual monitoring from 2022 to 2023 (from left to right). The blue line represents the perennial portion of Unnamed Drainage 68B (based on the most recent wet-dry mapping) while the dashed line represents intermittent portions. Orange circles represent the location and number of fish released, while grey circles represent the location and number of fish captured during monitoring.

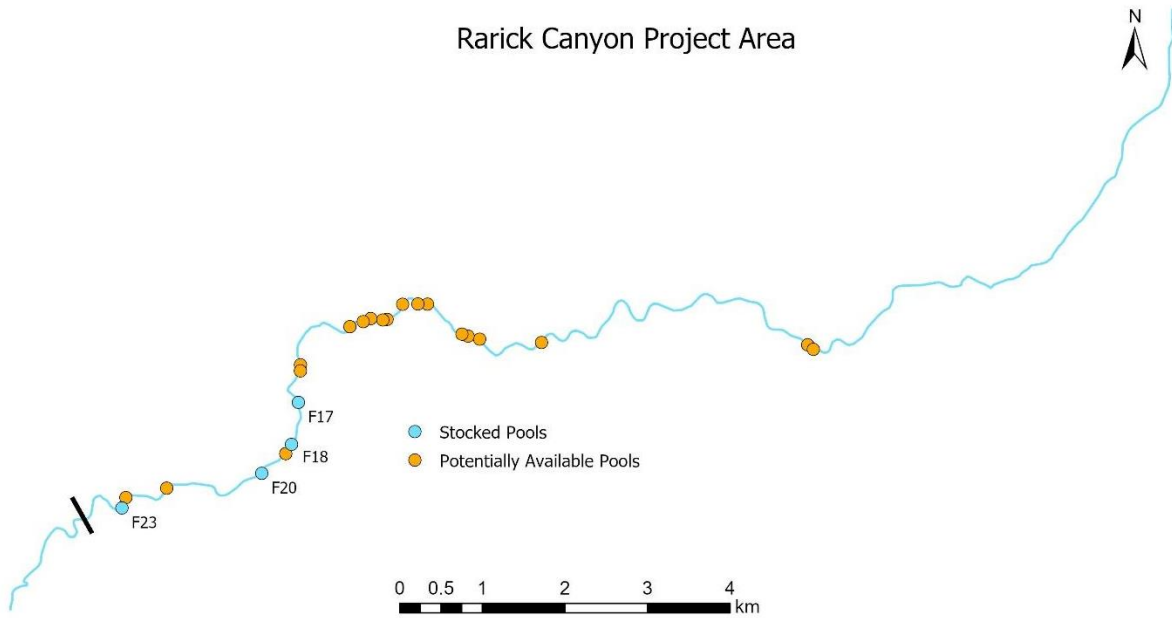


Figure 10.—Map of Rarick Canyon (blue line) including the location of the barrier falls (thick black line), names and locations of pools stocked with fish (blue dots), and the remaining potentially habitable pools in Rarick Canyon (orange dots).

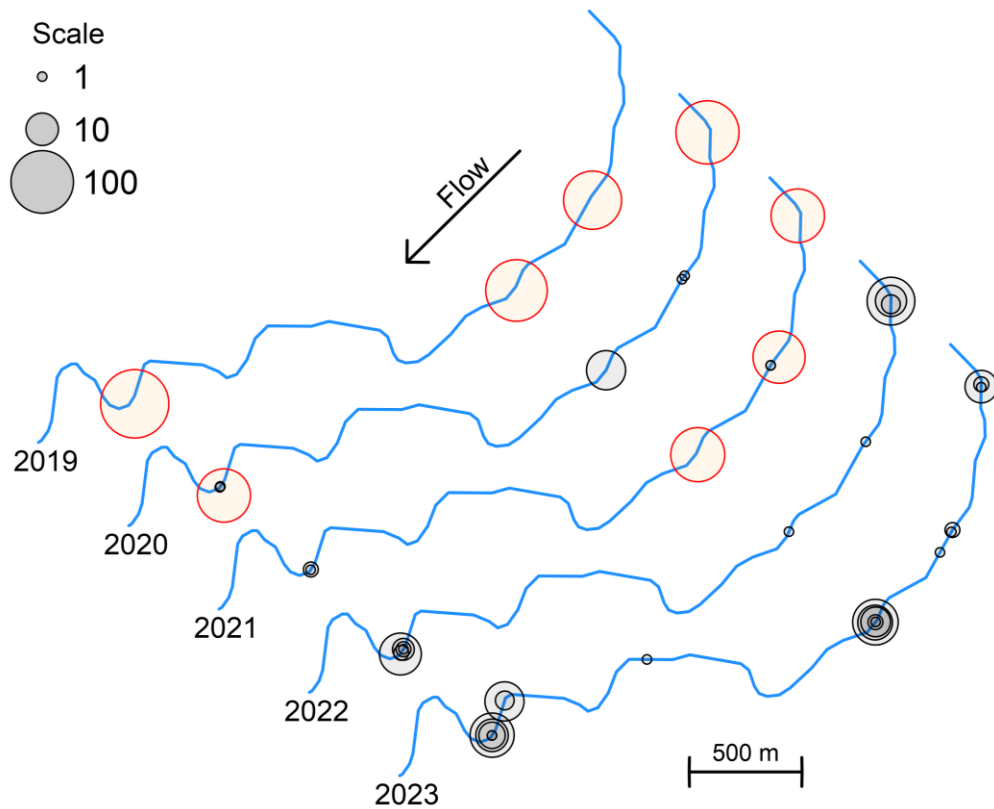


Figure 11.—Location and number of Roundtail Chub stocked and captured in Rarick Canyon from 2019 to 2023 (from top left to bottom right). Orange circles represent the location and number of fish released, while grey circles represent the location and number of fish captured during monitoring.

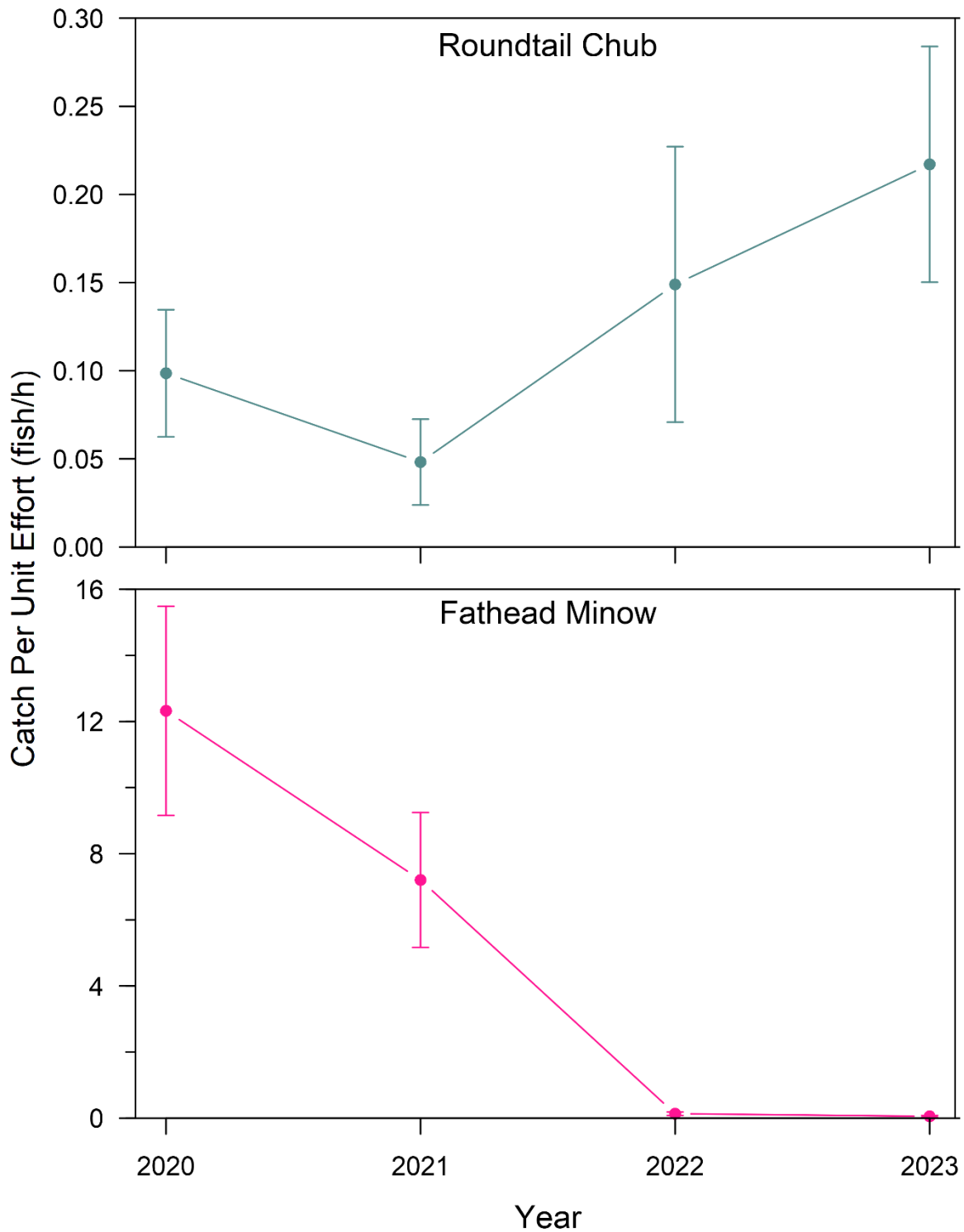


Figure 12.—Mean mini-hoop net and minnow trap catch per unit effort (fish/h) with standard error bars for Roundtail Chub (blue) and Fathead Minnow (pink) captured in Rarick Canyon, AZ from 2020 to 2023.

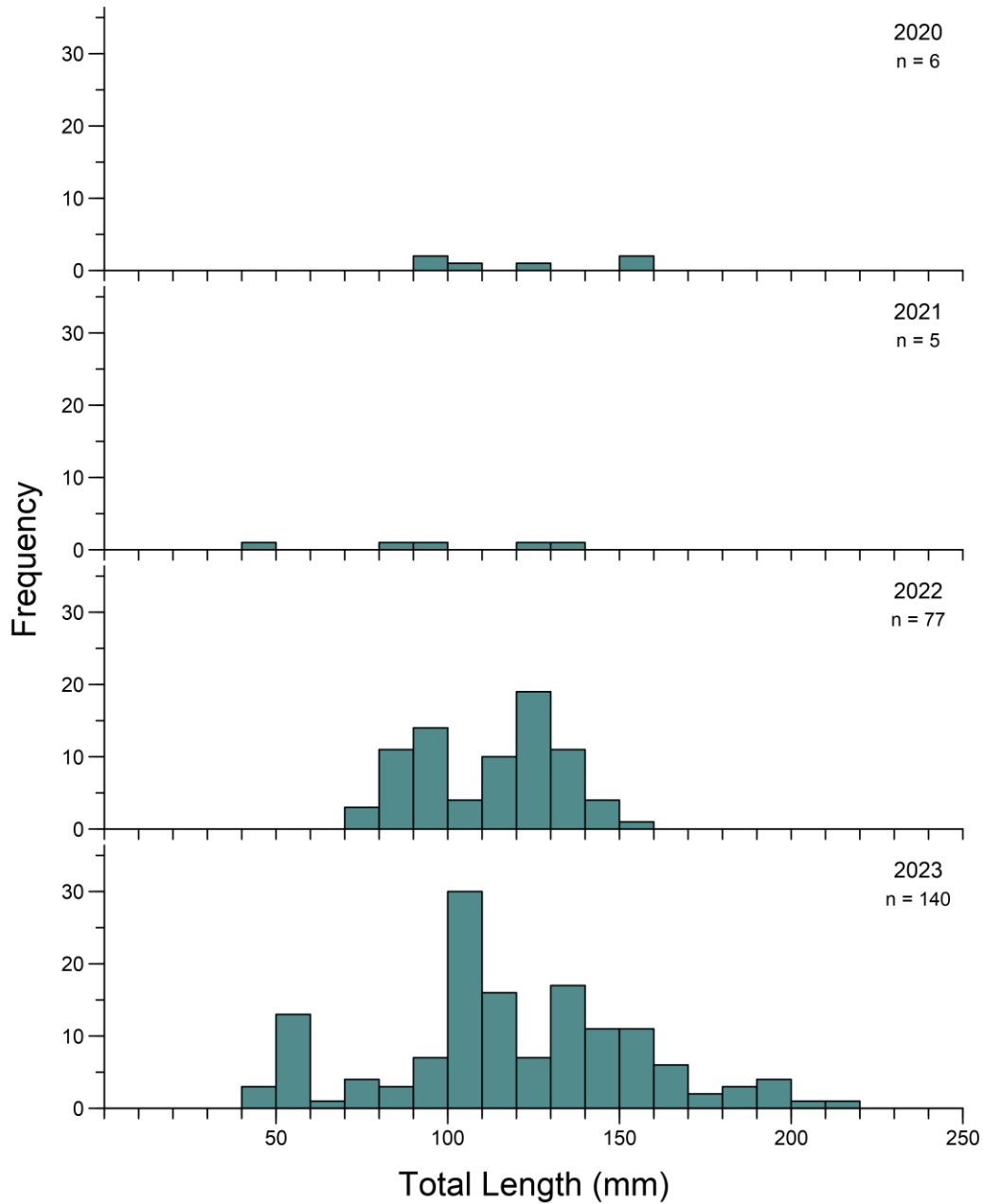


Figure 13.—Length frequency distribution of Roundtail Chub captured during annual monitoring in Rarick Canyon 2020 through 2023. Number of fish captured and measured each year is shown in the top right corner of each panel.



Figure 14.—Map of the reach of Willow Creek (light blue line) surveyed on May 31, 2023. Shown are the location of pools (blue dots), and fish barriers (orange dots) identified during the survey.

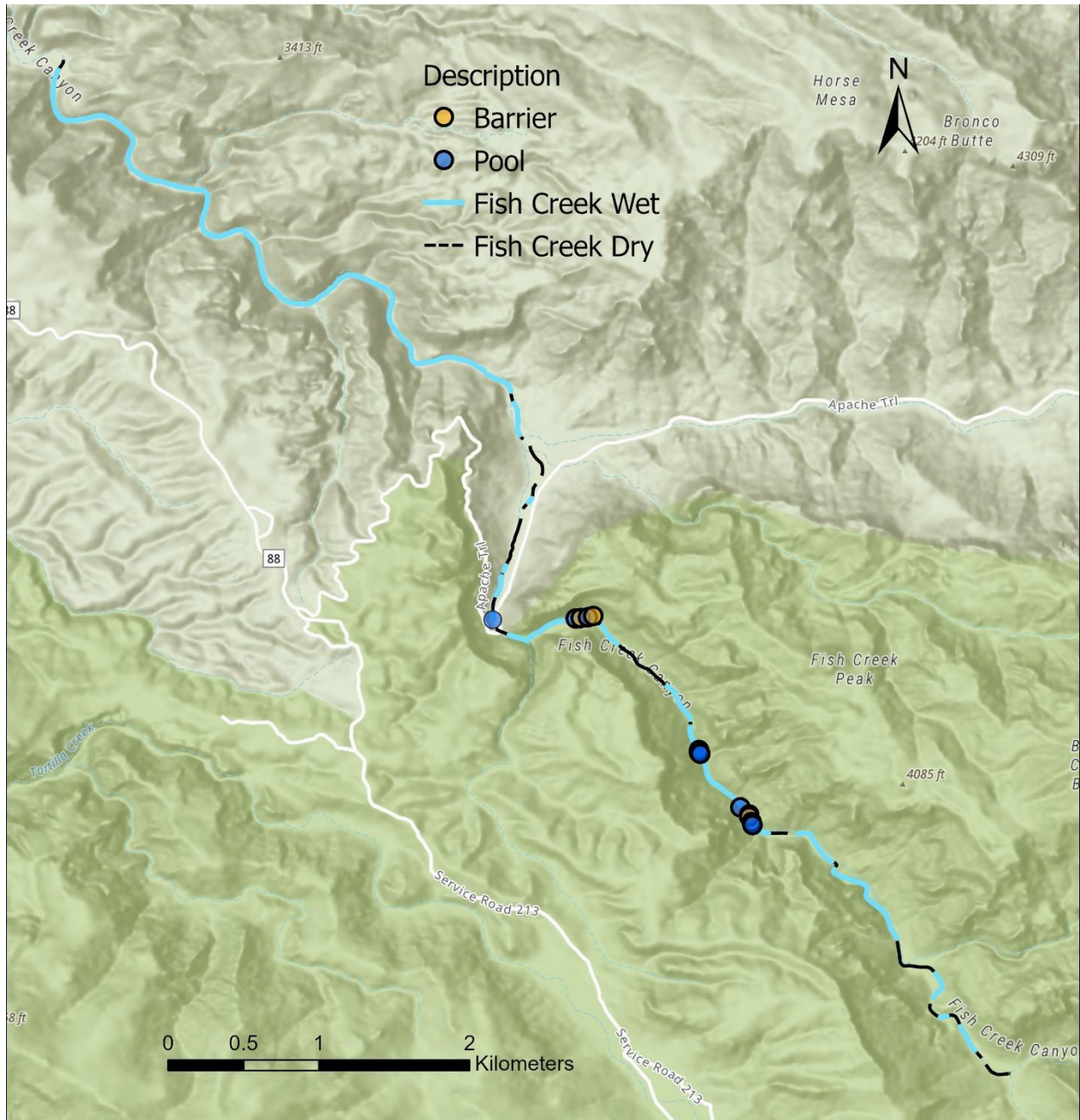


Figure 15.—Map of the reach of Fish Creek (black dashed line) surveyed on June 1, 2023. Shown is the extent of surface water (light blue line), and the location of pools (blue dots), and fish barriers (orange dots) identified during the survey.



Figure 16.—Photographs of stream habitat during habitat assessment efforts in Fish Creek on June 1, 2023. Shown is a shallow run flowing over deposited sediment, sand, and gravel, characteristic of the reach downstream of the Apache Trail bridge (A), an example of post-fire effected stream habitat in the reach upstream of the Apache Trail bridge (B), and an example of habitat that would normally create a bedrock pool, that is filled in by sediment, sand, and gravel (C).

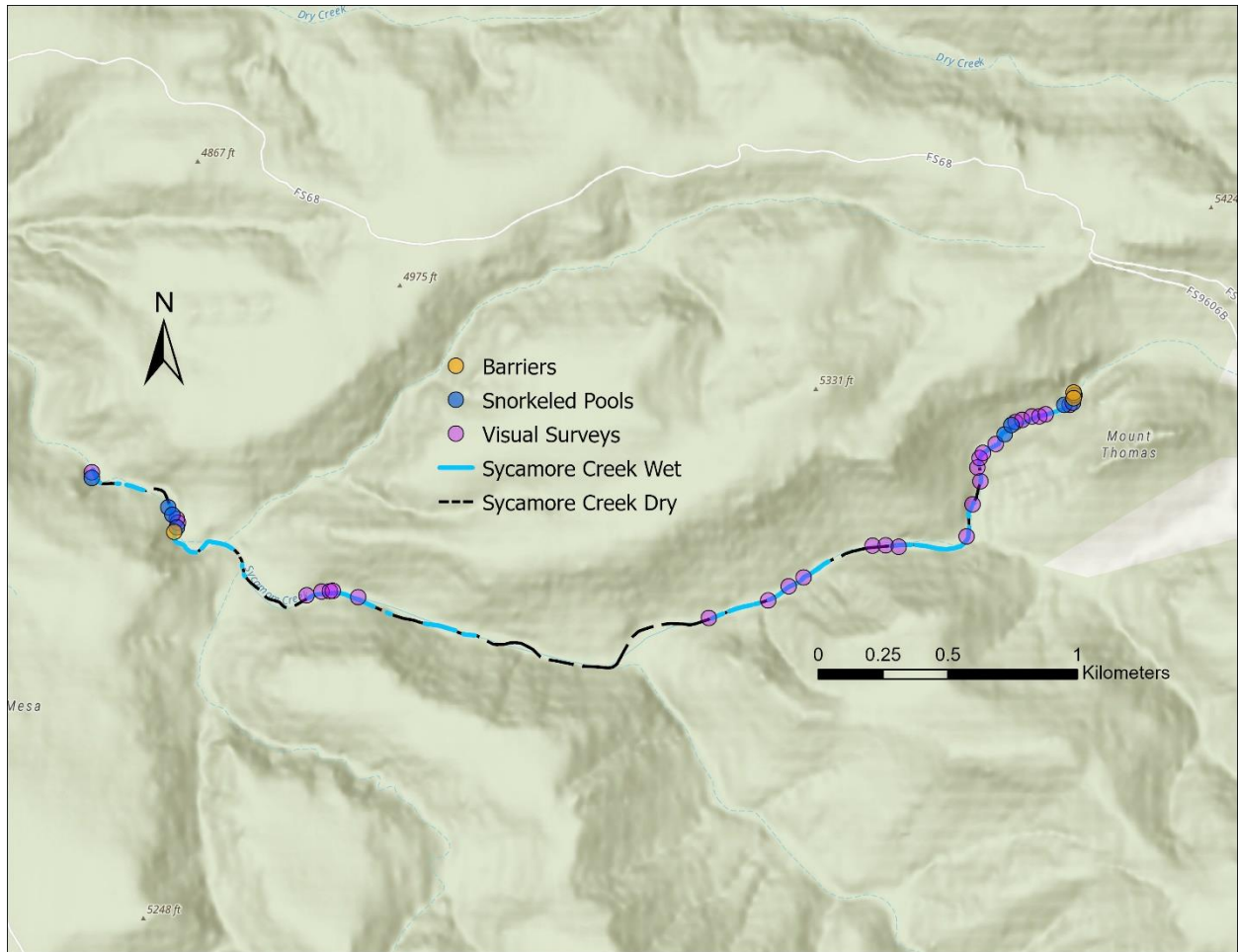


Figure 17.—Map of the reach of Sycamore Creek (black dashed line) surveyed on June 27, 2023. Shown is the extent of surface water (light blue line), and the location of visual surveys (purple dots), pools that were snorkeled (blue dots), and fish barriers (orange dots) identified during the survey.

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 Chub⁸, 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 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 (Shollenberger et al. 2023). Spikedace were initially stocked above the barrier in 2015, with additional stockings occurring in 2017, 2018, 2020, 2021, and 2022 (Figure 18). In total, 3,788 Spikedace have been translocated into Spring

⁸ Chub in Spring Creek were previously classified as Gila Chub.

Creek upstream of the barrier. Spikedace relative abundance was low during the first three years of annual monitoring, and remained low until reproduction was first documented in 2018, with peak relative abundance documented in 2019 (Figure 18).

Results:

On March 20, 2023, Department staff collected 34 Gila Topminnow from Sheepshead Canyon for a fish health assessment. The fish were transported to the fish health laboratory at Department headquarters for a fish health inspection, and were determined to be free of parasites or pathogens. The Sheepshead Canyon population was chosen as a Gila Topminnow donor site for Spring Creek because it is the same lineage as fish previously stocked in Spring Creek (Peck Canyon) and is close in proximity (approximately 2 km).

On May 16, 2023, Department staff translocated a total of 492 Gila Topminnow from Sheepshead Canyon to Spring Creek. Topminnow were stocked in the pool immediately upstream of the fish barrier, where topminnow previously established prior to flooding in 2021. There were 28 mortalities during capture and transport.

On September 8, 2023, 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 9 Spikedace were captured during the initial pass at each site, which is similar to the number of fish captured during first pass efforts in 2022 (Figure 18). In addition to Spikedace, 122 Roundtail Chub, 37 Desert Sucker, 133 Longfin Dace, and 185 Speckled Dace were captured during electrofishing (Table 2). Mean Spikedace relative abundance did not significantly decrease relative to 2022 (2023 = 10.38/h, 2022 = 11.77/h; $W = -55.5$, $P = 0.563$), however relative abundance has remained relatively low since the peak in 2019 (Figure 18). Mean size of Spikedace captured was 47.3 mm TL (min = 32, max = 67; Figure 19). A substantial portion of fish captured (approximately 41.7%) were young of year fish (<40 mm TL) that were likely spawned in 2023 (Figure 19).

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 sub-reach. Three additional Spikedace were captured during the two additional passes. Estimated abundance of Spikedace using a Carle-Strub method was 6 fish per hundred m (± 2.71) with an estimated capture probability of 0.55 (Table 3). The estimated abundance of Spikedace and the upper bound of the 95% confidence interval were slightly lower than the abundance estimate and lower bound of the confidence interval in 2022 ($N = 10 \pm 0.18$; Figure 20), which suggests there may have been a decrease in abundance. However, the capture probability of all taxa was relatively low this year compared to previous year which may partially explain the low estimated abundance of Spikedace (Table 3).

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 other native fish species in Spring Creek from 2015-2023 reveals that relative abundance of Spikedace actually has a significant, positive correlation with Roundtail Chub ($r = 0.855$, $t = 4.346$, $P = 0.003$) and Desert Sucker ($r = 0.900$, $t = 5.156$, $P = 0.001$; Figure 21). 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: Spikedace spawned for only the third time since monitoring efforts began in 2015. The relatively infrequent spawning of Spikedace in Spring Creek suggests that environmental conditions necessary for successful spawning are only intermittently available, and may sometimes occur less frequently than the typical lifespan for these fish in the wild (about 3-4 years). The monitoring information collected during the last nine years of monitoring suggests the Spikedace population in Spring Creek is not on a clear track for establishment or failure, but instead fluctuates somewhere between self-sufficient and dependent on translocations. Given the limited other options for Spikedace conservation on the landscape, and the substantial investment in securing this reach of stream from nonnative fish invasion, we recommend regular augmentation of this population with adult fish in the early spring as a necessary management strategy to ensure population persistence during periods without environmental conditions sufficient for successful spawning, and to provide the population an opportunity to substantially increase in abundance during periods when optimal conditions for successful spawning occur. Augmentations could occur as frequently as annually, or perhaps up to every three years pending coordination with partners and the availability of fish for translocations.

Tables and Figures:

Table 2.—Summary of fish captured during the first pass at three 100 m electrofishing sub-reaches in Spring Creek during annual monitoring on September 8, 2023. 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. Only data from the first pass are included.

