

Gila River Basin Native Fishes Conservation Program:
Arizona Game and Fish Department's Native Fish Conservation Efforts During 2021

Cooperative Agreement R16AC00077
Between Bureau of Reclamation and Arizona Game and Fish Department
Annual Report
March 7, 2022

Brian T. Hickerson, John E. Cleveland 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.

Civil Rights and Diversity Compliance

The Arizona Game and Fish Department prohibits discrimination on the basis of race, color, sex, national origin, age, or disability in its programs and activities. If anyone believes that they have been discriminated against in any of the AGFD's programs or activities, including its employment practices, the individual may file a complaint alleging discrimination directly with the AGFD Director's Office at (602) 942-3000 or by mail at 5000 West Carefree Highway, Phoenix, AZ 85086. Discrimination complaints can also be filed with the U.S. Fish and Wildlife Service, Office of Diversity and Inclusive Workforce, Attention: Public Civil Rights and Disability Coordinator, 5275 Leesburg Pike, Falls Church, VA 22041. If you require this document in an alternative format, please contact the AGFD Ombudsman as listed above or by calling TTY at 1-800-367-8939.

Americans with Disabilities Act Compliance

The Arizona Game and Fish Department (AGFD) prohibits discrimination on the basis of race, color, sex, national origin, age, or disability in its programs and activities. If anyone believes that they have been discriminated against in any of the AGFD's programs or activities, including its employment practices, the individual may file a complaint alleging discrimination directly with the AGFD Ombudsman, 5000 W. Carefree Highway, Phoenix, AZ 85086, (602) 942-3000 or U.S. Fish and Wildlife Service, 4040 N. Fairfax Dr. Ste. 130, Arlington, VA 22203. Persons with a disability may request a reasonable accommodation, such as a sign language interpreter, or this document in an alternative format, by contacting the AGFD Ombudsman, 5000 W. Carefree, Phoenix, AZ 85086, (602) 942-3000 or (623) 236-7373. Requests should be made as early as possible to allow sufficient time to arrange for accommodation.

Acknowledgements

The work described in this report was funded through Cooperative Agreement (No. R16AC00077) with the U.S. Bureau of Reclamation as part of the Central Arizona Project (CAP) Gila River Basin Native Fishes Conservation Program. Individuals that participated in monitoring, removal, and stocking activities are too numerous to list, however we could not have completed the work without their participation and involvement.

Recommended Citation:

Hickerson, B. T., J. E. Cleveland, and J. Walters. 2021. Gila River Basin Native Fishes Conservation Program: Arizona Game and Fish Department's native fish conservation efforts during 2021. An Arizona Game and Fish Department Annual Report for Cooperative Agreement No. R16AC00077 submitted to U.S. Bureau of Reclamation, Phoenix Area Office. Arizona Game and Fish Department, Aquatic Wildlife Branch, Phoenix.

Contents

Overview.....	1
Performance Measures.....	2
General Activities.....	3
Priority Actions.....	4
General Methods.....	4
Muleshoe ecosystem stream and spring repatriations (Task AZ-2003-1).....	8
Gila Topminnow stockings (Task AZ-2002-1).....	15
Spring Creek (Oak Creek tributary) repatriations (Task AZ-2013-1).....	30
Expand Roundtail Chub population in Harden Cienega Creek (Task AZ-2014-1).....	55
Red Tank Draw native fish restoration (Task AZ-2016-2).....	61
Sharp Spring native fish restoration (Task AZ-2016-3).....	70
Upper Verde River native fish restoration (Task AZ-2020-1).....	72
West Fork Black River Nonnative Fish Removal (Task AZ-2021-1).....	83
Aquatic Research and Conservation Center O&M (Task HA-2006-2).....	87
Projects Removed From Priority List In 2021.....	92
Literature Cited.....	93
Appendices.....	96

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

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

finalized and approved recovery plans for four of the five priority species, and a draft recovery plan for the Gila Chub (U.S. Fish and Wildlife Service 1983, 1991a, 1991b, 1998, 2002, 2015). There were several draft revised recovery plans for Gila Topminnow, one of which (USFWS 1999) was posted on the USFWS Ecological Services web site. The Loach Minnow and Spikedace recovery plans are being revised.

In addition to the fish and amphibian species specified above, other species mentioned in this report include: Longfin Dace *Agosia chrysogaster*, Speckled Dace *Rhinichthys osculus*, Roundtail Chub *Gila robusta*, Woundfin *Plagopterus argentissimus*, Desert Pupfish *Cyprinodon macularius*, Desert Sucker *Catostomus clarki*, Sonora Sucker *Catostomus insignis*, Apache Trout *Oncorhynchus apache*, Green Sunfish *Lepomis cyanellus*, Bluegill *Lepomis macrochirus*, Black Crappie *Pomoxis nigromaculatus*, Largemouth Bass *Micropterus salmoides*, Smallmouth Bass *Micropterus dolomieu*, Tilapia *Oreochromis* sp., Channel Catfish *Ictalurus punctatus*, Black Bullhead *Ameiurus melas*, Yellow Bullhead *Ameiurus natalis*, Fathead Minnow *Pimephales promelas*, Western Mosquitofish *Gambusia affinis*, Brook Trout *Salvelinus fontinalis*, and Brown Trout *Salmo trutta*. Other aquatic species mentioned include lowland leopard frog *Lithobates yavapaiensis*, Chiricahua leopard frog *Lithobates chiricahuensis*, American bullfrog *Lithobates catesbeiana*, Sonora mud turtle *Kinosternon sonoriense*, red-eared slider *Trachemys scripta*, northern crayfish *Faxonius virilis*, red swamp crayfish *Procambarus clarkia*, and Northern Mexican gartersnake *Thamnophis eques*.

This report summarizes Program work performed by the Department during 2021. For each priority action, work completed during 2021 is presented, followed by recommendations.

PERFORMANCE MEASURES

Cooperative Agreement R16AC00077 between Reclamation and the Department specified the following annual performance measures.

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

Results: During 2021 Department staff completed repatriation stockings into four waters (Appendix 1). Also during the performance period Department staff completed ten non-indigenous species control actions: four nonnative fish removal efforts in Red Tank Draw, two in Redfield Canyon, two in Harden Cienega Creek, and two in West Fork Black River.

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

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

Results: During 2021, Department staff conducted post-stocking monitoring of 18 populations (Appendix 2): 3 Spikedace, 1 Desert Pupfish, 9 Gila Topminnow, and 5 Roundtail Chub².

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

Results: During 2021, Department staff monitored five locations where nonnative fish removals have been implemented: Rarick Canyon, Redfield Canyon, Red Tank Draw, Spring Creek, and Harden Cienega Creek.

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

Results: In 2021, all Loach Minnow and Spikedace populations at ARCC spawned. ARCC produced 1,043 Aravaipa Creek Spikedace, 914 upper Gila River Spikedace, 203 Gila River Forks Spikedace, 919 Blue River Loach Minnow, 504 Aravaipa Creek Loach Minnow, 541 San Francisco River Loach Minnow, 196 Bear Creek Loach Minnow and 0 Gila River Forks Loach Minnow³

GENERAL ACTIVITIES

Department staff administered and managed Program projects identified in the agreement. 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. Department staff finalized the 2020 annual report, began analyzing data and drafting the 2021 annual report, and drafted the 2022 annual work plan, and Environmental Assessment Checklists. Staff coordinated with intra-agency staff, other agencies, and private landowners to continue work on existing projects and to develop potential new projects. The Program specialist also hired new seasonal staff and a replacement for the Program specialist.

¹ Including populations previously classified as Gila Chub.

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

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

PRIORITY ACTIONS

General Methods

Fish Stockings: The Department coordinates with USFWS about locations to stock and sources and lineages of fish to use. Fish for translocations were collected, transported, and stocked according to Department fish collection, transport, and stocking protocols (best management practice #4; AGFD 2011), and Hazard Analysis and Critical Control Point (HACCP) practices. Fish were collected from select waters inhabited by target lineages. Fish were collected 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, the fish were transferred from the transport cooler back to aerated 5-gallon buckets and carried to the stocking location. Water quality characteristics in the buckets and the stocking location were measured. Conductivity (μS), salinity (mg/L), total dissolved solids (mg/L), pH, and water temperature ($^{\circ}\text{C}$), 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 were within two degrees of the stream. Fish were sorted a final time to verify species identity, assess condition, and determine a final count before being released into the stream.

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

Fish Surveys: Backpack electrofishing was used at 100-m sub-reaches to survey translocated populations of Spikedace, Loach Minnow, and Roundtail Chub¹, and to assess habitats for fish translocations. The number of sub-reaches sampled was determined by length of target reach, with a minimum of three sub-reaches for short reaches and a goal of at least 10% of the reach length in longer streams (e.g., there were fifteen 100-m sub-reaches in the 14.6 km of the upper Blue River). A backpack electrofisher (Smith-Root; Model 12-B) was used to electrofish upstream through each sub-reach in a single pass. Stunned fish were netted with dip nets (tear-drop shaped, 0.43 m x 0.37 m with 2 or 3 mm mesh). At the upstream end of each major mesohabitat type (pool, run, riffle, or cascade) within each sub-reach, fish were processed and data were recorded. Captured fish were identified to species and counted. All Spikedace, Loach Minnow, and Roundtail Chub¹ were measured to the nearest millimeter in total length (mm TL). Other species were counted within two size classes for small bodied fishes (≤ 40 and >40 mm TL for Speckled Dace and Longfin Dace; <20 and ≥ 20 mm TL for Desert Pupfish and Gila Topminnow) and three size classes for

¹ Including chub populations previously classified as Gila Chub.

large bodied fish (<50, 50-100, and >100 mm TL; e.g. Desert Sucker, Smallmouth Bass). After processing, fish were released alive just downstream from where they were captured. Data recorded for each sampling effort included: site name, site location (GPS coordinates), length of site, date, time, participants, gear type, gear settings, gear dimensions, seconds shocked, species of fish captured, size class of fish, and counts of individuals within each species-size-class category.

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

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

Evaluation of Species Establishment: The goal of translocation efforts is to establish populations of Spikedace, Loach Minnow, Gila Topminnow, and Roundtail Chub¹ 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 1991) describes criteria for establishment with characteristics of abundance, age-class structure, and recruitment in the range of natural variation. To assess this goal, post-stocking monitoring data were collected for each 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 non-native fish species;
2. evaluate trends in relative abundance (estimated as catch-per-unit effort) of the translocated species, extant native fish species, and non-native piscivores;
3. evaluate size-structure of each population of fish species to detect reproduction and recruitment to the population;

¹ Including chub populations previously classified as Gila Chub.

4. determine if translocated species have dispersed outside of the stocking area.

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

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

Nonnative Piscivore Removal: Nonnative fishes were typically removed using traps and electrofishing. A variety of traps were used, depending on habitat size: mini-hoop nets (Promar® TR-502 collapsible traps; cylindrical, 0.85 m long x 0.3 m wide, with 9 mm mesh) and minnow traps (Promar® collapsible minnow traps; 0.46 m long x 0.3 m wide, with 2 mm mesh) baited with dry dog food (Gravy Train®). Traps were dispersed throughout the targeted reach and were primarily set in pools or runs that were more than 1-m deep. Traps were retrieved 2 to 22 hours later. For backpack electrofishing, typically the entire targeted reach was shocked, and any nonnative fish captured were removed. A single full pass is defined as electrofishing all water present from the downstream end to the upstream end of the target reach. An initial set of traps in the target reach is considered the first pass, with each reset within the same reach considered a subsequent pass.

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

¹ Including chub populations previously classified as Gila Chub.

goal is to suppress the nonnative population 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 will be examined, and a decrease in abundance of the target nonnative species to low levels or to zero will be evidence of control. Absence of target nonnative fishes confirmed by eDNA sampling is evidence of eradication.

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

Strategic Plan Goals:

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

Recovery Objectives:

- Spikedace recovery objective 6.3. Reintroduce Spikedace to selected reaches.
- Spikedace recovery objective 6.4. Monitor success/failure of reintroductions.
- Loach Minnow recovery objective 6.3. Reintroduce Loach Minnow to selected reaches.
- Loach Minnow recovery objective 6.4. Monitor success/failure of reintroductions.
- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 2.4 Protect habitats of reestablished or potential populations from detrimental nonnative aquatic species.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.
- Desert Pupfish recovery objective 2. Re-establish Desert Pupfish populations.
- Desert Pupfish recovery objective 5. Monitor and maintain natural, re-established, and refugia populations.
- Gila Chub draft recovery plan objective 1.3.1. Eliminate or control problematic nonnative aquatic organisms
- Gila Chub draft recovery plan objective 2. Ensure representation, resiliency, and redundancy by expanding the size and number of populations within Gila Chub historical range via replication of remnant populations within each RU.
- Gila Chub draft recovery plan objective 7. Monitor remnant, repatriated, and refuge populations to inform adaptive management strategies.

Background: The purpose of this action is to establish Spikedace, Loach Minnow, Gila Topminnow, and Desert Pupfish into various waters on the Muleshoe Ranch Cooperative Management Area (CMA). The Muleshoe Ranch CMA is located on the western slopes of the Winchester and Galiuro mountains. The various waters and stream reaches are described in Robinson et al. (2010), and Love-Chezem et al. (2015). Fish stockings began in 2007, when Spikedace and Loach Minnow were stocked into Hot Springs Canyon and Redfield Canyon; both species were again stocked into Redfield Canyon in 2008 and 2010. Both Spikedace and Loach Minnow failed to establish in Redfield Canyon. Gila Topminnow are established in Redfield

Canyon. In 2007, Roundtail Chub¹, Sonora Sucker, and Speckled Dace were translocated upstream of a waterfall in Redfield Canyon to expand their range in that system. Gila Topminnow were stocked into Bass Canyon in 2014, 2015, and 2018, and Double R Canyon in 2017 and 2018.

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

Results:

Nonnative Control. During April 28-29, 2021, Department staff completed the first Green Sunfish removal pass of the year in Redfield Canyon. The crew backpack electrofished Reaches 1 and 2, from the confluence with Swamp Springs (UTM 12S 563324/3588995) upstream to the waterfall barrier (563872/3589779; Figure 1). The crew electrofished for a total of 4,740 seconds and did not capture any Green Sunfish. Native fish were not counted and are typically counted during the June removal pass for comparison across years. The crew also set five mini-hoop nets in pools that have previously harbored the majority of the Green Sunfish in Reach 1. A total of five Roundtail Chub¹ and one Sonora Sucker were captured.

Department staff also completed one removal pass in Reach 3. The crew set 10 mini-hoop nets for two consecutive two hour sets and angled for Green Sunfish in the pools near the wilderness boundary. A total of 160 Green Sunfish were captured (47-196 mm TL) with 141 captured during the first set and 19 captured during the second set (Figure 2, Figure 3). An additional 10 Green Sunfish were captured by angling with fly rods while the traps soaked.

During June 9-10, 2021, Department staff removed Green Sunfish from Redfield Canyon by completing the second full electrofishing pass from the trail access in Reach 2 (562272/3588781) upstream to the waterfall barrier. Green Sunfish were not captured during 9,219 seconds of electrofishing. A total of 624 Roundtail Chub¹, 194 Sonora Sucker, 32 Gila Topminnow and 23 Longfin Dace were captured and returned to the stream. Department staff also set five mini-hoop nets in pools in Reach 1 where Green Sunfish have consistently been captured during previous removal efforts. The nets were retrieved after completion of electrofishing and a total of 13 Roundtail Chub¹ and 7 Sonora Sucker were captured with no Green Sunfish captured or observed in the pools.

A second Green Sunfish removal pass was also completed in Reach 3. Department staff set 10 mini-hoop nets for three consecutive 2-hour sets and angled for Green Sunfish in the pools near the wilderness boundary. A total of 132 Green Sunfish (63 – 183 mm TL) and one Sonora Sucker were captured in mini-hoop nets with 77 captured in the first set, 42 captured in the second set, and 13 captured in the third set (Figure 2, Figure 3). An additional 4 Green Sunfish were caught by angling with fly rods while traps soaked.

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

Overall, a total of 306 Green Sunfish were removed from Redfield Canyon in 2021 (Figure 2). Importantly, Green Sunfish were not captured in Reaches 1 or 2 for the first time since 2015, and only the second time since removal efforts began in 2007. It is unclear whether the absence of Green Sunfish can be attributed to the increased removal efforts in recent years or the lack of sufficient flow for dispersal this past year, but the combination of these factors seems to have resulted in successful suppression of Green Sunfish in the two upper reaches. While the total sunfish catch in Reach 3 declined this year, mean mini-hoop net catch per unit effort (6.00 fish/h) increased relative to 2020 (4.44 fish/h) which makes it difficult to assess the efficacy of removal efforts in this reach. One or two removal trips a year appears to be sufficient to effectively prevent Green Sunfish from reestablishing within Reaches 1 and 2 under current environmental conditions.

Monitoring of Repatriated Populations. On August 30, 2021, Department staff monitored Gila Topminnow in upper Bass Canyon. Eleven collapsible minnow traps were set in the vicinity of the most recent stocking location (572046/3579904) with traps being pulled after only an hour of soak time due to incoming thunderstorms. A total of 27 Gila Topminnow (8 < 20 mm TL, 19 ≥ 20 mm TL) and 38 Roundtail Chub¹ were captured. The crew also carried out seven dip net sweeps while traps soaked and captured an additional three Gila Topminnow and one Roundtail Chub¹. Bass Canyon continues to support a small population Gila Topminnow, despite the last stocking occurring in 2018. Topminnow catch during monitoring has remained relatively modest the last few years (2018 n = 3, 2019 n = 10, 2020 n = 53) with a relatively patchy distribution. The presence of juvenile fish and population persistence for multiple years indicates that this population is likely established, albeit at relatively low abundance. It is possible that the relatively low catch numbers during multiple surveys is due to low capture probability in this reach with relatively complex habitat, or perhaps the population of topminnow is truly small.

On August 30, 2021, Department staff monitored Gila Topminnow in Double R Canyon. Ten collapsible minnow traps were set from the confluence with Bass Canyon (571964/3579500) upstream to the most recent stocking location (571720/3579842). A total of 1 Gila Topminnow (1 ≥ 20 mm TL), 51 Roundtail Chub¹, and 9 Longfin Dace were captured. The crew also carried out two seine hauls and captured an additional 291 Gila Topminnow (213 < 20 mm TL, 78 ≥ 20 mm TL). There were few fish of any species in Double R Canyon upstream of the vicinity of the Bass Canyon confluence and it appeared that recent flooding may have pushed most of the fish downstream a few hundred meters. A total of 155 Gila Topminnow captured while seining were released a few hundred meters upstream at the most recent stocking location. Gila Topminnow catch has now increased with each year of monitoring since 2018 (2018 n = 0, 2019 n = 68, 2020 n = 218, 2021 n = 292). Gila Topminnow have been able to persist, reproduce, increase in abundance, and disperse within Double R Canyon since the most recent stocking in 2018, and the population should be considered established even though the total number of individuals captured has remained below 500.

¹ Chub in this location previously classified as Gila Chub.

Recommendations: Department staff will continue to contact the downstream private landowners in Redfield Canyon and attempt to gain permission to access the property and remove sunfish from Reach 3. If permission is granted, the goal of Green Sunfish removal efforts should shift from suppression to eradication, and the frequency and intensity of removal efforts should be increased. If the downstream landowners do not grant permission for access, eradication of Green Sunfish in Redfield Canyon will not be feasible. The current level of removal effort (1-2 removals per year) appears to be sufficient at suppressing the sunfish population in Reaches 1 and 2 and should be continued until the status of the downstream population changes.

The topminnow populations in both Bass Canyon and Double R canyon have persisted, increased in abundance and distribution, and meet our criteria for population establishment. Responsibility for monitoring the topminnow populations in Bass Canyon and Double R Canyon will be passed on to another program going forward.

Tables and Figures:

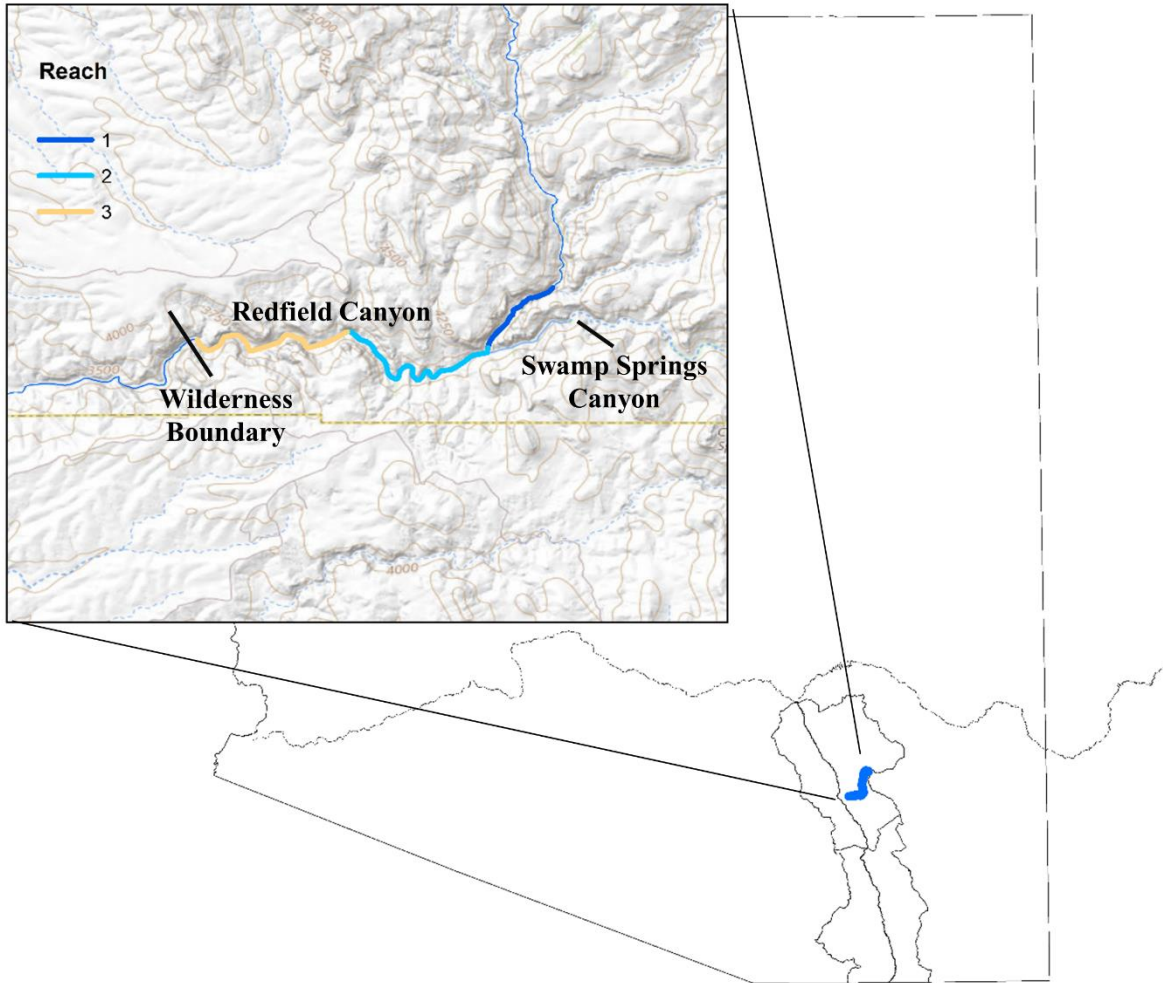


Figure 1.—Location of Redfield Canyon within the Gila River Basin and San Pedro River sub-basin. Inset map shows the location of sampling Reaches 1 (Swamp Springs Confluence upstream to Barrier), 2 (Rock House tributary upstream to Swamp Springs Confluence), and 3 (Wilderness Boundary upstream to Rock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

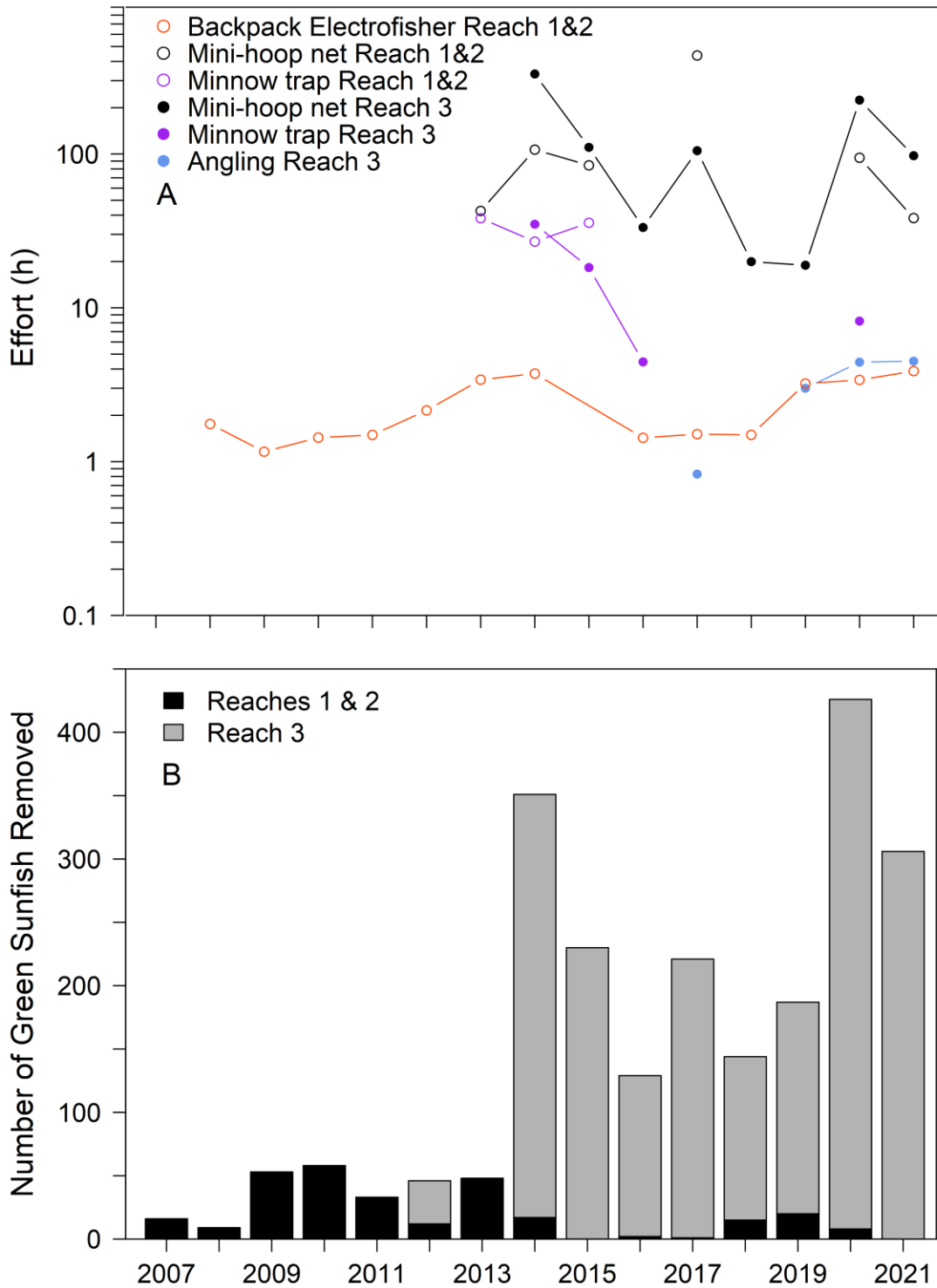


Figure 2.—Hours of removal effort by gear type and reach (A) and number of Green Sunfish removed during annual spring removal efforts and autumn monitoring from three reaches of Redfield Canyon, Arizona during 2007-2021 (B). Effort was not recorded for removals in 2007. Location and description of reaches within Redfield Canyon shown in Figure 1.