Sub-reach	Statistic	Roundtail Chub	Spikedace	Desert Sucker	Longfin Dace	Speckled Dace
Random-13	#Ind	53	4	2		46
	#Ind/h	133.32	11.80	4.23		96.67
Random-12	#Ind	47	2	8	1	33
	#Ind/h	164.45	8.83	30.36	3.75	238.71
Fixed-2	#Ind	22	3	27	132	106
	#Ind/h	101.67	13.86	124.78	610.01	489.86
Total	#Ind	122	9	37	133	185
	#Ind/h	147.06	10.38	32.14	69.86	219.27
	SE	(12.83)	(5.07)	(18.29)	(67.55)	(80.65)

Table 3.—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 2023. 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, CACL = Desert Sucker, 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	3	1	2	6	3.29	8.71	0.55	0.00	1.00
Spring Creek	Fixed-02	GIRO	22	28	25	250	0.00	750.13	0.11	0.00	0.36
Spring Creek	Fixed-02	AGCH	132	124	73	564	349.92	733.08	0.25	0.15	0.35
Spring Creek	Fixed-02	RHOS	106	114	10 6	174 2	0.00	4783.21	0.07	0.00	0.19
Spring Creek	Fixed-02	CACL	27	23	16	103	46.38	159.62	0.28	0.07	0.5

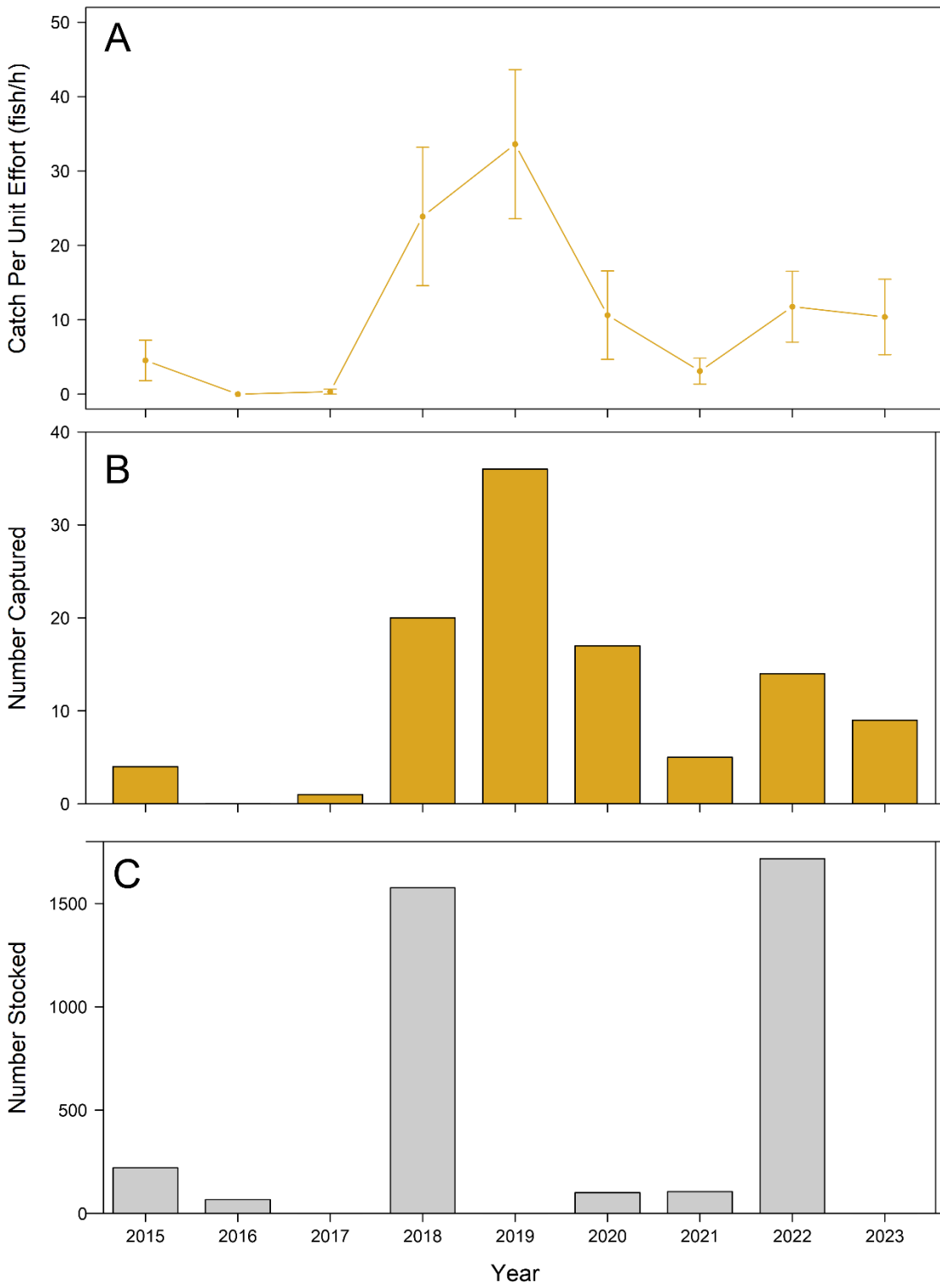


Figure 18.—Summary of Spikedace captured and stocked in Spring Creek, AZ, annually from 2015 to 2023 with (A) mean annual backpack electrofishing catch per unit effort (fish/h; first pass only) with standard error bars, (B) total number of fish captured (first pass), and (C) total number of fish stocked.

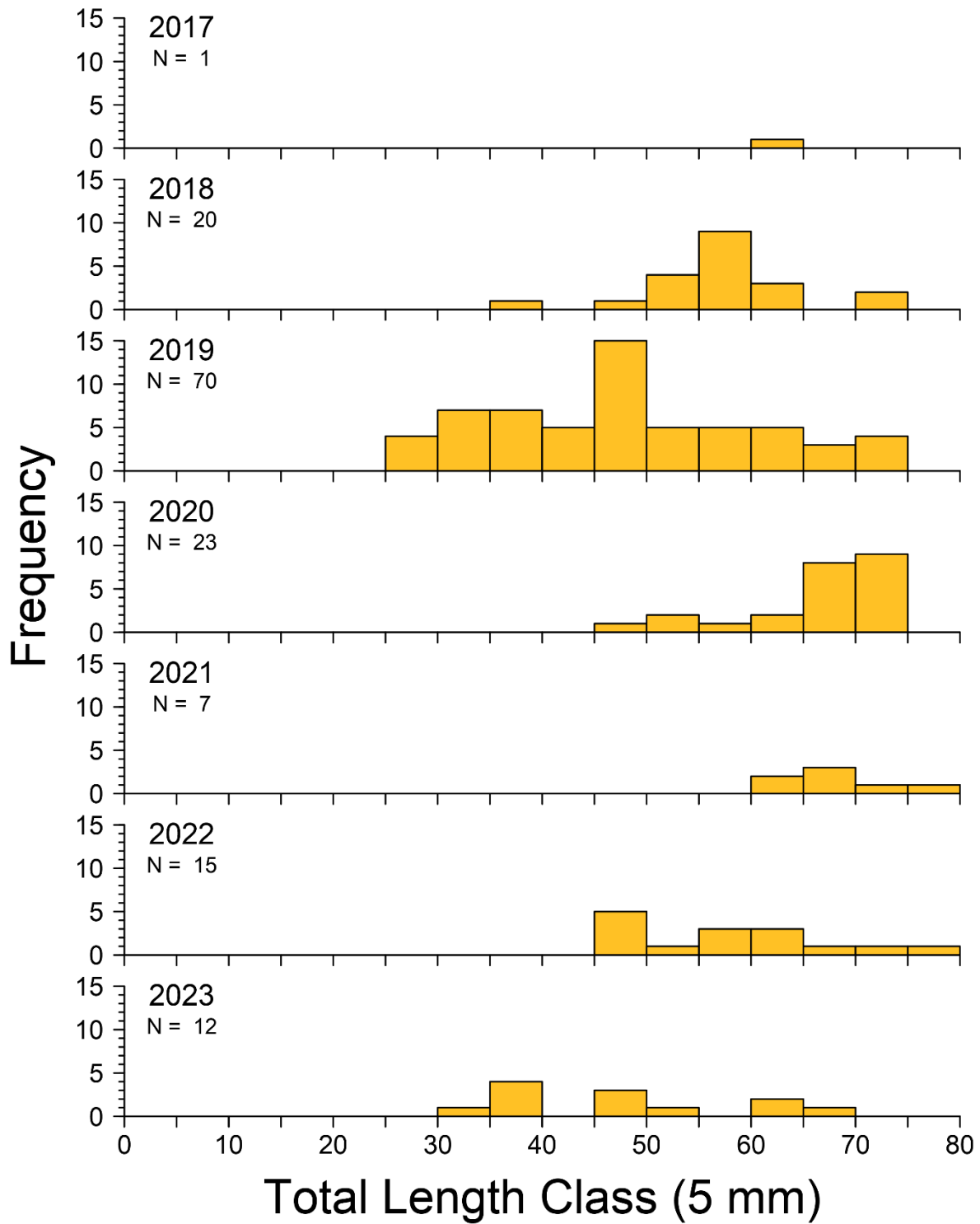


Figure 19.—Length frequency distribution of Spikedace captured during annual monitoring in Spring Creek, 2017 through 2023. Total number of fish captured and measured each among all passes each year is shown in the top left corner of each panel. Note depletion sampling did not occur in 2017 and 2018.

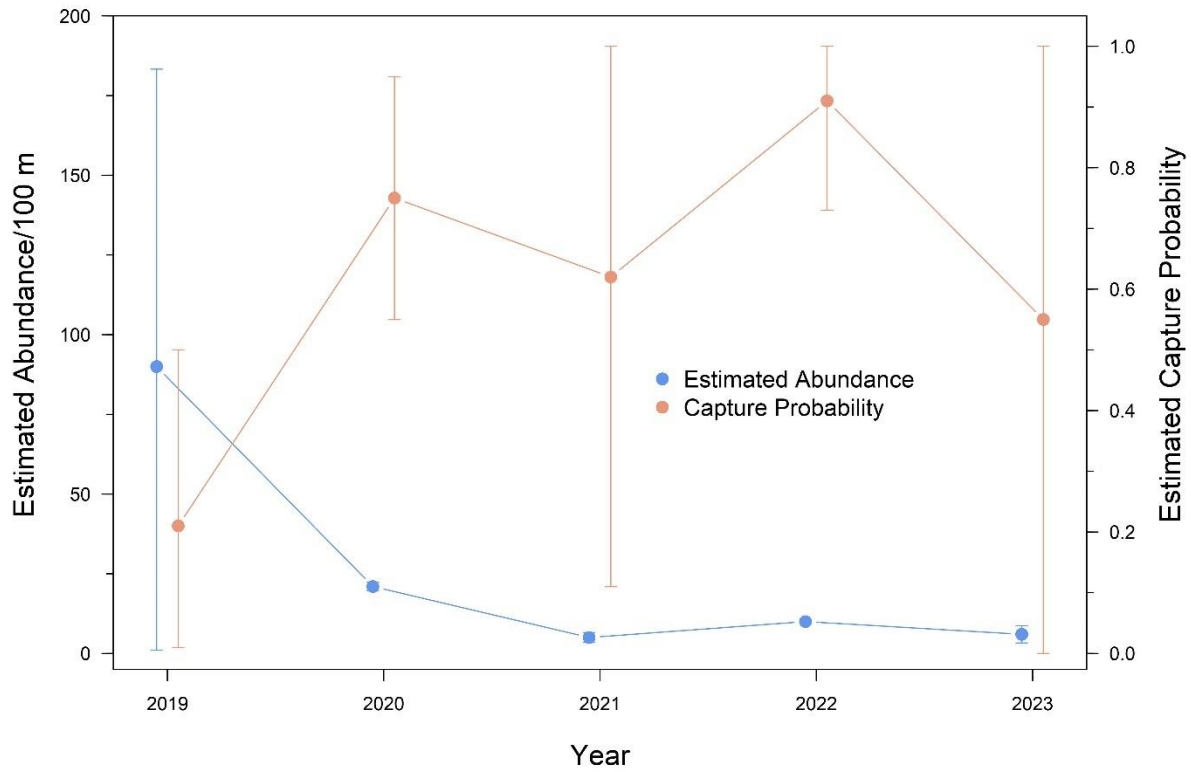


Figure 20.—Three-pass depletion estimates of Spikedace abundance per 100 m (blue points and lines) and capture probability (orange points and lines) at the fixed sub-reach in Spring Creek during annual monitoring from 2019-2023. Bars represent the lower and upper bounds of the 95% confidence interval of each estimate.

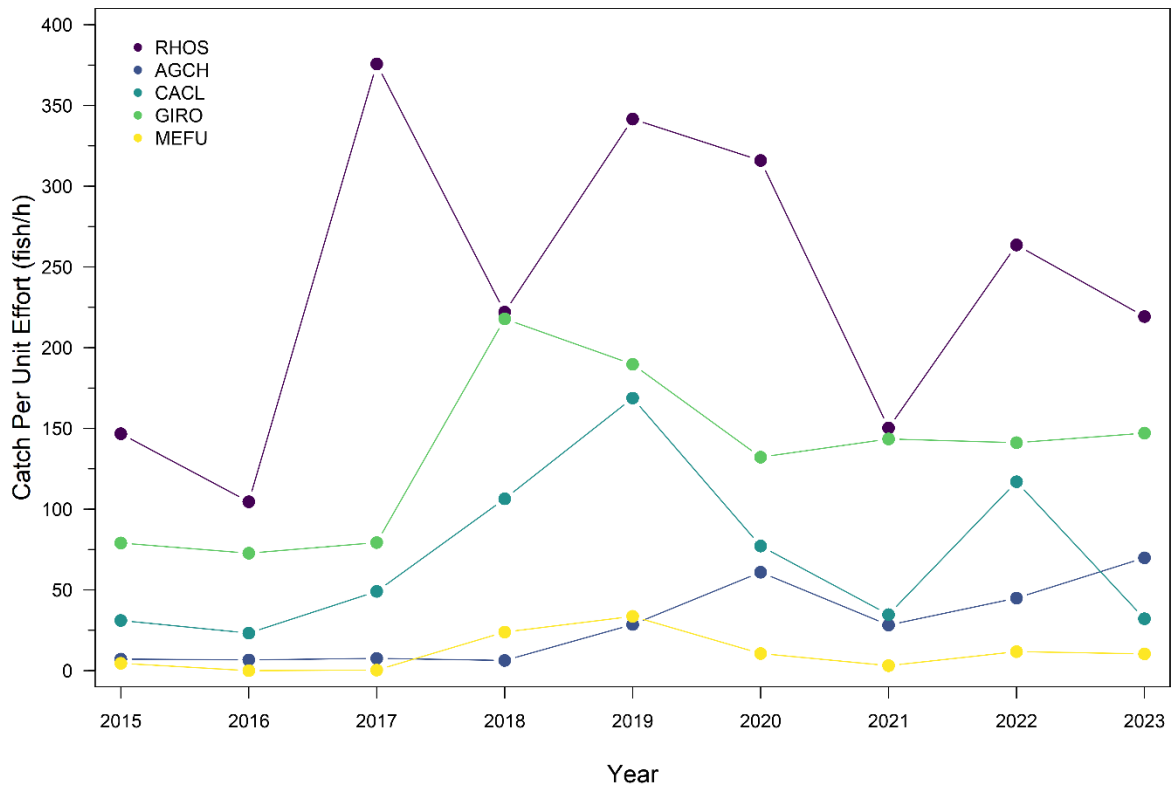


Figure 21.—Mean annual backpack electrofishing catch per unit effort (fish/h) of select native fish species (AGCH = Longfin Dace, CACL = Desert Sucker, GIRO = Roundtail Chub, MEFU = Spikedace, RHOS = Speckled Dace) captured in Spring Creek, AZ from 2015 to 2023. Standard error bars are not shown to improve 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 22). 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 22).

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 22). The downstream most 1.3 km of the project area is located on private property where permission to sample has not been granted since 2020. Roundtail Chub were initially stocked in the middle Blue River in 2016 (n = 1,194), with a subsequent augmentation in 2019 (n = 100; Figure 23). Spikedace were initially stocked in 2017 (n = 448), with a subsequent augmentation in 2018 (n = 291; Figure 24). 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 = 4), KP Creek to Cole Flat (n = 4), and Cole Flat to the natural waterfall barrier at The Box (n = 4). 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 the New Mexico state line (Figure 22). 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 February 21, 2023, Department staff translocated 650 Gila River lineage Spikedace and 2,496 Roundtail Chub into the lower Blue River near Juan Miller Crossing from ARCC (Appendix 2). Most of the Spikedace were adult fish, while the Roundtail Chub were juveniles between 50 and 100 mm TL. There were four Roundtail Chub mortalities during transport and stocking. The purpose of the translocation was to continue to augment the population after native fish population crashes following the Brigham and Cow Canyon fires.

Middle Blue River

On February 28, 2023, Department staff translocated 517 Gila River lineage Spikedace and 2,249 Roundtail Chub from ARCC into the middle Blue River just downstream of the final road crossing (667287/3713496; Appendix 2). Most of the Spikedace were adult fish, while the Roundtail Chub were juveniles between 50 and 100 mm TL. There was one Spikedace mortality during transport and stocking. The purpose of the translocation was to continue to augment the population after the native fish population crash following the Cow Canyon fire.

During July 19-20, 2023, Department and Apache-Sitgreaves National Forest staff salvaged Loach Minnow from Campbell Blue Creek due to post fire concerns from the Campbell Fire. The crew focused efforts on the reach upstream of the Turkey Creek confluence and captured a total of 134 Loach Minnow via electrofishing. The fish were translocated to the Blue River near the KP Creek confluence with one Loach Minnow mortality during transport.

During September 25-27, 2023, Department staff electrofished a total of ten randomly selected and two fixed 100 m long sub-reaches. A total of 280 Spikedace, 31 Roundtail Chub, 17 Loach Minnow, 118 Longfin Dace, 1,253 Speckled Dace, 1,450 Desert Sucker, and 293 Sonora Sucker

were captured during the first pass (Table 4). This is the greatest number of Spikedace captured within this reach since monitoring began in 2018 (Figure 24). Roundtail Chub captures increased slightly relative to 2022 ($n = 17$), but remain far below the peak in 2020 ($n = 180$; Figure 23). Loach Minnow continue to struggle in this reach since the Cow Canyon Fire, with only 17 individuals captured during the first pass, which is the second lowest total since monitoring began in 2018 and far below peak captures in 2020 ($n = 393$; Figure 25). The percent composition of Spikedace relative to all fish captured (8.13%) increased compared to 2022 (3.12%), while the percent composition of Roundtail Chub (0.90%) and Loach Minnow (0.49%) declined relative to 2022 (Roundtail Chub = 2.49%, Loach Minnow = 1.82%).

Mean relative abundance of all native fish, except Roundtail Chub and Loach Minnow, significantly increased from 2022 to 2023 (Table 5). Mean relative abundance of Roundtail Chub and Loach Minnow was not significantly different from 2022. Most native fish species had successful spawns and rebounded from the low relative abundance documented in 2022 (Figure 26).

Spawning success of Spikedace, Roundtail Chub, and Loach Minnow was variable this year. Spikedace had the strongest juvenile year class to date, with a majority of the fish (67.1%) captured being juveniles (Figure 27). Unsurprisingly, the mean size of Spikedace declined for the first time since 2020, when young of year fish were last captured (2020 = 38.35, 2021 = 54.66, 2022 = 65.21, 2023 = 39.30; Figure 27). Young of year Roundtail Chub were the most frequently captured year class, however the juvenile year class was not nearly as strong as the 2020 year class (Figure 28). There are two primary year classes of Roundtail Chub that have been consistently captured during monitoring; the chub initially stocked in 2016 which are still present and relatively large (~300-370 mm TL), and the strong year class of chub spawned in 2020, which are growing (~170-230 mm TL), but likely need additional time for most of the cohort to reach reproductive maturity (Figure 28). Loach Minnow continued to have limited reproductive success within this reach since 2020, which might be attributable to the increase in fine sedimentation since the Cow Canyon Fire. Consequently, mean size of Loach Minnow has increased each year (2020 = 39.30, 2021 = 51.10, 2022 = 53.75, 2023 = 54.5; Figure 29). The juxtaposition of the strong Spikedace year class with the relatively weak Loach Minnow year class suggests there can be substantial differences in the environmental conditions each species needs for successful spawning within the same reach.

Spikedace were captured at eight sub-reaches and Roundtail Chub at six sub-reaches, which is an increase in distribution relative to 2022 when Spikedace were captured at six sub-reaches and Roundtail Chub at four sub-reaches (Figure 30; Figure 31). Similarly, Loach Minnow were captured at six sub-reaches, compared to four sub-reaches in 2022 (Figure 32). Spikedace still seem to have difficulty persisting in 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, despite stocking at the upstream extent of the monitoring reach this spring. In contrast,

Loach Minnow were mostly found within the upper 1 km of the reach, suggesting habitat conditions in 2023 were causing Spikedace and Loach Minnow to essentially be spatially segregated, which is the first time we have observed this phenomena within the Middle Blue River.

Estimated abundance of Spikedace per 100 m was also the highest on record ($N = 126 \pm 20.0$) at the fixed site near the KP Creek confluence. Spikedace have only been captured at the downstream fixed site in 2020, 2021, and 2023, but capture probability has been remarkably consistent each year (0.43, 0.44, 0.46 respectively; Figure 33). The estimate of Roundtail Chub abundance ($N = 17 \pm 10.72$) at the upper fixed site was generally consistent with estimates since 2021 (Table 6; Figure 34). Estimated abundance of Loach Minnow at the upper fixed site has consistently declined since the peak in 2020, and Loach Minnow have not been captured at the lower fixed site since 2021 (Table 6; Figure 35).

Overall, most of the fish community was able to take advantage of above average flow this winter and spring and rebounded nicely from a few years of difficult drought and post-fire conditions, generally increasing in relative abundance and distribution (Figure 26; Figure 36). Some of the sedimentation that followed the Cow Canyon Fire seems to be slowly moving out of the system, further improving conditions for native fish. Importantly, Spikedace probably responded most strongly to these conditions, with the highest abundance and strongest year class documented to date in this reach. The strong response of Spikedace following the early spring stocking suggests that going forward translocations should take place in the early spring whenever possible so that stocked fish can immediately take advantage of spawning conditions without having to first survive through difficult winter conditions. Roundtail Chub and Loach Minnow did not respond as strongly as Spikedace, but were still able to change the declining population trajectory of the last two years. Population parameters for all three of these species will likely continue to improve given exposure to optimal, or at least non-extreme conditions over the next year or two.

Upper Blue River

On February 28, 2023, Department staff translocated 250 Gila River lineage Spikedace and 1,000 Roundtail Chub from ARCC into the upper Blue River in the vicinity of Bobcat Flat (680702/3732370; Appendix 2). An additional 250 Spikedace were stocked in the vicinity of the upper Blue Campground (678641/3729825; Figure 37). Most of the Spikedace were adult fish, while the Roundtail Chub were juveniles between 50 and 100 mm TL. There was one Spikedace mortality during transport and stocking. The purpose of the translocation was to augment the population after relatively few fish of both taxa were captured during the first two years of monitoring efforts.

During September 18-20, 2023, Department staff electrofished a total of 12 randomly selected and three fixed 100 m long sub-reaches starting at Blue Crossing Campground upstream to the New Mexico state line. A total of 2 Spikedace, 5 Roundtail Chub, 143 Loach Minnow, 691 Longfin Dace, 2,701 Speckled Dace, 978 Desert Sucker, 396 Sonora Sucker and 11 Brown Trout were

captured during the first pass (Table 7). Despite stocking this reach in February, the same number of Spikedace were captured on the first pass ($n = 2$), and there was not a significant difference in mean relative abundance compared to 2022 (Table 8; Figure 38). Roundtail Chub captures declined relative to 2022 ($n = 17$; Figure 39), but mean relative abundance did not significantly change (Table 8). Loach Minnow captures increased relative to 2022 ($n = 76$; Figure 40), however mean relative abundance did not significantly change (Table 8). Mean relative abundance of all other native fish taxa significantly increased from 2022 to 2023, with the exception of Longfin Dace which did not differ significantly (Table 8; Figure 41).

Young of year Spikedace were detected for the first time in this reach, which suggests that spawning habitat criteria are occasionally met within this reach. The two Spikedace captured were too small (33, 38 mm TL) to be individuals that were stocked in February (Figure 42). We have yet to capture any young of year Roundtail Chub within this reach, although we have been able to document survival of adults since the initial stocking in 2020 (Figure 43). Relative to 2022, we captured far more young of year individuals for all other native fish taxa. In particular, the greater number of Loach Minnow captured in 2023 appears to be largely driven by the strongest year class since monitoring began in 2021 (Figure 44).

Spatial distribution of both Spikedace and Roundtail Chub continues to be patchy within the upper Blue River. Spikedace were captured at two sub-reaches (1R44, 1F46) in Reach 1, which marks the first time Spikedace were captured outside the vicinity of the initial stocking locations (Figure 37). Roundtail Chub were only captured at three sub-reaches all located within Reach 3 (Figure 45), while Loach Minnow were captured only in Reach 2 and 3 (Figure 46). A large portion of Reach 1 was dry upon arrival, and several of the sites had to be re-selected to find wetted portions of stream. The tendency for intermittent flow in Reach 1 during periods of drought may partially explain why we have captured fewer fish of all taxa, and particularly the focal species, within this reach (Figure 47)

Three-pass depletions were carried out at one fixed sub-reach in each of the three monitoring reaches. Similar to 2022, Roundtail Chub were only captured at a single fixed site (3F44), with capture probability consistently low at depletion sites where chub have been captured (2022 $N = 0.15 \pm 0.48$, 2023 $N = 0.18 \pm 0.65$; Table 9). Roundtail Chub are likely more abundant within the upper Blue River than the first pass data alone suggests (Table 9). 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 9). Estimated abundance of Loach Minnow was the highest on record at the two depletion sites (2F47, $N = 43 \pm 40.73$; 3F44, $N = 104 \pm 101.22$) where Loach Minnow were captured (Figure 48).

The mean relative abundance of Brown Trout captured in 2023 was not significantly different than 2022 (Table 8). However mean size of Brown Trout significantly increased for the second consecutive year (2021 = 102.34, 2022 = 159.55, 2023 = 206.82 mm TL; $W = 326$, $P = 0.039$). Length-frequency data suggests that there was not a strong year class of juvenile fish compared to

2021 and 2022, possibly due to the elevated flows that occurred throughout the winter (Figure 49). Continued evidence that a 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.

Despite abundant winter precipitation and an extended period of spring runoff, the lack of typical monsoon moisture greatly reduced the discharge of the upper Blue River by the time monitoring occurred in September. The smaller size and higher watershed position of the upper Blue River reach may make it more susceptible to extreme changes in flow (both drought and flood) than downstream reaches, and the fish populations may be more susceptible to these events, as well as lag effects from past events.

Recommendations: Interannual survival and reproduction of Spikedace has already been documented during the first three years of monitoring in the upper Blue River, demonstrating that stream habitat can at least occasionally support these key life history requirements. The lack of captures of adult Spikedace in 2023 suggests that either survival within the reach or dispersal from it is limiting the population's ability to reproduce effectively and increase in abundance. Additional translocations of Spikedace are required in the upper Blue River to evaluate whether a population of Spikedace can establish under less extreme hydrologic conditions (both floods and drought) than those experienced since Spikedace were initially stocked in 2020. Without stocking, there is little opportunity for the Spikedace population to become established in the near term, given how few adults have been captured to date.

Evidence from monitoring throughout all three reaches of the Blue River suggests that Roundtail Chub reproduction occurs sporadically. It takes several years, likely five or more, to recruit into the population which makes assessing short-term population trends difficult. The continued persistence of adult Roundtail Chub in both the upper and middle Blue River reaches through extreme hydrologic conditions of the last few years is evidence that chub are resilient to these extreme conditions in both reaches and will likely be able to establish in time. Continued monitoring is needed to document reproduction and determine if additional supplemental stockings will be necessary in order for populations of chub to establish.

Preliminary reports from monitoring in the lower Blue River in 2023 suggests that many Spikedace from the stocking in February 2023 survived and likely reproduced. In addition, several different size classes of Roundtail Chub were captured, suggesting chub have been able to disperse from other portions of the river to recolonize this reach (P. Reap, Marsh and Associates, personal communication). The continued absence of Loach Minnow in the lower Blue River suggests that Loach Minnow have been unable to disperse to this reach and survive on their own since post-fire impacts from the Cow Canyon and Brigham Fires. Translocation of Loach Minnow to the lower Blue River either from ARCC or upstream reaches should be considered if stream habitat conditions continue to improve and Loach Minnow fail to recolonize this reach on their own, similar to efforts following the Wallow Fire in 2012.

Tables and Figures:

Table 4.—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 25-27, 2023. 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	Roundtail	Spikedace	Desert	Longfin	Sonora	Speckled
			Minnow	Chub		Sucker	Dace	Sucker	Dace
1	4	#Ind	1	9	162	569	50	97	392
		#Ind/h	1.39	7.51	143.28	354.09	31.73	67.04	264.78
		SE	(1.39)	(4.24)	(33.05)	(61.07)	(10.69)	(11.96)	(42.63)
2	4	#Ind	5	10	117	627	37	128	526
		#Ind/h	1.86	13.84	88.12	426.81	19.98	90.31	344.15
		SE	(1.86)	(12.53)	(19.57)	(65.68)	(7.69)	(23.26)	(52.93)
3	4	#Ind	11	12	1	254	31	68	335
		#Ind/h	9.22	8.26	0.84	153.14	23.46	44.06	208.95
		SE	(3.81)	(2.40)	(0.84)	(31.78)	(8.96)	(14.45)	(48.98)
Total	12	#Ind	17	31	280	1450	118	293	1253
		#Ind/h	3.63	10.20	84.02	329.91	24.91	70.18	281.42
		SE	(1.40)	(5.10)	(16.13)	(38.09)	(5.23)	(10.92)	(29.25)

Table 5.—Pairwise Wilcoxon signed rank test statistics evaluating changes in mean relative abundance of all fish taxa captured in the middle Blue River between 2022 and 2023. Two-sided tests were used for taxa where there was not an obvious change in mean relative abundance, and one-sided tests were used for taxa where mean relative abundance appeared to increase or decline from 2022 to 2023. Significant values are indicated with an asterisk (*).

Taxa	Alternative	<i>W</i>	<i>P</i>
Spikedace	One-sided	111	0.011*
Roundtail Chub	One-sided	89	0.150
Loach Minnow	One-sided	92	0.104
Longfin Dace	One-sided	117	0.005*
Speckled Dace	One-sided	144	<0.001*
Desert Sucker	One-sided	141	<0.001*
Sonora Sucker	One-sided	130	<0.001*

Table 6.—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 2023. Included is the number of fish caught in each pass (C1, C2, C3), Carle-Strub three pass abundance estimate (N), lower (N_LCI) and upper (N_UCI) 95% confidence interval of the abundance estimate, estimated capture probability (*p*), and the lower (*p*_LCI) and upper (*p*_UCI) 95% confidence interval of the estimate of capture probability. Species codes are MEFU = Spikedace, GIRO = Roundtail Chub, TICO = Loach Minnow, CACL = Desert Sucker, CAIN = Sonora Sucker, AGCH = Longfin Dace, and RHOS = Speckled Dace.

Sub-reach	Species	C1	C2	C3	N	N_LCI	N_UCI	<i>p</i>	<i>p</i> _LCI	<i>p</i> _UCI
Fixed-04	TICO	3	3	0	6	4.7	7.3	0.67	0.23	1.00
Fixed-04	GIRO	6	4	4	17	6.28	27.72	0.4	0.00	0.82
Fixed-04	RHOS	115	43	40	234	206.47	261.53	0.46	0.36	0.56
Fixed-04	CACL	70	30	21	139	121.02	156.98	0.49	0.37	0.61
Fixed-04	CAIN	25	10	10	53	39.35	66.65	0.45	0.24	0.67
Fixed-04	AGCH	18	6	4	29	25.22	32.78	0.62	0.41	0.84
Fixed-29	MEFU	59	29	19	126	106	146	0.46	0.33	0.6
Fixed-29	GIRO	0	0	1	1	0.00	4.97	0.33	0.00	2.32
Fixed-29	RHOS	167	58	62	345	308.45	381.55	0.45	0.36	0.53
Fixed-29	CACL	235	97	59	438	411.72	464.28	0.52	0.46	0.59
Fixed-29	CAIN	38	18	6	66	59.33	72.67	0.6	0.45	0.75
Fixed-29	AGCH	16	12	5	38	27.53	48.47	0.47	0.23	0.72

Table 7.—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 18-20, 2023. 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	303	112	121	708	2
		#Ind/h			1.24	204.63	88.30	74.43	529.25	1.07
		SE			(0.87)	(77.41)	(25.40)	(24.33)	(128.82)	(0.73)
2	5	#Ind	59			325	219	104	828	1
		#Ind/h	42.06			235.13	124.53	80.59	564.62	1.01
		SE	(10.65)			(54.77)	(42.94)	(17.75)	(55.49)	(1.01)
3	5	#Ind	84	5		350	360	171	1165	8
		#Ind/h	64.09	2.28		227.22	264.41	117.17	806.55	7.65
		SE	(18.03)	(1.31)		(37.26)	(51.75)	(29.95)	(129.79)	(4.64)
Total	15	#Ind	143	5	2	978	691	396	2701	11
		#Ind/h	37.90	0.85	0.37	233.14	165.99	92.53	664.35	3.50
		SE	(8.33)	(0.51)	(0.26)	(31.67)	(26.98)	(14.49)	(65.35)	(1.80)

Table 8.—Pairwise Wilcoxon signed rank test (non-parametric) and two-sample t-test (parametric) statistics evaluating changes in mean relative abundance of all fish taxa captured in the upper Blue River between 2022 and 2023. Two-sided tests were used for taxa where there was not an obvious change in mean relative abundance, and one-sided tests were used for taxa where mean relative abundance differed from 2022 to 2023. Significant values are indicated with an asterisk (*).

Taxa	Test Type	Alternative	<i>t</i>	<i>W</i>	<i>P</i>
Spikedace	Non-parametric	Two-sided		119	0.633
Roundtail Chub	Non-parametric	Two-sided		100	0.502
Loach Minnow	Non-parametric	Two-sided		147	0.156
Longfin Dace	Non-parametric	Two-sided		120	0.775
Speckled Dace	Non-parametric	One-sided		192	<0.001*
Desert Sucker	Parametric	One-sided	2.201		0.019*
Sonora Sucker	Parametric	One-sided	2.527		0.009*
Brown Trout	Non-parametric	Two-sided		71.5	0.076

Table 9.—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 2023. Included is the number of fish caught in each pass (C1, C2, C3), Carle-Strub three pass abundance estimate (N), lower (N_LCI) and upper (N_UCI) 95% confidence interval of the abundance estimate, estimated capture probability (*p*), and the lower (*p*_LCI) and upper (*p*_UCI) 95% confidence interval of the estimate of capture probability. Species codes are MEFU = Spikedace, GIRO = Roundtail Chub, TICO = Loach Minnow, CACL = Desert Sucker, CAIN = Sonora Sucker, AGCH = Longfin Dace, RHOS = Speckled Dace, SATR = Brown Trout.

Sub-reach	Species	C1	C2	C3	N	N_LCI	N_UCI	<i>p</i>	<i>p</i> _LCI	<i>p</i> _UCI
1-46F	AGCH	16	14	2	34	28.46	39.54	0.57	0.35	0.79
1-46F	RHOS	78	41	19	156	138.97	173.03	0.51	0.4	0.62
1-46F	CACL	11	7	5	28	15.68	40.32	0.42	0.1	0.73
1-46F	CAIN	4	2	1	7	5.3	8.7	0.64	0.21	1.00
1-46F	MEFU	1	0	0	1	1	1	1	1	1
1-46F	SATR	1	0	0	1	1	1	1	1	1
2-47F	TICO	10	9	8	43	2.27	83.73	0.27	0.00	0.62
2-47F	AGCH	43	22	16	98	77.22	118.78	0.44	0.27	0.6
2-47F	RHOS	213	120	58	457	421.22	492.78	0.47	0.4	0.54
2-47F	CACL	88	39	23	169	151.73	186.27	0.51	0.41	0.62
2-47F	CAIN	10	8	7	36	8.55	63.45	0.31	0.00	0.66
3-45F	TICO	23	12	19	104	2.78	205.22	0.21	-0.05	0.48
3-45F	AGCH	76	46	31	199	158.39	239.61	0.38	0.26	0.51
3-45F	RHOS	161	93	89	539	410.3	667.7	0.29	0.19	0.38
3-45F	CACL	59	32	35	199	118.78	279.22	0.28	0.12	0.44
3-45F	CAIN	37	29	20	129	73.42	184.58	0.3	0.12	0.49
3-45F	GIRO	2	2	6	21	0.00	85.91	0.18	0.00	0.83

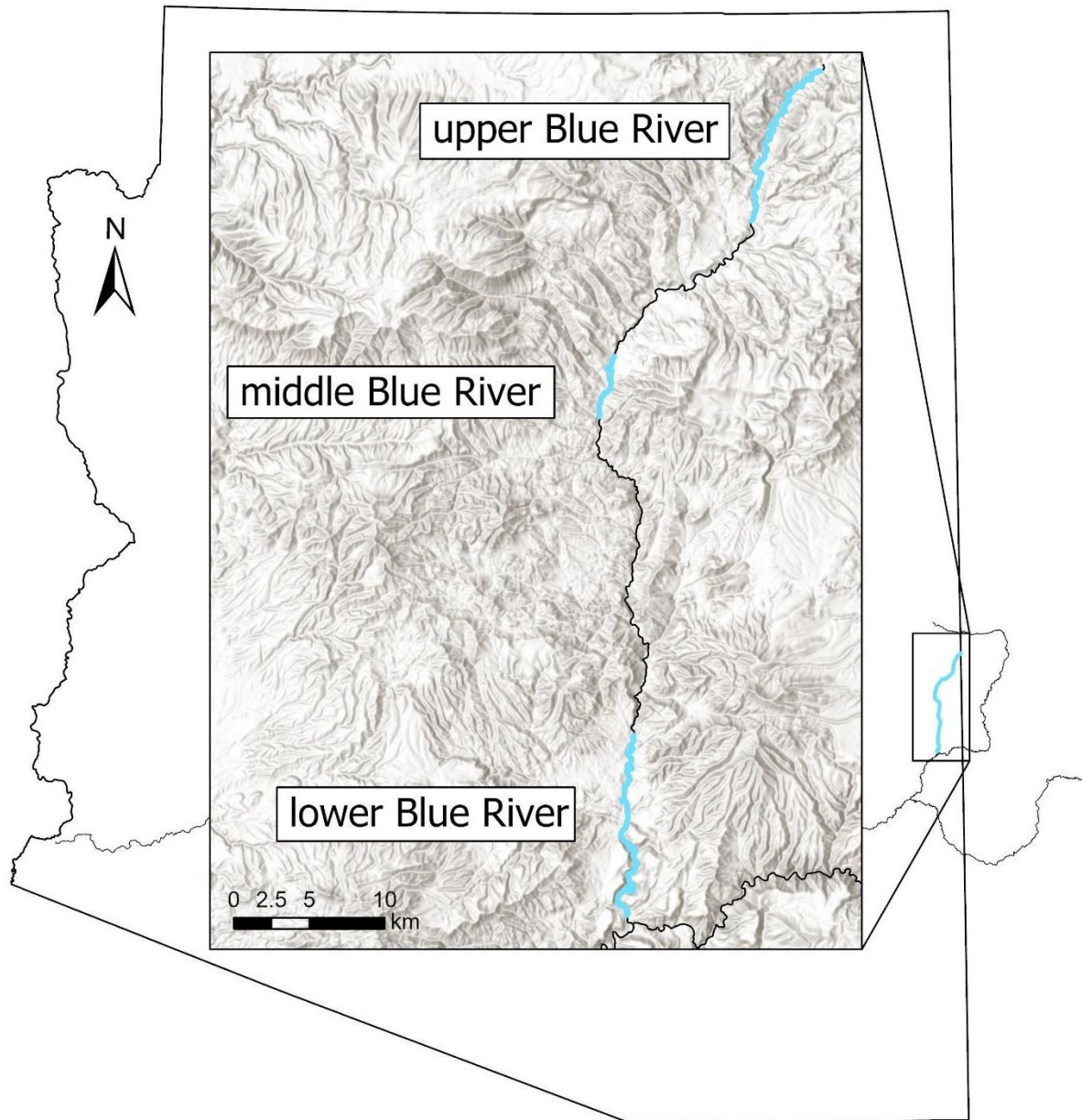


Figure 22.—Map showing the upper (New Mexico border downstream to Blue Crossing Campground), middle (The Box downstream to Fritz Ranch), and lower (Fritz Ranch downstream to the barrier) project areas of the Blue River.

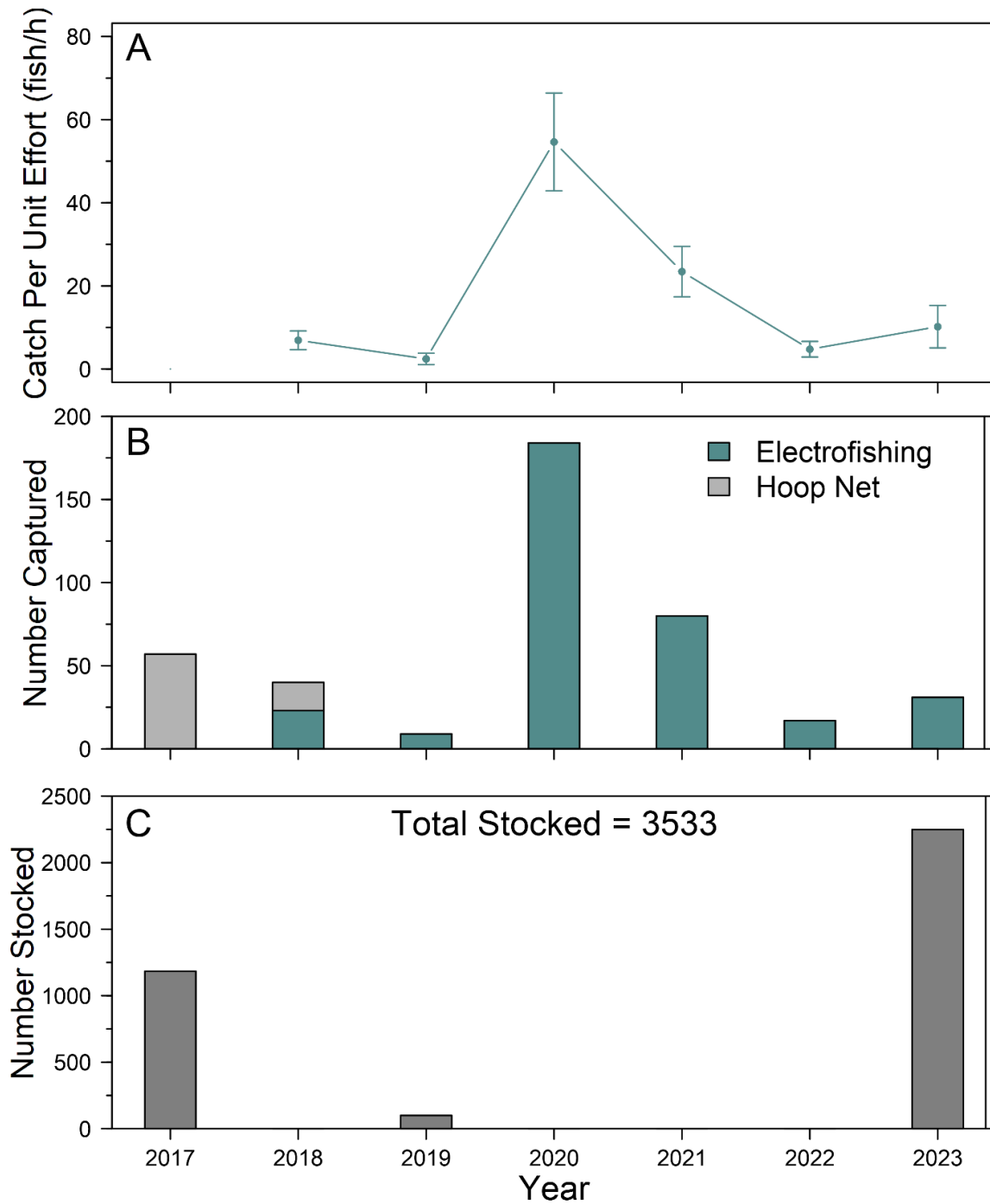


Figure 23.—Summary of Roundtail Chub captured and stocked in the middle Blue River, annually from 2017 to 2023 with (A) mean catch per unit effort (fish/h; first pass only) for backpack electrofishing with standard error bars, (B) total number of fish captured by gear type (hoop nets in gray, backpack electrofishing in black; first pass only), 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.

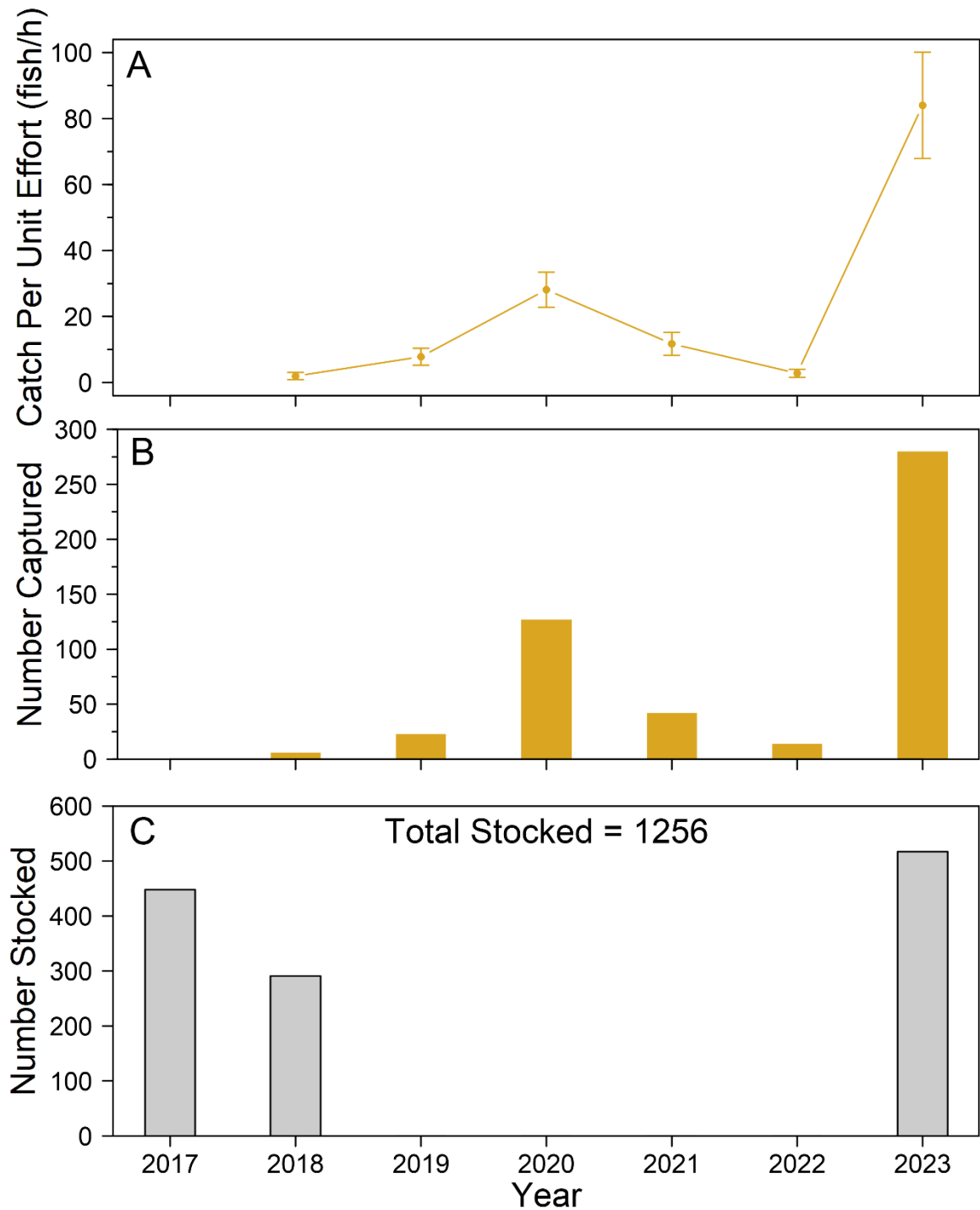


Figure 24.—Summary of Spikedace captured and stocked in the middle Blue River, annually from 2017 to 2023 with (A) mean annual backpack electrofishing catch per unit effort (fish/h; first pass only) with standard error bars, (B) total number of fish captured (first pass), and (C) total number of fish stocked.

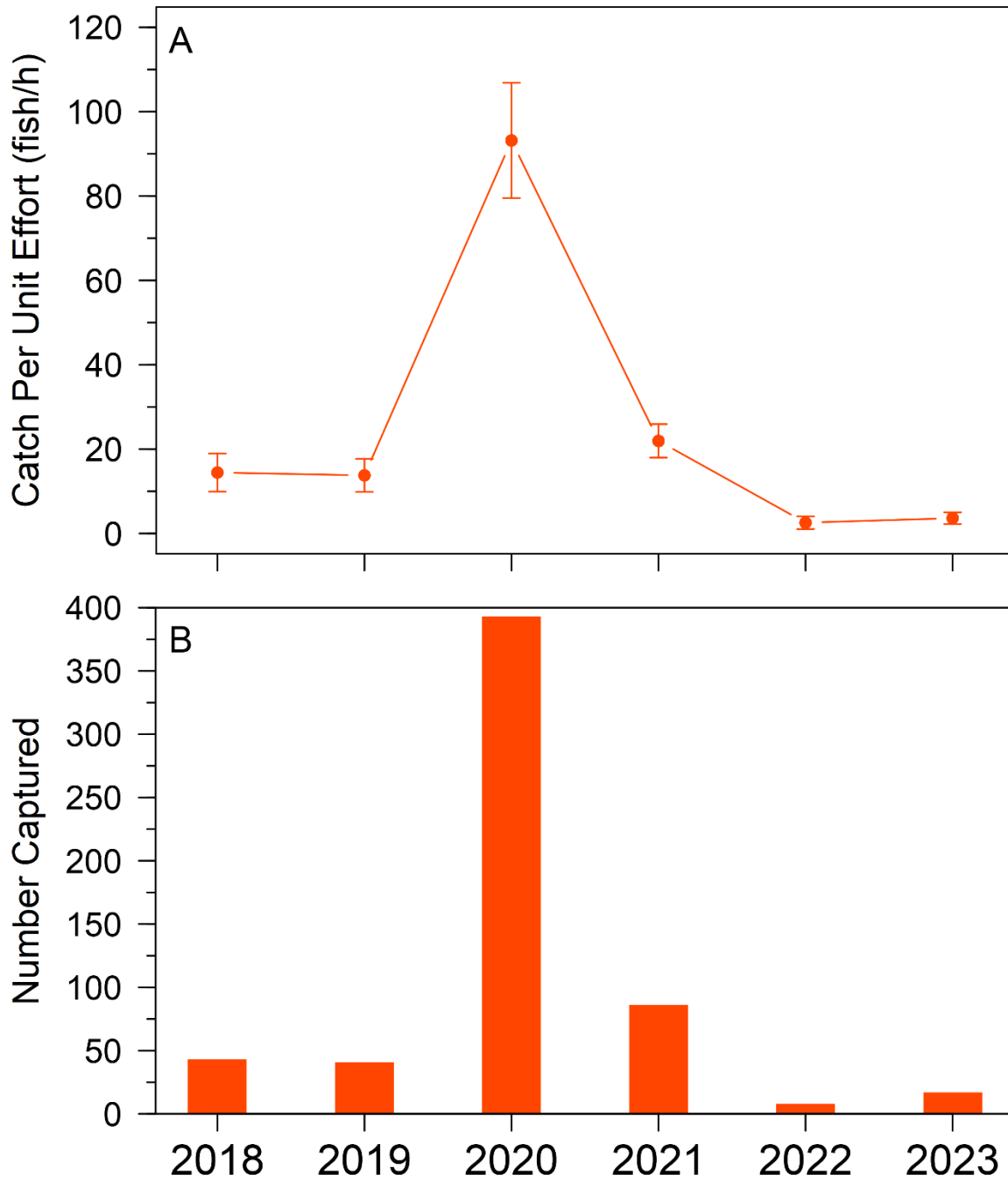


Figure 25.—Summary of Loach Minnow captured in the middle Blue River, annually from 2018 to 2023 with (A) mean annual backpack electrofishing catch per unit effort (fish/h; first pass only) with standard error bars, and (B) total number of fish captured (first pass).

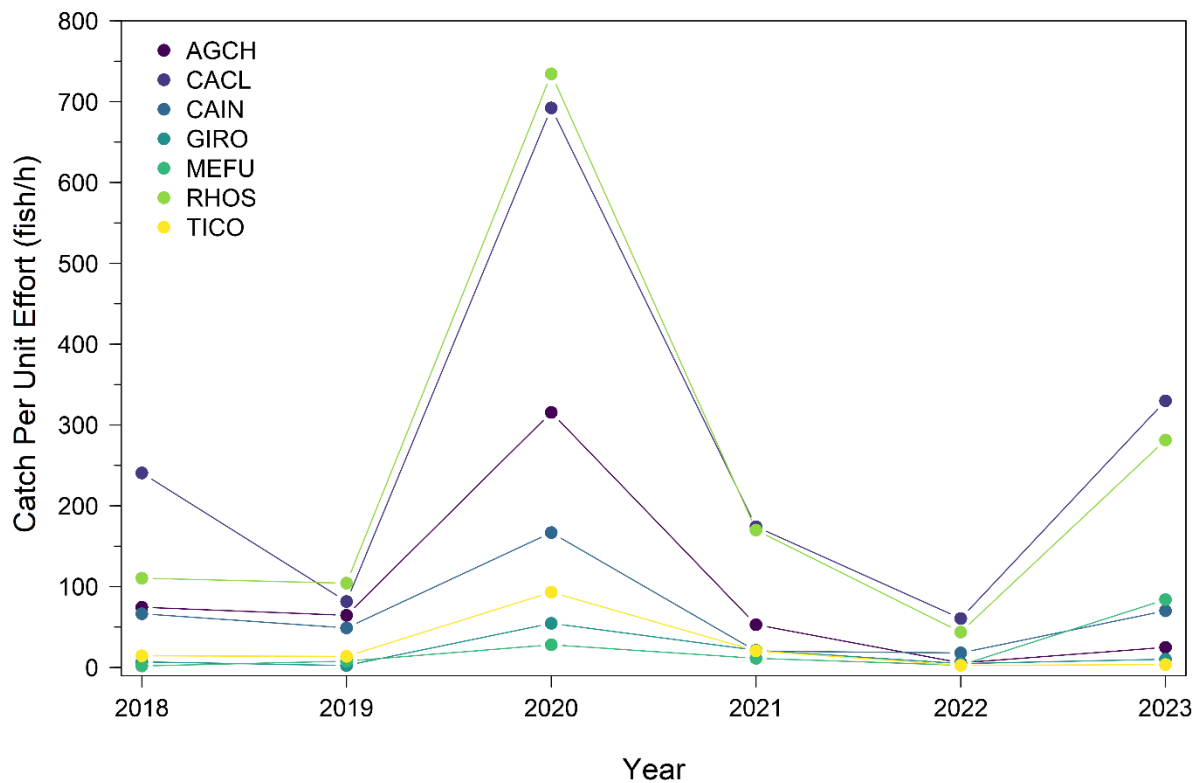


Figure 26.—Mean annual backpack electrofishing catch per unit effort (fish/h) of all native fish species (AGCH = Longfin Dace, CACL = Desert Sucker, CAIN = Sonora Sucker, GIRO = Roundtail Chub, MEFU = Spikedace, RHOS = Speckled Dace, TICO = Loach Minnow) captured in the middle Blue River, AZ from 2018 to 2023. Standard error bars are not shown to improve clarity of mean catch per unit effort trends.

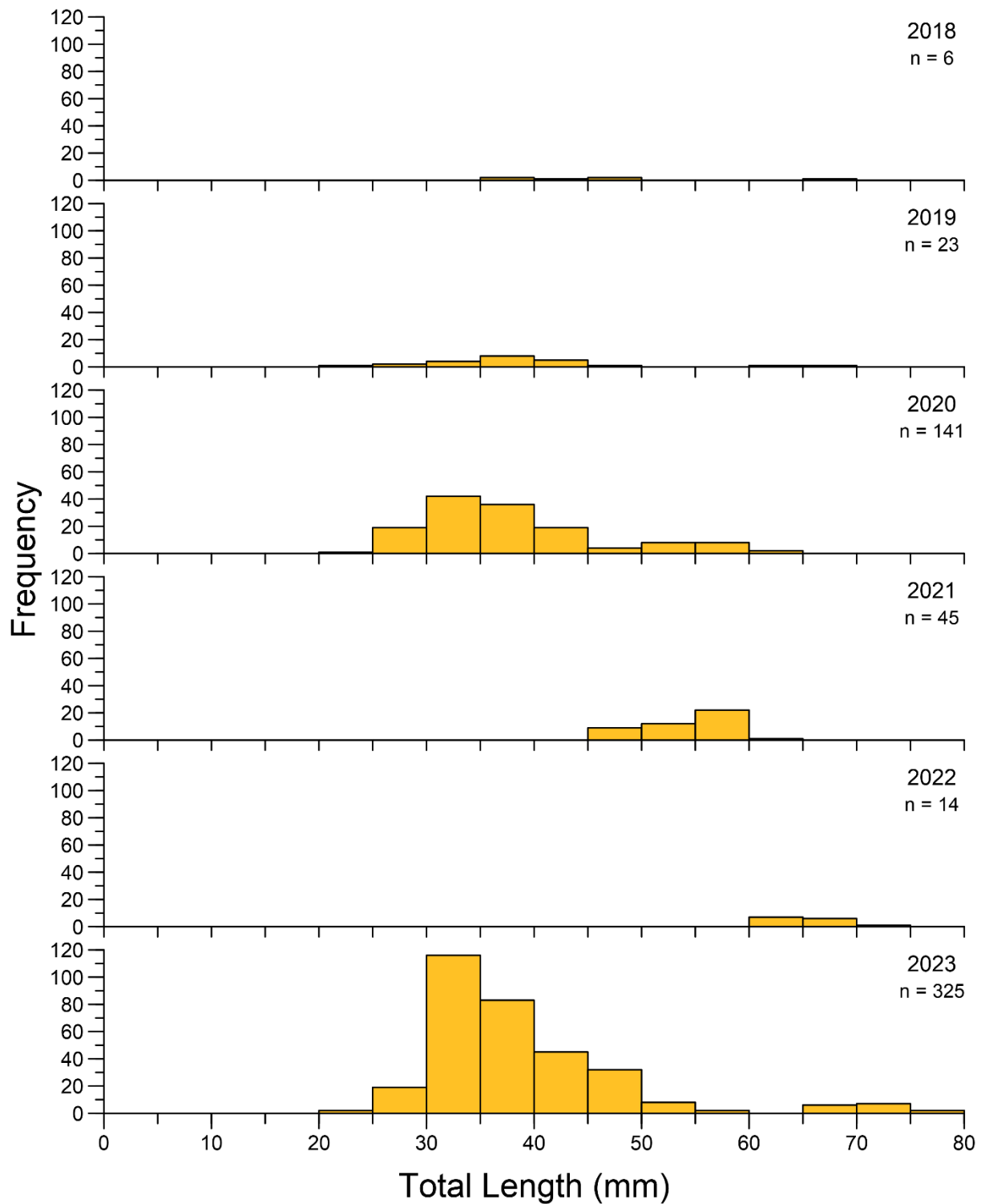


Figure 27.—Length frequency distribution of Spikedace captured during annual monitoring in the middle Blue River, from 2018 to 2023. Total number of fish captured and measured among all passes each year is shown in the top right corner of each panel. Note depletion sampling did not occur in 2018.