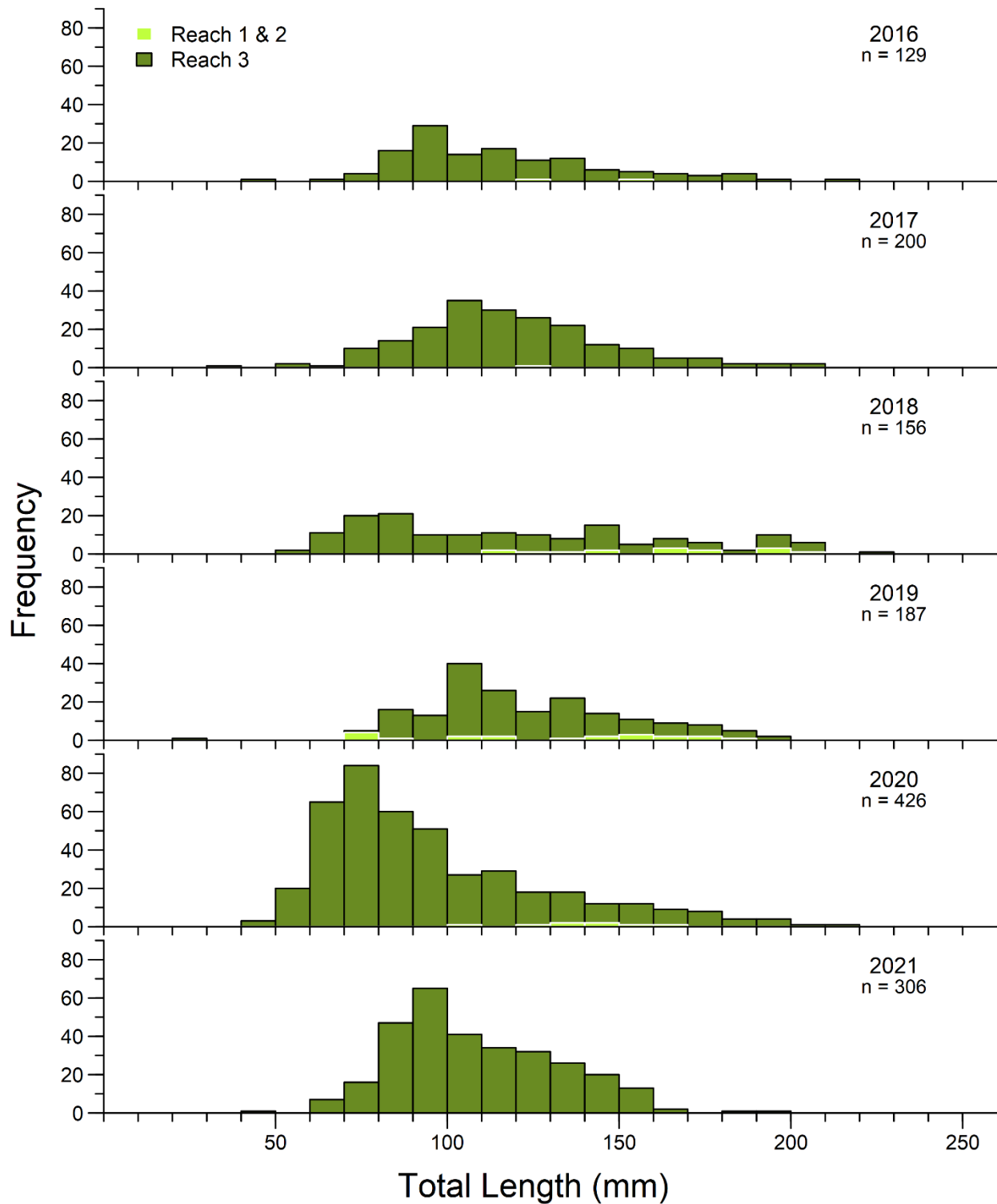


Figure 3.—Length frequency distribution of the number of Green Sunfish captured by reach during removal efforts and annual monitoring in Redfield Canyon, 2016 through 2021. Number of fish captured and measured each year is shown in the top right corner of each panel.

Gila Topminnow stockings (Task AZ-2002-1)

Strategic Plan Goals:

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

Recovery Objectives:

- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.
- Desert Pupfish recovery objective 2. Re-establish Desert Pupfish populations.
- Desert Pupfish recovery objective 5. Monitor and maintain natural, re-established, and refugia populations.
- 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. Desert Pupfish are sometimes stocked into the same sites because the species utilize similar habitats. Populations of Roundtail Chub¹ 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 a minimum of 10 baited minnow trap sets per site, often supplemented with dip netting or seining if habitat conditions allow.

¹ Populations of Roundtail Chub addressed by this project were formerly classified as Gila Chub.

Fish Health Assessments During 2021:

Walnut Spring #392. On February 8, 2021, Department staff collected 60 Gila Topminnow from Walnut Spring #392 for a fish health assessment. The fish were captured using a combination of minnow traps and dip net sweeps. All fish were transported back to the fish health laboratory at Department headquarters. Subsequent analyses determined the fish were free of parasites or pathogens.

Sites Monitored or Stocked During 2021:

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

Black Canyon City Heritage Park Pond

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

Western Mosquitofish and tilapia were illegally stocked into the pond, and in autumn 2016 the Department and Black Canyon City decided to drain and dry the pond to eliminate the nonnative fish. Before the drawdown, Desert Pupfish were salvaged from the pond and held overwinter at the Department headquarters. Gila Topminnow were not salvaged because of the close similarity to Western Mosquitofish. The pond was drained, left to dry for several weeks, and refilled. In March 2017, Department staff stocked 122 of the salvaged Desert Pupfish into Black Canyon City Heritage Park Pond. In November 2017, Department staff monitored the pond and captured 622 Desert Pupfish and 3 American bullfrogs (tadpoles) in collapsible minnow traps and seine hauls. In June 2018, Department staff collected 734 Sharp Spring lineage Gila Topminnow from Stop Sign Pond on Robbins Butte Wildlife Area and translocated the fish to Black Canyon City Heritage Park Pond. During follow up monitoring in August 2018, a total of 504 Desert Pupfish and 1,427 Gila Topminnow were captured. In August 2019, a total of 5,338 Gila Topminnow and 1,164 Desert Pupfish were captured. In July 2020, a total of 30 Gila Topminnow and 2 Desert Pupfish were captured

Results: On June 29, 2021, Department staff monitored Gila Topminnow and Desert Pupfish in Black Canyon City Heritage Park Pond. The crew set 10 minnow traps for a minimum soak time of two hours and captured a total of 479 Gila Topminnow (325 < 20 mm TL, 154 ≥ 20 mm TL) and 235 Desert Pupfish (63 < 20 mm TL, 172 ≥ 20 mm TL). The crew also carried out seven dip net sweeps and captured an additional 16 Desert Pupfish and 19 Gila Topminnow. Catch of Gila

Topminnow and Desert Pupfish suggests that populations rebounded from the apparent crash in 2020. Bullfrog tadpoles were abundant during sampling.

Recommendations: The Gila Topminnow and Desert Pupfish populations have persisted without augmentation in Black Canyon City Heritage Park Pond since 2018, and can now be considered established at this location. Augmentation of both populations with additional fish is recommended due to the low numbers of fish captured last year and the possible population bottleneck that occurred. Future monitoring can be transferred to another Department program.

Edgar Canyon

Background: Edgar Canyon is a tributary of the San Pedro River that originates near Mount Bigelow in the Santa Catalina Mountains. Edgar Canyon is primarily ephemeral but has a few short intermittent and perennial reaches. The furthest downstream perennial reach is located on Pima County lands approximately 5 km upstream of the confluence with the San Pedro River. This perennial reach was approximately 300 m long in September, 2019. Habitat in Edgar Canyon was determined to be suitable for Gila Topminnow in February, 2019. In April 2019, Department and Pima County staff stocked 564 Gila Topminnow (Redrock Canyon lineage) into Edgar Canyon (UTM 12S 543140/3590495). Previous monitoring efforts in September 2019 and September 2020 resulted in the capture of a total of 802 and 1,113 topminnow respectively. The Bighorn Fire burned a portion of the upper Edgar Canyon watershed in May and June, 2020.

Results: On August 31, 2021, Department staff monitored Gila Topminnow in Edgar Canyon. The crew set eight minnow traps in all available pool habitats throughout the perennial reach for a minimum soak time of two hours. The crew also carried out 13 dip net sweeps and 2 seine hauls. No fish were captured or observed during the survey. Several lowland leopard frogs and one Sonoran mud turtle were observed, indicating that some aquatic wildlife was able to persist through the flooding.

It was evident that substantial flooding occurred in Edgar Canyon during the 2021 monsoon season. Flood debris was observed at least 3 vertical meters above the base flow channel. It also appeared that there were multiple flood events, in which initial floods scoured out most of the grasses and cattails that previously constrained the channel and later floods deposited mostly sandy sediment in locations that were formerly pools. Burned wood was apparent in the flood debris, but the deposited sediment was not as black and ashy as other post-fire flood events. The absence of topminnow and evidence of extraordinary flooding suggest that the topminnow population may be extirpated.

Recommendations: Edgar Canyon should be revisited in 2022 to determine whether aquatic habitat has recovered sufficiently or if more time is necessary before considering restocking Gila Topminnow. This location was on a promising trajectory prior to the fire and subsequent flooding and it would be valuable to attempt another stocking of Gila Topminnow at this location once the watershed and stream habitat are able to recover.

La Barge Spring

Background: La Barge Spring is located in La Barge Canyon near the confluence with Bluff Spring Canyon. Nonnative guppies were detected in the spring box and several small pools downstream in 2017. The guppies were eradicated from the spring box in 2018 by diverting water from the spring box and manually pumping it dry. A total of 154 Gila Topminnow were stocked in La Barge Spring in April 2019. Nine topminnow were captured during the first monitoring effort in October 2019 and 26 were captured in October 2020. The Sawtooth Fire burned over the spring box in 2020.

Results: On November 4, 2021, Department staff monitored Gila Topminnow at La Barge Spring. The crew carried out four dip net sweeps in the spring box but failed to capture or observe any fish. The spring box is now mostly filled in with sediment, consistent with expectations following impacts from the Sawtooth Fire in 2020. The spring box had an average depth of 2.5-5 cm with a maximum depth of about 10 cm, so it is unlikely that fish were present and not observed.

Recommendations: The spring was stocked primarily for mosquito control and to prevent the restocking of nonnative fishes. Restocking of Gila Topminnow is not recommended unless conditions improve at the spring in the future. Additional post-stocking monitoring of the spring is not recommended due to the relatively small size of the spring and the high likelihood that the population is extirpated.

Las Cienegas National Conservation Area - Bill's Wildlife Pond

Background: Bill's Wildlife Pond is located in the Gardner Canyon drainage about 2.1 km upstream of the confluence with Cienega Creek. Bill's Wildlife Pond was initially stocked with 841 Gila Topminnow (Cienega Creek lineage) in 2016. Only 18 Gila Topminnow were captured during the first monitoring in 2017, and the population was augmented with an additional 636 topminnow later in the year. In May 2018, Department staff translocated 190 Gila Topminnow from Clyne Pond into Bill's Wildlife Pond as part of a salvage effort. Only five topminnow were captured during monitoring in August 2018, but captures increased to 519 individuals in 2019, and 3,858 in 2020.

Results: On August 2, 2021, Department staff monitored the Gila Topminnow population in Bill's Wildlife Pond. Ten minnow traps were set for a minimum soak time of two hours and a total of 3,457 Gila Topminnow (679 <20 mm TL, 2,778 ≥ 20 mm TL) were captured.

Recommendations: The Gila Topminnow population in Bill's Wildlife Pond appears to be established, with several thousand fish captured in each of the last two years. Because the last augmentation occurred in 2018, this population can now be considered established. Future monitoring can be transferred to another Department program or the Bureau of Land Management.

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.

Results: On April 21, 2021, Department staff translocated 248 Gila Topminnow from Empire Tank to Maternity Wildlife Pond. There were two mortalities during transport and stocking. Topminnow were in good condition and behaving normally at the time of release.

On August 2, 2021, Department staff monitored the Gila Topminnow population in Maternity Wildlife Pond. Ten minnow traps were set for a minimum soak time of two hours and a total of 1,554 Gila Topminnow (361 <20 mm TL, 1,139 ≥ 20 mm TL) were captured.

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

Las Cienegas National Conservation Area – Spring Water Wetland

Background: Spring Water Wetland is located just east of Cienega Creek about 0.4 km upstream of the confluence with Spring Water Canyon. Gila Topminnow were first stocked in Spring Water Wetland in 2013 and the population was determined to be established in 2016, with over 10,000 individuals captured during the final monitoring effort. In June of 2017, Department and USFWS staff salvaged 85 Roundtail Chub¹ from Cienega Creek and stocked them into Spring Water Wetland due to concerns about potential post-fire effects from the Sawmill Fire. Catch of Roundtail Chub¹ has consistently declined since 2018 (2018, n = 71; 2019 n = 40; 2020 n = 1). Bureau of Land Management staff reported that Spring Water Wetland was nearly dry during fall 2020 due to ongoing drought conditions.

Results: On August 3, 2021, Department staff set 10 mini-hoop nets for a minimum soak time of two hours and failed to capture any Roundtail Chub. Gila Topminnow were visually abundant and seemed to have rebounded well from the drought conditions.

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

Recommendations: Monitoring of Roundtail Chub¹ should be considered complete because chub were last stocked in 2017, and the population has steadily declined since 2018. Additional stocking of Roundtail Chub into Spring Water Wetland is not recommended because of the apparent susceptibility of this location to extraordinary drought and the lack of suitable conditions for Roundtail Chub.

Mud Spring-Coronado National Forest.

Background: Mud Spring is located on the southwest slope of the Huachuca Mountains in the Sycamore Canyon drainage within the upper San Pedro River drainage on the Coronado National Forest. The pond is located at 1,700 m elevation, has a surface area of about 255 m² and is about 2 m deep in the middle. Despite this relatively high elevation, winter water temperatures seem to be moderated by spring inputs and the south facing orientation of the pond. Vegetation (primarily sedges) line the perimeter of the pond and *Chara sp.* covers most of the bottom in the open water areas. The pond is occupied by Chiricahua leopard frog and is slated to be a Northern Mexican gartersnake translocation site. A total of 494 Sharp Spring lineage Gila Topminnow were translocated in August, 2018. Totals of 4,201 and 2,956 topminnow were captured during August 2019 and August 2020, respectively.

Results: On August 4, 2021, Department staff monitored Gila Topminnow in Mud Spring. Ten minnow traps were set for a minimum soak time of two hours and a total of 815 Gila Topminnow (429 < 20 mm TL, 386 ≥ 20 mm TL) were captured.

Recommendations: The Gila Topminnow population in Mud Spring appears to be established, with at least 800 fish captured during each monitoring effort. Because the last stocking occurred in 2018, this population can now be considered established. Future monitoring can be transferred to another Department program.

Milagrosa Canyon

Background: Milagrosa Canyon is a tributary to Agua Caliente Canyon that drains south from the Santa Catalina Mountains. Milagrosa Canyon was identified as a location that potentially had enough perennial aquatic habitat to support native fish conservation activities by Coronado National Forest staff. The extent of surface water and the fish community composition in the lower reaches were unknown and needed to be investigated before any potential conservation actions could take place.

Results: On April 21, 2021, Department staff assessed aquatic habitat in Milagrosa Canyon. The crew walked the stream channel from near the confluence of Milagrosa and Agua Caliente Canyons (UTM 12S 526715/3573397) upstream about 3 km (528834/ 3574623). Fewer than 30 meters of surface water were encountered within the surveyed reach. There were numerous fish barriers within the first kilometer of stream, as the channel passes through a high gradient boulder field. The two most downstream pools were also the largest and were located between a series of

large waterfalls (527325/ 3573834). The crew angled in the most downstream pools for about five minutes but did not capture or observe any fish. The surface of the pools were covered with numerous dead insects which is additional evidence that insectivorous fish like, Green Sunfish, were likely not present. The remaining four pools encountered were all very shallow (<0.5 m deep) and small (<3 m length), and likely dried before the onset of monsoon season.

Recommendations: There may be sufficient habitat to support a very small population of chub in the waterfall pools. Additional surveys would likely be required later in the year (May-June) to verify that perennial water exists year round. However, because of the limited potential and size of this location, it should not be a priority for this program to pursue any native fish projects in Milagrosa Canyon at this time.

Peterson Ranch Pond.

Background: Peterson Ranch Pond is located in Scotia Canyon (tributary to the Santa Cruz River in the San Rafael Valley) at 1,892 m elevation in the Coronado National Forest. The pond is about 670 m², has a maximum depth of about 3 m, and is fed by a spring which moderates winter water temperatures. The pond is surrounded by fencing to exclude livestock. Chiricahua leopard frogs, Longfin Dace (stocked in 2015), and Northern Mexican gartersnakes (introduced in 2018) also inhabit the pond. A total of 762 Gila Topminnow (Sharp Spring lineage) were translocated from Robbins Butte Stop Sign Tank and Swimming Pool Tank in August, 2018. Totals of 47 and 302 topminnow were captured during August 2019 and August 2020 respectively.

Results: On August 4, 2021, Department staff monitored Gila Topminnow in Peterson Ranch Pond. Ten minnow traps were set for a minimum soak time of two hours and a total of 613 Gila Topminnow (469 < 20 mm TL, 144 ≥ 20 mm TL) and 29 Longfin Dace were captured. Both size classes of topminnow were again visually more abundant than the capture data suggests.

Recommendations: The Gila Topminnow population in Peterson Ranch Pond appears to be established, with an increasing number of fish captured during each successive monitoring effort. Because the last stocking occurred in 2018, this population can now be considered established. Future monitoring can be transferred to another Department program.

Sabino Canyon

Background: Sabino Canyon is located northeast of Tucson, Arizona within the Coronado National Forest and Sabino Canyon Recreation Area. Sabino Canyon is a tributary to the Santa Cruz River and drains the Santa Catalina Mountains, flowing southwest to its confluence with Tanque Verde Wash in Tucson. Sabino Canyon was chemically treated in 1999 to remove nonnative Green Sunfish, and afterwards was stocked with salvaged Roundtail Chub¹ (Ehret and Dickens 2009).

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

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 (UTM 12S 520836/3581045). In October, 2019, Department staff collected 527 Gila Topminnow in three seine hauls from the pools immediately downstream of Sabino Dam. The fish were translocated to Sabino Canyon upstream of the confluence with East Fork Sabino Canyon (520784/3581144). A total of 350 Gila Topminnow were successfully stocked. No Gila Topminnow were detected during monitoring in May, 2020. A total of 15 Roundtail Chub¹ were captured during monitoring in May, 2020. The Bighorn Fire burned a substantial portion of the Sabino Canyon drainage in 2020.

Results: On April 20, 2021, Department staff monitored Gila Topminnow and Roundtail Chub in Sabino Canyon upstream of the East Fork of Sabino Canyon. The crew set 10 minnow traps in the vicinity of the topminnow stocking location and failed to capture or observe any topminnow. Topminnow have never been captured at this location following stockings in June, 2018 and October, 2019.

The crew also set 10 mini-hoop nets in the vicinity of the Roundtail Chub stocking location and captured a total of 10 chub. While the traps soaked the crew carried out four opportunistic seine hauls and captured eight additional chub. All fish captured were less than 100 mm TL, which is additional evidence that reproduction has occurred since the initial stocking in 2019 (Figure 4). Roundtail Chub were visually more abundant than the catch suggests. The crew also snorkeled through the pool where chub were initially stocked and observed 52 chub with multiple age classes present, including large adults.

The Bighorn Fire burned down to south/west bank of the stream and there visually appears to be more silt and fine substrate than during past surveys; however, the chub still seem to be surviving, reproducing and increasing in abundance despite these impacts.

Stream temperatures were generally higher this year than 2020, with an overwinter minimum temperature of 6.4°C. However, the temperature logger was partially buried in sediment when it was retrieved, and it appears that monsoon flooding in mid-August, 2020 may have contributed

¹ Chub in this location previously classified as Gila Chub.

the sediment, as daily fluctuations in temperature were greatly reduced for several months afterward (Figure 5).

Recommendations: Sabino Canyon upstream of the confluence with the East Fork still appears to have good potential for establishing a population of Gila Topminnow, but additional stockings probably should not occur until after the watershed has had more time to recover from the Bighorn Fire. The topminnow and chub populations should be monitored until at least 2022 and 2024, respectively. Roundtail Chub¹ were also stocked just downstream in 2019 and consideration should be given to translocating Roundtail Chub¹ further upstream to Hutch's Pool near West Fork Sabino Canyon. The Department also recommends a hike-through survey from the pools near East Fork Sabino down to The Crack to determine if any chub or topminnow have dispersed downstream and occupied the pools between the upper and lower stocking locations.

Tortilla Creek

Background: Tortilla Creek is located within the Salt River Drainage in the Tonto National Forest and flows into Canyon Lake near Tortilla Flat, AZ. Tortilla Creek has an established population of Gila Topminnow in the downstream reach of the creek near Tortilla Flat. Gila Topminnow in the lower reach of Tortilla Creek likely originated from a population stocked in 1982 in Mesquite Tank #2 (above Unnamed Drainage #68-B). A valve on the dam of Mesquite Tank #2 was opened, allowing it to drain and completely dry out. As a result, Gila Topminnow washed downstream and established a population in Unnamed Drainage #68-B and later dispersed into perennial pools in lower Mesquite Creek and lower Tortilla Creek. Due to the steep gradient and multiple waterfall barriers, Gila Topminnow never dispersed upstream into the upper perennial section of Tortilla Creek (about 4.3 km upstream of the confluence with Mesquite Creek). In March 2016, Department staff assessed habitat in the upper section, and deemed it suitable for Gila Topminnow. The only fish species present in the upper perennial section was nonnative Fathead Minnow, which is thought to have few negative interactions with Gila Topminnow. In June, 2017, Department staff stocked 548 Gila Topminnow (Peck Canyon lineage) into upper Tortilla Creek about 4.5 km upstream of the confluence with Mesquite Creek. A total of 829 Gila Topminnow were captured during the initial monitoring in November, 2017. During monitoring in 2018, a total of 2,020 Gila Topminnow and 65 Fathead Minnow were captured. Only 47 topminnow were captured in 2019. The Woodbury Fire began in June 2019 and burned 123,875 acres of the Superstition Mountains including the upper Tortilla Creek watershed. Evidence of substantial flooding in Tortilla Creek with some debris up to five vertical meters above the water surface was documented near the stocking location during monitoring in 2019, and likely contributed to the decline in catch at this location in 2019. The population was augmented with 374 topminnow in April, 2020 and a total of 322 topminnow were captured during monitoring in October, 2020.

Results: On October 26, 2021, Department staff monitored Gila Topminnow in Tortilla Creek near the original stocking location (UTM 12S 467239/3708608). The crew set 10 minnow traps for a minimum soak time of two hours and captured a total of 2,245 Gila Topminnow (959 <20 mm TL,

1,286 \geq 20 mm TL). The crew also carried out 14 opportunistic dipnet sweeps and captured an additional 145 Gila Topminnow (89 $<$ 20 mm TL, 56 \geq 20 mm TL). Gila Topminnow were captured and observed in pools farther downstream than in the past, which suggests that topminnow may be able to colonize more perennial pools downstream. Nonnative Fathead Minnow were not captured for the third consecutive year and may be extirpated from this location. More topminnow were captured at Tortilla Creek in 2021 than any previous year of monitoring efforts.

Recommendations: The population seems to be recovering from post-fire impacts of the Woodbury Fire, with evidence of reproduction, increasing abundance, and dispersal beyond the original stocking location. The Gila Topminnow population in upper Tortilla Creek should be monitored until at least 2022 to determine if the population is established since the population was augmented in 2020.

Telegraph Canyon

Background: Telegraph Canyon is a tributary to Arnett Creek and drains from the north side of Picketpost Mountain. In 1992, the Department, Tonto National Forest, and USFWS identified an opportunity to reestablish a native fish community in Arnett Creek and its tributary Telegraph Canyon. In the late 1990s, a fish barrier was built, the stream was chemically treated to remove nonnative fishes, and native fish were stocked. Unfortunately those fish did not establish populations, likely because too few were stocked and drought greatly reduced the amount of perennial water in the system. The partners re-evaluated the stream in 2007, and determined that the small amount of habitat was probably only suitable for Longfin Dace and Gila Topminnow. Longfin Dace were stocked in 2007 and established a population in Telegraph Canyon. During 2010-2015, Department staff surveyed the few tanks and potential perennial reaches upstream of the proposed stocking locations and did not detect any nonnative fish. In May 2017, a total of 522 Gila Topminnow were stocked into Arnett Creek just downstream of the Telegraph Canyon confluence. Only 74 Gila Topminnow were captured during post-stocking monitoring of Arnett Creek in October, 2017, with six captured in 2018 and none detected in 2019.

Results: On March 8, 2021, Department staff visited Telegraph Canyon to assess stream habitat for Gila Topminnow. The crew wet/dry mapped Telegraph Canyon from the confluence with Arnett Creek (UTM 12S 487288/3680525) upstream to the upstream extent of the riparian area (486760/3679608; Figure 6). Approximately 700 meters of surface water was present within this reach, with a few short reaches where surface water was absent. At least eight pools with a maximum depth of at least 0.5 m were documented. Five opportunistic dip net sweeps resulted in the capture of one adult Longfin Dace. Relatively few Longfin Dace ($<$ 50) were observed, consisting of mostly adults restricted to a few of the most downstream pools. The continued presence of Longfin Dace in Telegraph Canyon suggested that perennial water has been present since at least 2007, when Longfin Dace were initially stocked. Additionally, the relatively low abundance of Longfin Dace indicated that there would likely be limited initial competitive interactions between dace and topminnow.

On May 20, 2021, Department staff translocated 389 Redrock Canyon lineage Gila Topminnow from Walnut Spring #392 and one other location to the perennial portion Telegraph Canyon. Fish were stocked into the two deepest pools present within the perennial reach (487127/3680070; 486993/3679924). A total of 243 fish were collected from Walnut Spring #392 earlier in the day with five mortalities during transport. The remaining fish were collected from the other donor location a week prior and treated with an anti-parasitic before stocking because intestinal fluke were detected during the fish health assessment process. There was some expected mortality between the long holding time (9 days) and stress from treatment with a total of 151 fish stocked out from this location.

On October 28, 2021, Department staff monitored Gila Topminnow in Telegraph Canyon. The crew set 13 collapsible minnow traps from the downstream end of surface water (487203/3680205) up to the most upstream topminnow stocking location (486993/3679924) and captured a total of 398 Gila Topminnow (61 <20 mm TL, 337 ≥ 20 mm; Figure 6). The crew also carried out 28 dip net sweeps and captured an additional 165 Gila Topminnow (128 <20 mm TL, 37 ≥ 20 mm TL; Figure 6). Topminnow were present in virtually all surface water present downstream of the original stocking site, and even dispersed upstream about 100 m. A few Longfin Dace were observed just upstream of the stocking location. Lowland leopard frog tadpoles and adults were also abundant throughout the surveyed reach. The topminnow population managed to disperse within the stream, reproduce, and increase in numbers despite drought conditions at the time of stocking, the Telegraph Fire burning within the watershed in June, and some severe flooding during the monsoon season. All indicators are looking positive for the Gila Topminnow population in Telegraph Canyon at this time.

A few of the most downstream pools were isolated, very shallow, and appeared to be at risk of drying in the near future, so 128 of the topminnow captured in the pools were transported upstream to a pool at the most upstream extent of the perennial reach that was about 1.5 m in depth (486783/3679627). This pool was not initially stocked with topminnow because the maximum depth was only 0.58 m during the habitat assessment effort in March. The pool appears to have substantially increased in depth due to scouring from post-fire flooding. Moving these fish should extend the range of topminnow in Telegraph Canyon by about 350 m.

Following the monitoring effort in Telegraph Canyon, the crew walked downstream and carried out three dip net sweeps in Arnett Creek near the 2017 topminnow stocking location, and captured 21 Gila Topminnow (19 <20 mm TL, 2 ≥ 20 mm TL). This is the first time that topminnow have been captured in Arnett Creek since 2018 when it is believed that the original population failed due to drought. It appears that topminnow stocked in Telegraph Canyon may have dispersed downstream and colonized suitable habitats in Arnett Creek.

There appeared to be some substantial flooding in Telegraph Canyon, but with the exception of a few pools at the downstream end of the perennial reach that appeared to fill mostly with sand, flood flows mostly scoured out and increased the depth of pools. Limited post-fire evidence was

present in Telegraph Canyon mostly in the form of larger burnt logs without much if any ash or fine sediment present. Arnett Creek had much more post-fire debris present and seemed to have experienced flood flows of much greater magnitude than Telegraph Canyon. In general it seems like there was more deposition of sand and gravel in Arnett than scouring. Regardless, the fish seem to have persisted through the first (and hopefully worst) wave of post-fire impacts in both streams.

Recommendations: The Telegraph Canyon Gila Topminnow population should be monitored until 2024 to determine if the population is established. It would also be beneficial to monitor the perennial pools in Arnett Creek during future Telegraph Canyon monitoring efforts to determine whether topminnow will establish in both streams.