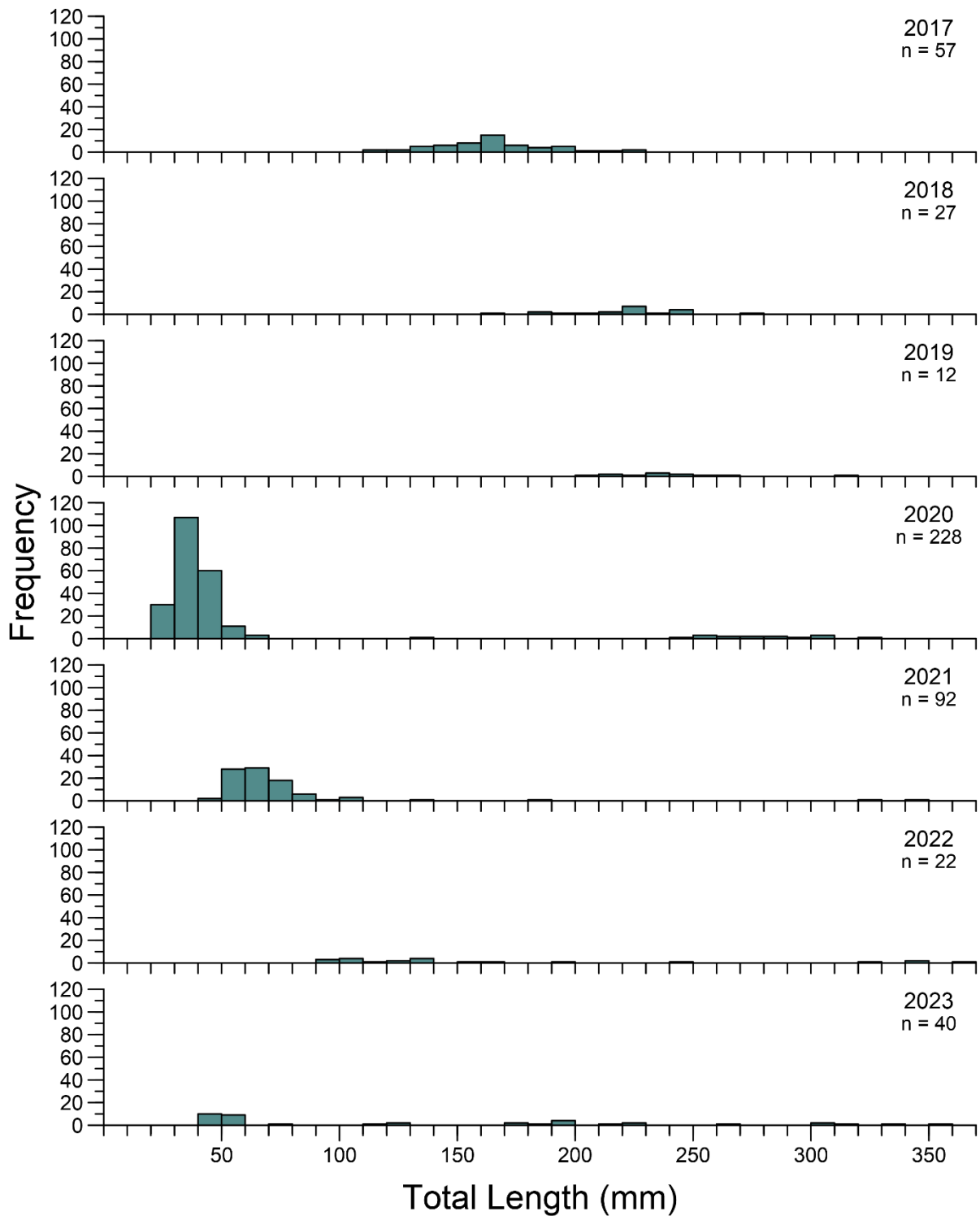


Figure 28.—Length frequency distribution of Roundtail Chub captured during annual monitoring in the middle Blue River, from 2017 to 2023. Total number of fish captured and measured among all passes each year is shown in the top right corner of each panel. Note depletion sampling did not occur in 2017 and 2018.

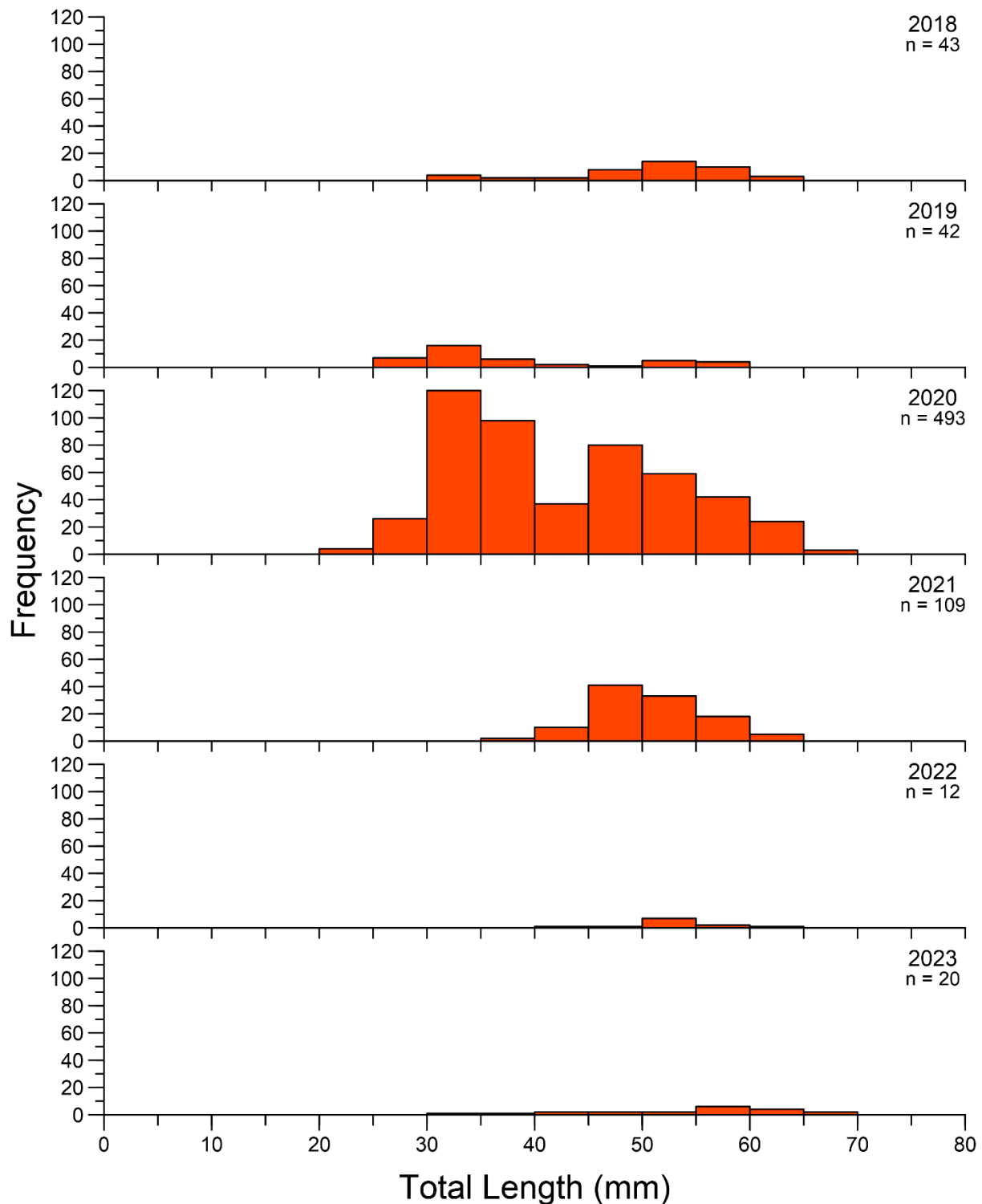


Figure 29.—Length frequency distribution of Loach Minnow captured during annual monitoring in the middle Blue River, from 2018 to 2023. Total number of fish captured and measured among all passes each year is shown in the top right corner of each panel. Note depletion sampling did not occur in 2018.

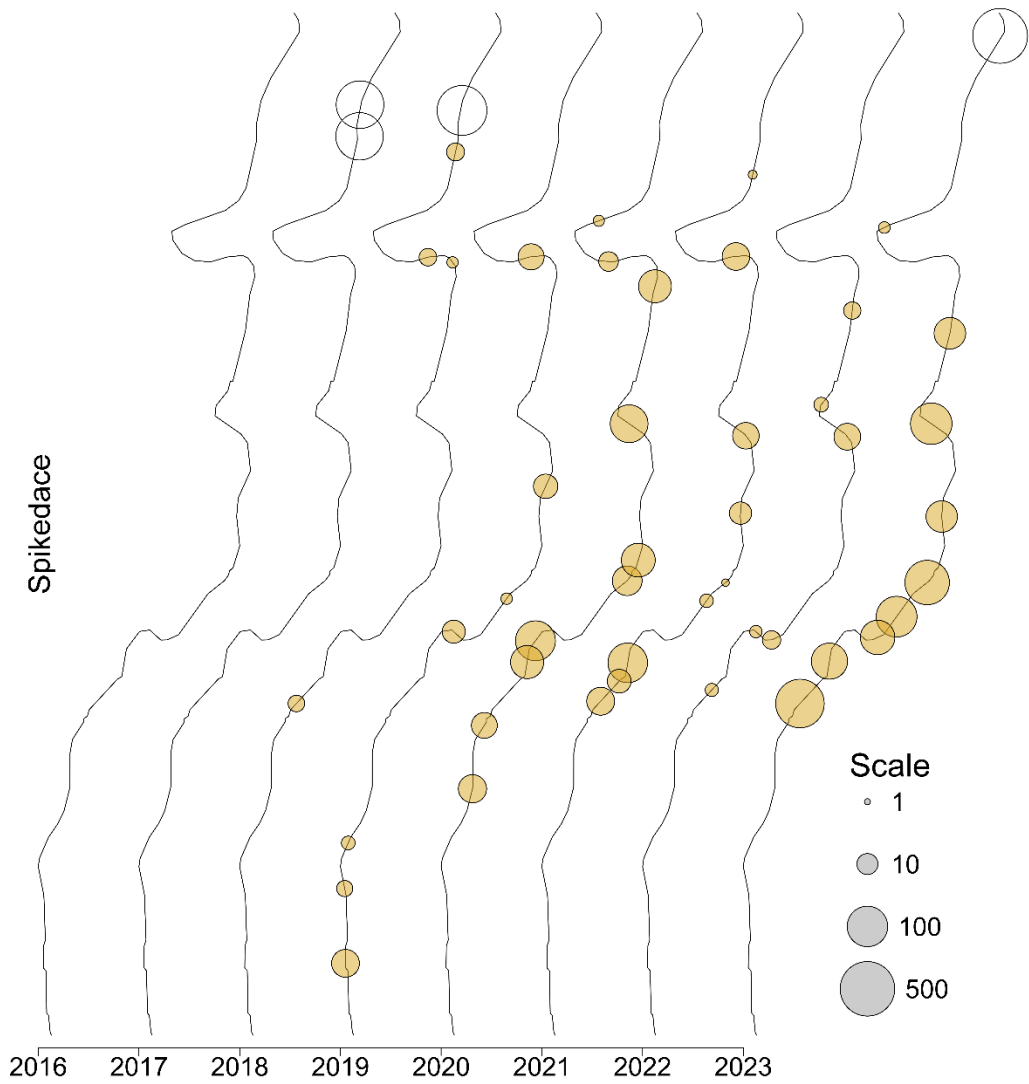


Figure 30.—Spikedace stocking locations (open circles) and mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the middle Blue River from 2016-2023. Size of points indicates either the number of fish stocked or the relative abundance during monitoring at a particular location.

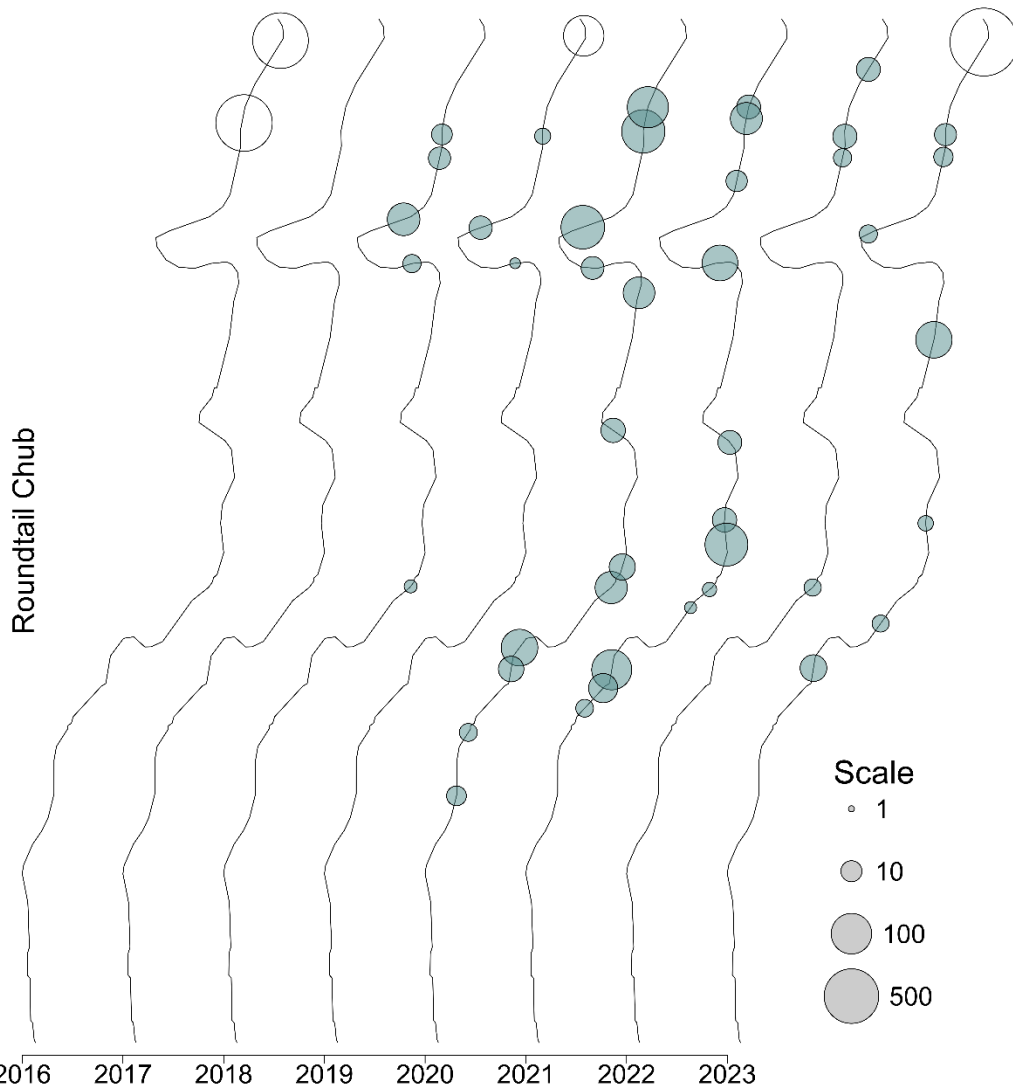


Figure 31.—Roundtail Chub stocking locations (open circles) and mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the middle Blue River from 2016-2023. Size of points indicates either the number of fish stocked or the relative abundance during monitoring at a particular location.

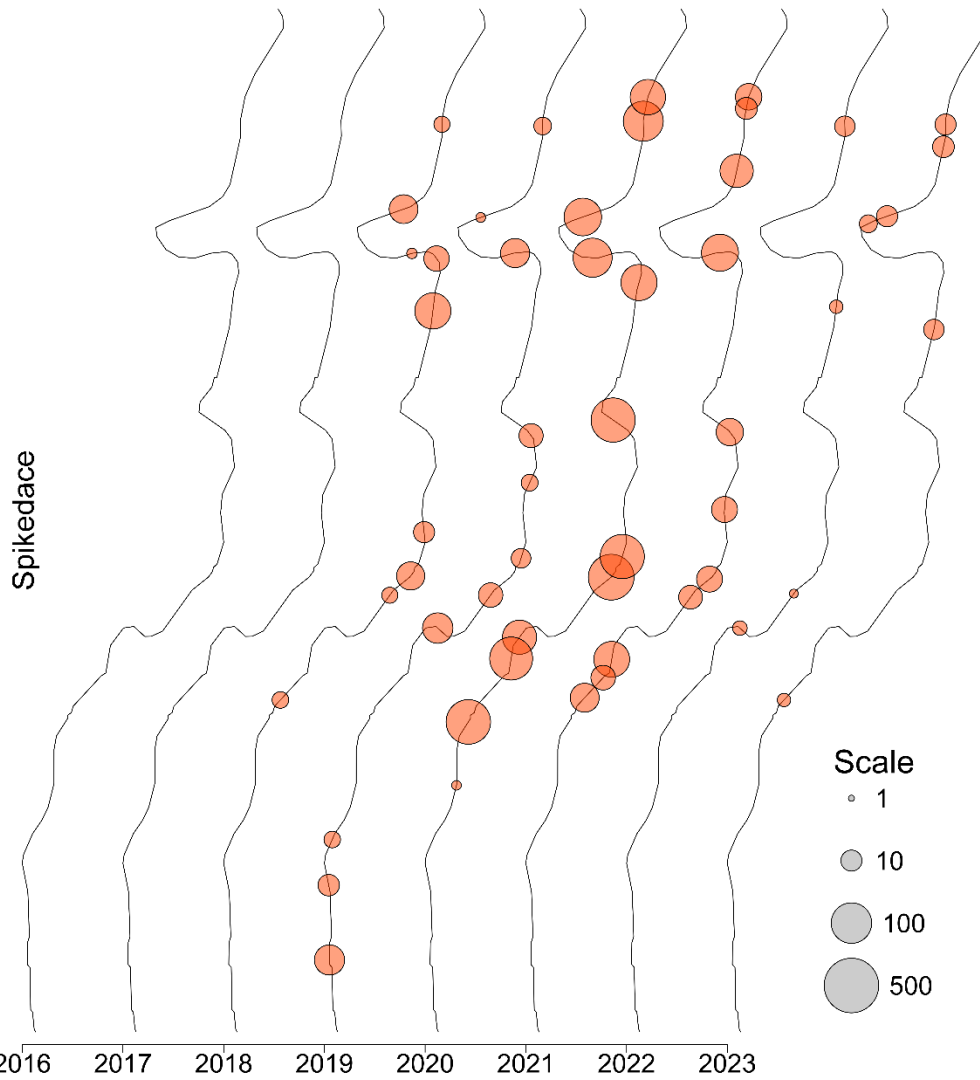


Figure 32.—Loach Minnow mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the middle Blue River from 2016-2023. Size of points indicates the relative abundance during monitoring at a particular location.

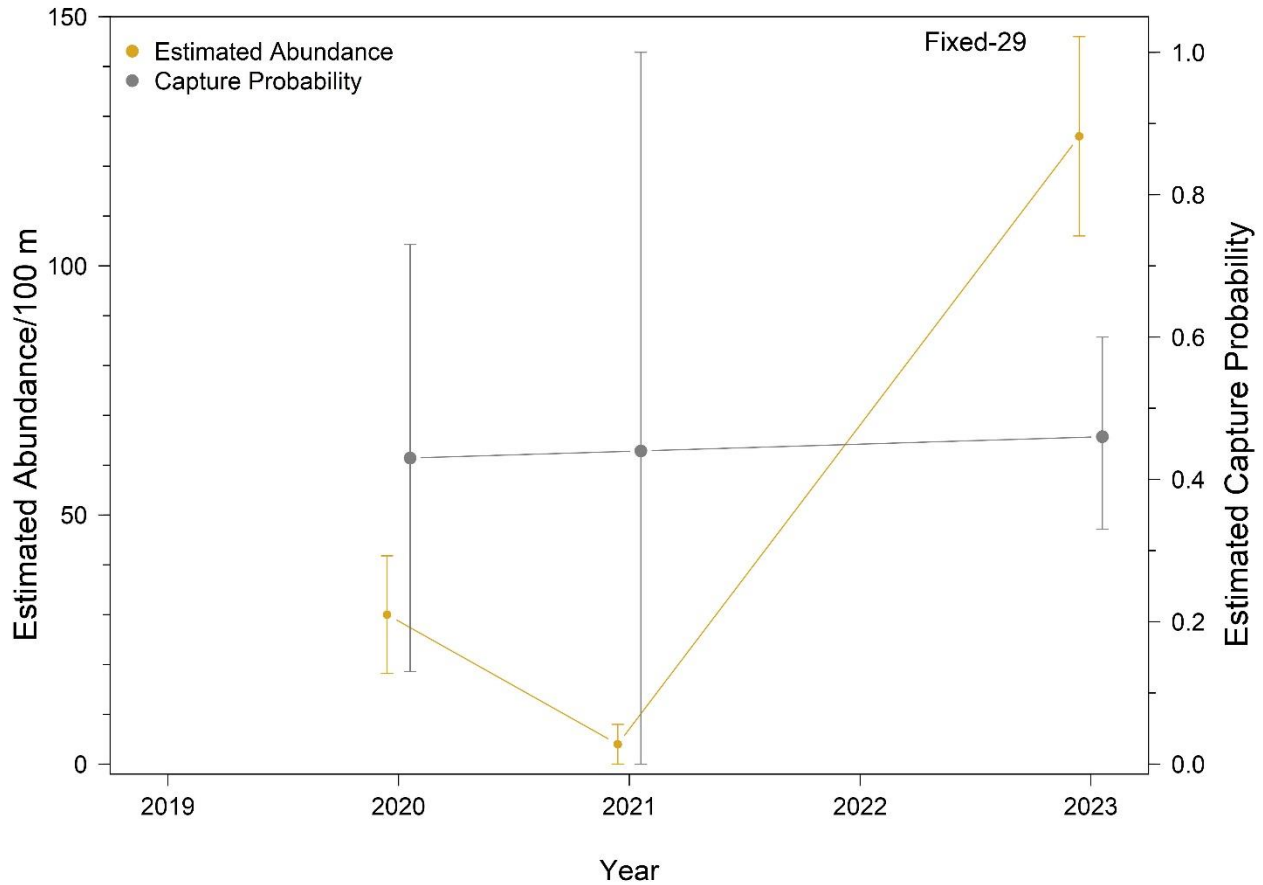


Figure 33.—Three-pass depletion estimates of Spikedace abundance per 100 m (yellow points and lines) and capture probability (grey points and lines) at the downstream (Fixed-29) sub-reach in the middle Blue River during annual monitoring from 2019-2023. Bars represent the lower and upper bounds of the 95% confidence interval of each estimate.

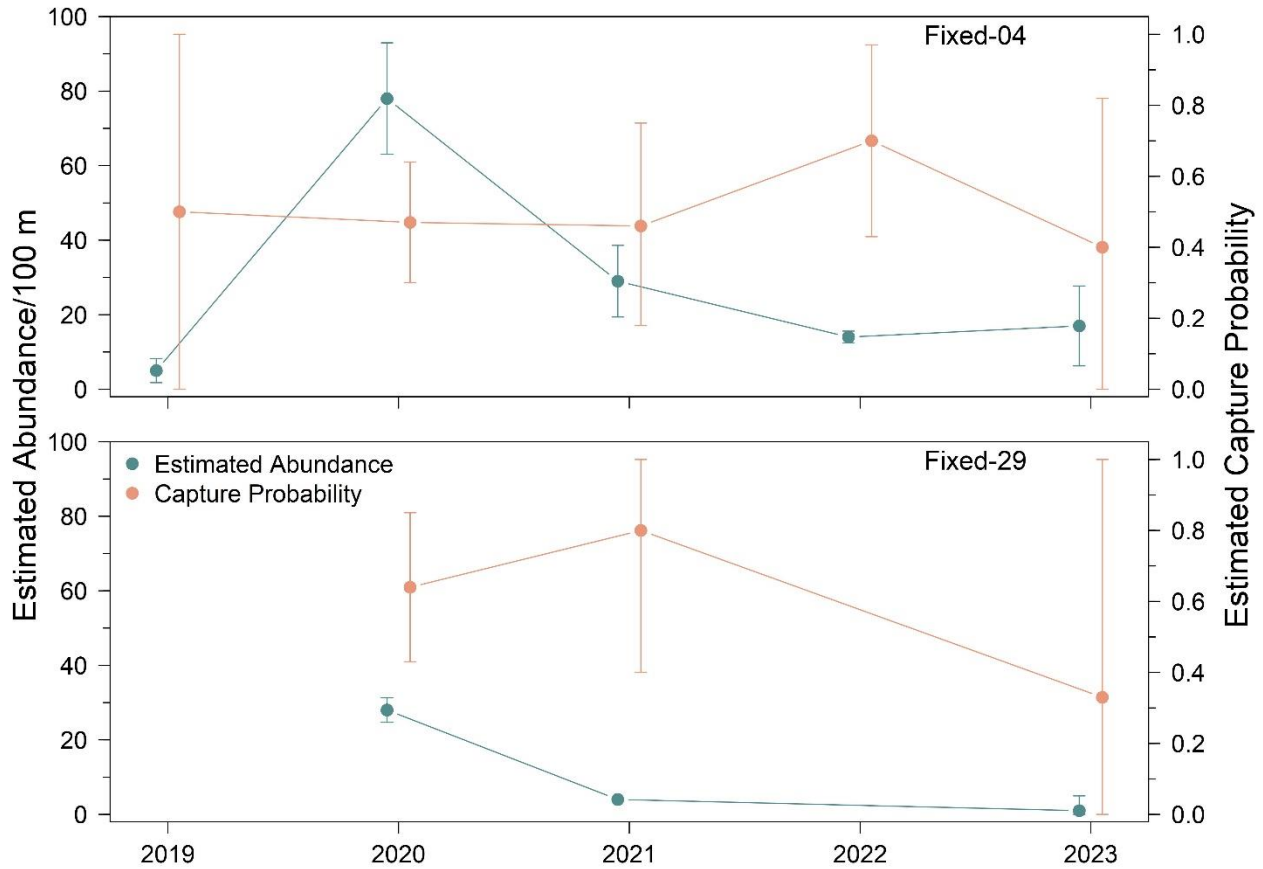


Figure 34.—Three-pass depletion estimates of Roundtail Chub abundance per 100 m (blue points and lines) and capture probability (orange points and lines) at the upstream (Fixed-04) and downstream (Fixed-29) sub-reaches in the middle Blue River during annual monitoring from 2019-2023. Bars represent the lower and upper bounds of the 95% confidence interval of each estimate.

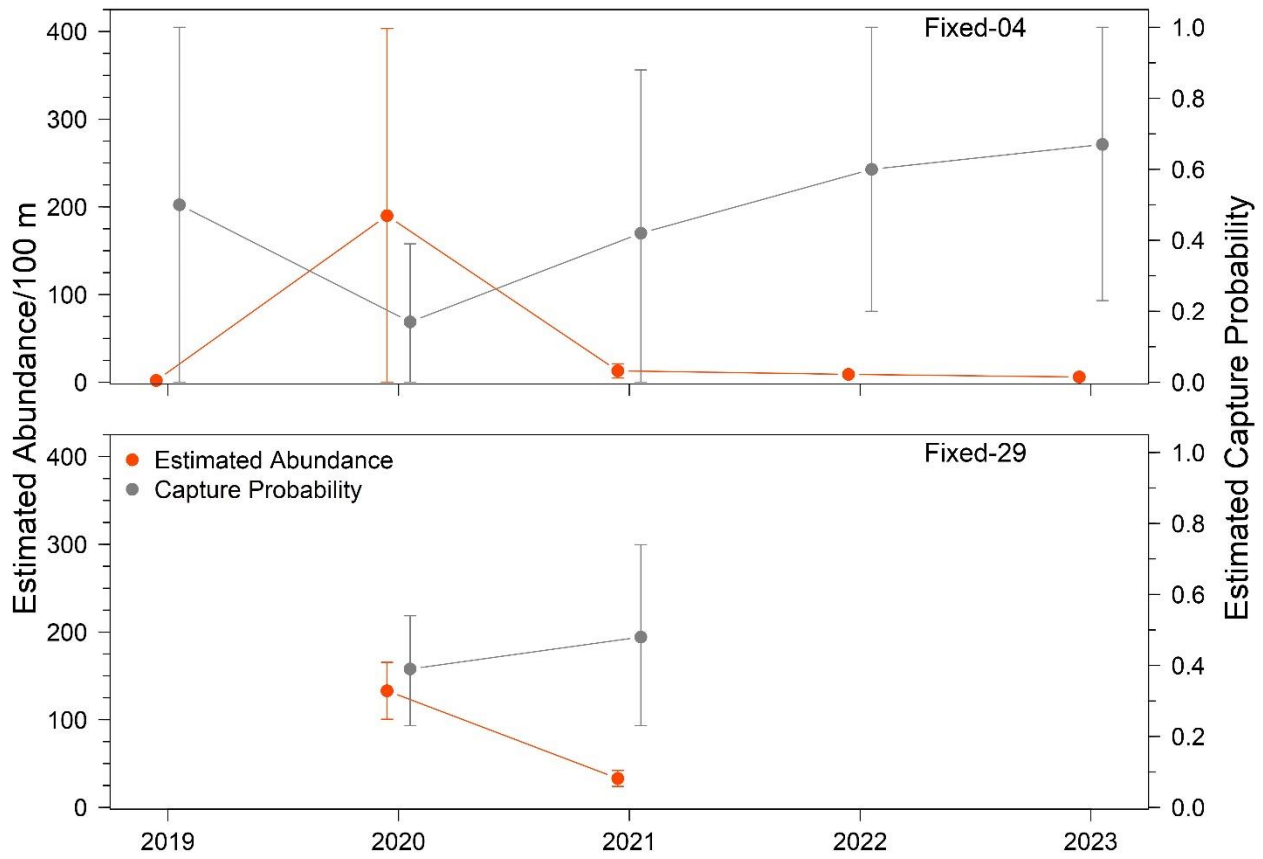


Figure 35.—Three-pass depletion estimates of Loach Minnow abundance per 100 m (orange points and lines) and capture probability (grey points and lines) at the upstream (Fixed-04) and downstream (Fixed-29) sub-reaches in the middle Blue River during annual monitoring from 2019-2023. Bars represent the lower and upper bounds of the 95% confidence interval of each estimate.

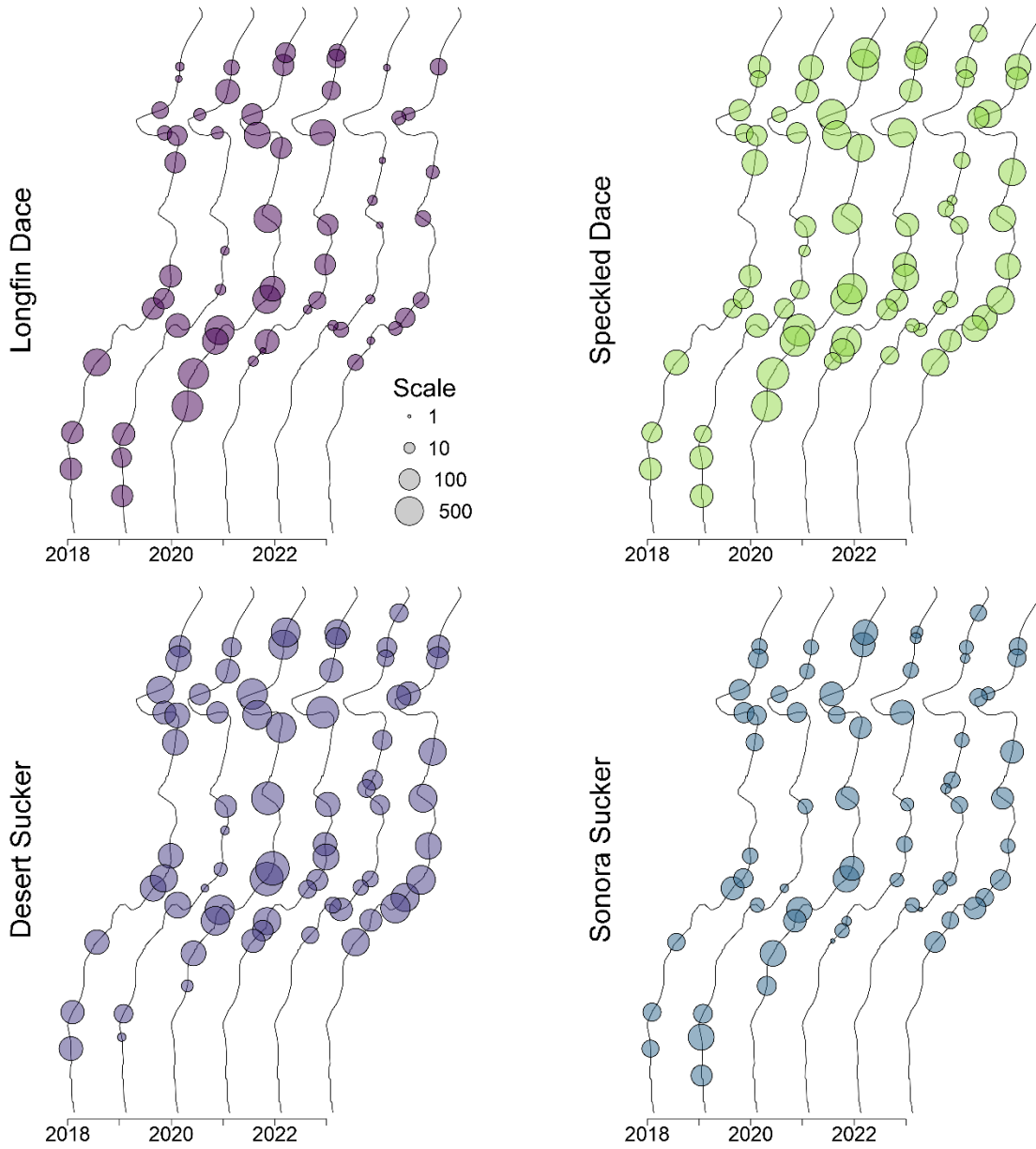


Figure 36.—Mean backpack electrofishing relative abundance (CPUE, fish/h) of Longfin Dace (top left), Speckled Dace (top right), Desert Sucker (bottom left), and Sonora Sucker (bottom right) at each monitoring sub-reach in the middle Blue River from 2018-2023. Size of points indicates the relative abundance during monitoring at a particular location.

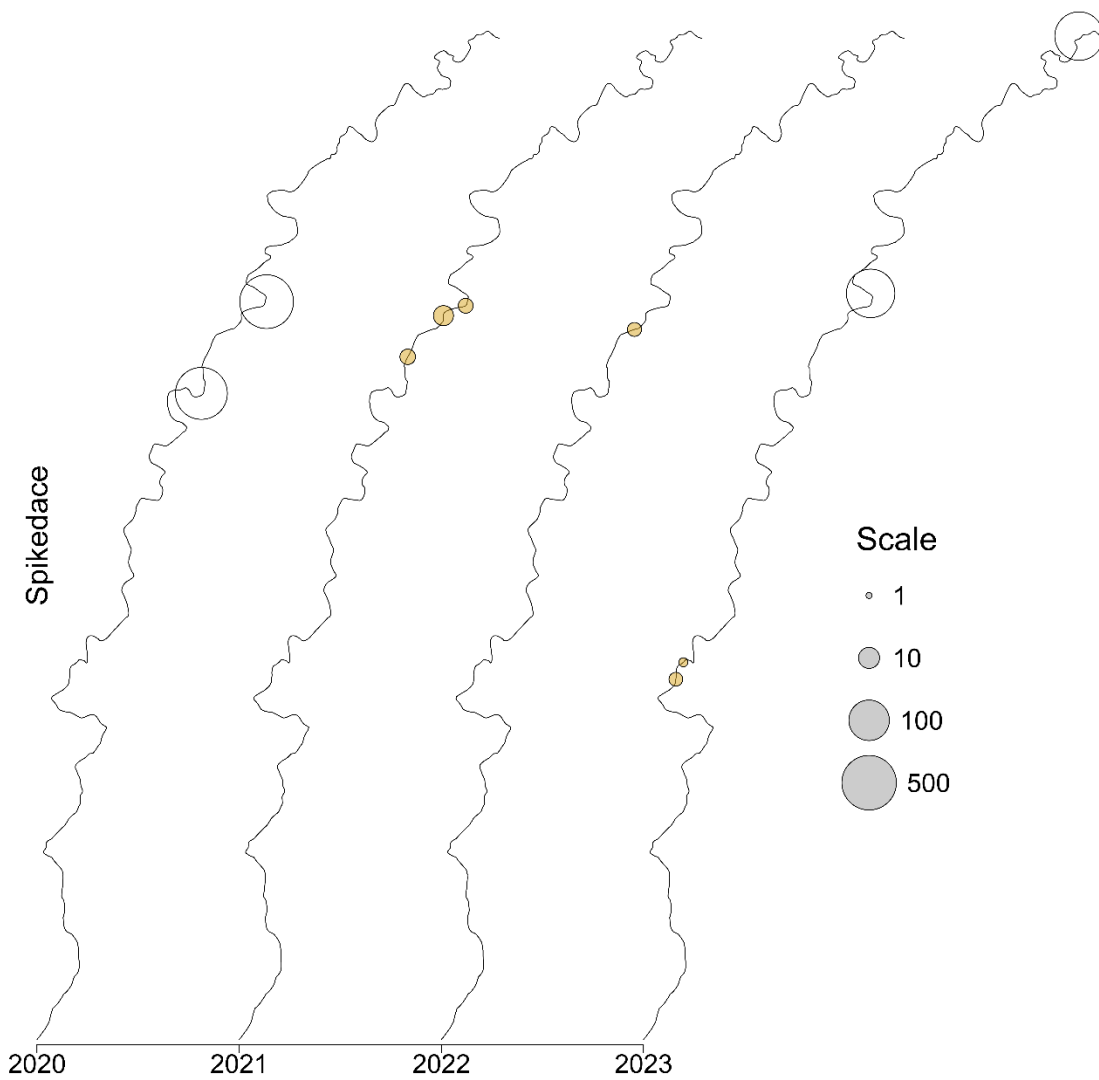


Figure 37.—Spikedace stocking locations (open circles) and mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the upper Blue River from 2020-2023. Size of points indicates either the number of fish stocked or the relative abundance during monitoring at a particular location.

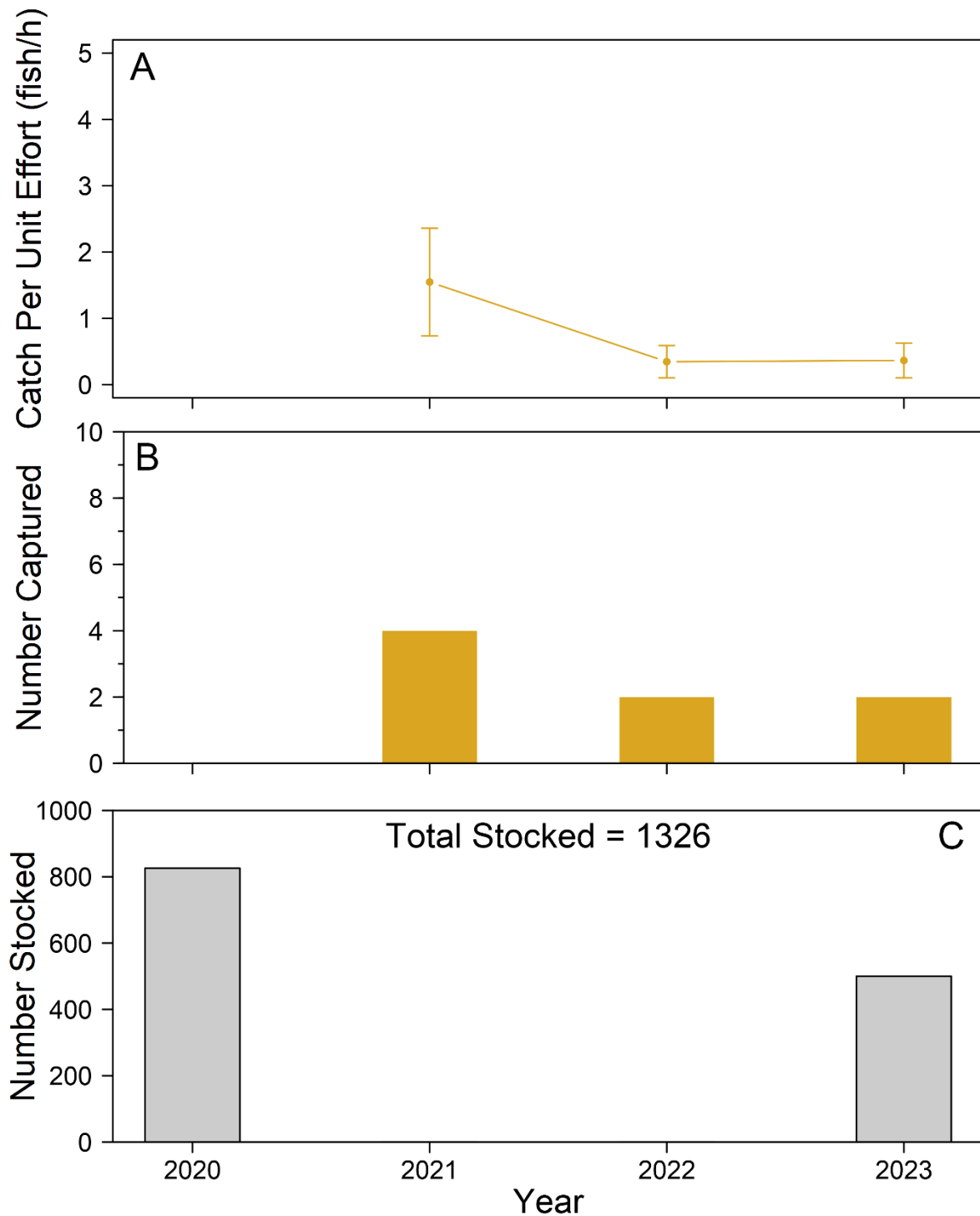


Figure 38.—Summary of Spikedace captured and stocked in the upper Blue River, annually from 2020 to 2023 with (A) mean annual backpack electrofishing catch per unit effort (fish/h; first pass only) with standard error bars, (B) total number of fish captured (first pass), and (C) total number of fish stocked.

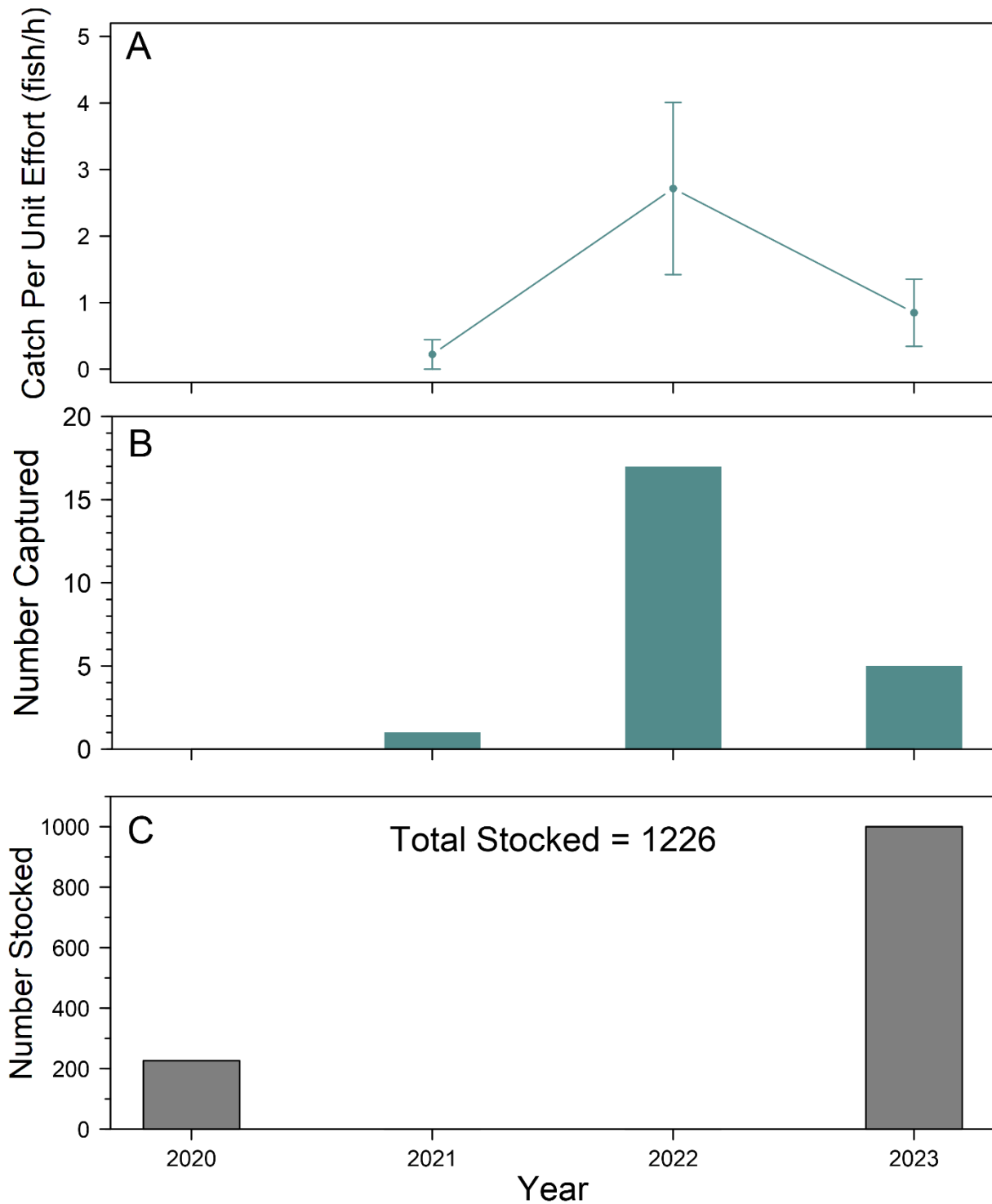


Figure 39.—Summary of Roundtail Chub captured and stocked in the upper Blue River, annually from 2020 to 2023 with (A) mean annual backpack electrofishing catch per unit effort (fish/h; first pass only) with standard error bars, (B) total number of fish captured (first pass), and (C) total number of fish stocked.

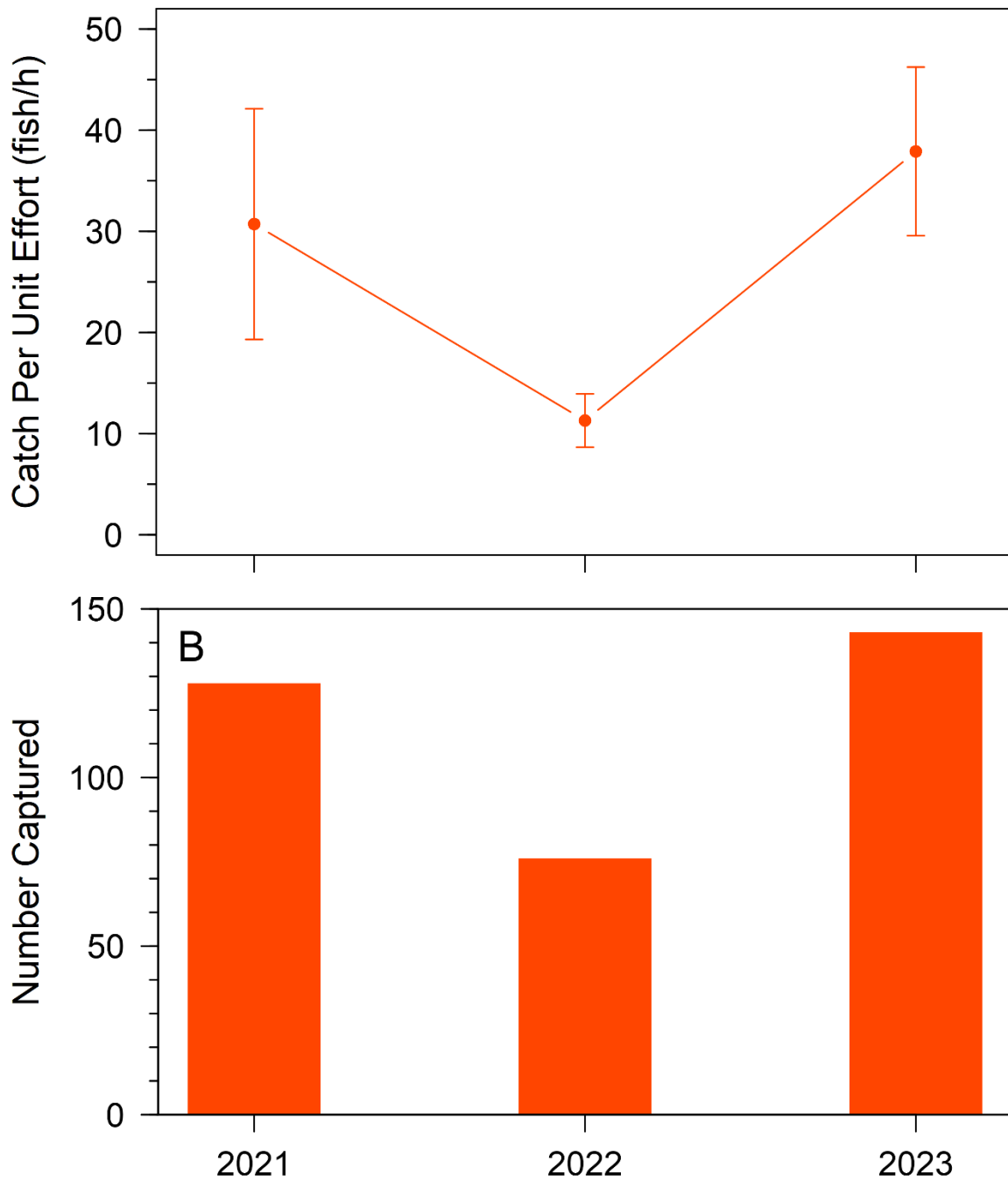


Figure 40.—Summary of Loach Minnow captured in the upper Blue River, annually from 2021 to 2023 with (A) mean annual backpack electrofishing catch per unit effort (fish/h; first pass only) with standard error bars, and (B) total number of fish captured (first pass only).

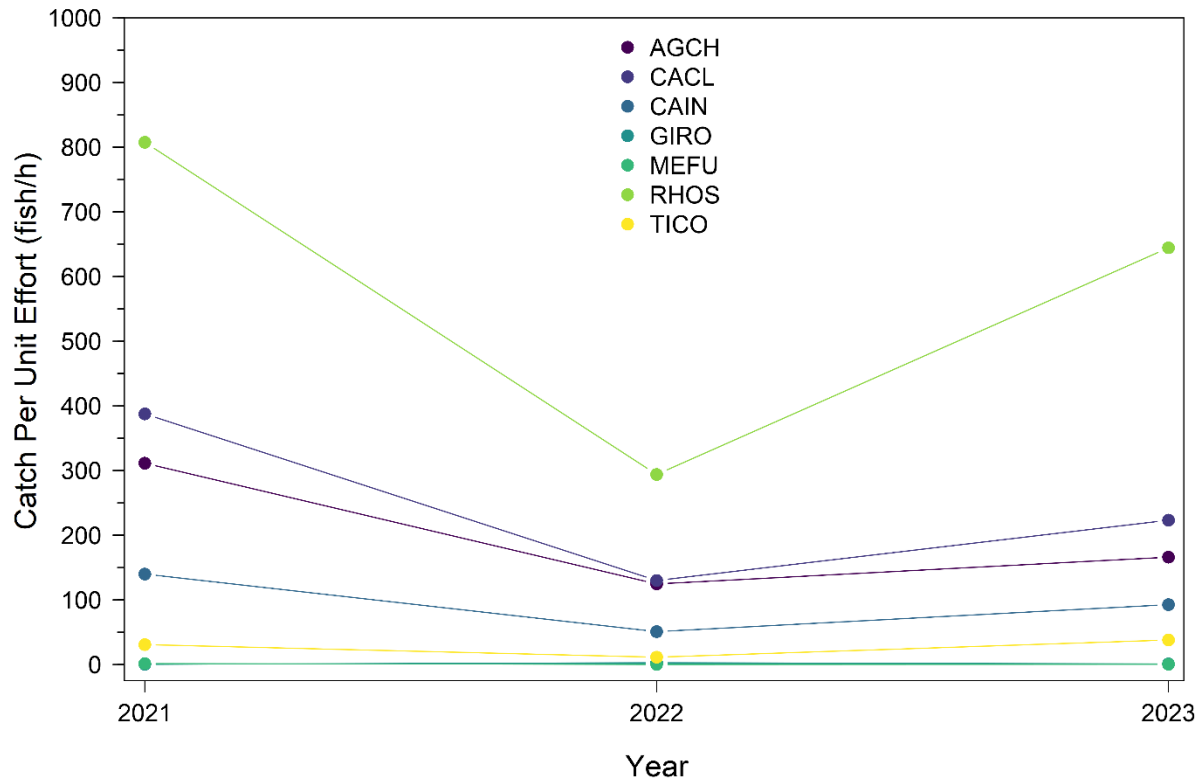


Figure 41.—Mean annual backpack electrofishing catch per unit effort (fish/h) of all native fish species (AGCH = Longfin Dace, CACL = Desert Sucker, CAIN = Sonora Sucker, GIRO = Roundtail Chub, MEFU = Spikedace, RHOS = Speckled Dace, TICO = Loach Minnow) captured in the upper Blue River, AZ from 2021 to 2023. Standard error bars are not shown to improve clarity of mean catch per unit effort trends.