Tables and Figures:

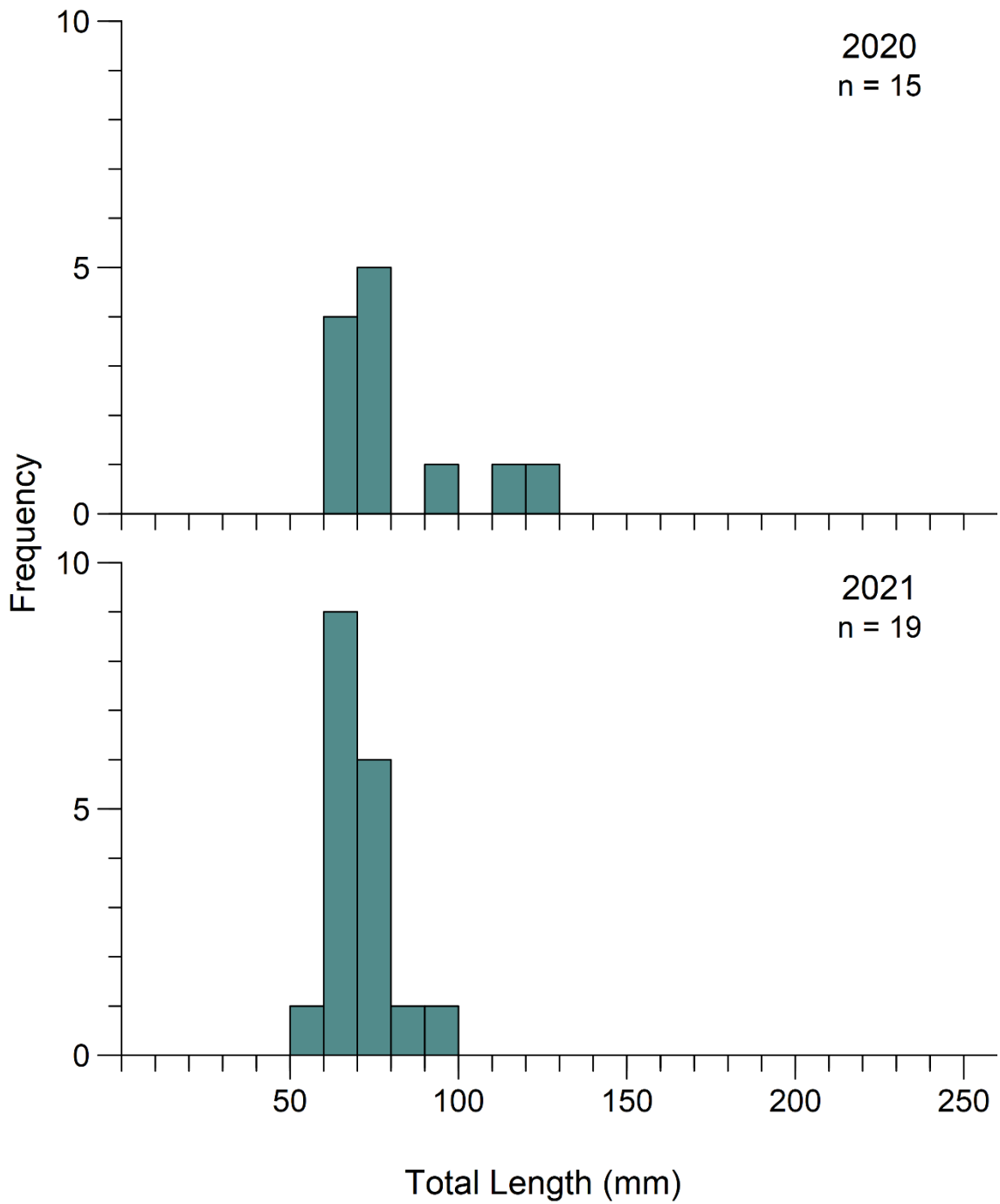


Figure 4.—Length frequency distributions of the number of Roundtail Chub captured during annual monitoring in Sabino Canyon upstream of the confluence with East Fork Sabino Canyon, 2020 through 2021. Number of fish captured and measured each year is shown in the top right corner of each panel.

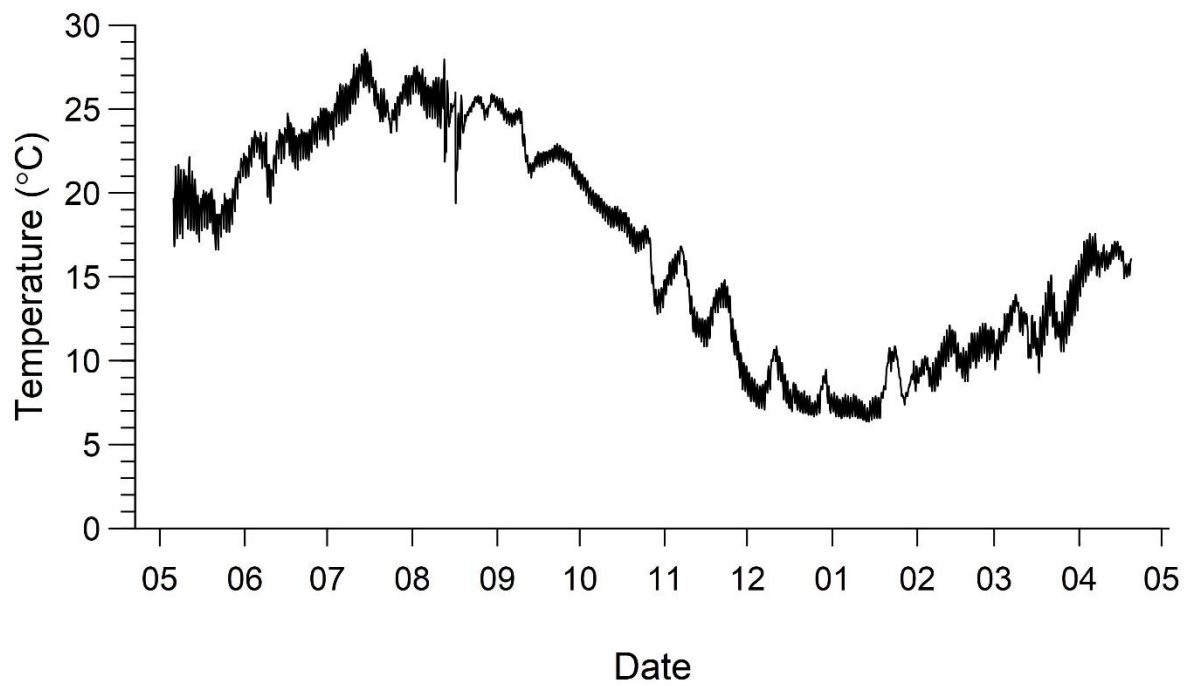


Figure 5.—Plot of stream temperature in Sabino Canyon at the Gila Topminnow stocking location upstream of the confluence with East Fork Sabino Canyon from May 6, 2020 to April 19, 2021.

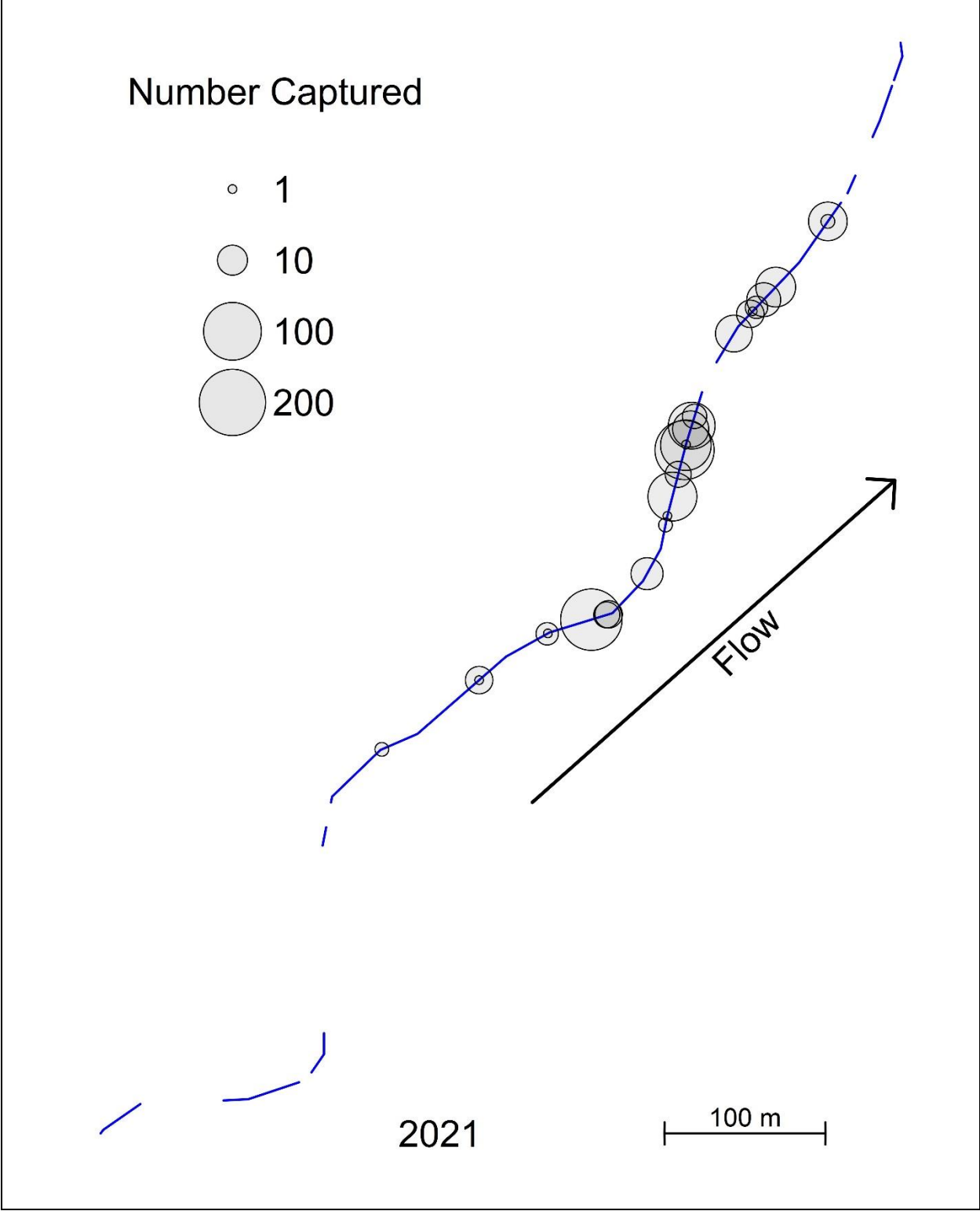


Figure 6.—Location and number of Gila Topminnow captured in Telegraph Canyon during annual monitoring on October 28, 2021. The blue line represents the perennial portion of Telegraph Canyon during the site visit on March 8, 2021.

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

Strategic Plan Goals:

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

Recovery Objectives:

- Spikedace recovery objective 6.2.5 Reclaim as necessary to remove non-native fishes.
- Spikedace recovery objective 6.3. Reintroduce Spikedace to selected reaches.
- Spikedace recovery objective 6.4. Monitor success/failure of reintroductions.
- Gila Topminnow 1999 draft revised recovery plan objective 2.2. Reestablish Gila Topminnow in suitable habitats following geographic guidelines.
- Gila Topminnow 1999 draft revised recovery plan objective 3. Monitor natural and reestablished populations and their habitats.
- Gila Chub draft recovery plan objective 1.3.1. Eliminate or control problematic nonnative aquatic organisms.
- 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 Chub¹, Speckled Dace, Longfin Dace, Sonora Sucker, Desert Sucker, and Northern Mexican gartersnake. A small diversion dam about 0.95 km upstream from the confluence with Oak Creek seemingly prevented most nonnative fishes from invading upstream, but there are records from the 1970s and 1980s of Smallmouth Bass and Fathead Minnow. Green Sunfish were detected below the diversion dam in 2011, and in May 2014 Green Sunfish were captured 2.5 km above the dam. Department staff began removal efforts immediately and completed seven removals during the summer of 2014, after which the Department's Conservation and Mitigation Program staff assumed responsibility of the removal efforts above the diversion dam. Reclamation finished construction of a fish barrier about 1.1 km upstream from Oak Creek in April 2015. Gila Topminnow were initially stocked in 2015 and were considered established above the barrier by 2019. In May 2015, 221 Spikedace (Aravaipa Creek lineage) were stocked above the barrier. Only three Spikedace were captured during the initial monitoring effort in 2015, and none were captured in 2016, so the population was augmented with 67 individuals in October, 2016. Spikedace captures increased to 11 individuals in 2017 and the population was augmented in February 2018

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

with 1,076 Spikedace as part of an eDNA research study. Spikedace captures increased again to 20 during annual monitoring in 2018 and an additional 500 Spikedace were stocked in December, 2018. Spikedace captures increased to 36 fish during the first pass in 2019, but declined to 17 in 2020. The first evidence of natural reproduction was documented in 2018 with more reproduction occurring in 2019. In 2020, ARCC staff and Kansas State Researchers released 101 PIT tagged fish into Spring Creek as part of an ongoing study on Spikedace and Loach Minnow survival and movement. Most of the Spikedace captured during monitoring in 2020 were PIT tagged before being released.

Results: On September 9, 2021, Department staff monitored Spikedace in Spring Creek. The crew targeted Spikedace by electrofishing one fixed 100-meter reach and two randomly selected 100-meter reaches. A total of five Spikedace were captured during the initial pass at each site, which is fewer than one-third the number of fish captured during first pass efforts in 2020 (Figure 7). Mean size of Spikedace captured was 68.0 mm TL (min = 61, max = 76; Figure 8). Both the decrease in catch and the increase in mean size compared to 2020 highlight that little to no reproduction occurred in Spring Creek in 2021. The absence of juvenile fish for two consecutive years further reduces the chances that the Spikedace population will establish without any additional stockings. In addition to Spikedace, 141 Roundtail Chub¹, 28 Desert Sucker, 78 Longfin Dace, and 185 Speckled Dace were captured during electrofishing (Table 1).

Three pass depletion electrofishing was carried out at the fixed site with block nets set at the downstream and upstream ends of the 100-m reach. A total of two Spikedace were captured during the two additional passes. Estimated abundance of Spikedace using a Carle-Strub method was 5 fish per hundred m with an estimated capture probability of 0.62 (Table 2; Carle and Strub 1978). The decrease in estimated abundance (2020 n = 21) suggests that true abundance of Spikedace in Spring Creek likely has continued to decline since 2019.

Of the seven Spikedace captured, four were PIT tagged with two tagged at ARCC and stocked in 2020, one tagged at ARCC and stocked in 2021 and one wild fish tagged in Spring Creek in 2020. Although relatively few fish were captured in 2021, the tag data suggests that Spikedace have inter-annual survival and that some fish are persisting that were not tagged or stocked in 2020 or 2021. The practice of tagging hatchery Spikedace prior to their release has improved understanding of post-stocking survival and movement of Spikedace and will allow continued identification of wild fish from hatchery fish.

An exceptional flood occurred in Spring Creek during the monsoon season which caused substantial changes to the stream channel with many large adult trees uprooted, changes to habitat types, some channels rearranged, and some previous pools entirely filled in. A standardized habitat survey was carried out at the fixed site at Willow Point Road in 2019 and the habitat consisted of 5% pool habitat, 41% run habitat and 54% riffle habitat, but following the floods 100% of the habitat at the fixed site consisted of riffle habitat. The flood impacts likely partially explain the decrease in Spikedace catch in 2021 as substantial declines in estimated abundance of all species

were observed at the fixed site compared to 2020 (Roundtail Chub 207-36, Speckled Dace 827-211, Longfin Dace 207-116, Desert Sucker 92-0; Table 2).

Recommendations: While declines in fish abundance following monsoon flooding in 2021 are likely to be short term for most species, an opportunity exists to stock more Spikedace in winter or spring 2022 (pending availability of fish) while the current abundance of large adult chub (which are potentially predators of naïve hatchery Spikedace) and nonnative crayfish are low relative to past surveys. Electrofishing monitoring should continue until at least 2023 regardless of whether more Spikedace are stocked.

Tables and Figures:

Table 1.—Summary of fish captured during the first pass at three 100-m electrofishing sub-reaches in Spring Creek during annual monitoring on September 9, 2021. Shown are the number of fish captured in each sub-reach (#Ind), the mean number of fish captured per hour of electrofishing effort (#Ind/h), and the overall mean and standard error of the catch rate.

Sub-reach	Statistic	Roundtail Chub ¹	Spikedace	Desert Sucker	Longfin Dace	Speckled Dace
Random-10	#Ind	64	2	15	3	76
	#Ind/h	184.19	3.39	42.50	7.42	180.81
Random-08	#Ind	48		13	1	9
	#Ind/h	188.66		55.72	2.50	59.60
Fixed-2	#Ind	29	3		74	100
	#Ind/h	124.73	12.90		318.28	430.11
Total	#Ind	141	5	28	78	185
	#Ind/h	176.02	4.33	43.74	133.70	226.76
	SE	(23.47)	(1.73)	(8.96)	(33.55)	(33.29)

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

Table 2.—Three-pass depletion estimates of abundance for all fish species captured per 100 m at the fixed sub-reach in Spring Creek during annual monitoring in 2021. Included is the number of fish caught in each pass (C1, C2, C3), Carle-Strub three pass abundance estimate (N), lower (N_LCI) and upper (N_UCI) 95% confidence interval of the abundance estimate, estimated capture probability (p), and the lower (p_LCI) and upper (p_UCI) 95% confidence interval of the estimate of capture probability. Species codes are MEFU = Spikedace, GIRO = Roundtail Chub, AGCH = Longfin Dace, and RHOS = Speckled Dace.

Stream	Site	Species	C1	C2	C3	N	N_LCI	N_UCI	p	p_LCI	p_UCI
Spring Creek	Fixed-02	MEFU	3	1	1	5	3.46	6.54	0.62	0.11	1.00
Spring Creek	Fixed-02	GIRO	29	6	1	36	34.98	37.02	0.82	0.69	0.95
Spring Creek	Fixed-02	AGCH	74	22	14	116	108.24	123.76	0.62	0.51	0.73
Spring Creek	Fixed-02	RHOS	100	47	32	211	185.19	236.81	0.46	0.36	0.57

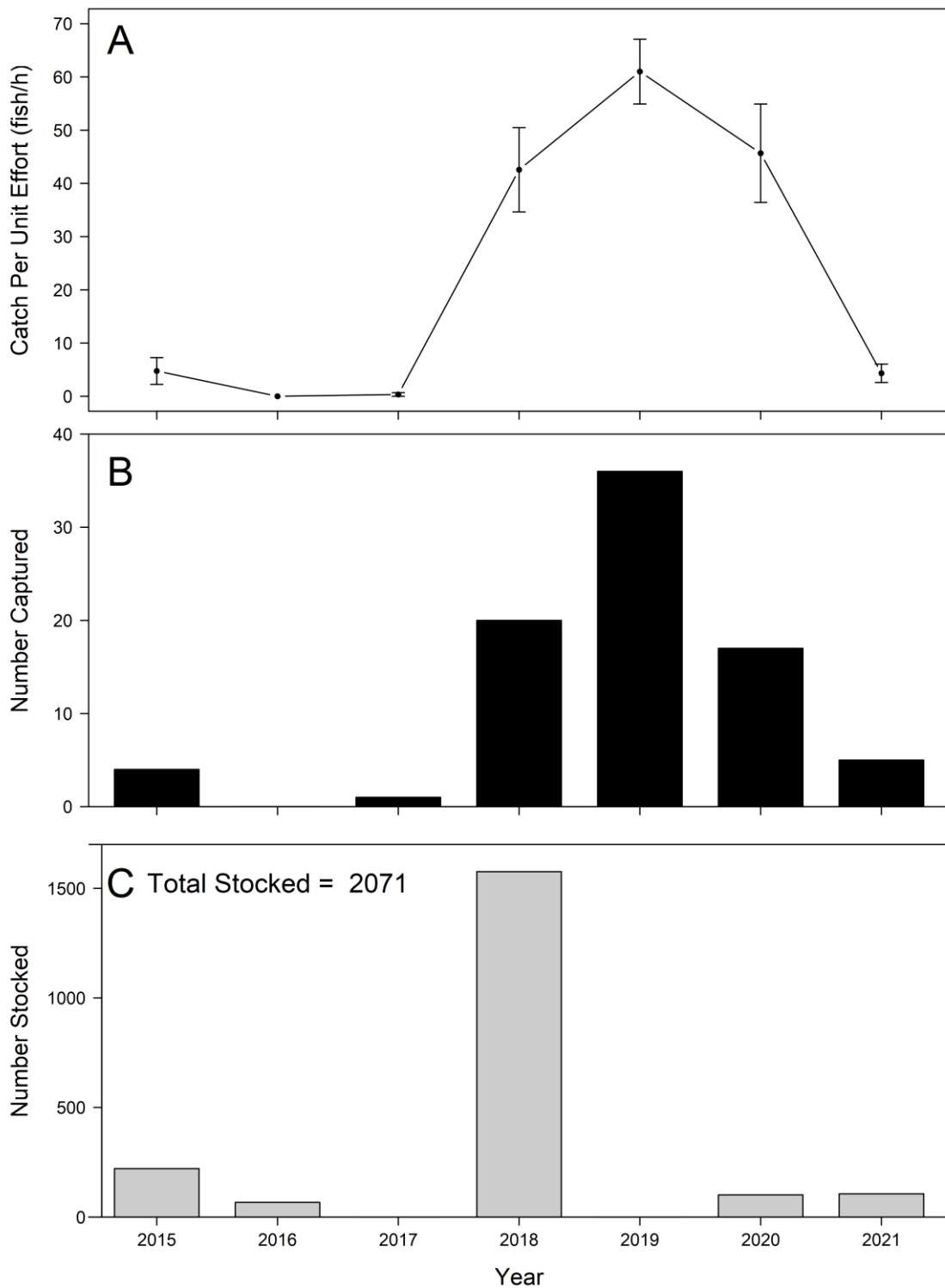


Figure 7.—Summary of Spikedace captured and stocked in Spring Creek, AZ, annually from 2015 to 2021 with (A) mean annual backpack electrofishing catch per unit effort (fish/h) with standard error bars, (B) total number of fish captured, and (C) total number of fish stocked.

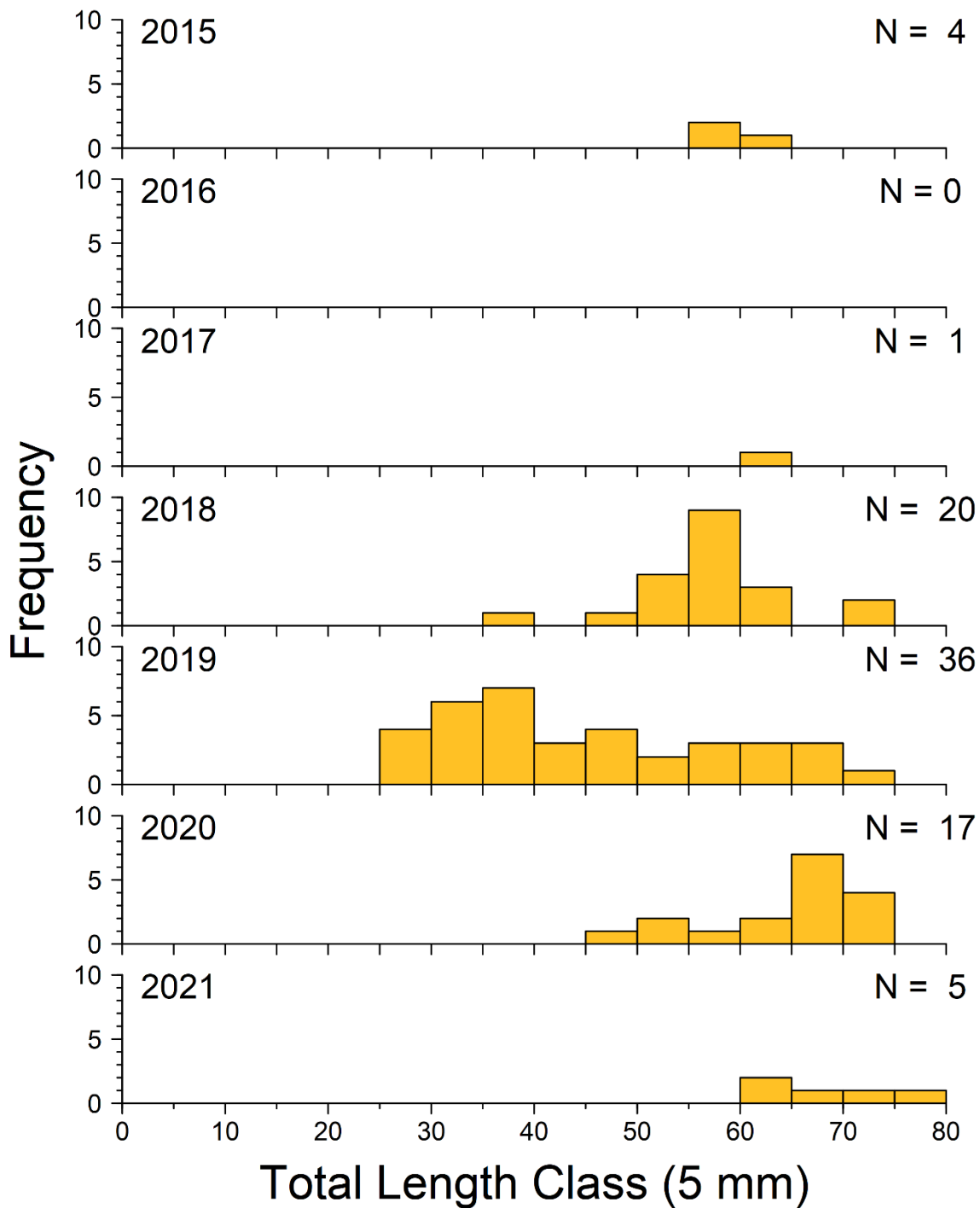


Figure 8.—Length frequency distribution of the number of Spikedace captured during annual monitoring in Spring Creek, 2015 through 2021. Only fish captured on the first pass are included. Number of fish captured and measured each year is shown in the top right corner of each panel.

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

Strategic Plan Goals:

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

Recovery Objectives:

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

Background: The Blue River Native Fish Restoration Project is implemented by the Department, Forest Service, Reclamation, and USFWS, with the goal of protecting and restoring the entire assemblage of native fishes within the Blue River drainage and benefiting their conservation status within the Gila River Basin (Reclamation 2010). The major components of the project are construction of a fish barrier, mechanical removal of non-native fishes, and 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 9). A synthesis of conservation efforts leading to the establishment of Spikedace and Roundtail Chub populations in the lower Blue River through 2019 can be found in Hickerson et al. (2021a). Additional efforts to establish populations of Spikedace and Roundtail Chub are now taking place in the middle and upper Blue River (Figure 9).

Efforts to remove non-native piscivorous fish from the lower Blue River began before barrier construction (Robinson et al. 2010) and continued until 2019. Nonnative fish were removed both during removal trips, and during annual post-stocking monitoring of native fishes. Catfish were the main targets of initial removal efforts and were removed by snorkeling and spearfishing. Channel Catfish have not been detected since 2013 and the most recent detection of Green Sunfish was in 2016.

Native fish conservation activities in the middle Blue River (McKittrick Creek confluence upstream to The Box; Figure 9) began in 2016 when 1,194 Roundtail Chub were stocked between The Box and Cole Flat. During the initial monitoring effort in 2017, a total of 57 Roundtail Chub

were captured with hoop nets. Immediately following the chub monitoring, 448 Spikedace were collected from the Blue River at Juan Miller crossing and translocated to the Blue River at Cole Flat. Spikedace were held in cages as part of an eDNA study before release, which may have contributed to some post-stocking mortality. In September, 2018, Department staff electrofished ten random and two fixed 100-m sub-reaches and captured a total of 12 Roundtail Chub and 6 Spikedace. In addition, large hoop nets were set overnight in 15 randomly selected pools throughout the monitoring reach resulting in the capture of 17 more Roundtail Chub. Following the monitoring, an additional 291 Spikedace were translocated from the Blue River near Juan Miller Crossing to the middle Blue River at Cole Flat. During annual monitoring in 2019, 23 Spikedace and 9 Roundtail Chub were captured by electrofishing only. Roundtail Chub catch declined each year from 2017 to 2019 despite additional electrofishing effort, and juvenile Roundtail Chub were not captured within the monitoring reach. Following annual monitoring in 2019, a total of 100 Roundtail Chub were collected near Juan Miller Crossing and translocated to the middle Blue River near Cole Flat. During annual monitoring in 2020, 117 Spikedace and 180 Roundtail Chub were captured, with substantial numbers of young-of-year fish for both species.