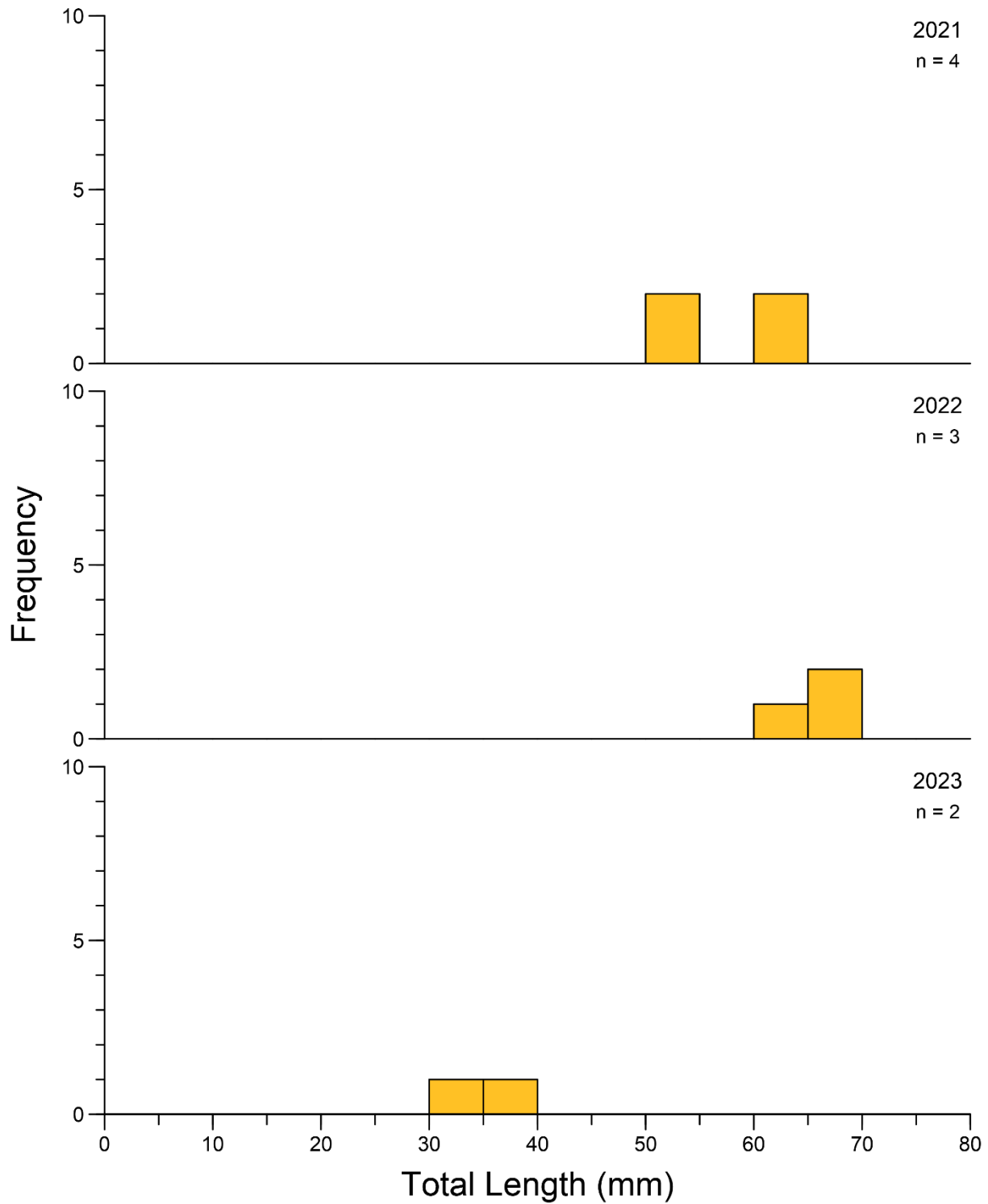


Figure 42.— Length frequency distribution of Spikedace captured during annual monitoring in the upper Blue River, from 2021 to 2023. Number of fish captured and measured each year is shown in the top right corner of each panel.

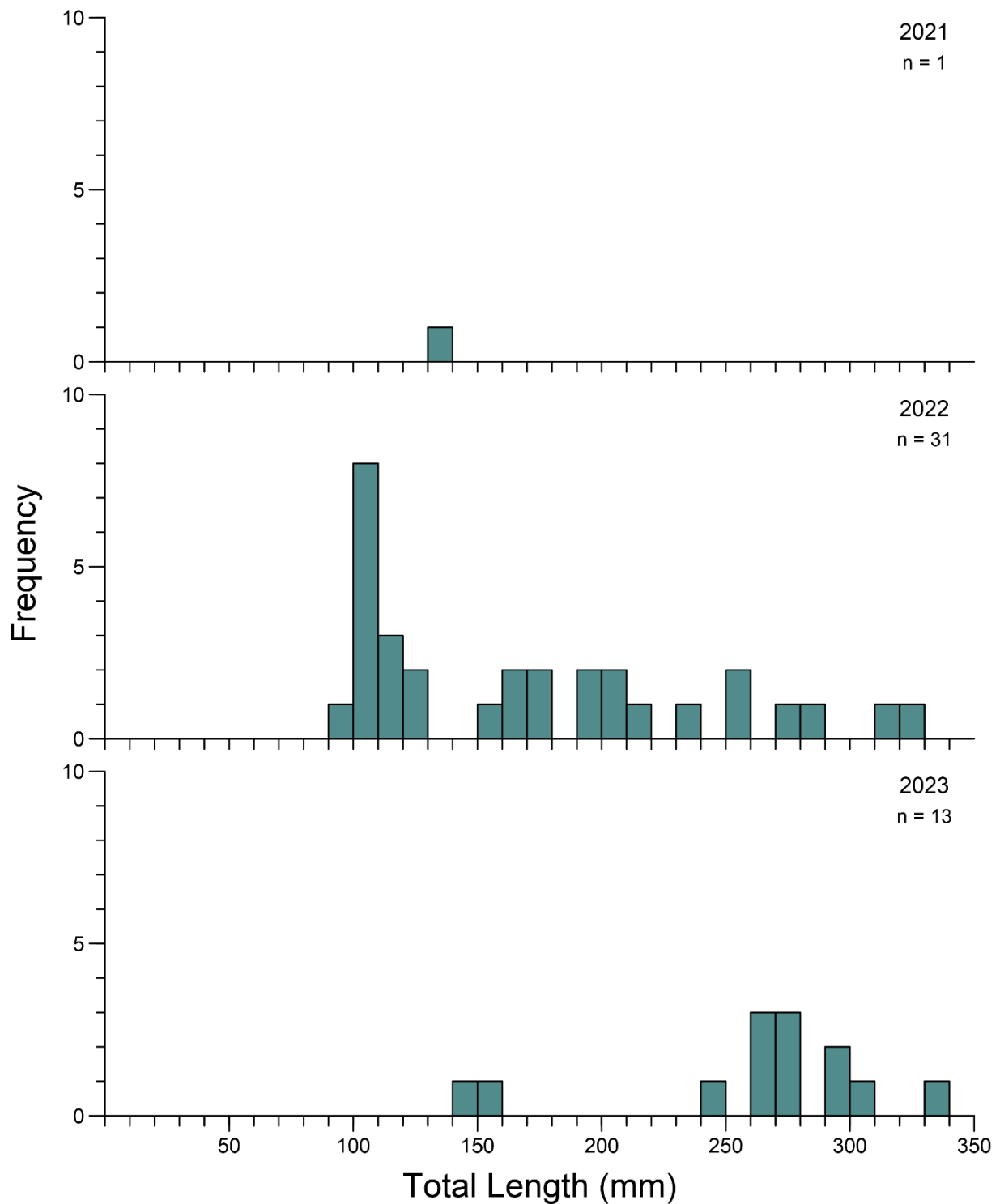


Figure 43.—Length frequency distribution of Roundtail Chub captured during annual monitoring in the upper Blue River, from 2021 to 2023. Number of fish captured and measured each year is shown in the top right corner of each panel.

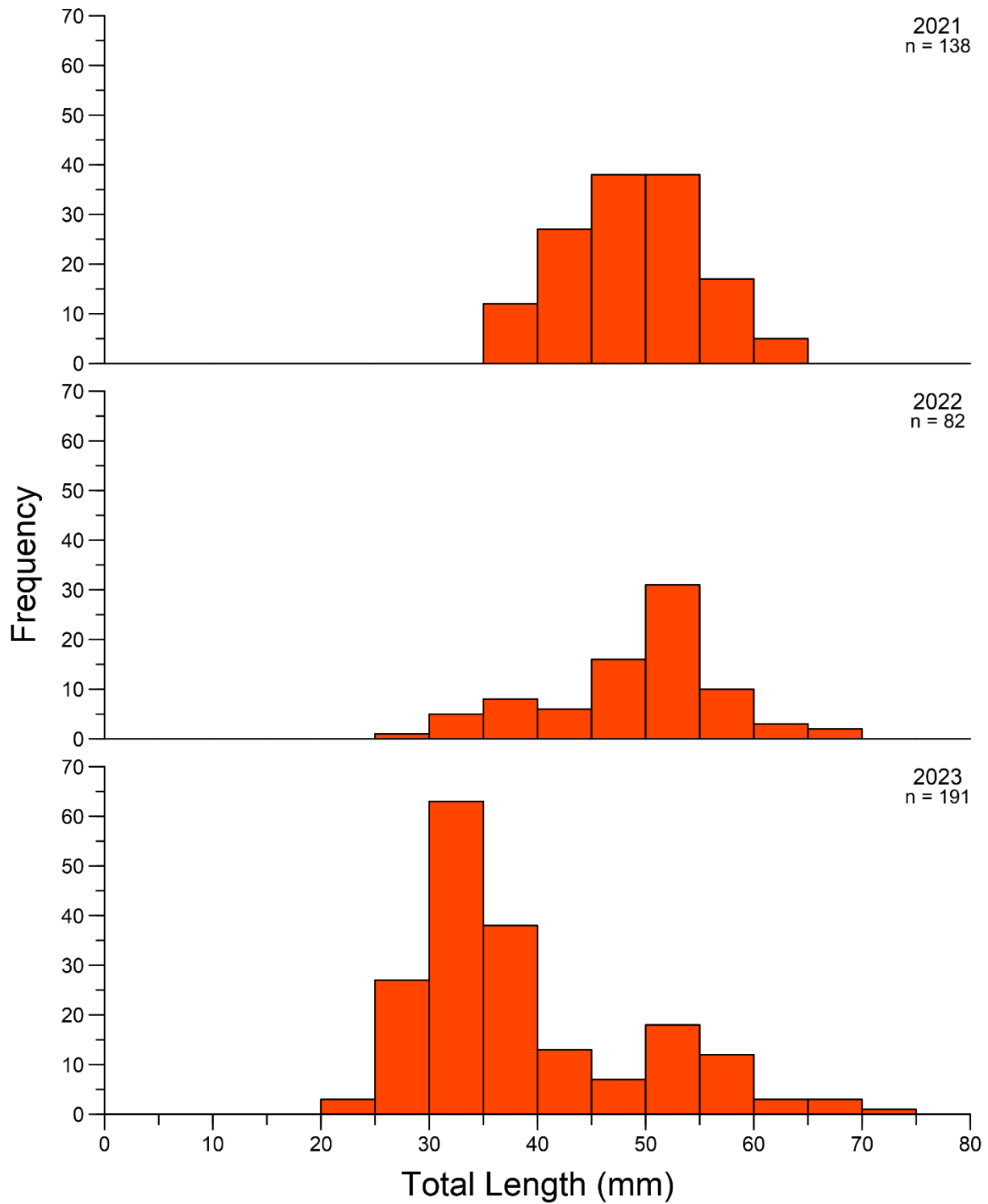


Figure 44.—Length frequency distribution of Loach Minnow captured during annual monitoring in the upper Blue River, from 2021 to 2023. Number of fish captured and measured each year is shown in the top right corner of each panel.

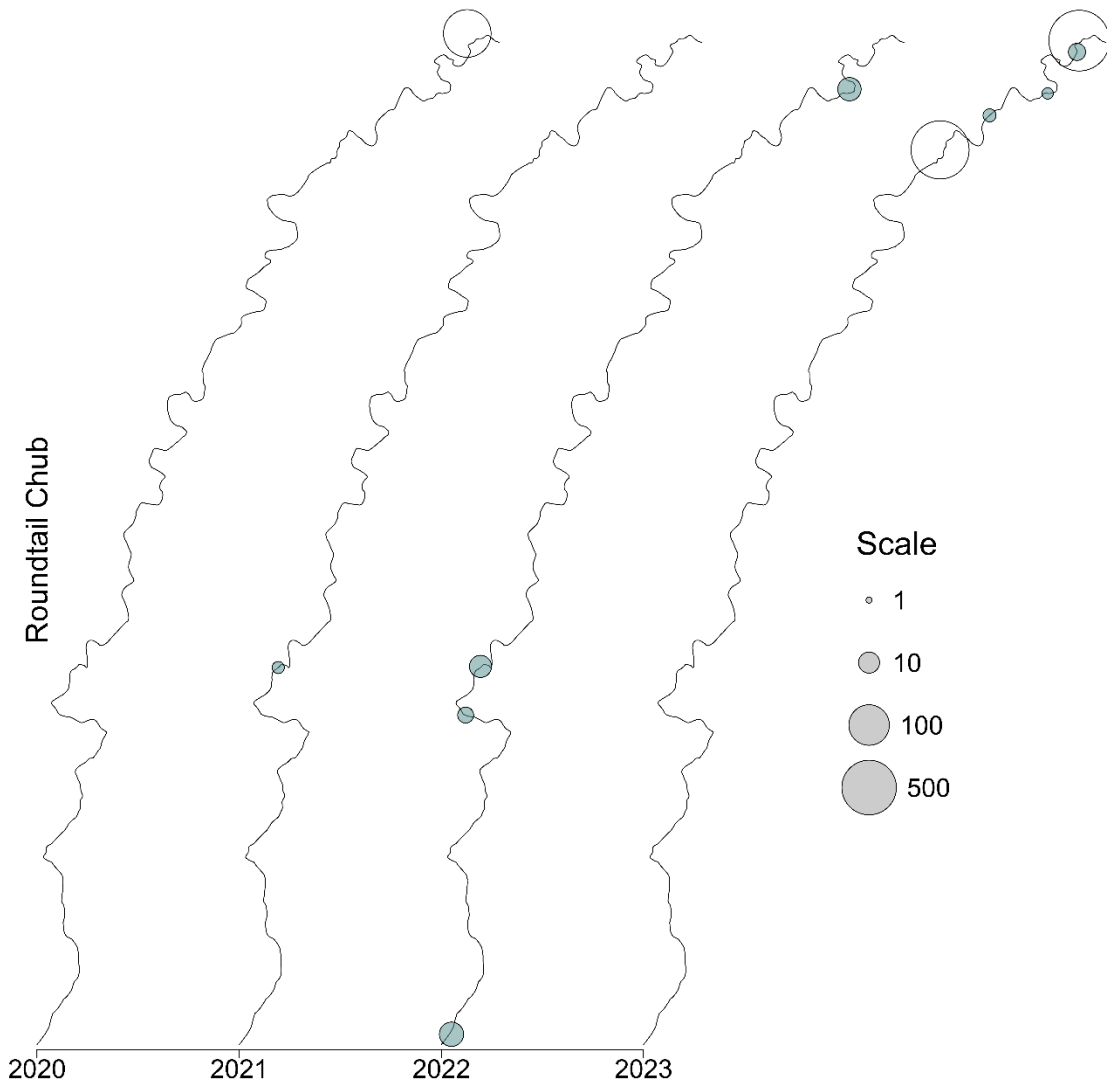


Figure 45.—Roundtail Chub stocking locations (open circles) and mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the upper Blue River from 2020 to 2023. Size of points indicates either the number of fish stocked or the relative abundance during monitoring at a particular location.

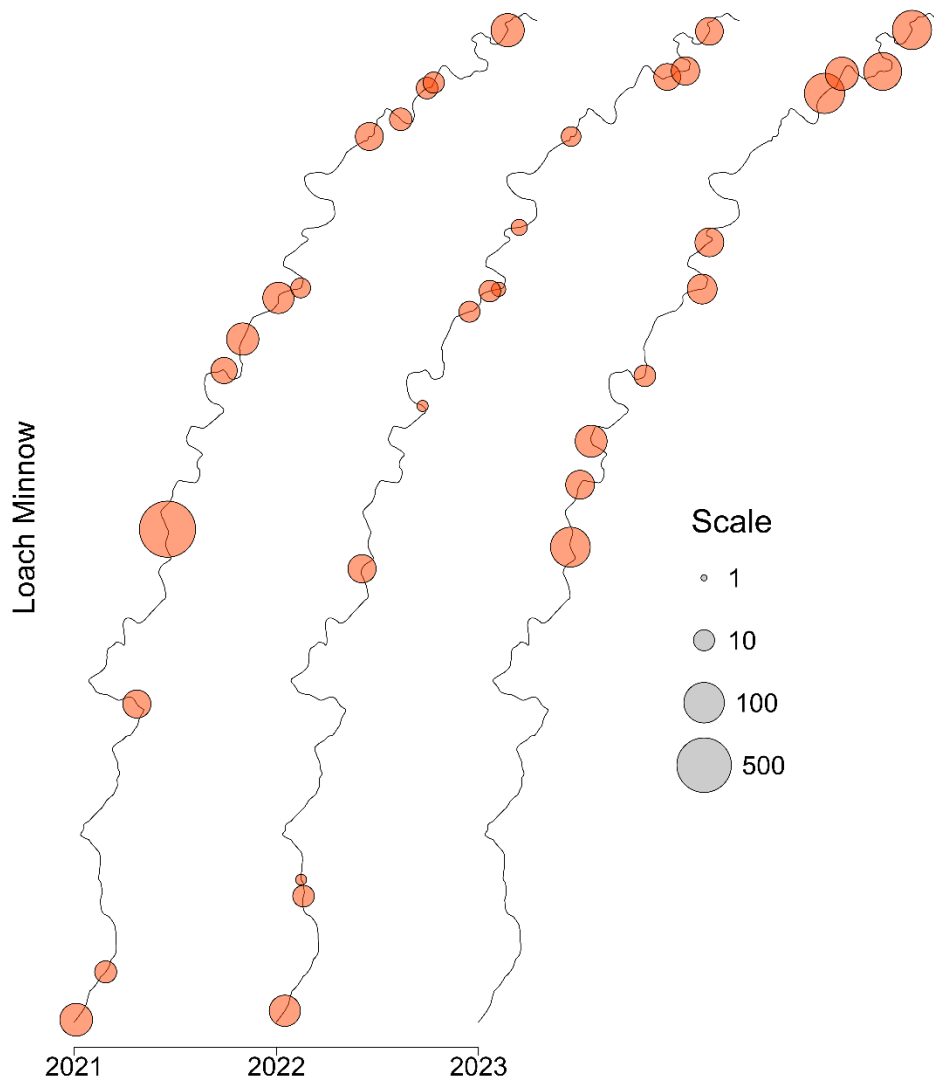


Figure 46.—Loach Minnow mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the upper Blue River from 2021 to 2023. Size of points indicates the relative abundance during monitoring at a particular location.

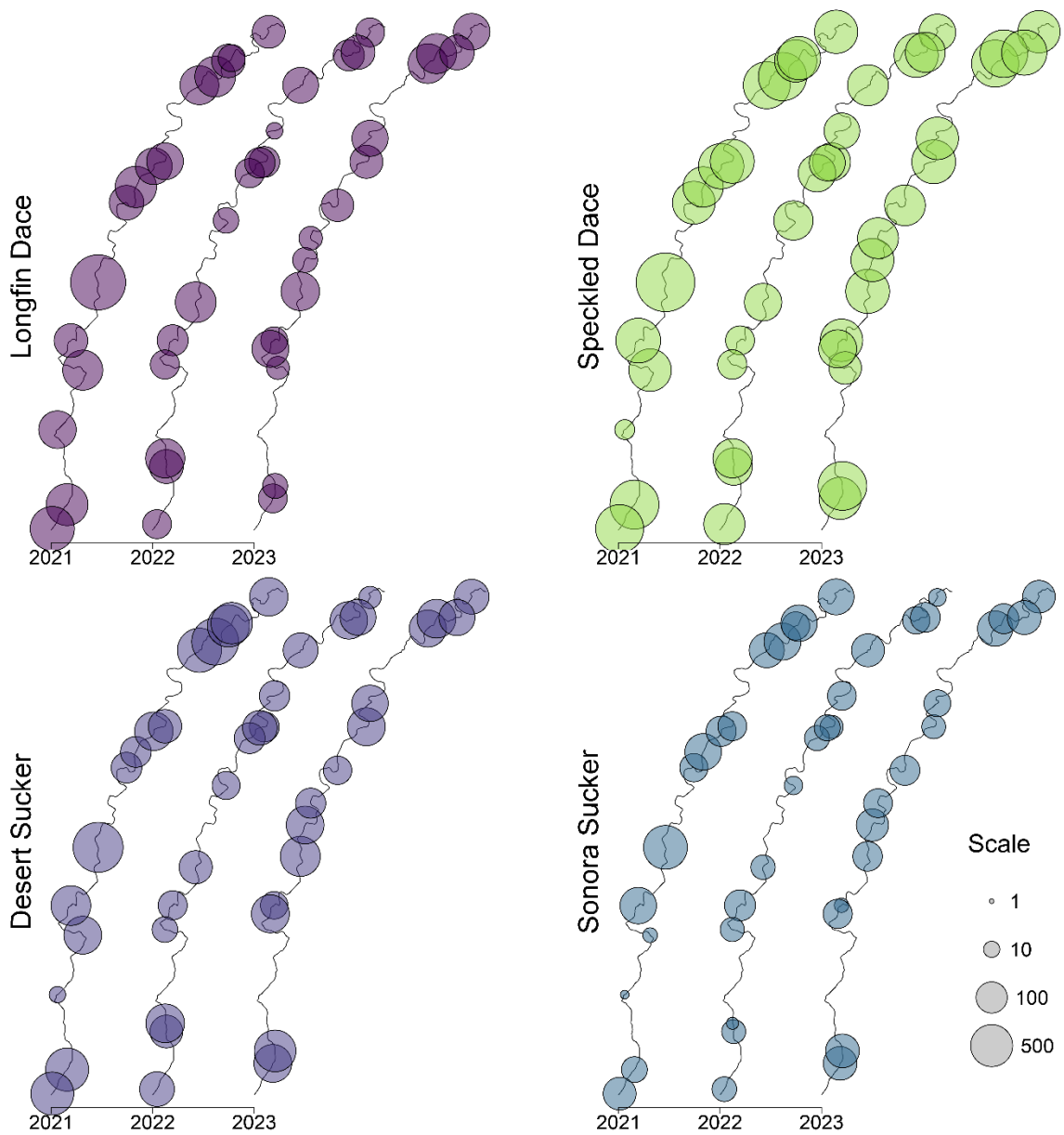


Figure 47.—Mean backpack electrofishing relative abundance (CPUE, fish/h) of Longfin Dace (top left), Speckled Dace (top right), Desert Sucker (bottom left), and Sonora Sucker (bottom right) at each monitoring site in the upper Blue River from 2021 to 2023. Size of points indicates the relative abundance during monitoring at a particular location.

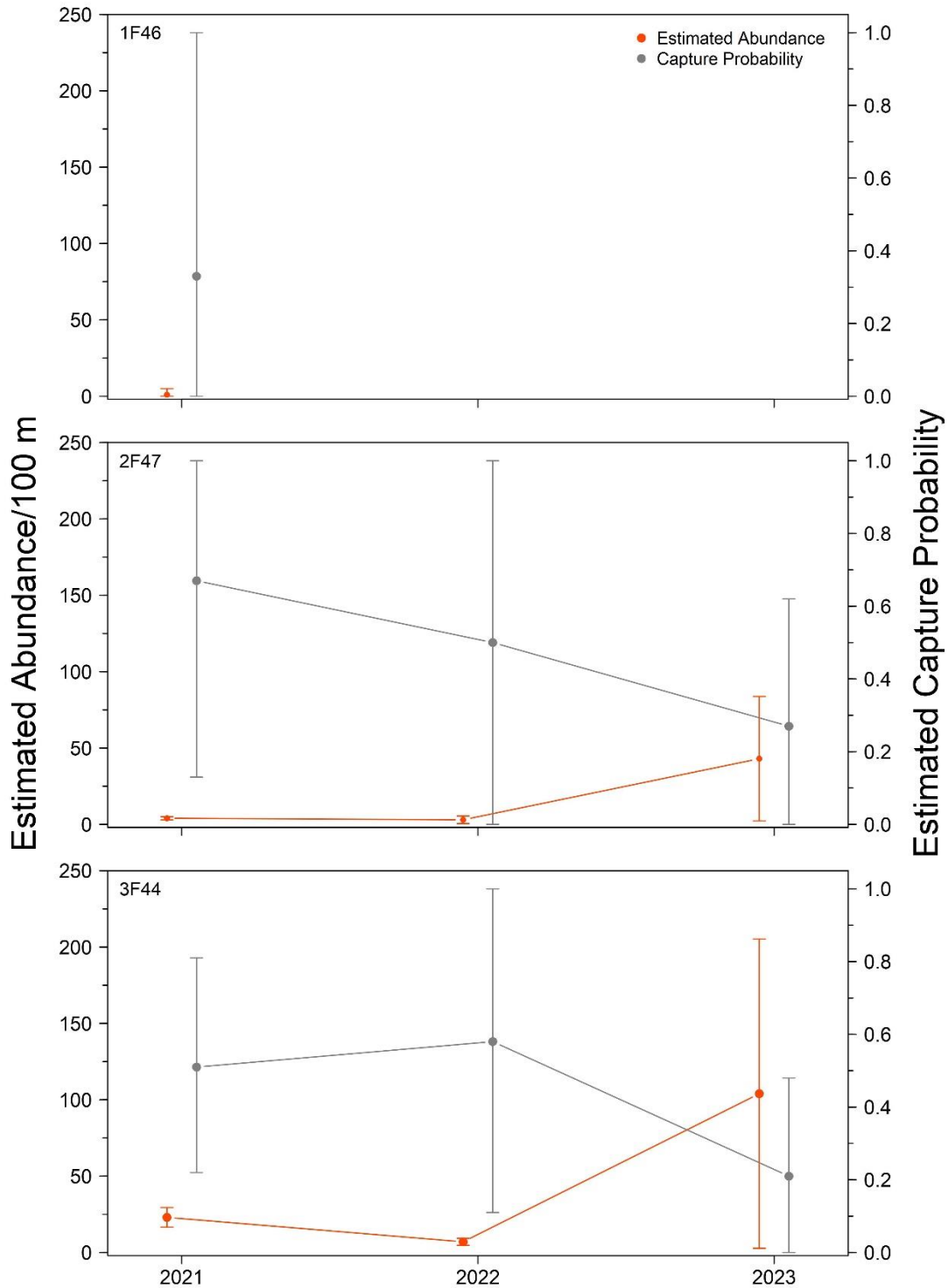


Figure 48.—Three-pass depletion estimates of Loach Minnow abundance per 100 m (orange points and lines and capture probability (grey points and lines) at the three fixed sub-reaches from downstream to upstream (1F46, 2F47, 3F44) in the upper Blue River during annual monitoring from 2021-2023. Bars represent the lower and upper bounds of the 95% confidence interval of each estimate.

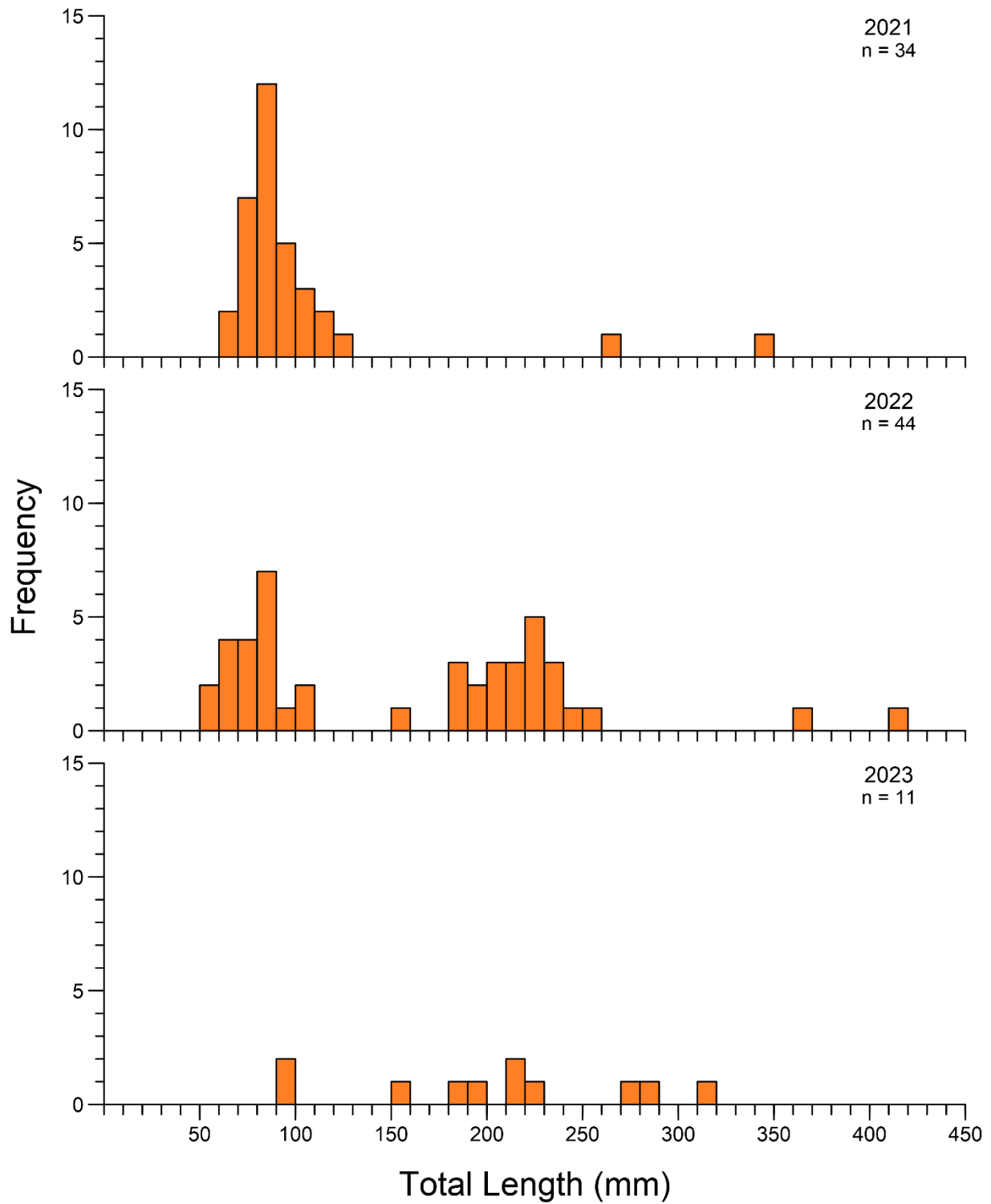


Figure 49.—Length frequency distribution of Brown Trout captured during annual monitoring in the upper Blue River, from 2021 to 2023. Number of fish captured and measured each year is shown in the top right corner of each panel.