Native fish conservations activities in the upper Blue River (Blue Crossing upstream to New Mexico state line; Figure 9) began in 2020 when 226 Roundtail Chub and 826 Spikedace were salvaged 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.

Results:

Lower Blue River

During May 10-12, 2021, Department staff collected eDNA samples from 19 sites on the lower Blue River from the fish barrier upstream to the confluence with Pigeon Creek (Figure 10). Previous research suggests that fish can be detected using eDNA sampling up to 500 m downstream from their location, so sample sites were spaced 500 m apart (Robinson et al. 2019). The crew planned to collect samples from more sites, but the stream was still relatively turbid and more filters were required at each site than initially anticipated. Green Sunfish were not detected in any of the samples.

On October 5, 2021, Department staff collected eDNA samples from 15 sites on the lower Blue River from the confluence with Pigeon Creek upstream to XXX Ranch (Figure 10). Much of the silt and sediment that was present during the May trip had been deposited above the current banks and in side channels following some severe scouring floods. Results from the eDNA samples are still pending at this time.

A manuscript detailing the success of the lower Blue River Native Fish Restoration Project was published in the North American Journal of Fisheries Management in June 2021 (Hickerson et al. 2021a).

Middle Blue River

During September 27-29, 2021, Department staff electrofished a total of 10 randomly selected and two fixed 100-meter long sub-reaches starting at the confluence with McKittrick Creek upstream to the Box. A total of 42 Spikedace, 80 Roundtail Chub, 86 Loach Minnow, 212 Longfin Dace, 622 Speckled Dace, 745 Desert Sucker, 90 Sonora Sucker, 1 Brown Trout, 1 hybrid Apache Trout and 1 Fathead Minnow were captured during the first pass (Table 3). The hybrid Apache Trout was likely an emigrant from Grant Creek that was able to take advantage of elevated flows during the monsoon season. Total catch and relative abundance of both Spikedace and Roundtail Chub declined compared to 2020, but still remained higher than any previous monitoring efforts in this reach (Figure 11, Figure 12). Spikedace were captured at 9 sub-reaches and Roundtail Chub were at all 12 sub-reaches (Figure 13). Spikedace still seem to have difficulty accessing the most upstream 400–500 m of the monitoring reach, probably due to the steeper gradient in this area, and have not yet been captured upstream of sub-reach 4. Roundtail Chub on the other hand, have been captured at all sub-reaches sampled each of the last two years. Most of the Roundtail Chub captured seem to be fish spawned in 2020 that are continuing to grow (40-100 mm TL; Figure 14), with few to no young of year fish captured this year. Similarly, Spikedace apparently failed to spawn within the middle Blue River in 2021 (Figure 15).

Three-pass depletion electrofishing was carried out at both fixed sites. Estimated abundance of Roundtail Chub per 100 m ranged from 4 to 29 individuals with an estimated capture probability of 0.46-0.80 (Table 4). Spikedace were not captured at the upper fixed site, and the estimate of abundance at the lower fixed site was 4 individuals per 100 m with an estimated capture probability of 0.44 (Table 4). The decrease in estimated abundance (2020 $n = 30$) suggests that true abundance of Spikedace in the middle Blue River likely has declined relative to last year.

Relative abundance and number of fish captured were down for all taxa this year compared to 2020. The decrease in fish numbers is not surprising considering in the last year the fish experienced a prolonged drought, followed by an intense monsoon season which brought with it flooding and post-fire debris from the Cow Canyon Fire (which was distributed throughout the sampled reach). Young of year Roundtail Chub and Spikedace were noticeably absent compared to 2020 (Figure 14, Figure 15), which could be attributed to the lack of sufficient flows for successful spawning this spring.

Despite a decrease in the number of fish captured, the proportion of all fish captured for both Spikedace (1.3 – 2.0%) and Roundtail Chub (2.2 – 4.1%) increased compared to 2020. Considering the disturbances both these fish populations experienced during the last year, both seem relatively resilient within this reach.

Upper Blue River

During September 20-22, 2021 Department staff electrofished a total of 12 randomly selected and three fixed 100 meter long sub-reaches starting at Blue Crossing Campground upstream to the New Mexico state line near Bobcat Flat. A total of 4 Spikedace, 1 Roundtail Chub, 128 Loach Minnow, 1,457 Longfin Dace, 3,829 Speckled Dace, 1,772 Desert Sucker, 681 Sonora Sucker and

29 Brown Trout were captured during the first pass (Table 5). While more Brown Trout were captured than expected, the mean size (102.34 mm TL, Figure 16) was relatively small. Young of year Spikedace (Figure 17) and Roundtail Chub (134 mm TL) were not detected. A relatively small proportion of the Spikedace and Roundtail Chub stocked in 2020 were captured in 2021 (Figure 18, Figure 19). However, the fact that individuals persisted in this reach since the initial stocking of salvaged fish in June 2020 is very promising, since fish have been exposed to extraordinary drought conditions for much of the year followed by extraordinary monsoon conditions.

Three-pass depletions were carried out at one fixed sub-reach in each of the three monitoring reaches. Only one Spikedace and one Roundtail Chub were captured at these sites, so estimates of abundance and capture probability of the target species are of limited utility at this time (Table 6).

Recommendations: The preliminary results from eDNA sampling suggest that Green Sunfish may have been successfully eradicated from the Blue River. Preliminary results from Reclamation's monitoring contractor indicate that monsoon floods and post-fire impacts in the lower Blue River had devastating impacts on native fish populations. Spikedace and Roundtail Chub should be stocked as soon as possible to help re-establish these populations.

The middle Blue River populations of Spikedace and Roundtail Chub should be monitored for five years after the final stocking to determine whether populations are established. If additional stockings do not occur, monitoring should continue through 2023 for Spikedace and 2024 for Roundtail Chub. Additional Spikedace and Roundtail Chub should be translocated as necessary to help establish populations. Population metrics should improve given relatively normal environmental conditions and a respite from any major disturbances.

Spikedace and Roundtail Chub reproduction was not documented in the upper Blue River in 2021. Pending availability of fish, it may be valuable to augment these populations should numbers remain low in 2022. Populations of Spikedace and Roundtail Chub should benefit from less extreme flow conditions. During the lower and middle Blue River projects, two stockings were typically required for populations to show substantial increases in abundance and distribution. Monitoring of Spikedace and Roundtail Chub in the upper Blue River should continue until at least 2025 unless more fish are stocked.

Tables and Figures:

Table 3.—Summary of fish captured during the first pass of backpack electrofishing within each monitoring reach in the middle Blue River during annual monitoring from September 27-29, 2021. 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		Desert Sucker	Longfin Dace	Sonora Sucker	Speckled Dace	Brown Trout	Apache	Fathead Minnow
			Minnow	Chub	Spikedace	Trout Hybrid							
1	5	#Ind	33	21	26	231	50	23	233			1	
		#Ind/h	29.50	38.47	33.44	187.97	59.97	16.64	279.38			0.89	
		SE	(2.70)	(6.93)	(5.36)	(23.43)	(15.90)	(5.40)	(45.19)			(0.89)	
2	3	#Ind	18	23	12	176	64	18	154				1
		#Ind/h	32.71	77.36	22.62	222.66	83.73	24.10	210.94				1.44
		SE	(3.42)	(10.98)	(3.82)	(20.11)	(21.24)	(9.42)	(17.75)				(1.44)
3	4	#Ind	35	36	4	338	98	49	235	1			
		#Ind/h	46.54	46.00	6.41	438.31	120.68	90.38	258.36	0.67			
		SE	(4.45)	(4.33)	(3.10)	(83.84)	(27.53)	(21.77)	(44.23)	(0.67)			
Total	12	#Ind	86	80	42	745	212	90	622	1	1	1	
		#Ind/h	36.80	52.34	25.70	278.26	85.19	47.91	255.81	0.20	0.43	0.33	
		SE	(2.28)	(4.44)	(3.49)	(32.11)	(13.00)	(10.60)	(24.56)	(0.20)	(0.43)	(0.33)	

Table 4.—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 2021. Included is the number of fish caught in each pass (C1, C2, C3), Carle-Strub three pass abundance estimate (N), lower (N_LCI) and upper (N_UCI) 95% confidence interval of the abundance estimate, estimated capture probability (p), and the lower (p_LCI) and upper (p_UCI) 95% confidence interval of the estimate of capture probability. Species codes are MEFU = Spikedace, GIRO = Roundtail Chub, RHCO = Loach Minnow, CACL = Desert Sucker, CAIN = Sonora Sucker, AGCH = Longfin Dace, and RHOS = Speckled Dace.

Sub-reach	Species	C1	C2	C3	N	N_LCI	N_UCI	p	p_LCI	p_UCI
Fixed-04	RHCO	4	5	2	13	4.85	21.15	0.42	0.00	0.88
Fixed-04	GIRO	14	5	6	29	19.39	38.61	0.46	0.18	0.75
Fixed-04	RHOS	58	22	14	103	92.06	113.94	0.55	0.42	0.68
Fixed-04	CACL	35	21	20	113	62.29	163.71	0.31	0.11	0.50
Fixed-04	CAIN	3	1	3	8	1.16	14.84	0.41	0.00	1.00
Fixed-04	AGCH	22	6	5	34	30.21	37.79	0.63	0.44	0.83
Fixed-28	RHCO	13	13	3	33	23.90	42.1	0.48	0.23	0.74
Fixed-28	GIRO	3	1	0	4	3.60	4.4	0.8	0.4	1.00
Fixed-28	MEFU	1	1	2	4	0.02	7.98	0.44	0.00	1.00
Fixed-28	RHOS	76	42	24	169	144.50	193.50	0.45	0.33	0.57
Fixed-28	AGCH	29	26	16	108	54.57	161.43	0.30	0.09	0.50
Fixed-28	CACL	68	69	27	230	171.64	288.36	0.34	0.21	0.47
Fixed-28	CAIN	12	2	1	15	14.15	15.85	0.79	0.58	1.00

Table 5.—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 20-22, 2021. 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	23	1		549	567	134	1296	8
		#Ind/h	29.74	0.80		442.13	343.75	142.45	844.51	8.37
		SE	(4.59)	(0.80)		(72.79)	(52.64)	(29.29)	(140.51)	(2.62)
2	5	#Ind	72		4	331	569	229	1295	11
		#Ind/h	253.71		4.81	459.30	789.20	188.42	1497.30	6.39
		SE	(31.47)		(2.39)	(97.33)	(217.40)	(41.10)	(330.01)	(1.66)
3	5	#Ind	33			892	321	318	1238	10
		#Ind/h	56.13			540.29	205.78	215.68	768.94	9.16
		SE	(7.51)			(62.76)	(34.03)	(28.31)	(74.94)	(2.82)
Total	15	#Ind	128	1	4	1772	1457	681	3829	29
		#Ind/h	148.66	0.22	1.55	486.85	432.98	188.39	1028.22	8.07
		SE	(17.88)	(0.22)	(0.81)	(44.29)	(76.69)	(19.50)	(121.80)	(1.44)

Table 6.—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 2021. Included is the number of fish caught in each pass (C1, C2, C3), Carle-Strub three pass abundance estimate (N), lower (N_LCI) and upper (N_UCI) 95% confidence interval of the abundance estimate, estimated capture probability (p), and the lower (p_LCI) and upper (p_UCI) 95% confidence interval of the estimate of capture probability. Species codes are MEFU = Spikedace, GIRO = Roundtail Chub, RHCO = Loach Minnow, CACL = Desert Sucker, CAIN = Sonora Sucker, AGCH = Longfin Dace, RHOS = Speckled Dace, and SATR = Brown Trout.

Sub-reach	Species	C1	C2	C3	N	N_LCI	N_UCI	p	p_LCI	p_UCI
1-46F	RHCO	0	0	1	1	0.00	4.97	0.33	0.00	1.00
1-46F	GIRO	1	0	0	1	1.00	1.00	1.00	NA	NA
1-46F	AGCH	53	16	25	120	90.63	149.37	0.39	0.24	0.55
1-46F	RHOS	275	109	84	542	505.25	578.75	0.48	0.42	0.55
1-46F	CACL	123	76	47	317	268.11	365.89	0.39	0.29	0.49
1-46F	CAIN	95	15	13	125	120.92	129.08	0.72	0.64	0.81
2-47F	RHCO	3	0	1	4	2.93	5.07	0.67	0.13	1.00
2-47F	MEFU	1	0	0	1	1.00	1.00	1.00	NA	NA
2-47F	AGCH	79	28	18	135	123.93	146.07	0.57	0.46	0.68
2-47F	RHOS	283	115	52	485	465.28	504.72	0.58	0.52	0.64
2-47F	CACL	64	31	16	124	109.83	138.17	0.52	0.40	0.65
2-47F	CAIN	31	17	5	57	50.02	63.98	0.58	0.41	0.74
2-47F	SATR	2	0	2	4	1.12	6.88	0.5	0.00	1.00
3-45F	RHCO	12	4	5	23	16.57	29.43	0.51	0.22	0.81
3-45F	AGCH	44	36	16	123	92.69	153.31	0.39	0.23	0.55
3-45F	RHOS	202	90	95	528	448.81	607.19	0.36	0.27	0.44
3-45F	CACL	97	59	49	299	223.00	375	0.32	0.20	0.44
3-45F	CAIN	70	33	24	151	127.83	174.17	0.45	0.33	0.58
3-45F	SATR	1	2	1	4	1.12	6.88	0.5	0.00	1.00

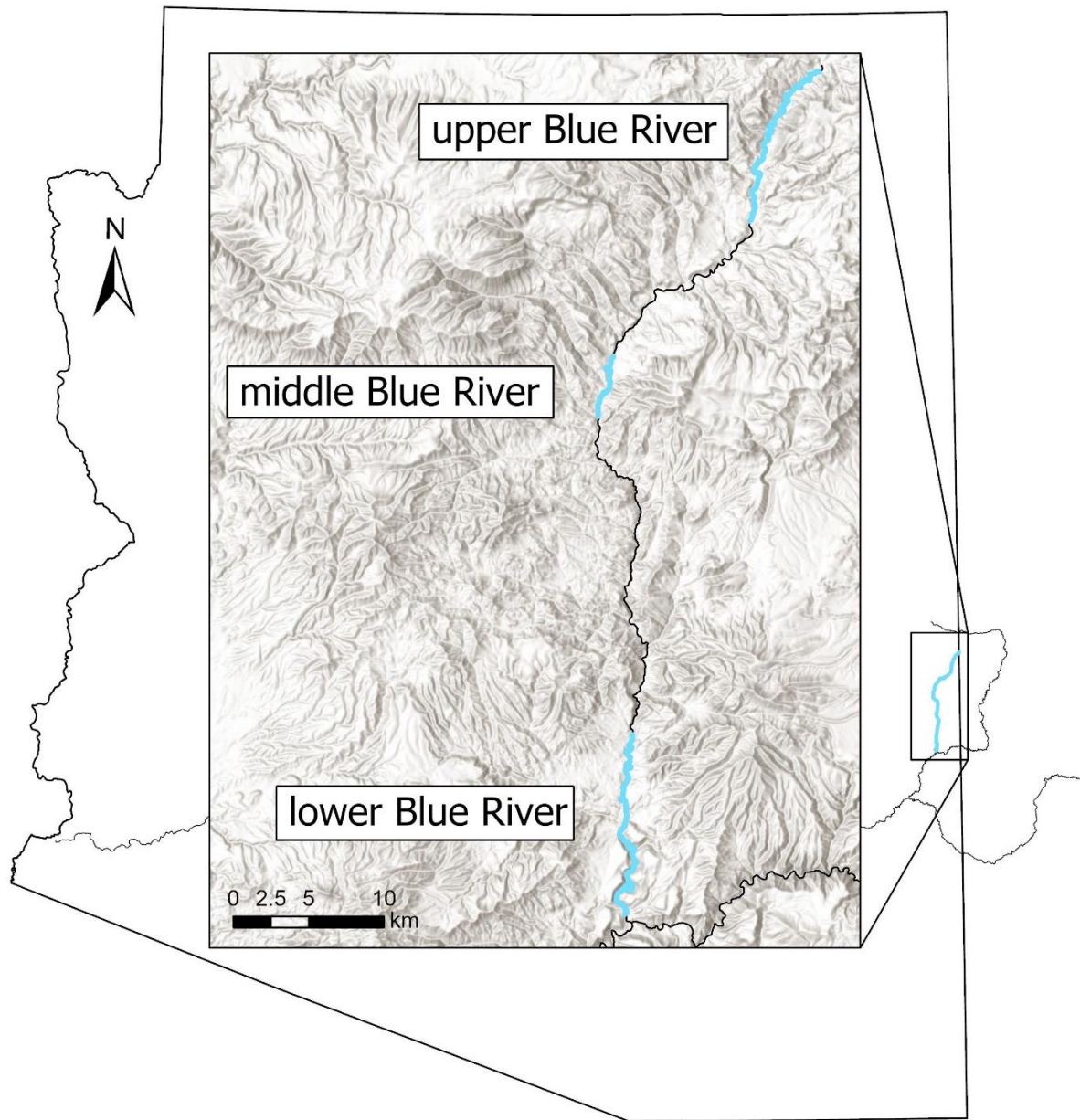


Figure 9.—Map showing the upper (New Mexico border downstream to Blue Crossing Campground), middle (The Box downstream to Fritz Ranch), and lower (Fritz Ranch downstream to the barrier) project areas of the Blue River.

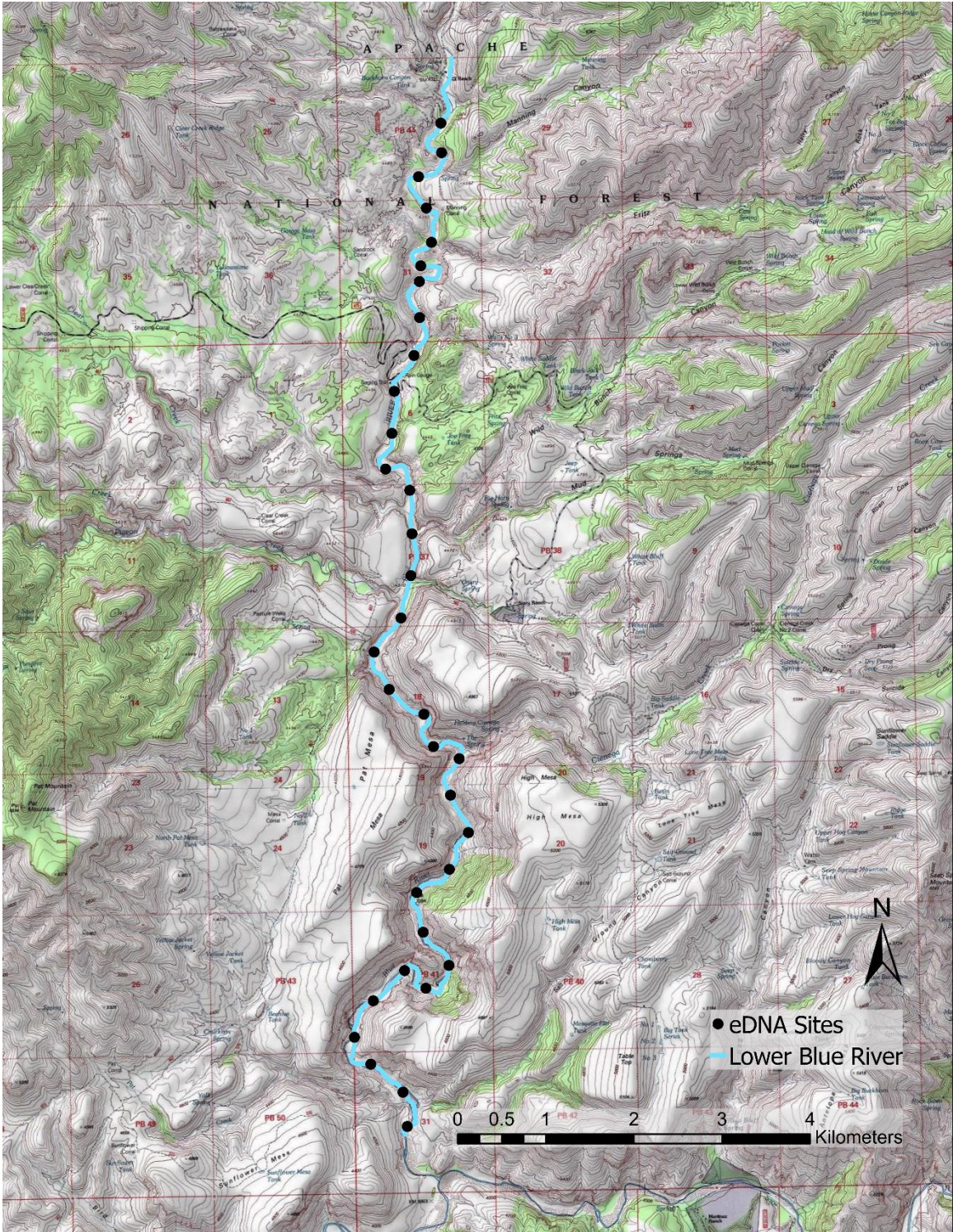


Figure 10.—Map showing the location of eDNA samples collected from the lower Blue River (Fritz Ranch downstream to the barrier) during May 10-12 and October 5, 2021. Sample locations were spaced 500 m apart.

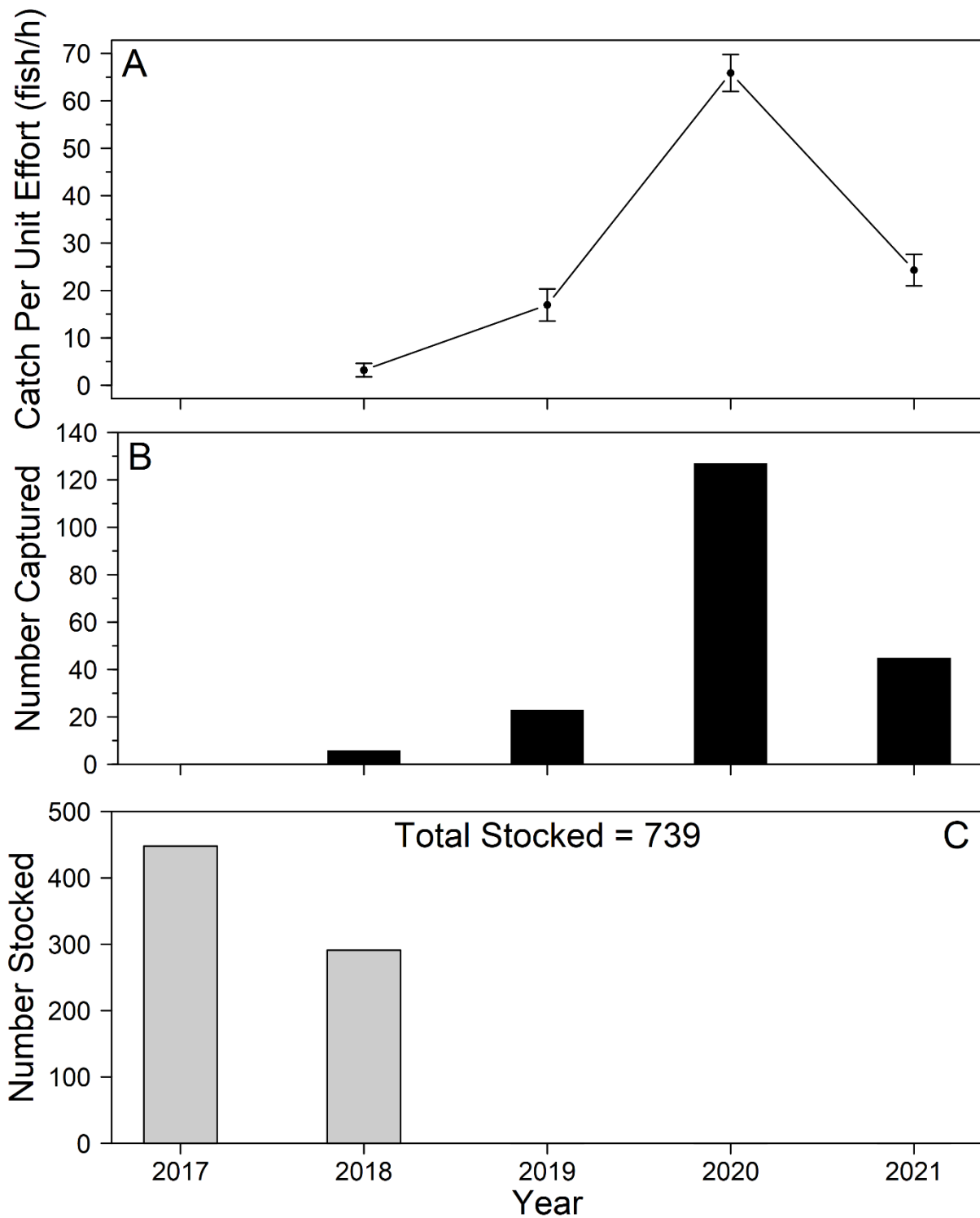


Figure 11.—Summary of Spikedace captured and stocked in the middle Blue River, annually from 2017 to 2021 with (A) mean catch per unit effort (fish/h) with standard error bars, (B) total number of fish captured, and (C) total number of fish stocked.

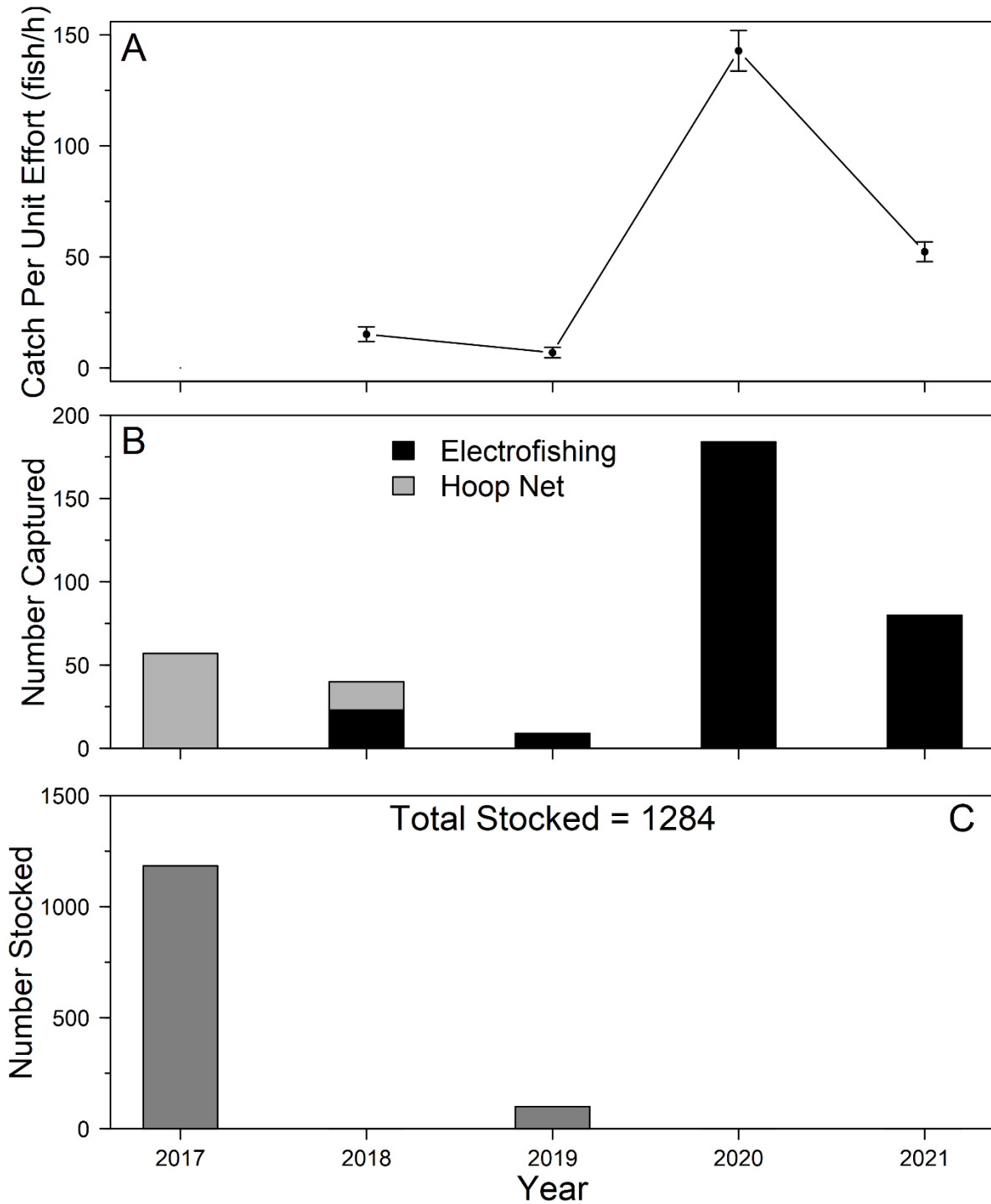


Figure 12.—Summary of Roundtail Chub captured and stocked in the middle Blue River, annually from 2017 to 2021 with (A) mean catch per unit effort (fish/h) for backpack electrofishing with standard error bars, (B) total number of fish captured by gear type (hoop nets in gray, backpack electrofishing in black), and (C) total number of fish stocked. Catch per unit effort is not displayed for hoop nets in panel A because it was less than one fish per hour.

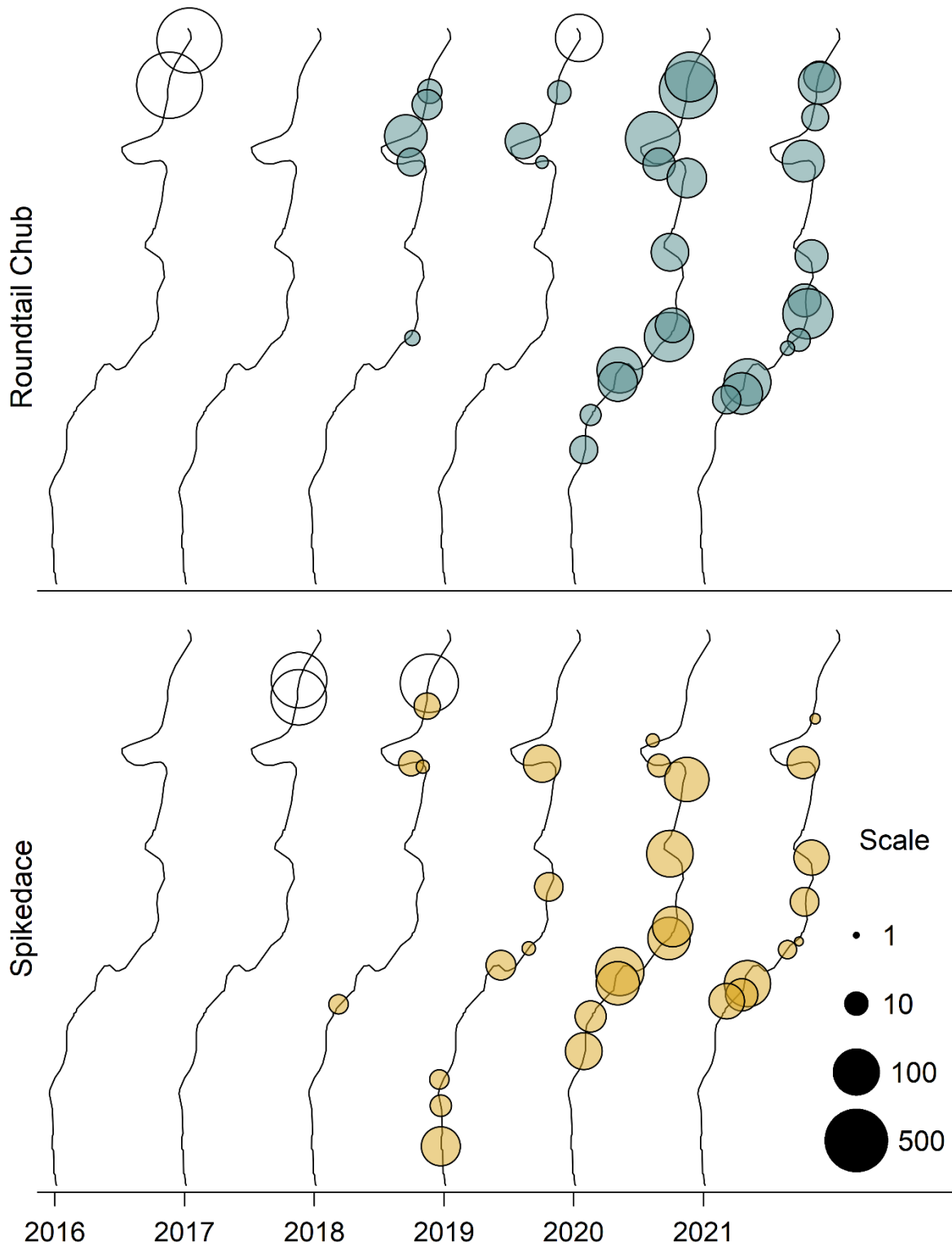


Figure 13.—Roundtail Chub (top row) and Spikedace (bottom row) stocking locations (open circles) and mean backpack electrofishing relative abundance (CPUE, fish/h) at each monitoring site in the middle Blue River from 2016-2021. Size of points indicates either the number of fish stocked or the relative abundance during monitoring at a particular location.

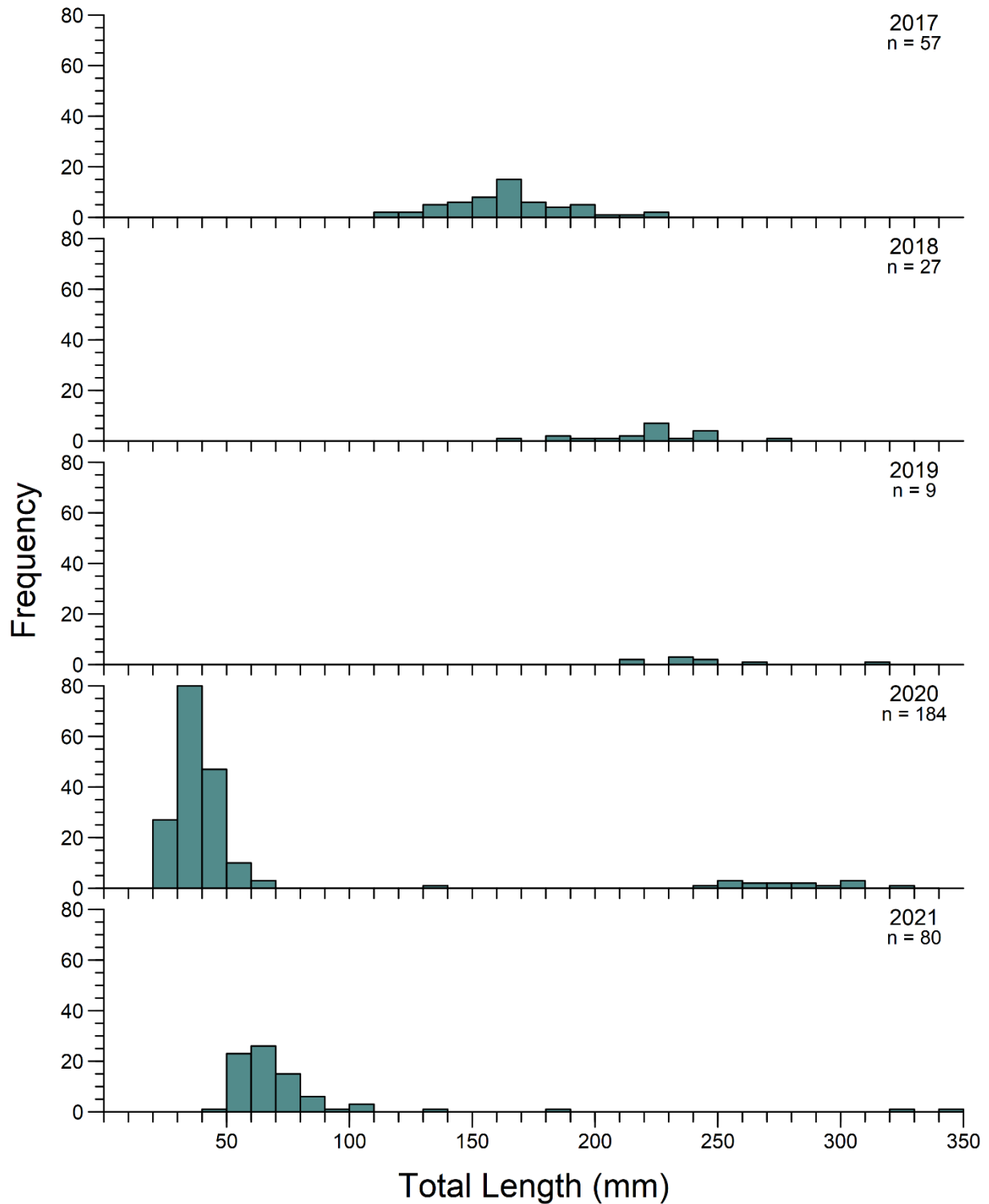


Figure 14.—Length frequency distribution of the number of Roundtail Chub captured during annual monitoring in the middle Blue River, from 2017 to 2021. Only fish captured on the first pass are included. Number of fish captured and measured each year is shown in the top right corner of each panel.

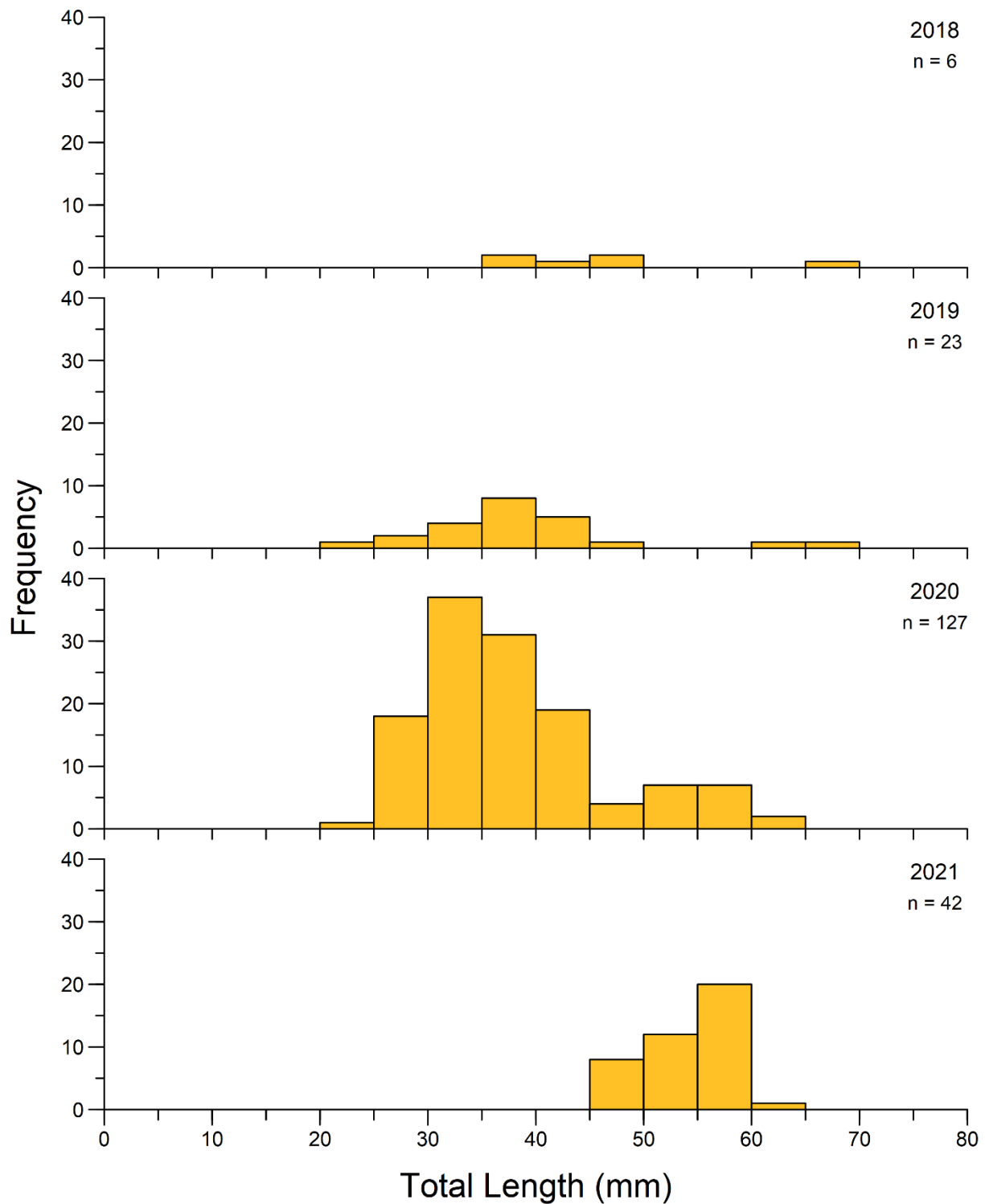


Figure 15.—Length frequency distribution of the number of Spikedace captured during annual monitoring in the middle Blue River, from 2018 to 2021. Only fish captured on the first pass are included. Number of fish captured and measured each year is shown in the top right corner of each panel.

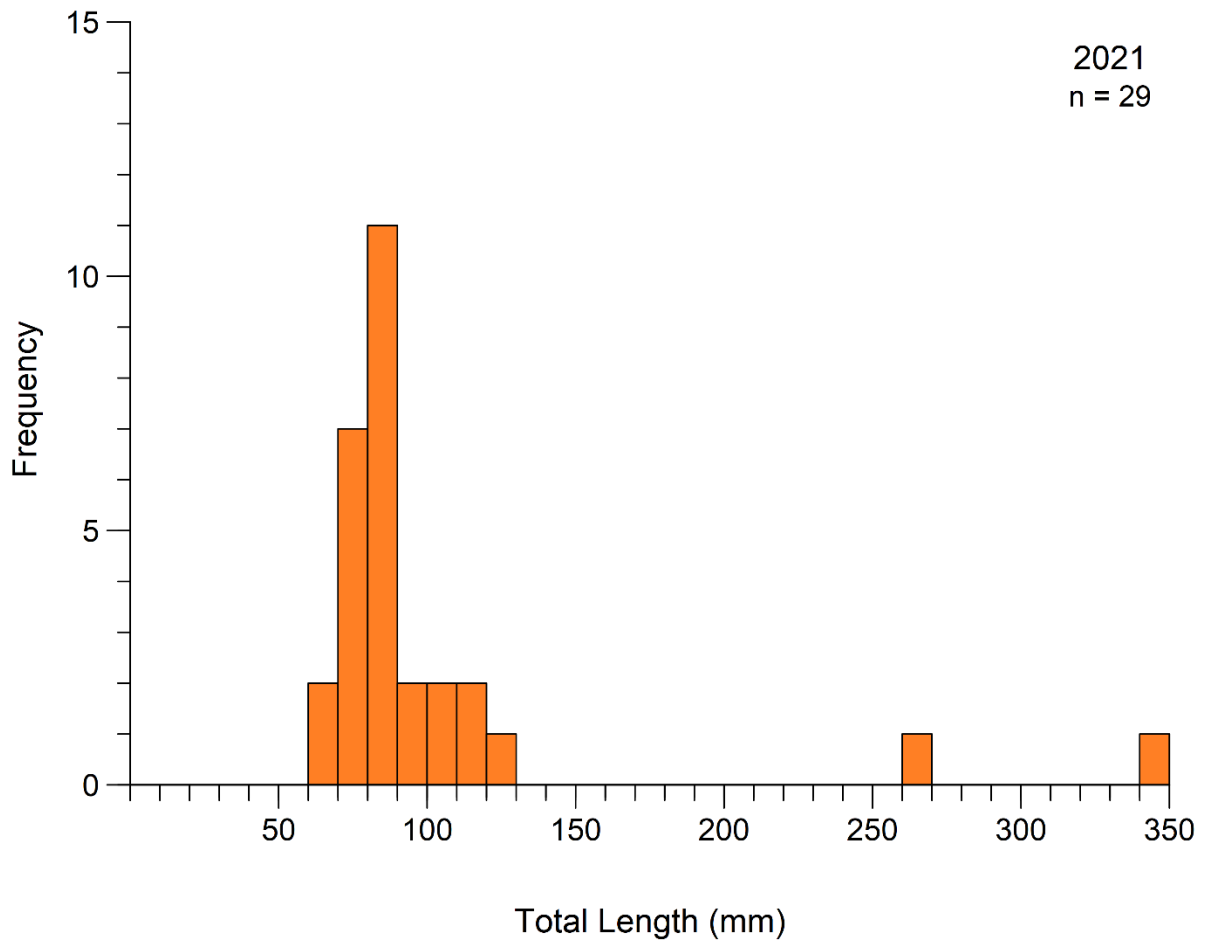


Figure 16.—Length frequency distribution of the number of Brown Trout captured during annual monitoring in the upper Blue River in 2021. Number of fish captured and measured each year is shown in the top right corner of each panel.

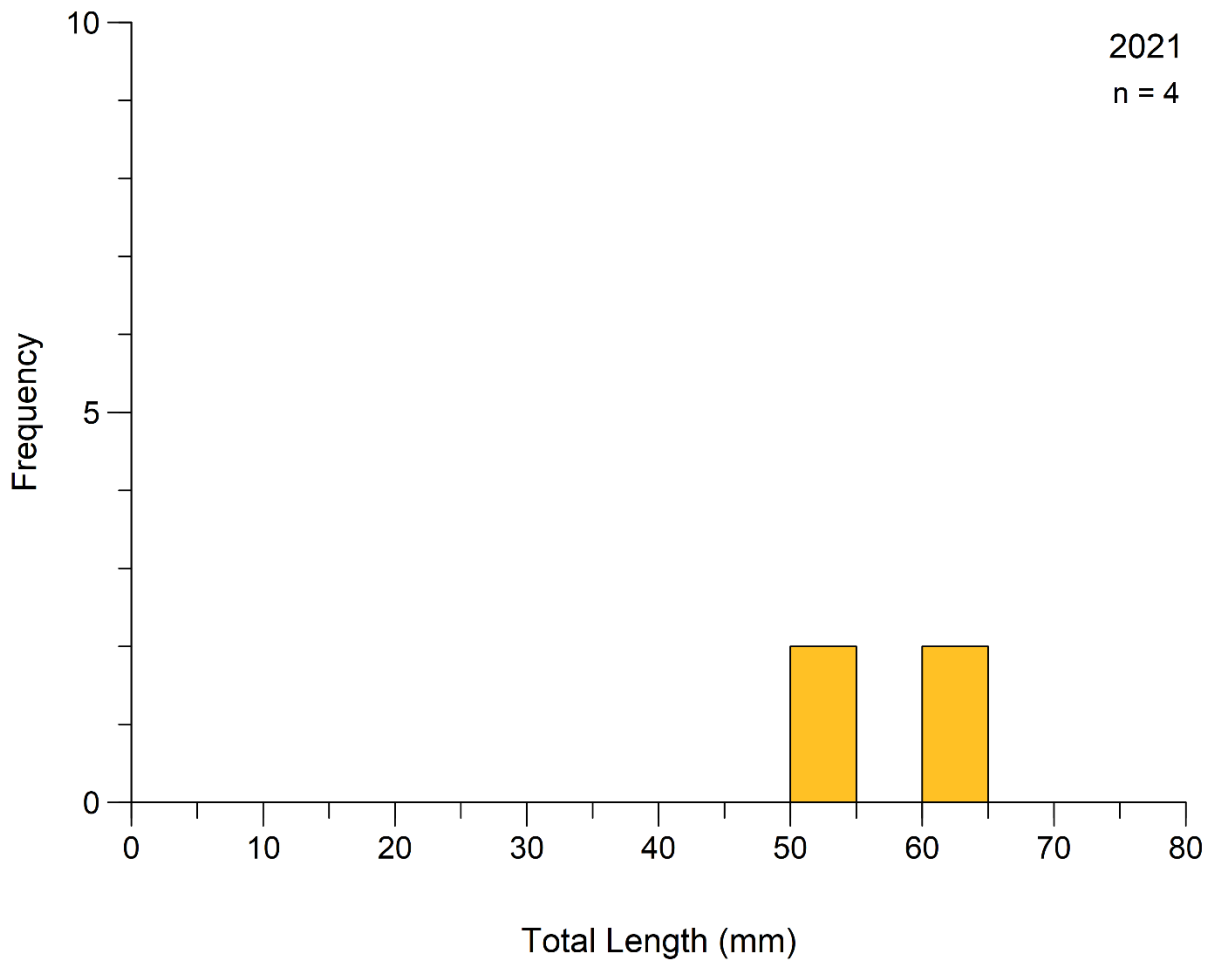


Figure 17.—Length frequency distribution of the number of Spikedace captured during annual monitoring in the upper Blue River in 2021. Number of fish captured and measured each year is shown in the top right corner of each panel.

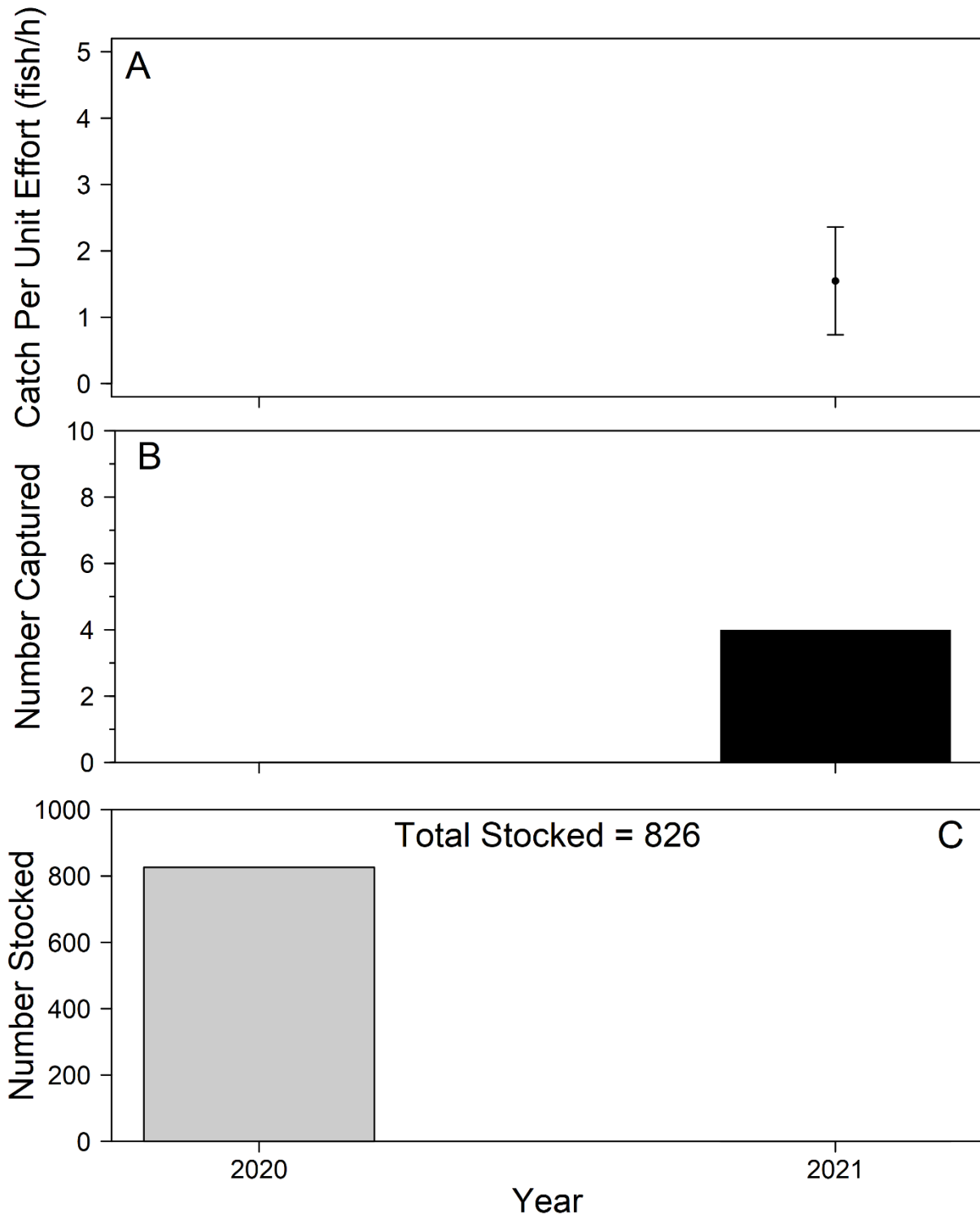


Figure 18.—Summary of Spikedace captured and stocked in the upper Blue River, annually from 2020 to 2021 with (A) mean catch per unit effort (fish/h) with standard error bars, (B) total number of fish captured, and (C) total number of fish stocked.

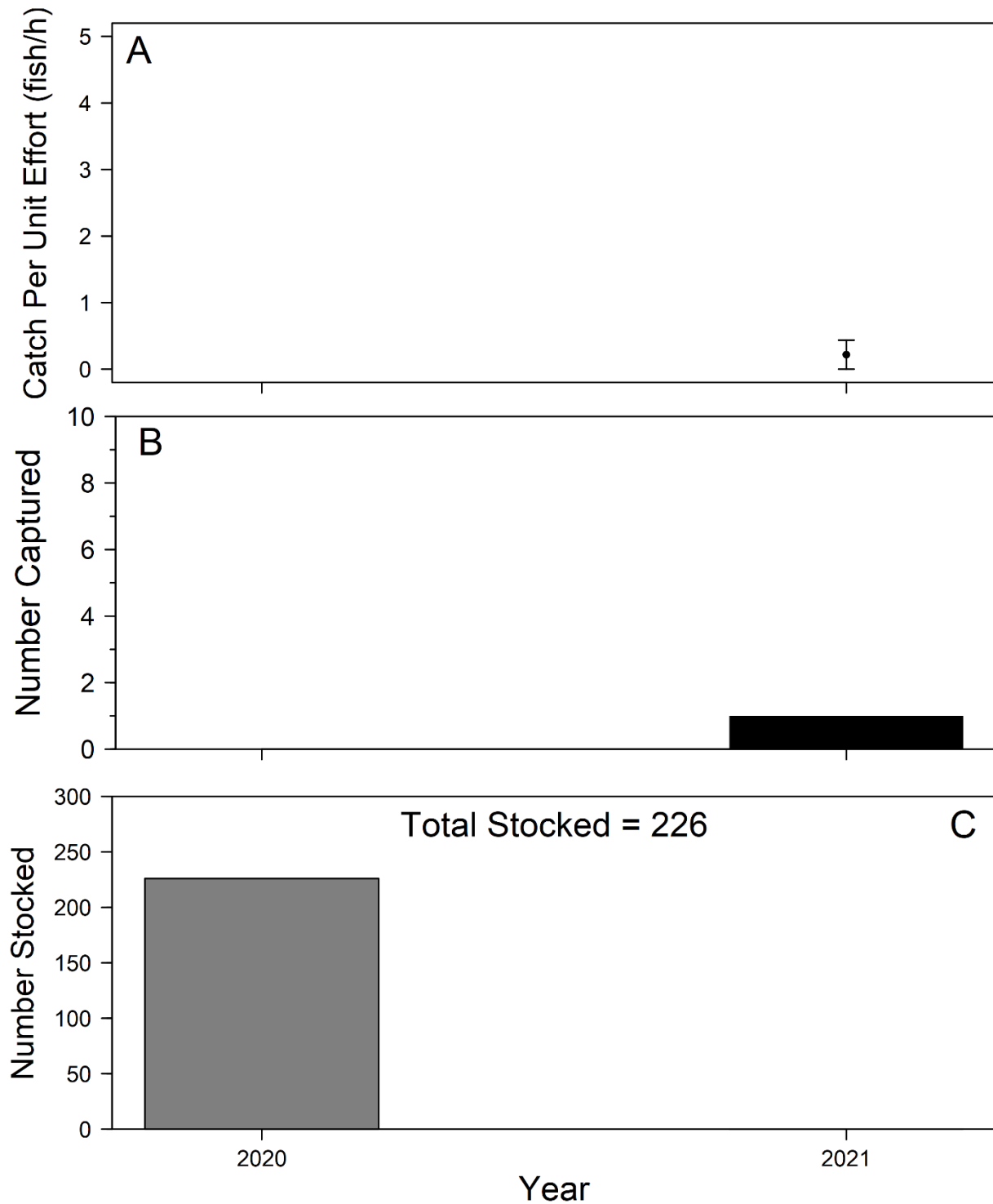


Figure 19.—Summary of Roundtail Chub captured and stocked in the upper Blue River, annually from 2020 to 2021 with (A) mean catch per unit effort (fish/h) with standard error bars, (B) total number of fish captured, and (C) total number of fish stocked.