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 Chub⁹ occupy the stream, although their 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 upstream 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 occurring in 2018 and 2019. Monitoring from 2017 to 2020 detected several hundred chub representing all size classes upstream of the waterfall 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. 2020a) 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 Creek drainage.

⁹ Chub in Harden Cienega Creek were previously classified as Gila Chub.

Surveys of all 43 stock tanks in the Arizona portion 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 50).

Results:

Gila Topminnow Monitoring

On May 24, 2023, Department staff monitored Gila Topminnow in Harden Cienega Creek by setting ten minnow traps in the vicinity of the stocking location for a soak time of approximately six hours. A total of seven Roundtail Chub, one Speckled dace, two Longfin Dace, three Sonora Sucker, and four Desert Sucker were captured. No Gila Topminnow were captured or observed during the monitoring or removal efforts. Evidence of substantial flooding during the winter and spring was observed, with the channel rerouting in several locations, and many of the pools near the stocking location changing to riffles or run, or greatly decreasing in depth and width (Figure 51)

Green Sunfish Removal

During May 24-25, 2023, Department staff completed two Green Sunfish removal passes in the perennial reach of Harden Cienega Creek. The stream was electrofished for 19,020 seconds from the start of flow (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 adult Brown Trout (211, 305 mm TL) were captured approximately 700 m upstream of the confluence with the San Francisco River (674270/3674551 and 674515/3674700) and removed from the stream. A search of available records suggests this is the first time that Brown Trout have been captured in Harden Cienega Creek. These fish likely dispersed from the Blue River (or its tributaries) or from tributaries to the San Francisco River in New Mexico, a journey of more than 40 km in either case, made possible by the exceptional spring runoff in 2023.

The crew also set six mini-hoop nets overnight in the pool where most of the Green Sunfish have been captured in the past during the first pass and captured 3 Green Sunfish, 15 Roundtail Chub, 1 Desert Sucker, 1 Sonora Sucker. Three of the mini-hoop nets were reset in the same pool for approximately 7 hours during the second pass and failed to capture any additional fish. One mini hoop-net each was set in two additional pools that were too deep to sample effectively with backpack electrofishing equipment and failed to capture any fish. 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; 2023 pass 1 = 3, 2023 pass 2 = 0; Figure 52). All Green Sunfish captured were of adult, or possibly sub-adult size, consistent with trends from the past few years (Figure 52). In addition, Green Sunfish still do not appear to be spawning in Harden Cienega Creek, as young of year fish have not been captured during removal passes. 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 does not occur.

Recommendations: Topminnow have not been captured following the initial stocking in 2019 and subsequent augmentation in 2021. It is not clear what limited survival of Gila Topminnow in Harden Cienega Creek, but predation pressure from the existing chub population may have been a primary factor based on observations from underwater video taken during the 2021 stocking. In addition, flooding in 2023 seems to have reduced the quality and quantity of pool habitat in the vicinity of the stocking location. Given these factors, translocation of additional Gila Topminnow into Harden Cienega Creek is not recommended at this time.

Green Sunfish removal efforts should continue in Harden Cienega Creek in 2024. If two successive removal passes are completed in the perennial reach without capturing additional Green Sunfish, a pass of eDNA samples should be collected to confirm eradication in 2025. Surveys of Harden Cienega Creek upstream of the perennial reach to the New Mexico state line should be undertaken in order to determine whether any isolated pools containing Green Sunfish exist. If isolated pools containing Green Sunfish are detected, removal efforts should be expanded to target these pools.

Tables and Figures:

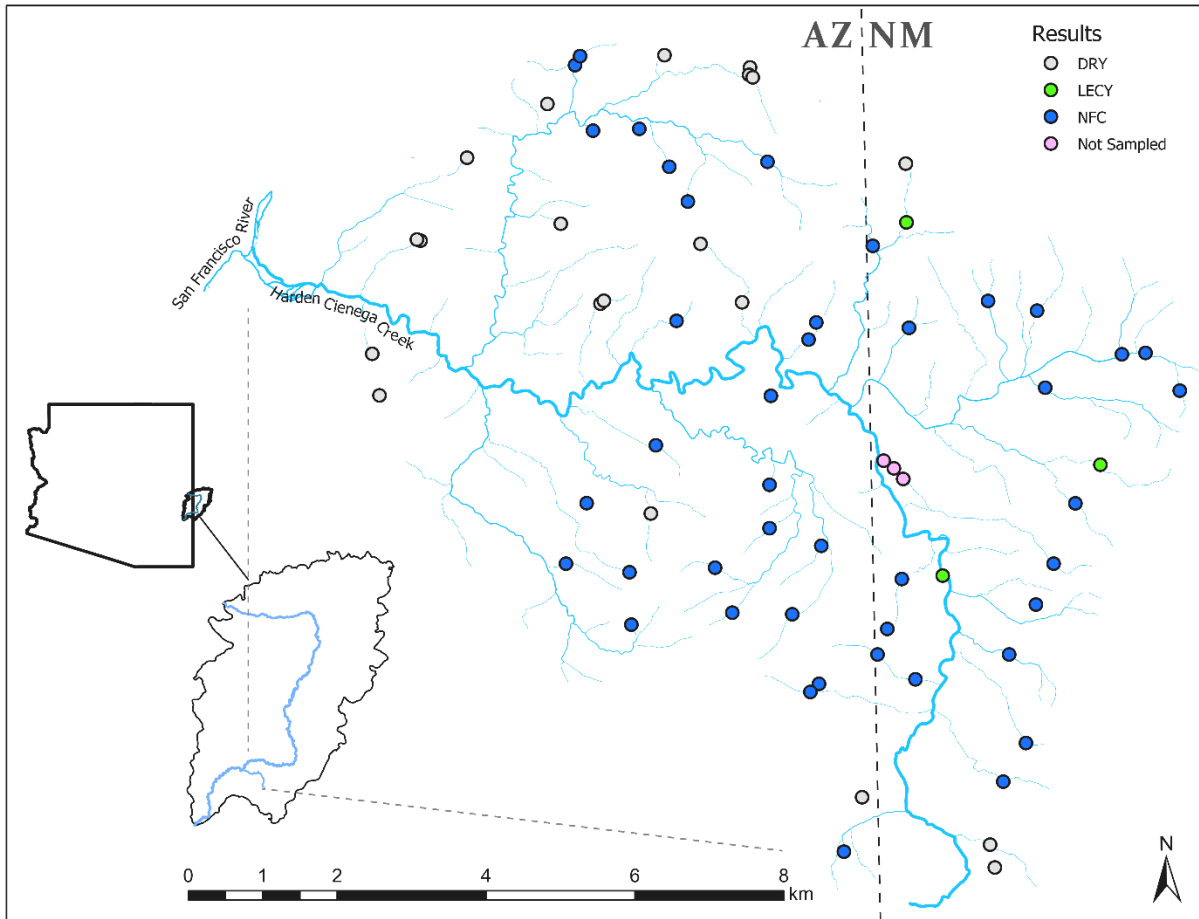


Figure 50.—Map of all tanks surveyed in the Arizona and New Mexico portions of the Harden Cienega Creek drainage during 2020 and 2021. Shown are tanks that were dry upon arrival (grey points), tanks that contained water and were sampled by bag seine, straight seine or dip net (blue points). Also shown are Ditch Tank, Distill Tank and California Tank where Green Sunfish were detected (green points). Three tanks on private property in New Mexico remain to be sampled (pink points).



Figure 51.—Example of channelization and pool habitat reduction in Harden Cienega Creek in the vicinity of the stocking location in June 2021 (left, looking upstream) and May 2023 (right, looking downstream) following flooding during the winter and spring of 2023. Note the tree in the center of the photographs is the same tree.

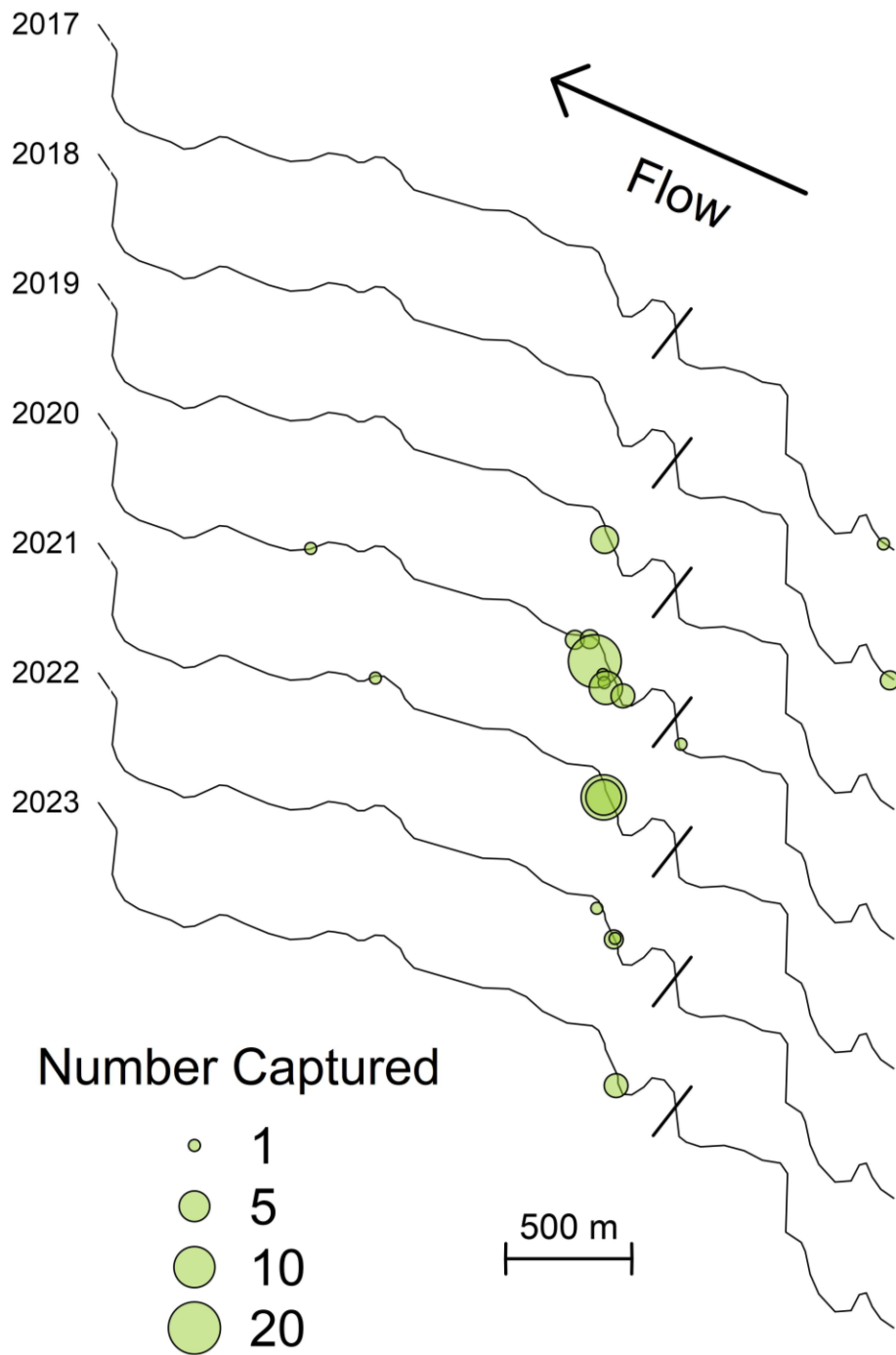


Figure 52.—Locations of Green Sunfish captured in the perennial portion of Harden Cienega Creek from 2017 to 2023. The barrier location is indicated by a diagonal line. Size of points indicates number of fish captured at a particular location during nonnative removal or monitoring efforts. Monitoring during 2017-2019 was only upstream of the barrier.

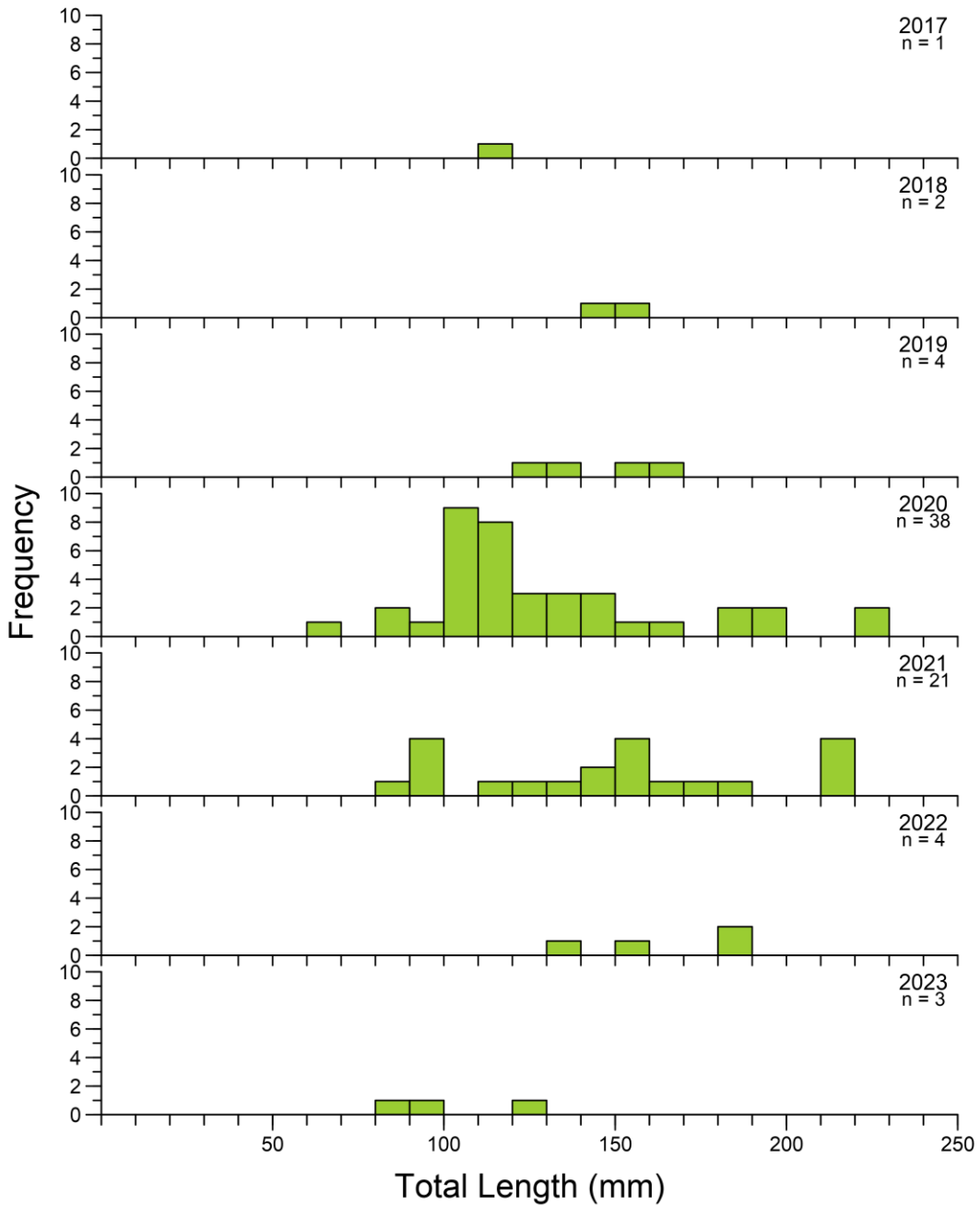


Figure 53.—Length frequency distribution of the number of Green Sunfish captured and removed during annual monitoring and nonnative removal efforts in Harden Cienega Creek, from 2017 to 2023. Number of fish captured and measured each year is shown in the top right corner of each panel.

Remote Site Inventory San Francisco River (Task AZ-2023-1)

Strategic Plan Goals:

- Build the Scientific Foundation for Recovery Efforts
 - Goal 1. Identify critical streams and populations in need of protection and replication.
 - Goal 5. Survey poorly studied stream systems to document existing fish communities.

Recovery Objectives:

- Spikedace recovery objective 1.1.1. Identify all populations and determine level of protection
- Loach Minnow recovery objective 1.1.1. Identify all populations and determine level of protection
- Gila Chub draft recovery plan objective 3. Monitor remnant and replicated populations to ensure they are persisting and threats are being managed.

Background: The San Francisco River is a 256 km long tributary to the Gila River that originates in the White Mountains of Arizona near the town of Alpine (Figure 54). Sampling in recent years detected both Roundtail Chub and Spikedace in the vicinity of the Blue River confluence and upstream near the confluence with Big Dry Creek in New Mexico (Hickerson et al. 2020b, Shollenberger et al. 2021). Smallmouth Bass were also recently detected in the San Francisco River near Glenwood, NM for the first time since 1990's (Ferguson and Zeigler 2021) and the source of these fish and their distribution throughout the river is unknown. Little sampling has occurred within a remote 15 km reach of the river between the Harden Cienega Creek confluence upstream to the New Mexico border since 1977 (Anderson and Turner 1977). Due to the lack of recent fish sampling data, little current information exists about the distribution and abundance of focal native fish species (Spikedace, Loach Minnow, and Roundtail Chub) and potentially emerging nonnative fish threats (Smallmouth Bass) within this remote reach.

The 15 km reach identified for sampling was split into three reaches of roughly equal length (~4.9 km) to stratify sampling efforts (Figure 54). A total of 13, 100 m long sub-reaches were randomly chosen for sampling within each of the three reaches. Several seine hauls were carried out within each sub-reach to sample as much habitat as possible.

Results: During June 5-7 and 20-22, 2023, Department and Reclamation staff surveyed fish populations in the San Francisco River from the confluence with Harden Cienega Creek upstream to the Arizona/New Mexico border. The crew carried out 172 seine hauls and captured a total of 2 Channel Catfish, 12 Common Carp, 1 Flathead Catfish, 99 Red Shiner, 2 Roundtail Chub and 4 Sonora Sucker (Table 10). Both Roundtail Chub were captured within a few hundred meters of the confluence with Harden Cienega Creek, which supports a robust chub population. Opportunistic

dip net sweeps in a few isolated pools resulted in the capture of 12 young of year Common Carp and an observation of a recently deceased Brook Stickleback. A query of available fish sampling records suggests that the Brook Stickleback observation may be the first occurrence of the species in Arizona. Stickleback have previously been documented in the Tularosa River in New Mexico (Ferguson and Zeigler 2021) which is a tributary far upstream in the watershed. One night of angling effort in Reach 2 resulted in the capture of two additional Channel Catfish. Nonnative fish accounted for 95% of the total catch in the San Francisco River with over 90% of the total catch in each of the three sub-reaches comprising nonnative species (Figure 54).

Evidence of severe flooding was observed throughout the surveyed reach, likely as a result of runoff from the substantial snowpack in the watershed during the winter and spring. The crew observed deeply scoured banks with uprooted mature trees, large off channel piles of woody debris, and evidence of flood debris several meters above the normal channel. The flooding likely also contributed to the extremely low density of fishes, both native and nonnative, throughout the reach. Flooding may have displaced fish downstream (as might be the case for the lone Brook Stickleback observed), the flooding and debris may have caused mortality of many fish that could not find refuge, or possibly a combination of the two.

Recommendations: The current fish community in the San Francisco River from Harden Cienega Creek upstream to the New Mexico border is dominated by nonnative fish. However, the relative abundance of all fishes is extremely low. Surveys of this reach should occur in the future to determine how the fish community recovers, and whether conditions become more favorable for native fishes (i.e., nonnative fishes struggle to reestablish).

Tables and Figures:

Table 10.—Summary of fish captured within each sampling reach of the San Francisco River during June 5-22, 2023. Shown are the number of individual seine hauls within each reach (N), number of fish captured in each reach (#Ind), the mean number of fish captured per square m seined (#Ind/m²), and standard error of mean relative abundance.

Reach	N	Statistic	Sonora Sucker	Red Shiner	Common Carp	Roundtail Chub	Channel Catfish	Flathead Catfish
Reach 1	55	#Ind	1	11	5		1	
		#Ind/m ²	0.00030	0.00391	0.00144		0.00021	
		SE	(0.00030)	(0.00158)	(0.00078)		(0.00021)	
Reach 2	61	#Ind	3	48	4			
		#Ind/m ²	0.00068	0.00977	0.00033			
		SE	(0.00048)	(0.00354)	(0.00028)			
Reach 3	56	#Ind		40	3	2	1	1
		#Ind/m ²		0.00457	0.00013	0.00018	0.00004	0.00040
		SE		(0.00177)	(0.00013)	(0.00018)	(0.00004)	(0.00040)
Total	172	#Ind	4	99	12	2	2	1
		#Ind/m ²	0.00033	0.00618	0.00061	0.00006	0.00008	0.00003
		SE	(0.00019)	(0.00147)	(0.00027)	(0.00006)	(0.00007)	(0.00003)

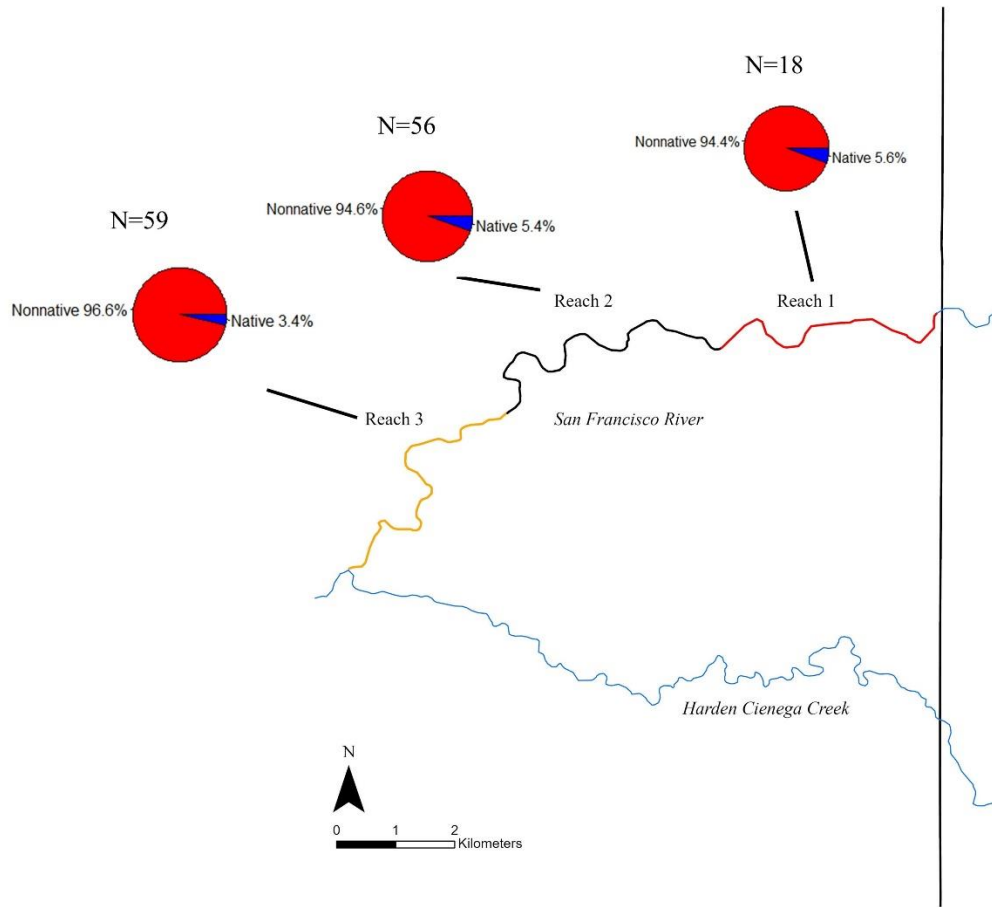


Figure 54.—Map of the three survey reaches (Reach 1, Reach 2, Reach 3) surveyed within the San Francisco River from the confluence with Harden Cienega Creek upstream to the New Mexico border during June 5-22, 2023. Shown for each reach is a pie chart with the percentage of native and nonnative fish captured in each reach, and the number of total fish captured in each reach (N).

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 by and competition with nonnative mosquitofish. In June 2013, Department staff attempted to eradicate Western Mosquitofish by draining the pools in Sharp Spring with gasoline powered pumps, but the effort was abandoned because the pools could not be completely dried. The purpose of this project is to eradicate Western Mosquitofish from Sharp Spring and reintroduce Sharp Spring lineage Gila Topminnow. Eradication of Western Mosquitofish will also create an opportunity to potentially replicate a population of upper Santa Cruz River Roundtail Chub¹⁰ in Sharp Spring.

¹⁰ Roundtail Chub in the upper Santa Cruz River previously classified as Gila Chub.

Department staff conducted a rotenone treatment in June 2022 and successfully eradicated Western Mosquitofish. Following confirmation of eradication, 659 Gila Topminnow from two donor locations were stocked into two pools of Sharp Springs in June 2022. Nine additional topminnow were stocked in October 2022. A total of 135 Gila Topminnow were captured in the two pools that were initially stocked during the first post-stocking monitoring effort in October 2022.

Results:

On February 27, 2023, Department staff collected 36 Gila Topminnow (Sharp Spring lineage) from Cottonwood Tank at Robbin's Butte 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, 27, 2023, Department staff collected 34 Gila Topminnow (Sharp Spring lineage) from Peterson Ranch Pond 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 May 18, 2023, Department staff translocated 686 Gila Topminnow into three pools in Sharp Spring. A total of 200 fish were translocated into each of the pools that were previously stocked with Gila Topminnow in 2022 (SP02, SP10; Figure 55). The remaining 286 fish were stocked in a pool (SP13) that had not received any topminnow since the treatments took place. The fish were collected earlier in the day from Cottonwood Tank (n = 744) and Twin Tanks (n = 25) at Robbins Butte Wildlife Area. There were 3 mortalities during collection, and another 83 mortalities during transport and stocking.

On August 29, 2023, Department staff monitored the Gila Topminnow population at Sharp Springs. The crew set a total of 26 minnow traps for a minimum soak time of two hours in 11 of the pools, with five minnow traps set in each of the three pools that were previously stocked (SP02, SP10, SP13). A total of 117 Gila Topminnow (3 <20 mm, 114 ≥20 mm) were captured in SP02, with no fish captured or observed in any of the other pools.

It is not clear why Gila Topminnow are struggling to persist and thrive in historically occupied habitat free of Western Mosquitofish, since all of the stocked pools supported Western Mosquitofish up until the rotenone treatment. It is possible that low dissolved oxygen may be limiting survival of topminnow during the summer months. Duckweed also seems to cover nearly the entire surface of some of the pools, which might contribute to dissolved oxygen problems. American bullfrog also seem to be very abundant at the site, and although topminnow seem to persist fine with bullfrogs at other locations, high densities of bullfrogs could be problematic for a topminnow population in the establishment phase when numbers of topminnow are inherently low.

On August 30, 2023, Department staff collected 244 Gila Topminnow from Peterson Ranch Pond and translocated them to Sharp Springs. A total of 29 fish were stocked in SP02, 70 in SP04, 68 in SP10, and 77 in SP13 with eight mortalities during transport and stocking.

Recommendations: Additional Gila Topminnow from other Sharp Spring lineage donor populations should be translocated in 2024 to recreate as much of the original genetic diversity as possible in Sharp Spring. Dissolved oxygen loggers should be deployed during the spring, summer, and early fall of 2024 to determine whether oxygen concentration is a limiting factor in pools other than SP02. Having continuous dissolved oxygen information will allow staff to determine if water quality is impeding establishment of topminnow or whether other potential limiting factors (e.g., duckweed, bullfrogs) need to be evaluated further.