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

Strategic Plan Goals:

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

Recovery Objectives:

- Gila Chub draft recovery plan objective 1.3.1. Eliminate or control problematic nonnative aquatic organisms
- Gila Chub draft recovery plan objective 2. Ensure representation, resiliency, and redundancy by expanding the size and number of populations within Gila Chub historical range via replication of remnant populations within each RU.

Background: Harden Cienega Creek is a tributary to the San Francisco River near the New Mexico state line. Roundtail Chub¹ distribution was historically limited to approximately 2 km of stream below a natural waterfall barrier. In April 2013, Department staff surveyed above the waterfall and determined that about 1.4 km of perennial water existed above the waterfall that was suitable for Roundtail Chub¹. In April, 2015, a total of 102 Roundtail Chub¹ were translocated from lower Harden Cienega Creek to the previously unoccupied reach upstream of the waterfall. Monitoring from 2017 to 2020 detected several hundred chub representing all size classes above the barrier. The population was augmented with five individuals from below the barrier in 2018 and 104 in 2019 in an effort to increase the genetic diversity above the barrier. In October 2019, Gila Topminnow (n = 631; Bylas Spring lineage) were first stocked in lower Harden Cienega Creek downstream of the waterfall barrier. A temperature logger was also installed at the stocking location at the time of stocking to track whether winter water temperatures were sufficient for topminnow establishment. Unfortunately, Green Sunfish were detected above the barrier during post-stocking monitoring, with one removed in 2017 and two in 2018. Four Green Sunfish were captured and removed downstream of the barrier in 2019, suggesting the population was increasing in abundance and distribution within Harden Cienega Creek. A removal plan was drafted (Hickerson et al. 2020) and Green Sunfish removal efforts were initiated in 2020. A total of 38 sunfish were removed during the first removal pass in 2020, with only one individual captured above the barrier. 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

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

drainage. Surveys of all 43 stock tanks in the Arizona portion of the Harden Cienega Creek watershed failed to detect any fish.

Results:

Green Sunfish Removal

During May 3-5, 2021, Department staff completed the first Green Sunfish removal pass of the year in Harden Cienega Creek. The stream was electrofished from the start of flow (UTM 12S 673650/3674847; approximately 200 m upstream from the confluence with the San Francisco River) upstream to the terminus of perennial water near Prospect Canyon for 12,139 seconds, resulting in the capture of three Green Sunfish in two separate pools downstream of the barrier (Figure 20). Native fish captured were not counted during electrofishing due to the time required to count fish and Green Sunfish are probably not sufficiently abundant or broadly distributed enough to make detectable changes to the fish community. The crew also set eight mini-hoop nets in pools too deep to sample effectively with backpack electrofishing equipment and captured an additional 13 Green Sunfish, 73 Roundtail Chub and 3 Desert Sucker.

During June 21-23, Department staff completed the second Green Sunfish removal pass of the year in Harden Cienega Creek. The stream was electrofished from the start of flow upstream to the terminus of perennial water near Prospect Canyon for 13,269 seconds with no sunfish captured. The crew also set 16 mini-hoop nets in pools where Green Sunfish were previously captured or were too deep to effectively electrofish, and captured a total of 7 Green Sunfish, 53 Roundtail Chub and 1 Sonora Sucker. All sunfish were captured in the same pool downstream of the barrier that has harbored the majority of Green Sunfish during the two previous removal passes (Figure 20). One Green Sunfish was observed in the pool that was not captured.

Green Sunfish captures have consistently declined with each full removal pass (2020 pass 1 = 38, 2021 pass 1 = 16, 2021 pass 2 = 7). This decline has probably been aided by the drought conditions which have likely prohibited movement of sunfish into Harden Cienega Creek from upstream sources. In addition, Green Sunfish still do not appear to be spawning in Harden Cienega Creek, as only adult fish have been captured during removal passes (Figure 21). Current removal efforts seem to be on track to eradicate Green Sunfish from Harden Cienega Creek in the near future, as long as the upstream sources of Green Sunfish can be eradicated in the near future.

Tank Surveys

During July 12-14, Department staff assisted New Mexico Department of Game and Fish (NMDGF) staff with surveys of 22 stock tanks in the New Mexico portion of the Harden Cienega Creek drainage. Green Sunfish were detected in two tanks on Gila National Forest lands; Distill Tank (n = 675) and California Tank (n = 98; Figure 22). A single bag seine haul was carried out in each of these tanks because presence of Green Sunfish was confirmed on the first haul. No live Green Sunfish were captured in Ditch Tank where sunfish were visually detected in 2020. However, several recently deceased Green Sunfish were discovered during seine hauls. There was evidence that substantial flooding occurred immediately before our surveys which appears to have

caused either a partial or total fish kill in Ditch Tank, likely through rapid changes in water quality conditions (i.e., low dissolved oxygen). Additional surveys will be required in the future to confirm whether sunfish still persist in Ditch Tank. The remaining 20 tanks were fishless. Two additional tanks were confirmed to have dried earlier in the year during a conversation between NMDGF staff and a private landowner. Three tanks on private property in New Mexico remain to be sampled, but NMDGF staff were unable to make contact with the landowner in 2021.

Topminnow Stocking

During May 3-5, 2021, Department staff attempted to collect Gila Topminnow to translocate to Harden Cienega Creek. Unfortunately, after several hours of effort very few fish were observed at the donor site and the collection attempt was postponed to the following trip.

During June 21-23, Department staff collected 516 Gila Topminnow (Bylas Spring lineage) and transported the fish in an aerated cooler to Frisco Camp on the San Francisco River. A total of 469 Gila Topminnow were stocked into Harden Cienega Creek at the same location originally stocked in 2019 (674768/3674598). There were a total of 47 mortalities during collection and transport. Prior to stocking the topminnow, the crew electrofished approximately 80 meters of stream between a small waterfall and the stocking location and relocated all adult Roundtail Chub (>100 mm TL) captured below the small waterfall, in the hopes of improving the short-term survival of topminnow. Topminnow were in good condition at the time of release and were observed at the stocking location the day after the stocking.

Recommendations: Continued nonnative removal effort is warranted in Harden Cienega Creek in 2022 because Green Sunfish are still present, and more fish may have dispersed into this reach with monsoon rains. Both backpack electrofishing and mini-hoop nets proved effective at capturing and removing Green Sunfish, and this combination approach should continue. Eradication seems possible to achieve if the upstream sources can be eradicated in the near future.

The topminnow population in Harden Cienega Creek should be monitored annually until 2024 unless more topminnow are stocked. Monitoring will involve a combination of setting minnow traps and backpack electrofishing.

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

Tables and Figures:

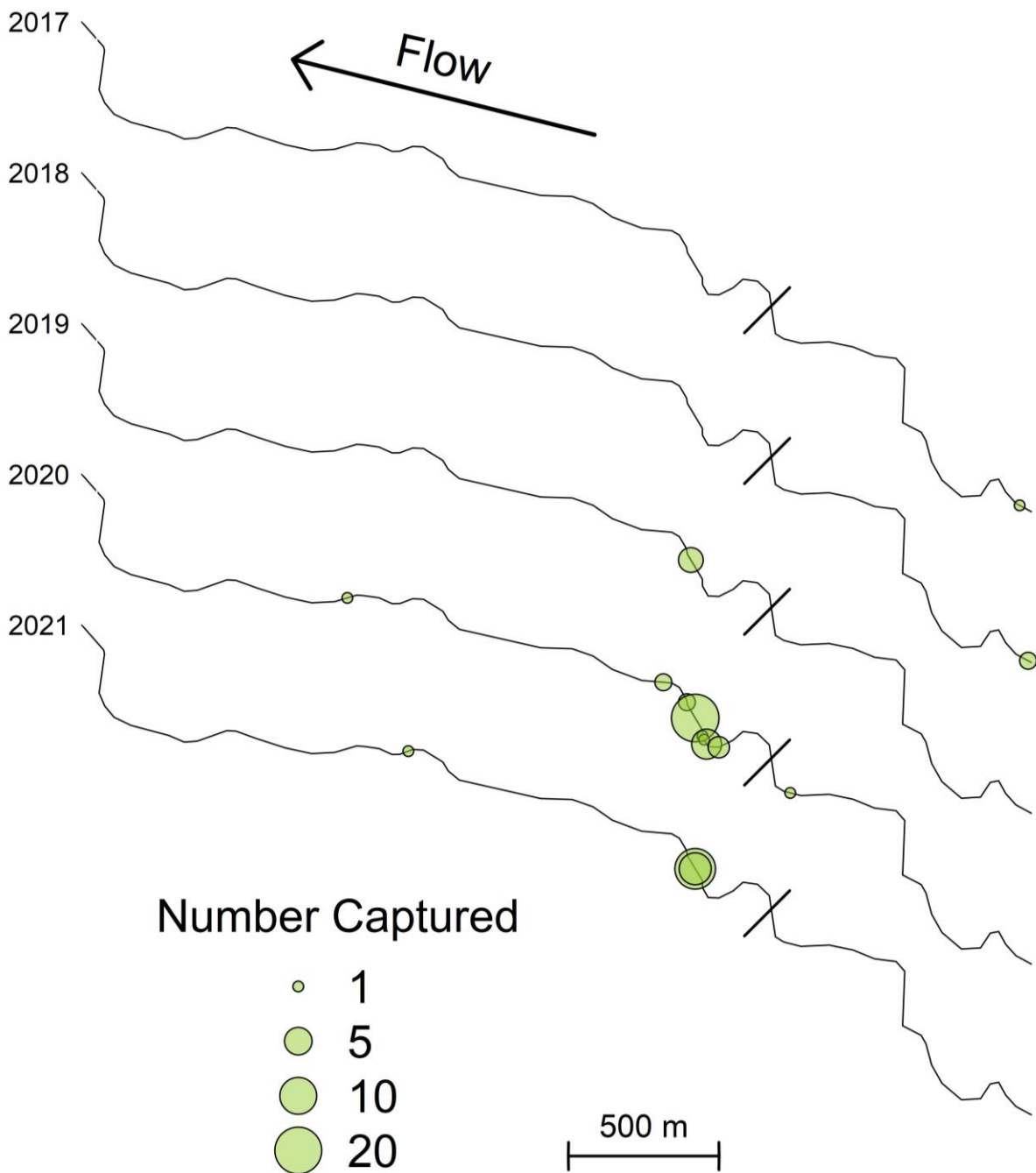


Figure 20.—Locations of Green Sunfish captured in the perennial portion of Harden Cienega Creek from 2017-2021. The barrier location is indicated by a diagonal line. Size of points indicates number of fish captured at a particular location during nonnative removal or monitoring efforts. Monitoring during 2017-2019 was only above the barrier.

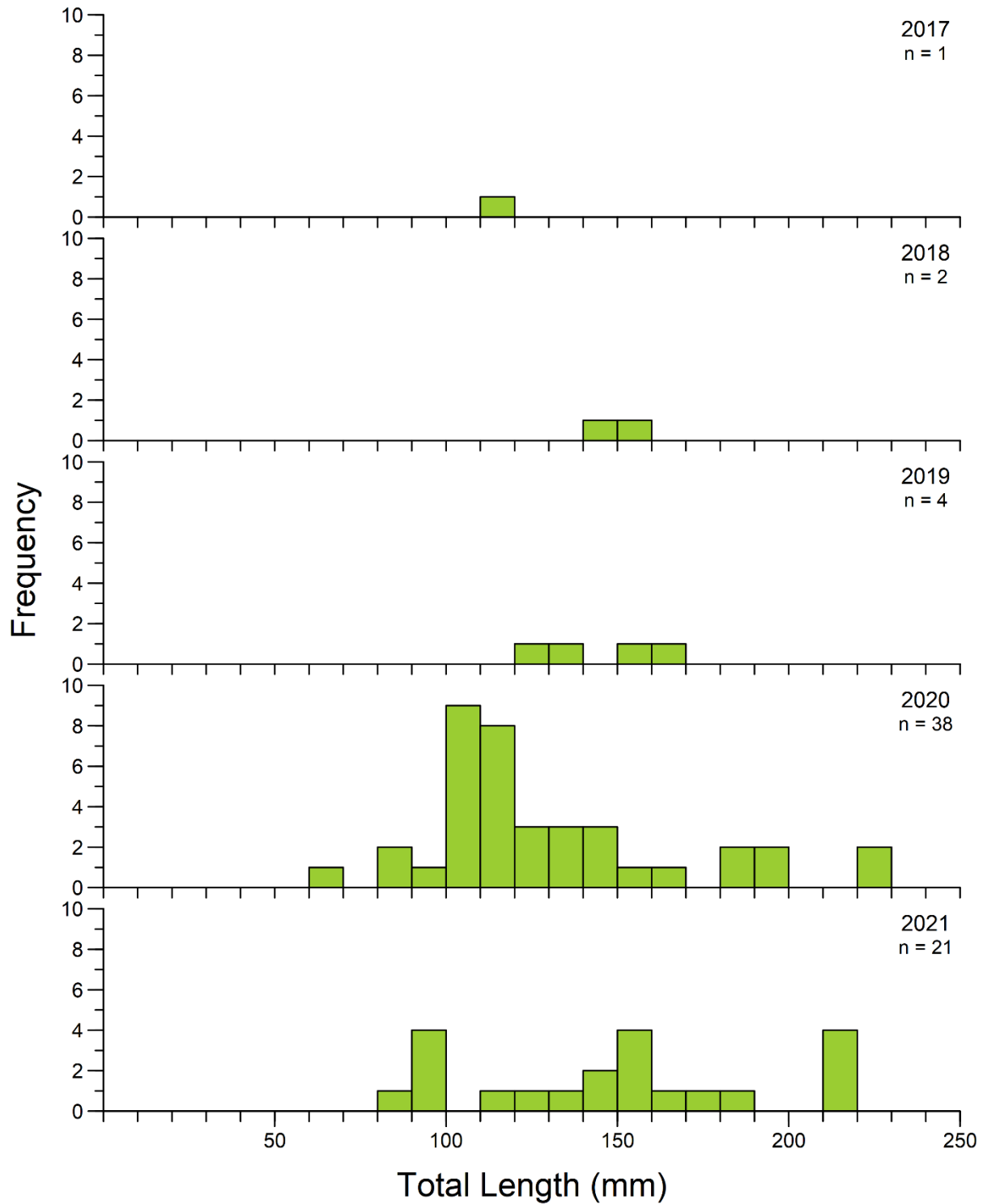


Figure 21.—Length frequency distribution of the number of Green Sunfish captured and removed during annual monitoring and nonnative removal efforts in Harden Cienega Creek, from 2017 to 2021. Number of fish captured and measured each year is shown in the top right corner of each panel.

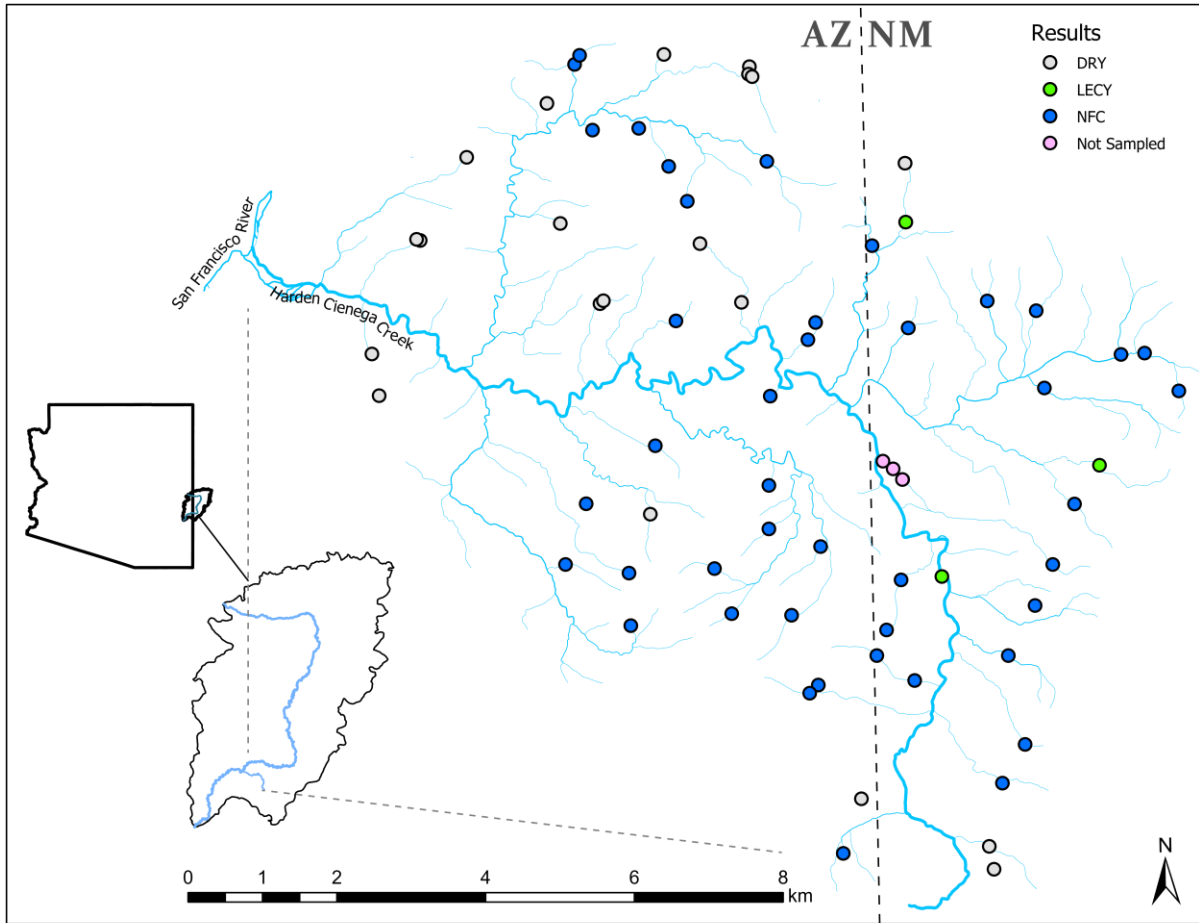


Figure 22.—Map of all tanks surveyed in the Arizona and New Mexico portions of the Harden Cienega Creek drainage during 2020 and 2021. Shown are tanks that were dry upon arrival (grey points), tanks that contained water and were sampled by bag seine, straight seine or dip net (blue points). Also shown are Ditch Tank, Distill Tank and California Tank where Green Sunfish were detected (green points). Three tanks on private property remain in New Mexico remain to be sampled (pink points).

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

Strategic Plan Goals:

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

Recovery Objectives:

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

Background: Red Tank Draw is a tributary to Wet Beaver Creek on the Coconino National Forest. Red Tank Draw is occupied by Roundtail Chub¹, Longfin Dace, Desert Sucker, Sonora Sucker, and several nonnative species including Green Sunfish, Black Bullhead, Fathead Minnow, and Northern crayfish. Roundtail Chub¹ inhabit a fragmented perennial reach between the USGS gage and the confluence of Rarick and Mullican Canyons. Perennial pools exist in the tributaries Rarick Canyon and Mullican Canyon that support nonnative fishes. The total perennial portion of Red Tank Draw is about 2.4 km long and is isolated from upstream invasion of nonnative fish from Wet Beaver Creek by an intermittent reach that is 7.7 km long, but may be passable during continuous flows. The purpose of this project is to remove Green Sunfish and Black Bullhead from the Roundtail Chub¹ occupied reach, and the entire drainage above the chub occupied reach if possible.

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

A comprehensive survey of stock tanks in the Red Tank Draw drainage above the chub occupied reach in 2017 found only Fathead Minnow occurred in the Rarick Canyon drainage (Rarick Tank and Gnat Tank). Unfortunately, Green Sunfish and Black Bullhead were detected in Mullican Place Tank in the Mullican Canyon drainage. Mullican Place Tank is immediately downstream of Bruce Place Tank which is on private property. The landowner indicated that fish were present in Bruce Place Tank, but denied access for sampling in 2017. An impassable waterfall barrier (UTM 12S 437657/3843902) was documented in Rarick Canyon in 2018. The barrier is approximately 10 m in height and is located about 2 km upstream from the confluence with Mullican Canyon. Isolated perennial pools upstream of the barrier in Rarick Canyon were visually assessed during 2017 and 2018 and a total of 23 perennial pools were identified, with Fathead Minnow observed throughout the wetted reach. Unfortunately, two Black Bullhead were also observed in a single pool upstream of the barrier in the 2018 surveys. A removal plan for the Red Tank Draw drainage, including Rarick and Mullican Canyons, was drafted in March, 2019 (Hickerson and Robinson 2019). Intensive trapping efforts in Rarick Canyon in 2019 resulted in the removal of 13 Black Bullhead from the isolated pools above the waterfall barrier. By August 2019, Black Bullhead were determined to be eradicated from Rarick Canyon. In October 2019, a total of 315 Roundtail Chub¹ were collected from Red Tank Draw and translocated to a series of three isolated pools above the barrier in Rarick Canyon. In April 2020, a total of 649 Gila Topminnow were stocked into a single pool in Rarick Canyon (F18, Figure 23) upstream of the barrier falls. In October 2020, a total of 154 Roundtail Chub¹ were translocated to two isolated pools in Rarick Canyon. A total of 23 Roundtail Chub and 447 Gila Topminnow were captured during the first annual monitoring effort in October 2020.

Results:

Red Tank Draw

During June 1-3 and 14-16, 2021, Department staff completed four full removal passes in Red Tank Draw. A combination of backpack electrofishing, mini-hoop nets, and minnow traps were used to target Green Sunfish. A total of 564 Green Sunfish were removed during all four passes (Table 7). Total Green Sunfish catch declined with each successive pass (Table 8, Figure 24). Across all four passes, total Green Sunfish catch (2021 n = 531, 2020 n = 749) and mean relative abundance of fish captured by backpack electrofisher (fish/h; 2021 = 155.52, 2020 = 254.50) substantially decreased compared 2020. Because the removals occurred within a two week period and surface water was isolated to a few short reaches, the sunfish population can reasonably be considered a closed population and the initial population size in Red Tank Draw in 2021 can be estimated. The initial population size of the Green Sunfish population in Red Tank Draw was likely between 627 and 726 individuals at the start of removal efforts, however capture probability was somewhere between 0.31 and 0.41 for all passes (Table 8). While some measures indicate the sunfish population is declining, these estimates suggests that it would still require a substantial

¹ Roundtail Chub in this location were previously classified as Gila Chub.

amount of effort to eradicate the remaining sunfish in Red Tank Draw even if additional spawning does not occur.

Mean size of Green sunfish remained virtually the same with each successive pass, and overall size structure was not meaningfully different from 2020 (Figure 25). Green Sunfish as small as 74 mm TL have been observed to be ripe with eggs, which will likely make suppression of spawning much more difficult if sunfish are able to produce viable gametes at such a small size. Removal efforts may have successfully eradicated Black Bullhead from this reach since bullhead have not been captured since 2018. Unfortunately, bullhead will likely have the opportunity to recolonize this reach in the future as they disperse from upstream sources in the Mullican Canyon drainage.

Across all four passes, total Roundtail Chub captured by backpack electrofishing in 2021 ($n = 1606$) declined relative to the number captured 2020 ($n = 2096$). However, mean relative abundance of chub captured by backpack electrofishing (fish/h) substantially increased (2020 = 254.17, 2021 = 388.44; Table 7). Chub numbers appear to have declined slightly compared to 2020, but still remain higher than nearly any other year since removal efforts began, despite removing several hundred individuals to augment Rarick Canyon each of the last three years (Table 7, Figure 24). Fewer young of year chub (< 50 mm TL) were captured than the past two years, which could be partially attributable to the extraordinary drought conditions through the spring that may not have facilitated ideal spawning conditions (Figure 26). Juvenile Roundtail Chub (50-100 mm TL) remain relatively abundant in Red Tank Draw despite Green Sunfish still being present in high numbers (Figure 26).

Rarick Canyon Monitoring

On October 12, 2021, Department staff monitored translocated populations of Gila Topminnow and Roundtail Chub in Rarick Canyon. The crew set a combination of 12 collapsible minnow traps and 15 mini-hoop nets in the four pools previously stocked with chub (F23, F20, F18, F17; Figure 23) for a soak time of about four hours. A total of 5 Roundtail Chub and 755 Fathead Minnow were captured. Gila Topminnow were not captured or observed in the pool where they were initially stocked (F18) or any other pool downstream, although conditions were not ideal for detecting topminnow (air temps in the 40's F and overcast skies). During augmentation efforts two days later, dozens of chub were observed in F17 where none were captured during the monitoring effort. Contrary to 2020, flow was present throughout nearly the entire surveyed reach, and most pools were at or near capacity. Capture probability was likely lower this year since a similar number of traps were used to sample a much greater volume of water, which may partially explain the lower number of chub captured compared to 2020 ($n = 23$). Also, a single seine haul in pool F20, which was reduced to a few square meters in size at the time, accounted for a majority of the fish captured in 2020 ($n = 17$). Given these conditions, the chub population was probably substantially undercounted. It is possible that topminnow were still present, but not detected during

monitoring. However, the population is near the extreme upper range of the elevation tolerance of the species and winter water temperature could be a limiting factor.

Rarick Canyon Translocations

On October 21, 2021, Department staff translocated Roundtail Chub from Red Tank Draw to two isolated pools in Rarick Canyon. A total of 150 Roundtail Chub were collected from Red Tank Draw by backpack electrofishing and transported in aerated buckets to three pools above the waterfall barrier in Rarick Canyon. A total of 51 chub were translocated to the most upstream pool that has been stocked with chub to date (F17), 46 to the next pool downstream (F18), and the remaining 53 to the next downstream pool (F20). There were no mortalities during collection, transport or stocking.

Recommendations: Removal efforts in Red Tank Draw will not continue next year due to anticipated changes in funding and Program priorities. Removal efforts in Red Tank Draw have successfully increased the numbers of both Roundtail Chub and Desert Sucker, which has allowed for translocation of some chub isolated habitats upstream in Rarick Canyon that are now free of nonnative predatory fish. If possible, Roundtail Chub¹ should also be stocked into Gnat Tank with the goal of reducing or eliminating the upstream source of Fathead Minnow to Rarick Canyon. Translocations of some Desert Sucker to Rarick Canyon should potentially be considered by other Department programs or agencies in the future, due to the relatively low abundance of the sucker population in Red Tank Draw. In the absence of additional removal efforts, Green Sunfish numbers will likely increase and Roundtail Chub number will likely gradually decrease to the low numbers observed at the onset of removal efforts. A survey should be conducted by Department staff in the future to determine the status of the chub population in the absence of annual removal efforts.

Nonnative fish should be eradicated from the two tanks in Mullican Canyon drainage where nonnative fish are present (Mullican Place Tank and Bruce Place Tank) should permission be obtained from the Bruce Place Tank landowner. If eradication of nonnative fish from these tanks can be achieved, removal efforts in Red Tank Draw should be re-initiated with the goal of eradicating the Green Sunfish population.

The Rarick Canyon population of Roundtail Chub should be monitored for at least five years after the most recent augmentation (2026) to determine whether the population establishes. Additional augmentation of the topminnow population in Rarick Canyon should be discussed with partners due to the potential for minimum winter water temperatures to prevent population establishment at this location. If additional stockings do not occur, the topminnow population should be monitored through 2023. Monitoring of the Roundtail Chub and Gila Topminnow populations in Rarick Canyon will be reported under the ‘Gila Topminnow stockings’ subproject in future years.

Tables and Figures:

Table 7.—Summary of fish captured by gear type during each year of nonnative fish removal efforts in Red Tank Draw from 2016-2021. Shown for each year and gear type is the total number of fish captured (#Ind), the mean relative abundance (number of fish captured per hour of electrofishing effort, or per hour of trap effort; #Ind/h) and standard error of mean relative abundance (SE).