Tables and Figures:

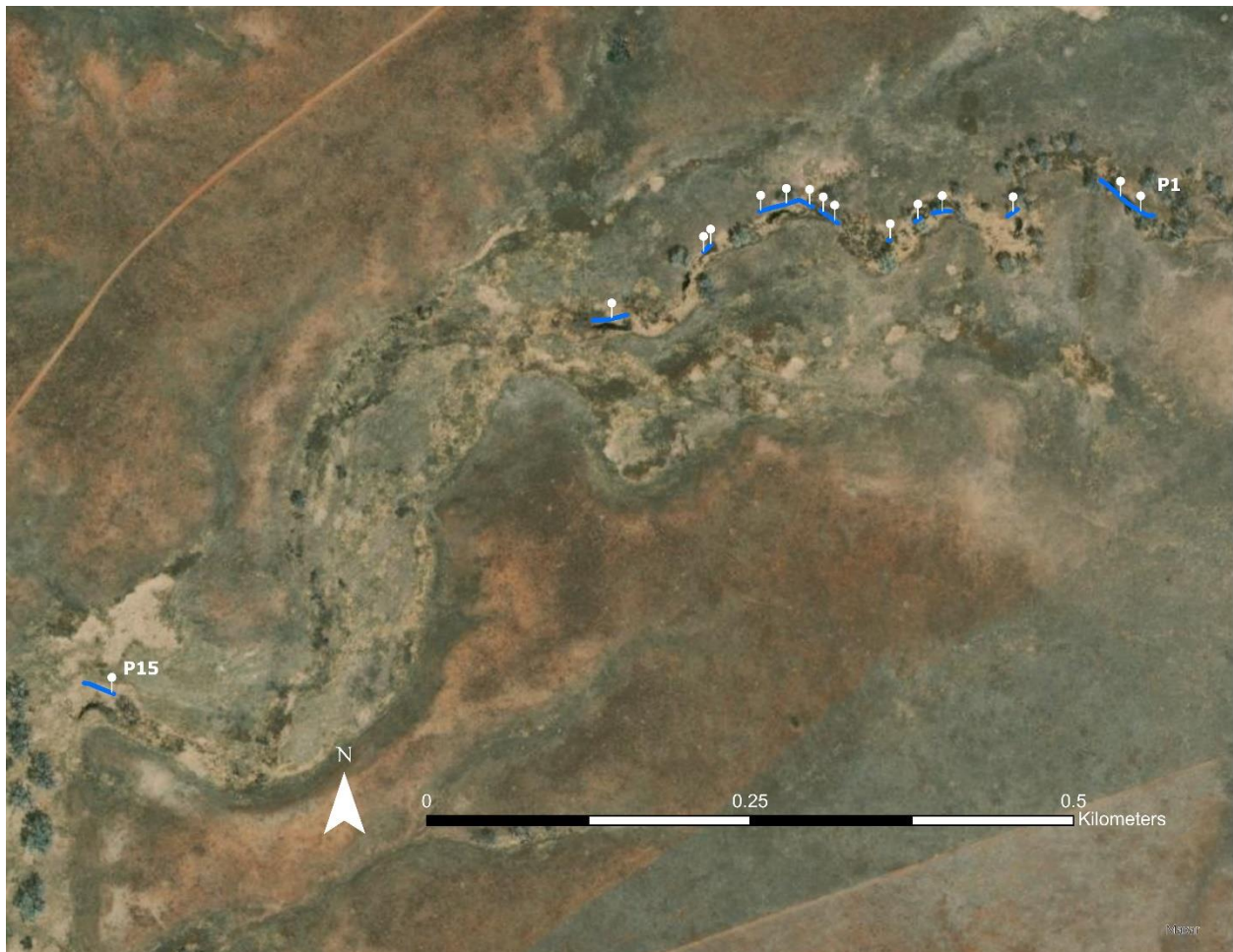


Figure 55.—Detail of Sharp Spring including the approximate location of each of the 15 pools (white dots) from upstream (P1) to downstream (P15).

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 distribution and assemblage of nonnative fish communities within the watershed to inform potential planning for 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. A total of 87 tanks were surveyed, with 19 containing nonnative fishes. The remaining 59 tanks were either dry, or access for sampling was denied. Green Sunfish is the most common species detected in stock tanks to

date, with Yellow Bullhead, Black Crappie, Largemouth Bass, Western Mosquitofish, Bluegill and Western Mosquitofish also detected.

With the detection of nonnative fish in a relatively high proportion of tanks sampled, and the observation of Green Sunfish actively dispersing through several ephemeral stream channels during the summer of 2021, surveys of ephemeral and intermittent stream channels that could be utilized as conduits for dispersal by nonnative fish (particularly downstream of nonnative fish occupied stock tanks) was identified as a critical information gap. Priority streams identified for sampling in 2023 were lower Hell Canyon (from the Verde River confluence to Hell Canyon Tank), which has at least 12 nonnative fish occupied stock tanks within the watershed, and Big Chino Wash and Williamson Valley Wash where permission to survey stock tanks within each watershed was largely denied (Figure 56).

Results:

On March 20, 2023, Department staff surveyed Big Chino and Williamson Valley immediately upstream of their confluence. Greater than average winter precipitation caused continual streamflow to be present nearly continuously in the normally dry Williamson Valley Wash from November 5, 2022 until May 23, 2023, peaking at over 5,200 cfs on March 22. Similarly, streamflow was also present in Big Chino Wash during most of this time period, although revised gauge data does not accurately reflect the presence of flow for a majority of the same time period.

The crew carried out seven seine hauls in Williamson Valley Wash (sampled length of 441 m) and captured 102 Fathead Minnow (101 <40 mm, 1 \geq 40 mm). Detection of Fathead Minnow in Williamson Valley Wash is evidence that nonnative fish present further upstream in the watershed can use this typically intermittent stream channel to disperse into the Verde River.

The crew also carried out five seine hauls in Big Chino Wash (sampled length of 186 m) and captured five Green Sunfish (78-167 mm TL). Detection of Green Sunfish in Big Chino Wash is evidence that nonnative fish present further upstream in the watershed, likely in inaccessible stock tanks, can use this typically intermittent stream channel to disperse into the Verde River.

On August 7, 2023, Department and Prescott National Forest staff surveyed approximately 20.3 km of Hell Canyon downstream of Hell Canyon Tank. Five pools were detected and sampled with minnow traps, seines, and dip nets. A total of 144 Green Sunfish, 25 Yellow Bullhead, and 4 Fathead Minnow were captured (Figure 57). All pools were relatively shallow and appeared likely to dry in the coming weeks without substantial precipitation.

During September 13-14, 2023 Department and U.S. Fish and Wildlife Service staff surveyed approximately 11.9 km of Hell Canyon from pool 5 to the Verde River confluence (Figure 57). The surveyed reach was entirely dry (including pool 5), with the exception of King Spring. The crew resampled King Spring (which was also sampled in 2021) by setting three mini-hoop nets

and two collapsible minnow traps and captured 40 Green Sunfish. The crew also observed Red Shiner and Yellow Bullhead despite failing to capture these species in the traps.

Recommendations: Nonnative fish appear to be able to readily move within tributaries to the upper Verde River, specifically Williamson Valley Wash, Big Chino Wash, and Hell Canyon. Movement of nonnative fish in Big Chino and Williamson Valley Washes is almost certainly from upstream sources, since Sullivan Dam is immediately downstream of the sampling locations and acts as a barrier to upstream movement from fish in the Verde River. Dispersal of Green Sunfish from these tributaries is an important consideration for developing potential nonnative fish removal goals for the upper Verde River watershed.

Nonnative fish seem to be able to disperse upstream from the Verde River into Hell Canyon potentially as far as the dam below Hell Canyon Tank. The presence of Red Shiner in King Spring is likely evidence that nonnative fishes can disperse upstream at least 6.8 km from the Verde River. It is less clear if the Green Sunfish and Yellow Bullhead detected in pools 1-5 came from upstream or downstream sources. Regardless of the source of the fish in pools 1-5, their presence suggests that all ephemeral or intermittent stream channels downstream of stock tanks with known nonnative fish populations should be considered potentially occupied by nonnative fishes. Other ephemeral and intermittent tributaries to the upper Verde River downstream of stock tanks with populations of nonnative fish should be surveyed to better understand the distribution and dispersal of nonnative fish within the watershed. Specifically, these tributaries include upper Hell Canyon (upstream of Hell Canyon Tank), Grindstone Wash, MC Canyon, and Bear Canyon.

Tables and Figures:

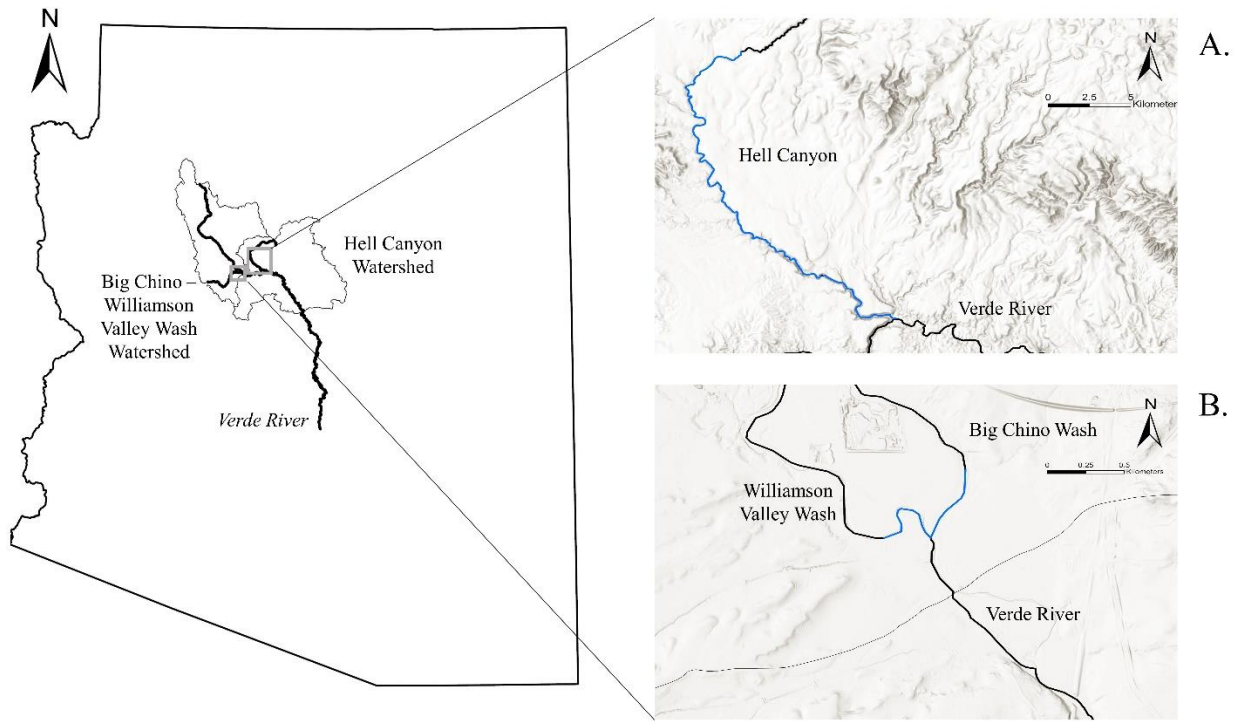


Figure 56.—Location of the major tributaries to the upper Verde River sampled in 2023. Insets show the sampled reaches of Hell Canyon (A) and Big Chino and Williamson Valley Washes (B) with dark blue lines.

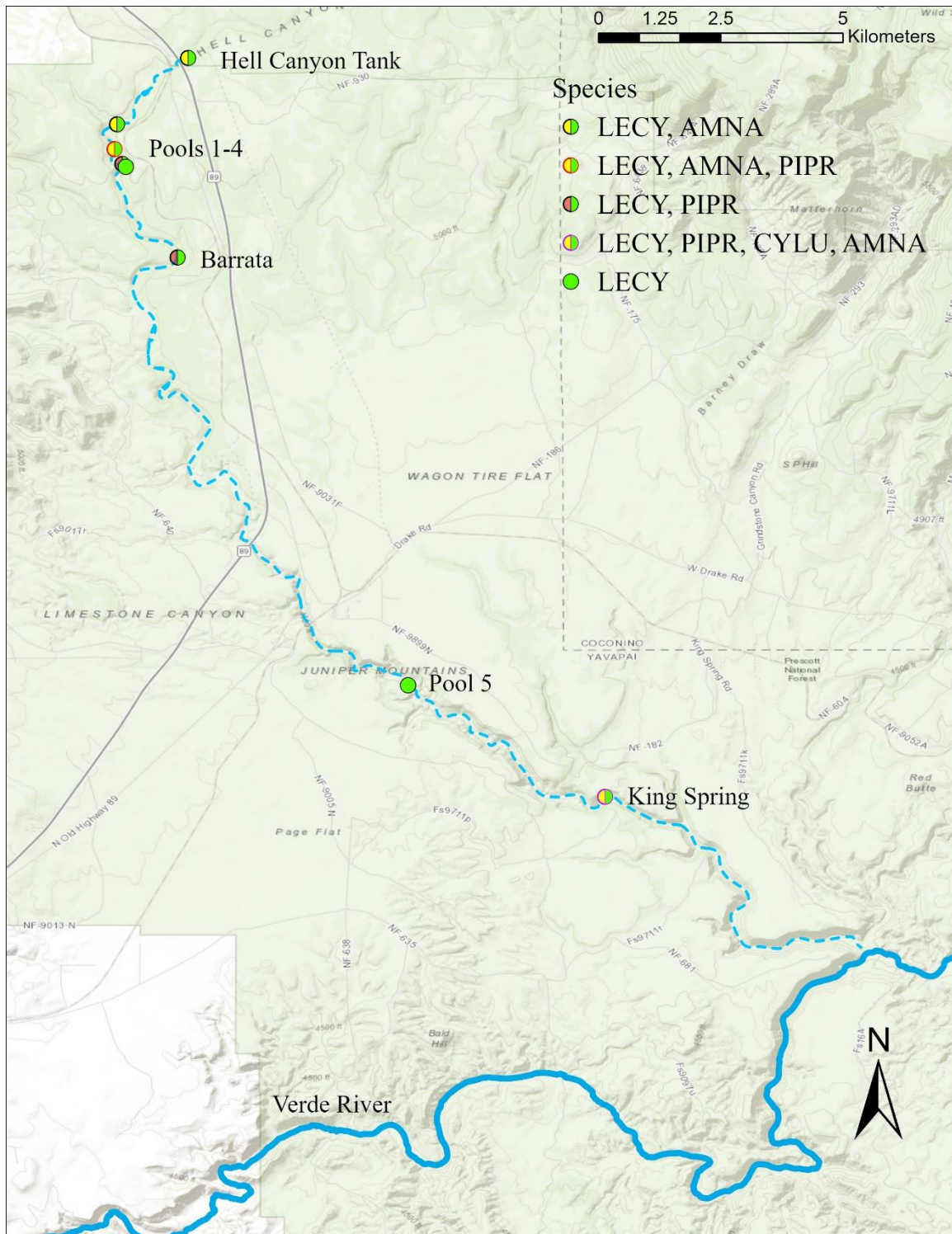


Figure 57.—Map of the reach of Hell Canyon surveyed during August 7 and September 13-14, 2023. Shown is the location of pools detected during surveys and their species composition (LECY = Green Sunfish, AMNA = Yellow Bullhead, PIPR = Fathead Minnow, CYLU = Red Shiner). Note Hell Canyon Tank, Barrata, and King Spring were previously sampled in 2021 and 2022.

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. New individuals should be 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 11.

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 2017, 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 continues to operate ARCC in 2023. Department staff maintained 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). All Bear Creek Loach Minnow, including individuals salvaged in 2020 and 41 offspring were repatriated into Bear Creek, thereby removing this lineage from the ARCC. The putative Parker Canyon lineage of topminnow also continued to persist at the facility as of the end of 2023.

During the 2023 spawn season, ARCC staff continued the Loach Minnow nest spacing study started in 2022. The 2023 spawn season was set up to investigate Loach Minnow nest spacing as a potential means of increasing the number of broodstock individuals used in spawning, as well as

the offspring produced. Tanks were established for all Loach Minnow, with one spawning pair per nest. This approach was adopted to eliminate the unnecessary variable of having extra Loach Minnow males in a spawning raceway, which could potentially impact the study by not providing a nest to defend and by distracting other males.

Using the Blue River lineage of Loach Minnow, three nest spacings were established, each with two replicates. Two raceways had nests spaced 25.4 cm apart, resulting in a total of 18 nests and 36 fish. Another two raceways had nests spaced 38 cm apart, totaling 13 nests and 26 fish. Finally, two raceways had nests spaced 50.8 cm apart, with a total of 12 nests and 24 fish. All nests had a diameter of 15 cm. All other Loach Minnow spawning tanks were setup the same as previous years but implemented the new ratio of one spawning pair per nest for a total of 13 nests spaced at 38 cm from edge to edge and using 26 fish.

Unfortunately, due to raccoon activity which resulted in broken nest structures and missing fish, all spawning tanks were dismantled shortly after the initial setup. Emergency lids were constructed for four spawning raceways, with two allocated for Spikedace and two for Loach Minnow. Unfortunately, by the time this was completed, the necessary spawning cues were missed, resulting in no spawning in the remaining raceways and none from the original setup before the raccoon issues were detected. Consequently, ARCC produced no Spikedace or Loach Minnow of any lineage held on station (Table 11).

No new large-scale physical improvements to ARCC were completed in 2023.

Acquire Spikedace, Loach Minnow, and rare populations of other native fish

On July 17, 2023, Department staff and a volunteer collected 37 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 two seine hauls to capture the required number of Longfin Dace for the assessment and also captured and released 33 juvenile Loach Minnow.

On July 7, 2023, Department staff collected 40 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 that were collected later in the year to augment the existing hatchery stock at ARCC. The fish were transported to the fish health laboratory at Department Headquarters for a fish health inspection.

On November 21, 2023, Department staff collected Loach Minnow from the upper Blue River. The crew electrofished three locations in the reach for a total of 13,943 seconds and captured a total of 95 Loach Minnow. All fish were transported to ARCC with one Loach Minnow mortality

during transport. Unfortunately, due to miscommunication about a permitting issue 50 of the Loach Minnow had to be returned to the Blue River on December 19, 2023.

On November 14, 2023, Department staff collected Loach Minnow and Spikedace from Nature Conservancy property on the east side of Aravaipa Creek. The crew collected a total of 67 Spikedace and 56 Loach Minnow in 11 seine hauls. All fish were transported to ARCC in aerated coolers with three Loach Minnow mortalities during transport.

Recommendations: For 2024, ARCC staff will focus on running all raceways at the lowest density identified in 2018 with a third 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 2024 include continuing to collect Spikedace and Loach Minnow from remnant populations, with goals to minimize impacts on the remnant population while also acquiring the number of fish necessary to maintain a refuge population of at least 500 adults. ARCC staff should coordinate with NMDGF regarding acquiring more stock of the New Mexico lineages.

ARCC staff have also taken steps to help address the issues with raccoons experienced in 2023. After the initial raccoon issue was identified, ARCC staff purchased wood and fencing to create emergency lids in an attempt to secure raceways to continue spawning. These lids are sufficient to keep raceways safe should raccoons find their way into the spawning cage in 2024 but complicate spawn and feeding. Further steps have been taken to limit the possibility of raccoons finding their way back into the spawn cage, such as the removal of a large tree that had grown in cage one, the construction of lids to be used if necessary, as well as the addition of fencing to reduce gate gaps. 2024 spawn will be closely monitored through daily checks and the use of trail cameras.

Tables and Figures:

Table 11.—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 2016 through 2023. Data for years prior to 2016 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		2016	2017	2018	2019	2020	2021	2022	2023	
Spikedace	upper Gila River	#B	531	267	159	254	219	176	131	72	
		#P	0	384	352	2404	408	914	466	0	
		#S	0	327	0	0	0	0	0	0	1667
		#A	0	0	0	0	0	0	0	0	0
Spikedace	Gila River Forks	#B	138	122	83	71	76	151	120	96	
		#P	0	1183	195	1132	833	203	1252	0	
		#S	0	1000	0	0	0	0	705	0	
		#A	0	0	1	0	0	52	0	0	
Spikedace	Aravaipa Creek	#B	262	382	331	523	529	379	158	180	
		#P	120	1347	3214	4250	2182	1032	393	0	
		#S	67	0	2234	0	2897	106	1707	0	
		#A	80	160	0	322	49	0	27	67	
Loach Minnow	Bear Creek	#B						112	66	0	
		#P						196	65	0	
		#S						0	0	41	
		#A						0	0	0	
Loach Minnow	Gila River Forks	#B	96	128	97	169	121	0	58	40	
		#P	220	7	1207	665	15	0	475	0	
		#S	0	159	0	0	0	0	0	0	
		#A	0	110	145	0	0	102	0	0	
Loach Minnow	San Francisco R.	#B	215	314	318	231	208	173	92	51	
		#P	26	177	1627	601	3	541	310	0	
		#S	0	243	0	0	0	0	0	205	
		#A	0	0	0	0	0	0	0	0	
Loach Minnow	Blue River	#B	214	156	117	290	266	364	244	172	
		#P	426	47	6	713	16	919	278	0	
		#S	390	0	0	0	500	400	0	0	
		#A	12	0	223	80	269	130	4	45	
Loach Minnow	Aravaipa Creek	#B	297	490	439	354	337	261	200	174	
		#P	265	305	1848	1398	57	504	168	0	
		#S	0	0	0	0	300	0	0	0	
		#A	200	100	0	57	82	0	23	56	
Roundtail Chub	Eagle Creek	#B	101	99	99	99	98	84	81	58	
		#P	0	57	0	0	0	0	7405	0	
		#S	1194	0	0	0	0	0	0	6640	
		#A	0	0	0	0	0	0	0	0	

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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 2007-2023.

Year	Citation
2007	C. Carter. 2007. Three Forks Loach Minnow Survey, August 28-30, 2007. <i>Arizona Game and Fish Department</i> , Phoenix, AZ.
2008	Robinson, A.T. 2008. Arnett Creek and Telegraph Canyon 1-Year Post-Stocking Monitoring, July 23, 2008. <i>Arizona Game and Fish Department</i> , 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. <i>Arizona Game and Fish Department</i> , Phoenix, AZ.
2008	Robinson, A.T. 2008. Mineral Creek Fish Survey, April 21-22, 2008. <i>Arizona Game and Fish Department</i> , Phoenix, AZ.
2008	Robinson, A.T. 2008. Mud Springs #18: Gila Topminnow and Desert Pupfish Monitoring on November 6, 2008. <i>Arizona Game and Fish Department</i> , 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. <i>Arizona Game and Fish Department</i> , 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. <i>Arizona Game and Fish Department</i> , Phoenix, AZ.
2009	Robinson, A.T. 2009. Repatriation of Native Fishes to Fossil Creek: Summary of Monitoring and Stocking During 2008. <i>Arizona Game and Fish Department</i> , Phoenix, AZ.
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Appendix 2.—Summary of native fish translocated in Arizona during 2023 by the Department under the Gila River Basin Native Fishes Conservation Program. Easting and Northing are in UTM's (NAD 83; zone 12S).

Taxa	Water Name	Easting	Northing	Date	Lineage	# Stocked	# Mortalities
Roundtail Chub	Blue River	667900	3684053	2/21/2023	Eagle Creek	2496	4
Spikedace	Blue River	667954	3685062	2/21/2023	Gila Mainstem	650	0
Roundtail Chub	Blue River	680704	3732366	2/28/2023	Eagle Creek	1000	0
Roundtail Chub	Blue River	667286	3713496	2/28/2023	Eagle Creek	2249	0
Spikedace	Blue River	680702	3732370	2/28/2023	Gila Mainstem	500	0
Spikedace	Blue River	667287	3713496	2/28/2023	Gila Mainstem	517	1
Gila Topminnow	Spring Creek	416613	3845826	5/16/2023	Peck Canyon	492	28
Gila Topminnow	Sharp Spring	540496	3468806	5/18/2023	Sharp Spring	686	86
Gila Topminnow	Sharp Spring	540496	3468806	8/30/2023	Sharp Spring	236	8
Gila Topminnow	Unnamed Drainage 68B	464695	3711289	10/25/2023	Monkey Spring	500	0
Gila Topminnow	Telegraph Canyon	486905	3679666	10/26/2023	Redrock Canyon	100	0

Appendix 3.—Summary of monitoring results during 2023 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). Note, statistics reflect only the first pass of electrofishing at the Blue River and Spring Creek.

Taxa	Location	Date	Gear Type	Sample Size	Statistics	2023
Gila Topminnow	Aravaipa Creek	10/2/2023	Minnow Trap	10	#Ind	193
					%YOY	20
					Mean CPUE	8.12
					SE	3.04
Gila Topminnow	Aravaipa Creek	10/2/2023	Dip Net	14	#Ind	36
					%YOY	42
					Mean CPUE	3.67
					SE	3.27
Gila Topminnow	Aravaipa Creek	10/2/2023	Seine	5	#Ind	0
					%YOY	0
					Mean CPUE	0
					SE	0
Gila Topminnow	Arnett Creek	10/26/2023	Minnow Trap	2	#Ind	212
					%YOY	19
					Mean CPUE	61.75
					SE	61.75
Gila Topminnow	Maternity Wildlife Pond	7/31/2023	Minnow Trap	10	#Ind	6618
					%YOY	34
					Mean CPUE	253.23
					SE	30.52
Gila Topminnow	Sharp Spring	8/29/2023	Minnow Trap	26	#Ind	117
					%YOY	3
					Mean CPUE	2.16

					SE	1.99
Gila Topminnow	Telegraph Canyon	10/26/2023	Minnow Trap	10	#Ind	38
					% YOY	0
					Mean CPUE	1.85
					SE	0.92
Gila Topminnow	Telegraph Canyon	10/26/2023	Dip Net	16	#Ind	109
					% YOY	10
					Mean CPUE	18.41
					SE	9.64
Gila Topminnow	Unnamed Drainage 68b	10/25/2023	Minnow Trap	15	#Ind	1434
					% YOY	6
					Mean CPUE	36.32
					SE	13.90
Gila Topminnow	Unnamed Drainage 68b	10/25/2023	Dip Net	1	#Ind	1
					% YOY	0
					Mean CPUE	5.41
					SE	0
Loach Minnow	upper Blue River	9/18/2023	Backpack Electrofisher	15	#Ind	143
					% YOY	66
					Mean CPUE	37.90
					SE	8.33
Loach Minnow	middle Blue River	9/25/2023	Backpack Electrofisher	12	#Ind	17
					% YOY	6
					Mean CPUE	3.63
					SE	1.40
Roundtail Chub	upper Blue River	9/18/2023	Backpack Electrofisher	15	#Ind	5
					% YOY	0
					Mean CPUE	0.85
					SE	0.51
Roundtail Chub	middle Blue River	9/25/2023	Backpack Electrofisher	12	#Ind	31

					% YOY	19
					Mean CPUE	10.20
					SE	5.10
Roundtail Chub	Rarick Canyon	10/11/2023	Angling	5	#Ind	10
					% YOY	0
					Mean CPUE	15.73
					SE	2.22
Roundtail Chub	Rarick Canyon	10/11/2023	Minnow Trap	8	#Ind	28
					% YOY	7
					Mean CPUE	0.13
					SE	0.09
Roundtail Chub	Rarick Canyon	10/11/2023	Mini-Hoop Net	16	#Ind	102
					% YOY	0
					Mean CPUE	0.27
					SE	0.09
Roundtail Chub	Sabino Canyon	5/10/2023	Mini-Hoop Net	10	#Ind	3
					% YOY	0
					Mean CPUE	0.12
					SE	0.12
Roundtail Chub	Sabino Canyon	5/10/2023	Seine	3	#Ind	5
					% YOY	20
					Mean CPUE	0.05
					SE	0.02
Roundtail Chub	Sabino Canyon	5/10/2023	Dip Net	2	#Ind	2
					% YOY	50
					Mean CPUE	3.38
					SE	2.03
Spikedace	upper Blue River	9/18/2023	Backpack Electrofisher	15	#Ind	2
					% YOY	100
					Mean CPUE	0.37

Spikedace	middle Blue River	9/25/2023	Backpack Electrofisher	12	SE	0.26
					#Ind	280
					% YOY	65
					Mean CPUE	84.02
					SE	16.13
Spikedace	Spring Creek	9/7/2023	Backpack Electrofisher	3	#Ind	9
					% YOY	42
					Mean CPUE	10.38
					SE	5.07