Year	Gear	Statistic	Roundtail Chub	Desert Sucker	Green Sunfish	Black Bullhead	Fathead Minnow
2016	Backpack Electrofisher	#Ind	78		205	122	23
		#Ind/h	109.17		472.22	55.22	16.53
		SE	(11.34)		(26.12)	(9.70)	(2.95)
2016	Mini-hoop Net	#Ind	12		72	23	3
		#Ind/h	0.11		0.46	0.23	0.20
		SE	(0.01)		(0.02)	(0.03)	(0.14)
2017	Backpack Electrofisher	#Ind	96	4	185	10	54
		#Ind/h	37.02	3.48	45.03	3.63	19.25
		SE	(1.62)	(0.00)	(1.58)	(0.57)	(2.56)
2017	Mini-hoop Net	#Ind			27		2
		#Ind/h			1.29		0.11
		SE			0.14		0.01
2018	Backpack Electrofisher	#Ind	570	5	894	26	371
		#Ind/h	152.73	3.82	242.09	15.14	66.07
		SE	(13.95)	(0.82)	(5.14)	(1.21)	(15.92)
2018	Mini-hoop Net	#Ind	1		4	6	
		#Ind/h	0.05		1.07	1.16	
		SE	(0.00)		(0.00)	(0.18)	
2018	Collapsible Minnow Trap	#Ind	20		148	43	7
		#Ind/h	0.18		1.28	2.90	0.31
		SE	(0.03)		(0.13)	(0.33)	(0.13)
2019	Backpack Electrofisher	#Ind	845	14	188		67
		#Ind/h	399.96	7.37	203.93		53.87
		SE	(11.10)	(0.45)	(15.49)		(17.35)
2019	Mini-hoop Net	#Ind	1		8		1
		#Ind/h	0.21		1.71		0.21
		SE	(0.00)		(0.00)		(0.00)
2019	Collapsible Minnow Trap	#Ind	1		6		
		#Ind/h	0.33		0.44		
		SE	(0.00)		(0.08)		
2020	Backpack Electrofisher	#Ind	2096	38	749		232
		#Ind/h	254.17	15.49	254.50		90.90
		SE	(10.75)	(1.67)	(8.44)		(27.85)
2020	Mini-hoop Net	#Ind	4		21		11
		#Ind/h	0.16		0.86		0.42
		SE	(0.03)		(0.17)		(0.30)
2020	Collapsible Minnow Trap	#Ind	10		101		14
		#Ind/h	0.35		4.31		0.69
		SE	(0.13)		(0.29)		(0.34)

2021	Backpack Electrofisher	#Ind	1606	19	531	220
		#Ind/h	388.44	5.81	155.52	101.68
		SE	(7.30)	(2.99)	(4.46)	(41.45)
2021	Mini-hoop Net	#Ind	1		10	
		#Ind/h	0.06		0.86	
		SE	(0.00)		(0.16)	
2021	Collapsible Minnow Trap	#Ind	3		23	
		#Ind/h	0.53		0.86	
		SE	(0.18)		(0.12)	

Table 8.—Four-pass depletion estimates of abundance for Green Sunfish and Fathead Minnow within the removal reach of Red Tank Draw during 2021. Included is the number of fish caught in each pass (C1, C2, C3, C4), Carle-Strub four pass abundance estimate (N), 95% confidence interval of the abundance estimate (N_LCI, N_UCI), estimated capture probability (p), and 95 % confidence interval of estimate of capture probability (p_LCI, p_UCI).

Taxa	C1	C2	C3	C4	N	N_LCI	N_UCI	p	p_LCI	p_UCI
Green Sunfish	264	128	93	79	677	627.61	726.39	0.36	0.31	0.41
Fathead Minnow	75	56	59	30	338	244.94	431.06	0.23	0.14	0.32

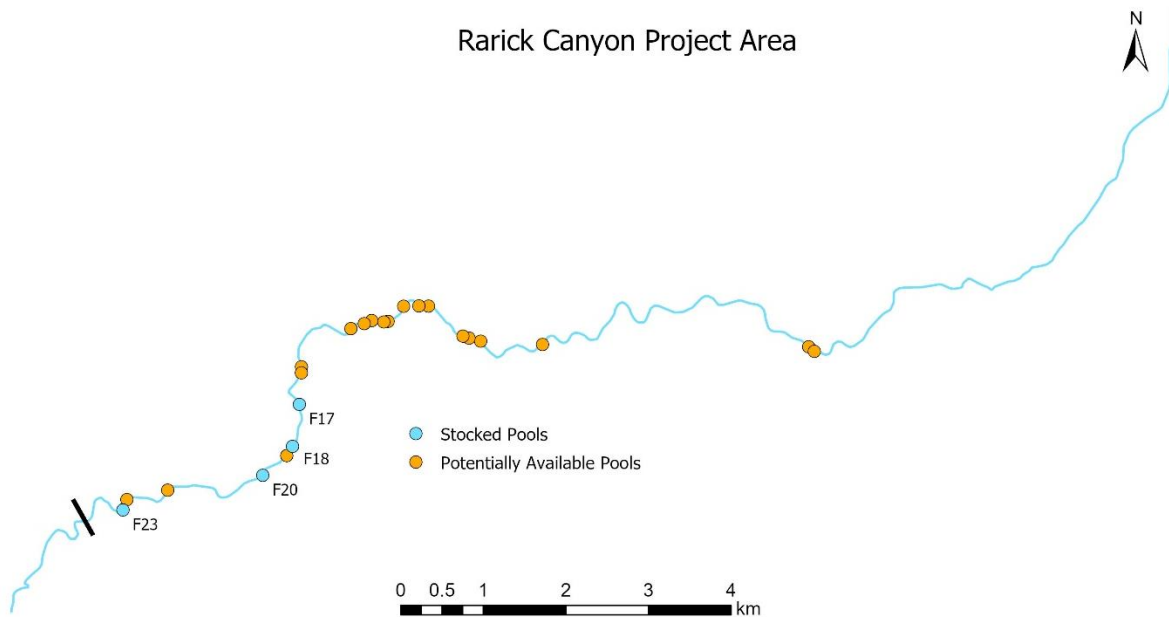


Figure 23.—Map of Rarick Canyon (blue line) including the location of the barrier falls (thick black line), names and locations of pools stocked with fish (blue dots), and the remaining potentially habitable pools in Rarick Canyon (orange dots).

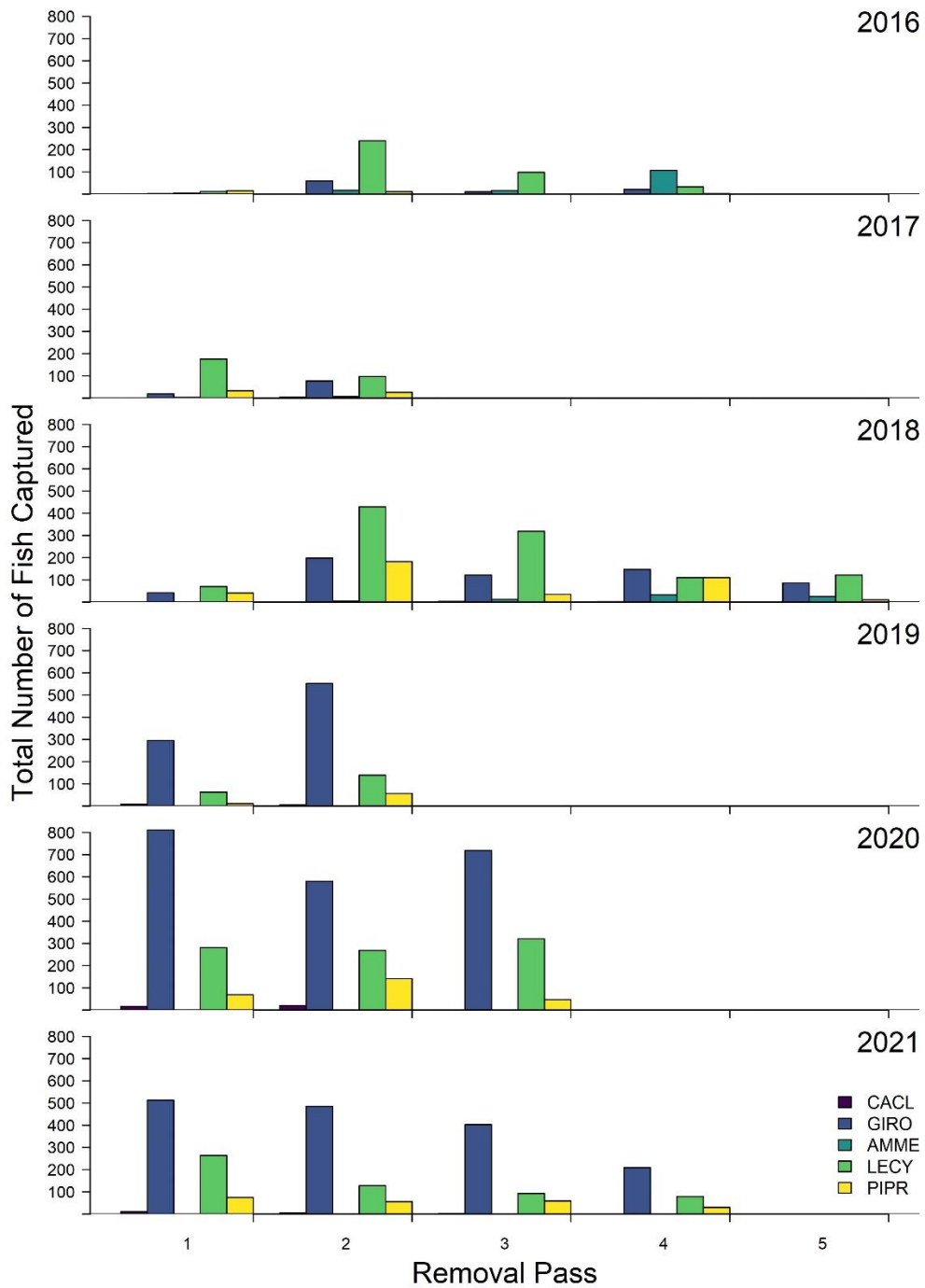


Figure 24.—Summary of species (CACL = Desert Sucker, GIRO = Roundtail Chub¹, AMME = Black Bullhead, LECY = Green Sunfish, PIPR = Fathead Minnow) captured in each removal pass in Red Tank Draw from 2016 to 2021. Total number of fish captured includes fish captured by backpack electrofishing, mini-hoop nets, minnow traps and angling.

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

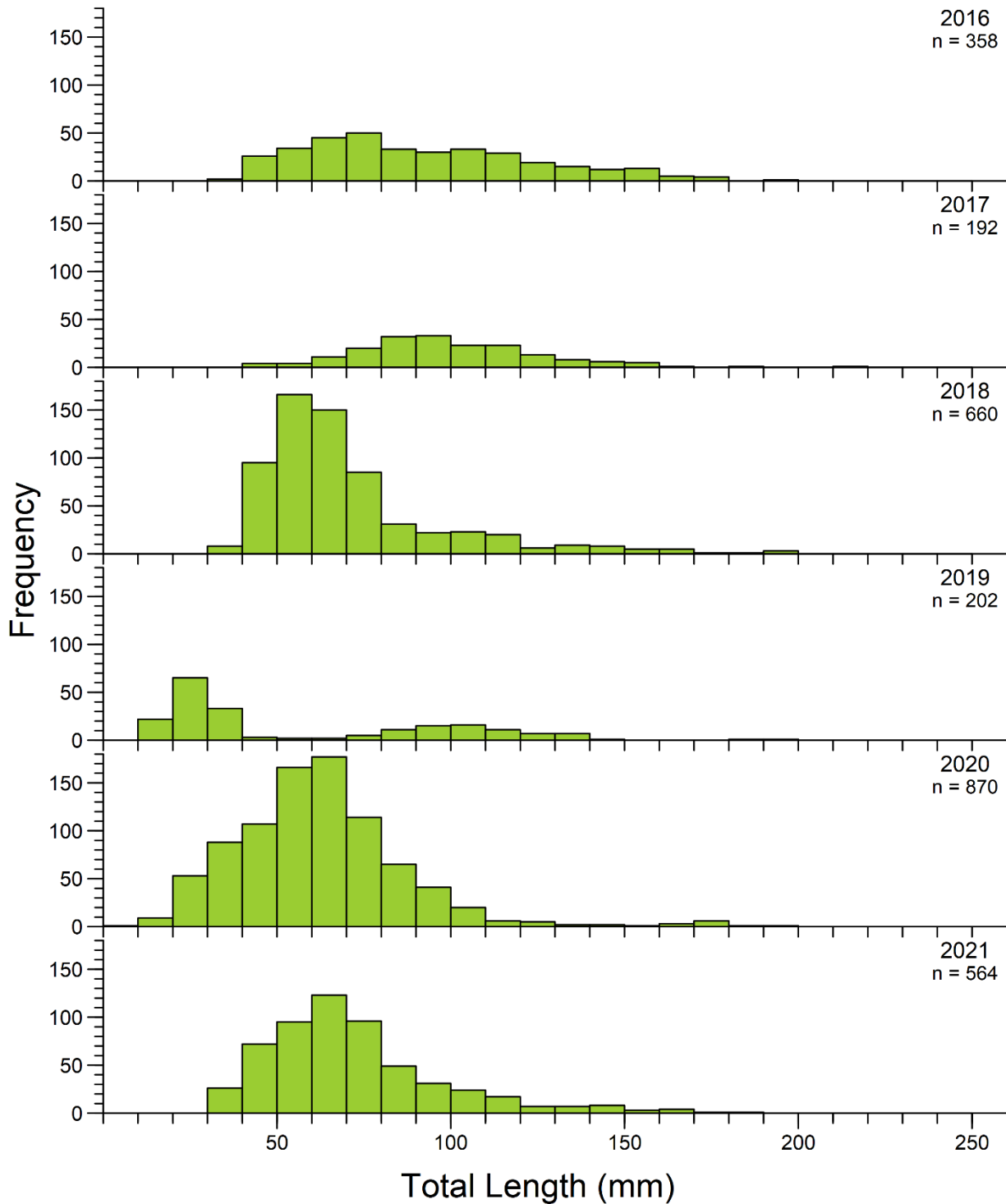


Figure 25.—Length frequency distribution of the number of Green Sunfish captured during nonnative removal efforts in Red Tank Draw, from 2016 to 2021. Number of fish captured and measured each year is shown in the top right corner of each panel.

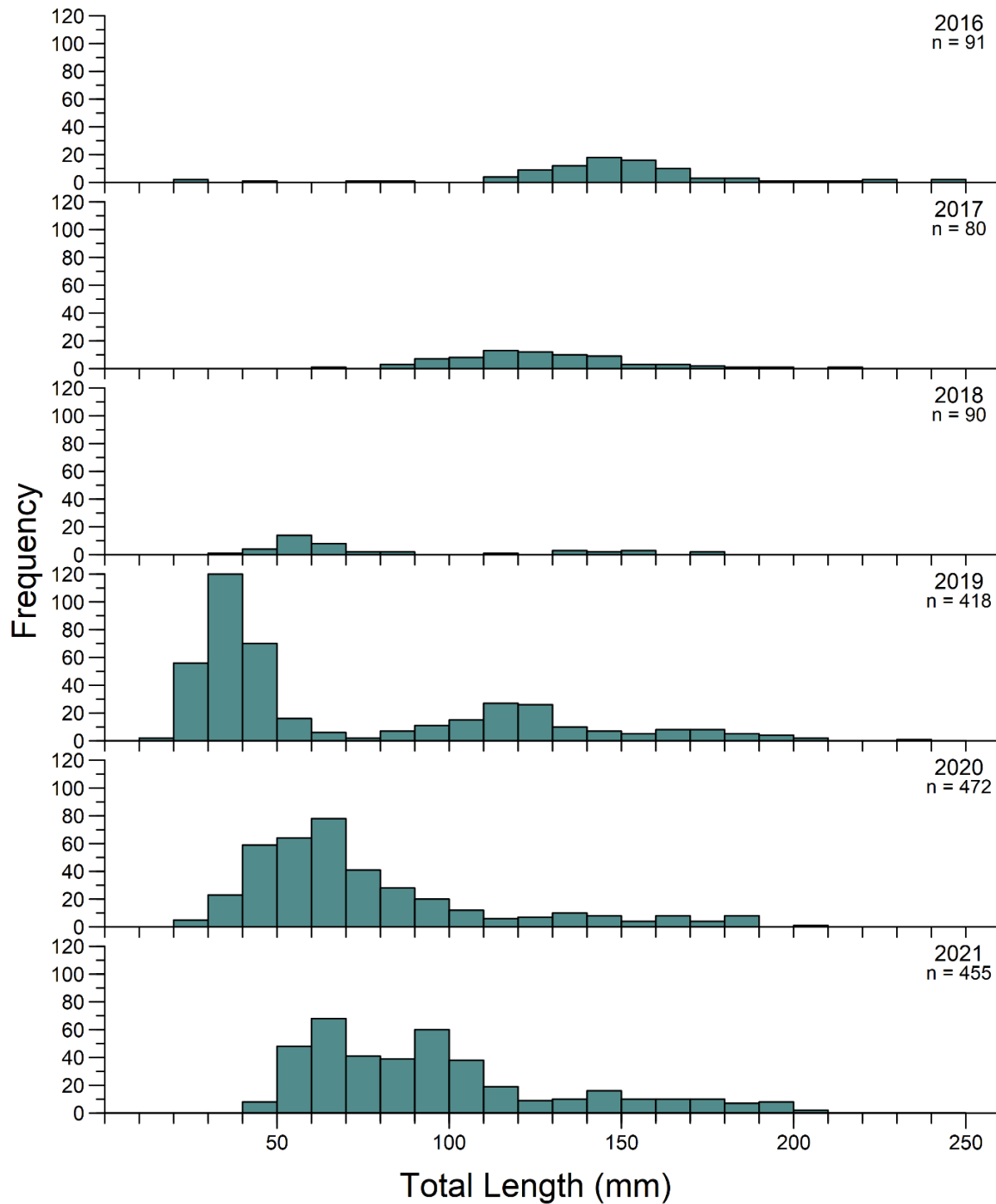


Figure 26.—Length frequency distribution of the number of Roundtail Chub¹ captured during nonnative removal efforts in Red Tank Draw, from 2016 to 2021. In general, only the first 100 Roundtail Chub¹ captured per pass were measured between 2018 and 2021. Number of fish captured and measured each year is shown in the top right corner of each panel.

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

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

Strategic Plan Goals:

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

Recovery Objectives:

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

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 has not been detected since 2001. Extirpation of topminnow has primarily been attributed to predation and competition with nonnative mosquitofish. The purpose of this project is to eradicate Western Mosquitofish from Sharp Spring and reintroduce Sharp Spring lineage Gila Topminnow. Eradication of Western Mosquitofish would also create an opportunity to potentially replicate a population of upper Santa Cruz River Roundtail Chub¹ in Sharp Spring.

¹ Roundtail Chub in the upper Santa Cruz River previously classified as Gila Chub.

In June 2013, Department staff attempted to eradicate Western Mosquitofish by draining the pools in Sharp Spring with gasoline powered pumps. The two most upstream pools were pumped down, but not entirely drained due to the unexpected depth of fine sediment in the bottom of the pools, which retained water (and fish) and fouled the pumps. The pools partially refilled overnight, and live mosquitofish were observed the following morning. The pumping effort was abandoned because the pools could not be completely dried. The Department met with AZSP staff in January 2017 to discuss options for nonnative fish eradication in Sharp Spring. Following the meeting, communication broke down between AZSP staff the Department with little progress made until 2020.

Results: During 2021, Department staff submitted a commercial rental permit for research and monitoring and received approval from AZSP to move forward with Sharp Spring treatment planning. Department staff also completed Stage 1 (project proposal, internal review and approval) and Stage 2 (preliminary treatment plan, public involvement plan, internal review and approval) of the Department's piscicide treatment planning and procedures manual. In June 2021, Department staff visited Sharp Spring to obtain volumetric measurements of the pools to inform treatment planning documents. Department staff also made progress on the third and final stage (Stage 3) of treatment planning (intermediate treatment plan, public meetings, environmental compliance, commission review). On November 10, 2021 Department staff hosted a public meeting to brief the public on the proposed rotenone treatment in Sharp Spring, as required in Stage 3 of the treatment planning process. As of the end of 2021, internal environmental compliance documentation (Environmental Assessment Checklist) was submitted. An intermediate treatment plan was also drafted and approved by Department leadership. The proposed treatment is on track to occur in June 2022 pending approval by Department staff and the Arizona Game and Fish Commission.

Recommendations: Coordination and planning efforts for the proposed rotenone treatment of Sharp Spring should continue in 2022. If the proposed treatment is approved and successfully executed, Sharp Spring Gila Topminnow should be stocked as soon as possible.

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

Strategic Plan Goals:

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

Recovery Objectives:

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

Background: The upper Verde River Native Fish Restoration Project is a multi-agency effort focused on protecting and restoring the native fish assemblage within the upper Verde River drainage in central Arizona. The project is currently focused on assessing the feasibility of nonnative fish control efforts should Reclamation construct fish barriers on the Verde River. In 2019, Department staff assessed the feasibility of surveying stock tanks in the upper Verde River drainage for presence of nonnative fishes. Tanks most likely to support nonnative fish were identified using an automated approach developed in Program R to classify tanks as wet or dry using normalized difference water index values, and a scoring system based on perennial status, previous nonnative fish records, and distance to the Verde River. A total of 146 tanks received a score of 2 or greater which corresponded to the greatest risk categories. The goal of tank surveys

is to understand the species composition and distribution of nonnative fish in the upper Verde River drainage and the potential for nonnative fish to disperse to the Verde River.

Results: Of the 146 tanks identified as having the greatest risk to the Verde River, a total of 52 were surveyed by Department Gila River Basin Native Fish Program staff in 2021. All tanks with water present were sampled with three hauls of a bag seine (see general methods) unless noted otherwise. The total number of fish captured at each location, mean relative abundance (CPUE, fish/m²), coordinates, and water presence for each tank surveyed in 2021 can be found in Table 9. Map of the location and species composition of tanks sampled in 2021 can be found in Figure 27.

In addition to the 52 tanks surveyed, Department regional staff were able to document dry tanks as they patrolled their units and made contact with private landowners and public land lessees to request permission to sample tanks. Some landowners and lessees chose to provide information about whether fish were known to be present, or if the tank ever spills which will help inform future planning efforts. Department regional staff identified an additional 14 tanks as dry and 17 tanks where permission to sample was denied (Table 10, Figure 27).

During July 19-21, 2021, Department staff conducted the first tank surveys in the upper Verde River drainage. A total of 15 tanks were visited on the Kaibab National Forest, three of which (Lawless Tank, Tule Tank, and Station Tank) were dry upon arrival. Green Sunfish were detected in Burro Tank (n = 2,119), Bear Tank 2 (n = 102) and Split Tank (n = 816). Yellow Bullhead were detected in Dutch Kid Tank (n = 119). The remaining tanks were fishless.

During July 26-28, 2021, Department staff conducted tank surveys in the upper Verde River drainage. A total of 14 tanks were visited on the Kaibab National Forest. Green Sunfish were detected in Big T Tank (n = 286), MC Tank (n = 219), Thirty-Six Tank (n = 340), and DT Tank (n = 1). Green Sunfish (n = 617) and Northern crayfish (n = 5) were detected in Barney Tank. Largemouth Bass (n = 13), Black Crappie (n = 46), Bluegill (n = 332), hybrid sunfish (Bluegill x Green Sunfish; n = 1) and Green Sunfish (n = 2) were detected in Mesa Tank. Red-eared slider (n = 4) were detected in Hat Tank. The remaining tanks were fishless. There was a substantial amount of flooding prior to the surveys from monsoon rains and many tanks were still filling and spilling or there was evidence of a recent spill. Green Sunfish were detected several hundred meters upstream and downstream of Barney Tank, MC Tank, and Thirty-six Tank, indicating that these tanks occasionally serve as a source of Green Sunfish dispersal both upstream and downstream in their respective drainages. In addition, the crew walked MC Canyon and an unnamed tributary to MC Canyon (from Burro Tank) and visually observed Green Sunfish throughout the stream and in a natural pool called MC Canyon Natural Tank. Sunfish appeared to be dispersing out of Burro, Big T and/or MC Tank to reach this location.

During August 9-11, 2021, Department staff conducted tank surveys in the upper Verde River drainage. A total of 10 tanks were visited on the Kaibab and Prescott National Forests, one of which (Rattlesnake Tank) was dry upon arrival. Green Sunfish (n = 15) and Yellow Bullhead (n = 4) were detected in Grindstone Tank on the Prescott National Forest. The remaining tanks were fishless.

During August 16-18, 2021, Department staff conducted tank surveys in the upper Verde River drainage. A total of 13 tanks were visited on the Prescott National Forest and State Trust Lands. Yellow Bullhead (n = 20) were detected in Turkey Tank on the Prescott National Forest. The remaining tanks were fishless.

On August 26, 2021, Department staff completed tank and stream surveys in the upper Verde River drainage. One tank was visited on the Kaibab National Forest. Green Sunfish were detected in Lower Bass Pond Tank (n = 31) using a straight seine. Green Sunfish were also visually observed in nearly all pools in Hell Canyon downstream of Lower Bass Pond Tank (upstream of Hell Canyon Tank). The crew also surveyed a perennial spring-fed portion of Hell Canyon called King Spring (downstream of Hell Canyon Tank) using a backpack electrofisher and detected Green Sunfish (n = 64), Fathead Minnow (n = 9), and Red Shiner (n = 4). An isolated pool in Grindstone Wash 4.5 km downstream of Grindstone Tank was surveyed with a straight seine resulting in the capture of Green Sunfish (n = 3). Yellow Bullhead were also visually observed in the same pool. An isolated pool in MC Canyon 4.5 km upstream of the confluence with the Verde River was sampled with a straight seine resulting in the capture of Fathead Minnow (n = 1). Green Sunfish and Red Shiner were also visually observed in the same pool. Results of these surveys suggest that nonnative fish, particularly Green Sunfish, are able to disperse out of stock tanks and travel relatively close to the Verde River in multiple drainages.

Overall, a total of 53 tanks were surveyed in 2021 with nonnative fishes being detected in 13 of the 52 tanks (Table 9). Of the tanks where nonnative fish were detected, Green Sunfish were by far the most common (n = 11), followed by Yellow Bullhead (n = 3), with a single tank supporting Largemouth Bass, Black Crappie, Bluegill, and hybrid sunfish. Far more tanks contained nonnative fishes than originally anticipated. Multiple size classes of Green Sunfish were detected in many of the tanks which suggests that these populations are self-sustaining and can persist even through the extraordinary drought conditions of early 2021. The heavy monsoon rains in 2021 caused many tanks to fill and spill and allowed for observations of nonnative fish movement within the upper Verde River drainage during ideal flow conditions for dispersal. The presence of Green Sunfish and Yellow Bullhead in isolated pools within the stream channels of Hell Canyon, Grindstone Wash, and MC Canyon suggests that dispersal of nonnative fish from stock tanks within the drainage to the Verde River is a very real concern for any future attempts to reintroduce native fishes to the Verde River (Figure 28).

Department staff also participated in several monthly barrier meeting updates in 2021.

Recommendations: Tank surveys in the upper Verde River drainage should continue in 2022. A total of 83 tanks were surveyed, verified as dry or denied access to sample in 2021. Access to the remaining 63 tanks within 30 km of the Verde River should be pursued, and all tanks where permission is granted should be surveyed in 2022. In addition, stream channels downstream of stock tanks containing nonnative fishes should be surveyed to determine the community composition and distribution of nonnative fish in these drainages. It may be valuable to survey additional tanks farther than 30 km (straight line distance) away from the Verde River if there are concerns about additional tanks potentially serving as sources of nonnative fish dispersal to the Verde River. Information about the frequency and duration of hydrologic connections between tributary streams (Hell Canyon, Chino Valley Wash, etc.) and the Verde River should also be evaluated with trail camera or modified conductivity loggers to better understand the risk of dispersal of nonnative fishes from stock tanks to the Verde River. Department staff will continue to participate in Verde River barrier calls and will begin internal discussions about how to manage nonnative fish in stock tanks and tributaries to the upper Verde River.

Tables and Figures:

Table 9.—Summary of stock tanks and stream sites surveyed in the upper Verde River drainage in 2021. Included for each location is the site easting and northing (UTM NAD83 12S), whether water was present (status), gear type, species captured, total number of individuals captured, and the catch per unit effort (CPUE; fish/m² for bag seine and straight seine, fish/h for backpack electrofishing) for each species at each location.

Water Name	Easting	Northing	Status	Gear	Taxa	Catch	CPUE
Barney Tank	394820	3892130	Wet	Bag Seine	Green Sunfish	670	3.62
Barney Tank	394820	3892130	Wet	Bag Seine	Northern Crayfish	5	0.01
Bean Dam Tank	375359	3879829	Wet	Bag Seine	No Fish	0	0
Bear Tank 2	388412	3890527	Wet	Bag Seine	Green Sunfish	102	0.4
Betty Tank	377818	3865971	Wet	Bag Seine	No Fish	0	0
Big T Tank	391105	3888621	Wet	Bag Seine	Green Sunfish	286	1.5
Black Mesa No2	386687	3859831	Wet	Bag Seine	No Fish	0	0
Bodkin Tank	389372	3848918	Wet	Bag Seine	No Fish	0	0
Borrow Tank	389665	3892785	Wet	Bag Seine	No Fish	0	0
Bottom Tank	385836	3890206	Wet	Bag Seine	No Fish	0	0
Boundary Tank	373370	3854448	Wet	Bag Seine	No Fish	0	0
Burro Tank	386742	3887263	Wet	Bag Seine	Green Sunfish	2119	9.81
Buzzard Tank	389507	3883873	Wet	Bag Seine	No Fish	0	0
Cow Tank #1	383255	3883721	Wet	Bag Seine	No Fish	0	0
Davenport Tank	392805	3885397	Wet	Bag Seine	No Fish	0	0
DT Tank	388955	3880628	Wet	Bag Seine	Green Sunfish	1	0
Dutch Kid Tank	383290	3888212	Wet	Bag Seine	Yellow Bullhead	119	0.21
Elk Tank	384297	3889888	Wet	Bag Seine	No Fish	0	0
First Tank	391958	3888015	Wet	Bag Seine	No Fish	0	0
Gas Tank	382444	3850577	Wet	Bag Seine	No Fish	0	0
Grindstone Tank	381538	3872573	Wet	Bag Seine	Yellow Bullhead	4	0.01
Grindstone Tank	381538	3872573	Wet	Bag Seine	Green Sunfish	15	0.06
Grindstone Wash	381512	3867991	Wet	Straight Seine	Green Sunfish	3	0.12
Harolds Tank	388443	3848834	Wet	Bag Seine	No Fish	0	0
Juniper Tank	383072	3862397	Wet	Bag Seine	No Fish	0	0

King Spring	378785	3867835	Wet	Backpack Electrofisher	Red Shiner	4	15.52
King Spring	378785	3867835	Wet	Backpack Electrofisher	Green Sunfish	64	744.83
King Spring	378785	3867835	Wet	Backpack Electrofisher	Fathead Minnow	9	69.83
King Tank	376705	3852274	Wet	Bag Seine	No Fish	0	0
Lawless Tank	379912	3889172	Dry	Visual Survey	No Fish	0	
Little Page Tank	374882	3867179	Wet	Bag Seine	No Fish	0	0
Lower Bass Pond Tank	374990	3884146	Wet	Straight Seine	Green Sunfish	31	1.16
MC Canyon Tributary	387591	3885447	Wet	Visual Survey	Green Sunfish	8	
MC Canyon	382011	3867788	Wet	Straight Seine	Fathead Minnow	1	0.01
MC Tank	389070	3886496	Wet	Bag Seine	Green Sunfish	219	1.44
McClure Tank	381227	3868024	Wet	Bag Seine	No Fish	0	0
Mesa Tank	392110	3892829	Wet	Bag Seine	Green Sunfish	2	0.01
Mesa Tank	392110	3892829	Wet	Bag Seine	Bluegill	332	2.96
Mesa Tank	392110	3892829	Wet	Bag Seine	Bluegill x Green Sunfish Hybrid	1	0.01
Mesa Tank	392110	3892829	Wet	Bag Seine	Largemouth Bass	13	0.07
Mesa Tank	392110	3892829	Wet	Bag Seine	Black Crappie	46	0.3
Metate Tank	389932	3889151	Wet	Bag Seine	No Fish	0	0
Middle Crossing Tank	380414	3888197	Wet	Bag Seine	No Fish	0	0
Midway Tank	384439	3860079	Wet	Bag Seine	No Fish	0	0
Mudd Tank	391023	3886889	Wet	Bag Seine	No Fish	0	0
Rattlesnake Tank	378068	3874053	Wet	Bag Seine	No Fish	0	0
Rattlesnake	387821	3884406	Dry	Visual Survey	No Fish	0	
Road Canyon Tank	393034	3852961	Wet	Bag Seine	No Fish	0	0
Roy Tank	378361	3881206	Wet	Bag Seine	No Fish	0	0
Sams Dam Tank	374269	3876601	Wet	Bag Seine	No Fish	0	0
Sand Flat Tank	392263	3870302	Wet	Bag Seine	No Fish	0	0
Shellrock Tank	378009	3878971	Wet	Bag Seine	No Fish	0	0

Split Tank	386790	3888884	Wet	Bag Seine	Green Sunfish	816	3.35
Station Tank	384412	3884811	Dry	Visual Survey	No Fish	0	
Thirty-Six Tank	386441	3882453	Wet	Bag Seine	Green Sunfish	340	2.27
Tule Tank	379996	3886414	Dry	Visual Survey	No Fish	0	
Turkey Tank	392374	3842844	Wet	Bag Seine	Yellow Bullhead	20	0.1
Upper Mormon Pocket Tank	395669	3857108	Wet	Bag Seine	No Fish	0	0
West Ike Tank	382281	3885627	Wet	Bag Seine	No Fish	0	0
West St Matthews Tank	381770	3847511	Wet	Bag Seine	No Fish	0	0

Table 10.—Summary of stock tanks in the upper Verde River drainage visited by Regional staff in 2021. Included for each location is the water name (Water Name), the site easting and northing (UTM NAD83 12S), whether water was present during the visit or reported present by property owner or lessee (Status), species reported as present by landowner or lessee (Taxa Reported), and whether the landowner or lessee was willing to request access for further surveys (Access Permission). Tanks reported as well fed without a spillway are indicated with an asterisk following the water name.

Water Name	Easting	Northing	Status	Taxa Reported	Access Permission
Unnamed Tank	349033	3875728	Dry	NA	No
Unnamed Tank	348559	3851095	Dry	NA	No
Green	369730	3890624	Dry	NA	Yes
Unnamed Tank	345909	3853490	Dry	NA	No
Unnamed Tank	346374	3853517	Dry	NA	No
North	369164	3890948	Dry	NA	Yes
Pipeline	371000	3884963	Dry	NA	Yes
Unnamed Tank	346370	3859822	Dry	NA	NA
Rafael Tank	340434	3852590	Dry	NA	NA
Stringfield Tank	351795	3841016	Dry	NA	No
Stringfield Tank	366699	3880822	Dry	NA	Yes
Stringfield Tank	359974	3836349	Dry	NA	No
Tank No 1	347021	3843201	Dry	NA	Yes
Winter Camp Tank	380469	3875872	Dry	NA	Yes
Lakin Tank	339838	3853066	Wet	Largemouth Bass	No
Unnamed Tank*	350205	3854010	Wet	Largemouth Bass	No
Unnamed Tank*	349545	3854052	Wet	Largemouth Bass	No
Unnamed Tank	346075	3839971	Wet	Largemouth Bass	No
Unnamed Tank *	339234	3853209	Wet	NA	No
Sampson Well	341598	3859721	Wet	NA	No
Section 5 Tank	343895	3852282	Wet	NA	No
Unnamed Tank	345857	3858100	Wet	Lepomis sp.	No
Unnamed Tank	351418	3869061	NA	NA	No
Unnamed Tank	351078	3866444	NA	NA	No

Unnamed Tank	354733	3866625	NA	NA	No
Unnamed Tank	365162	3834222	NA	NA	No
Unnamed Tank	349974	3861253	NA	NA	No
Unnamed Tank	346936	3876899	NA	NA	No
Unnamed Tank	346787	3865256	NA	NA	No
Unnamed Tank	348599	3873080	NA	NA	No
Unnamed Tank	354640	3857205	NA	NA	No

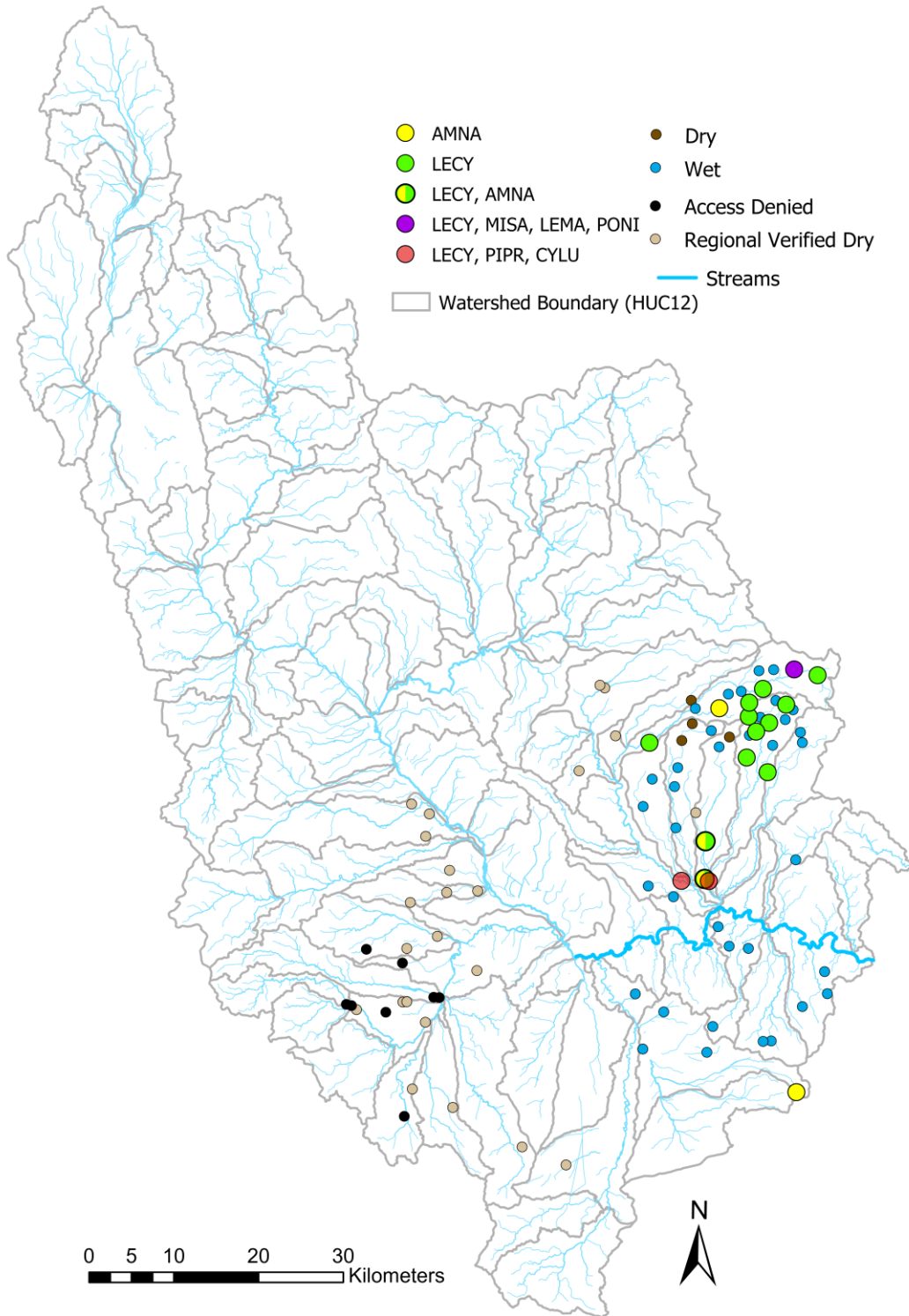


Figure 27.—Map of tanks sampled within the upper Verde River watershed and the assemblage of fish species detected (AMNA = Yellow Bullhead, LECY = Green Sunfish, MISA = Largemouth Bass, LEMA = Bluegill, PONI = Black Crappie, PIPR = Fathead Minnow, CYLU = Red Shiner) during the 2021 field season.

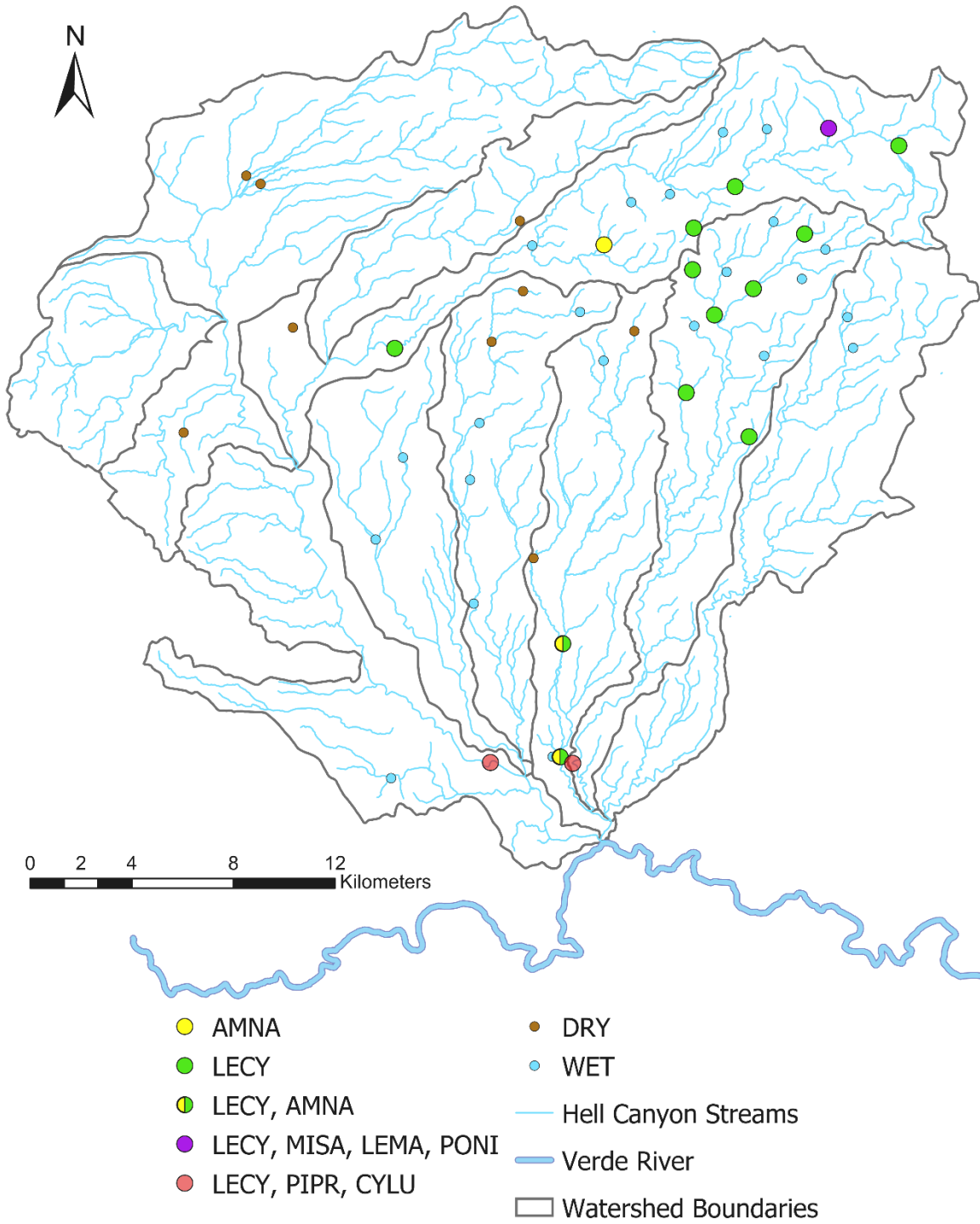


Figure 28.—Detail of tanks sampled within the Hell Canyon watershed and the assemblage of fish species detected (AMNA = Yellow Bullhead, LECY = Green Sunfish, MISA = Largemouth Bass, LEMA = Bluegill, PONI = Black Crappie, PIPR = Fathead Minnow, CYLU = Red Shiner) during the 2021 field season.

West Fork Black River Nonnative Fish Removal (Task AZ-2021-1)

Strategic Plan Goals:

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

Recovery Objectives:

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

Background: West Fork Black River and a major tributary, Thompson Creek, originate on the Fort Apache Indian Reservation near Baldy Peak and flow downstream for approximately 11 km on reservation lands before reaching Apache-Sitgreaves National Forest lands. West Fork Black River and Thompson Creek then flow through Apache-Sitgreaves National Forest lands for approximately 7 km to the original fish barriers and support populations of Apache Trout, Speckled Dace, and Desert Sucker. West Fork Black River and Thompson Creek were initially treated with antimycin in 1996 to remove nonnative Brown Trout and Brook Trout and reintroduce Apache Trout above the fish barrier. The treatment was thought to be successful until Brook Trout were detected above the original fish barriers in 2006. Department Regional and Native Trout Program staff opportunistically carried out removals of Brook Trout on Apache-Sitgreaves National Forest Lands until 2014 when removal effort was increased to multiple passes each year consistent with a removal plan.

Reclamation constructed a fish barrier on the West Fork Black River 0.6 km from the confluence with the East Fork Black River in 2016 to secure habitat for Loach Minnow between the fish barriers. Removal of nonnative fish from the West Fork Black River between the barriers has not occurred because of the persistent population of Brook Trout above the original fish barriers. The White Mountain Apache Tribe has been opposed to chemical renovations of streams on their lands in recent years, which has necessitated the use of mechanical removals to attempt to eradicate Brook Trout from the West Fork Black River. The purpose of this project is to assist the Department's Region I Aquatic Wildlife Program and the Native Trout Program by carrying out additional mechanical removal passes on the Apache-Sitgreaves reaches of West Fork Black River and Thompson Creek upstream of the original fish barrier to potentially accelerate eradication of Brook Trout from West Fork Black River. The removal protocol involved a crew of 2-4 personnel backpack electrofishing a 500 m reach between two block nets. Typically three electrofishing passes were carried out within each 500 m reach before proceeding upstream to the next 500 m reach, unless no Brook Trout were captured during a previous pass. A total of 14 reaches occur within the removal area with four in Thompson Creek and the remaining ten in West Fork Black River. All Brook Trout were measured to the nearest millimeter total length (mm TL) before being removed from the stream. Only Apache Trout captured during the first pass were measured. Ultimately the goal is to prepare West Fork Black River upstream of the Reclamation constructed fish barrier for translocation of Loach Minnow.

Results: The Program assisted the Department’s Region 1 Aquatic Wildlife Program and Native Trout Program with two of four total removal passes on the West Fork Black River and Thompson Creek in 2021. Pass 1 occurred between May 24 and June 3, Pass 2 occurred between June 14 and June 23, Pass 3 occurred between August 3 and August 11, and Pass 4 occurred between September 13 and September 16. A total of 805 Brook Trout were removed from West Fork Black River and 344 Brook Trout were removed from Thompson Creek (Table 11). Contrary to expectations, the number of Brook Trout captured substantially increased from the first pass to the fourth pass in both West Fork Black River (Pass 1, n = 249; Pass 4, n = 321) and Thompson Creek (Pass 1, n = 52; Pass 4, n = 105). The mean size of Brook Trout also substantially decreased from the first pass to the fourth pass in both the West Fork Black River (Pass 1 = 134.3 mm TL, Pass 4 = 98.9 mm TL; Figure 29) and Thompson Creek (Pass 1 = 135.4 mm TL, Pass 4 = 119.27 mm TL; Figure 30). The decrease in mean length of Brook Trout may be attributable to the presence of young of year fish that dispersed downstream from reaches on reservation land with elevated flows during the monsoon season. Brook Trout smaller than 90 mm TL were rarely captured during the first two passes, but comprised a majority of the catch during passes three and four (Figure 29, Figure 30). Mean Brook Trout CPUE was also consistently highest in the 500 m sub-reaches closest to the reservation boundary in both West Fork Black River and Thompson Creek (Figure 31).

Recommendations: Additional mechanical removal effort will be required on both sides of the forest boundary in order to successfully eradicate the Brook Trout population on West Fork Black River and Thompson Creek. At least the same amount of effort or more will be required to achieve eradication since the number of Brook Trout captured did not decline with each successive pass. Funding for this project was not allocated for FY22 and removals will continue to be led by other Department programs.

Tables and Figures:

Table 11.—Number of Brook Trout captured and removed and Apache Trout captured and released by pass in West Fork Black River and Thompson Creek during 2021. Included is total hours of electrofishing effort for each pass.

Water Name	Dates	Pass	Effort (h)	Apache Trout	Brook Trout
West Fork Black River	5/24/2021-6/03/2021	1	26.99	424	249
West Fork Black River	6/14/2021-6/23/2021	2	18.23	345	111
West Fork Black River	8/03/2021-8/11/2021	3	26.04	252	124
West Fork Black River	9/13/2021-9/16/2021	4	22.37	577	321
Thompson Creek	5/24/2021-6/03/2021	1	6.44	41	52
Thompson Creek	6/14/2021-6/23/2021	2	4.23	4	56
Thompson Creek	8/03/2021-8/11/2021	3	8.48	11	131
Thompson Creek	9/13/2021-9/16/2021	4	7.83	18	105

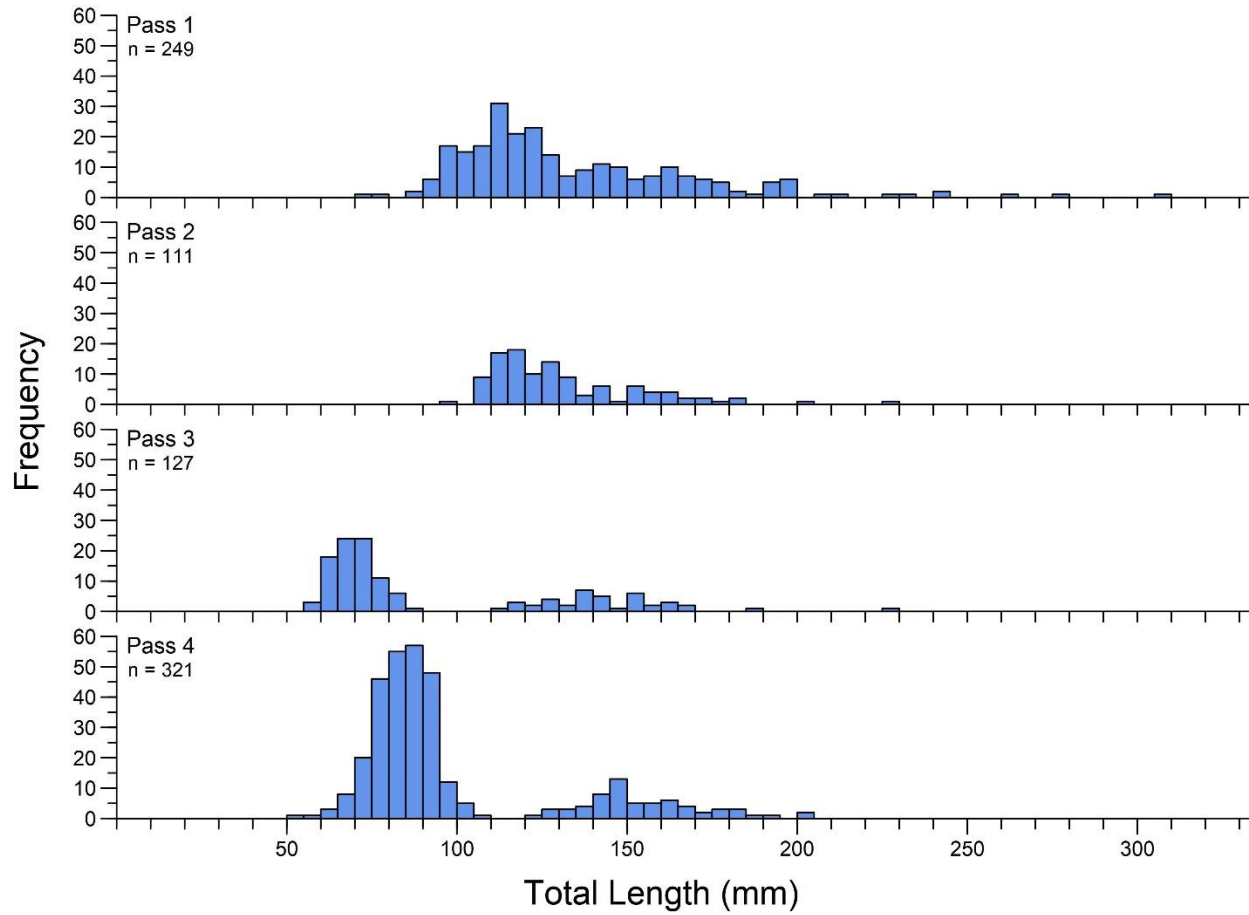


Figure 29.—Length frequency distribution of the number of Brook Trout captured by pass during nonnative removal efforts in West Fork Black River in 2021. Included in the top left of each pane is the number of fish captured and measured by pass.

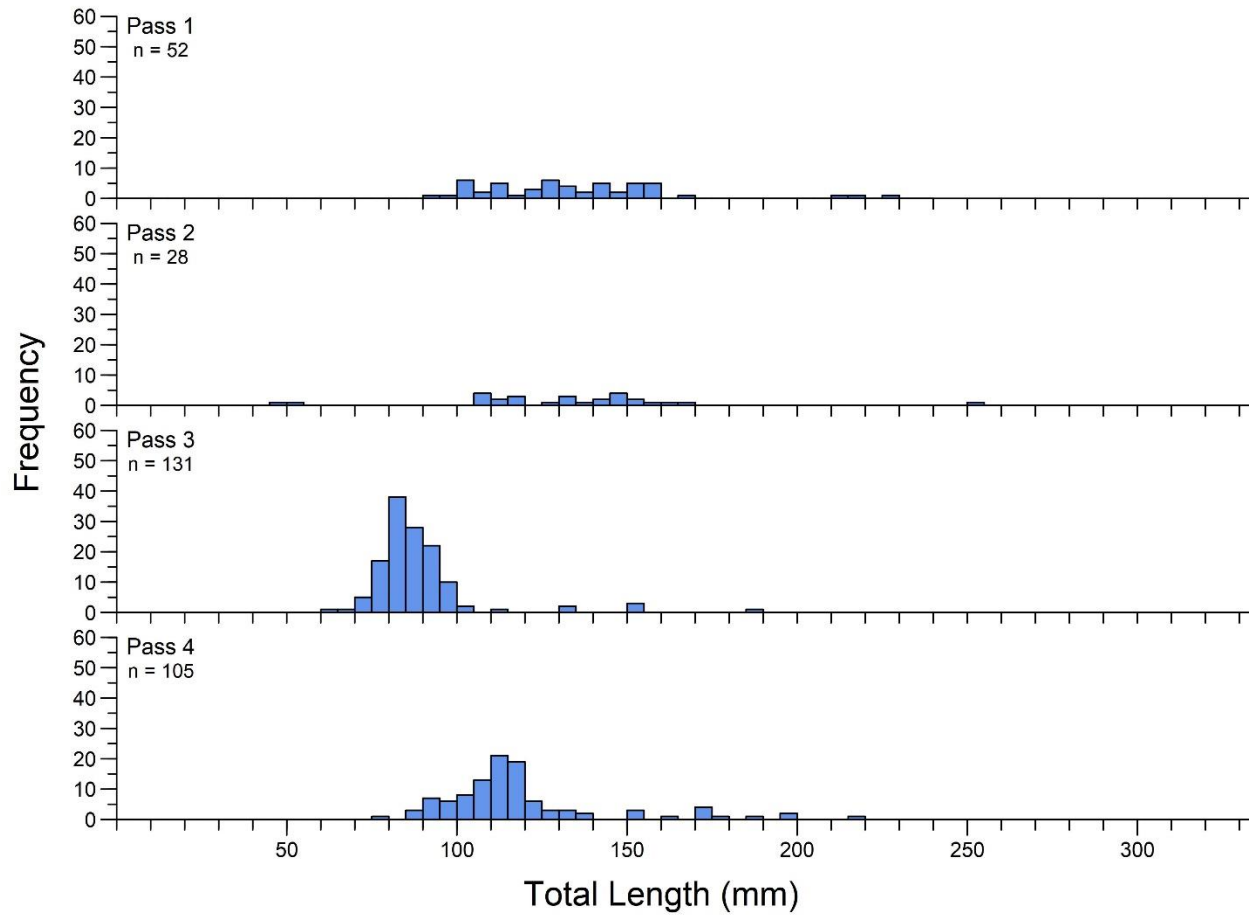


Figure 30.—Length frequency distribution of the number of Brook Trout captured by pass during nonnative removal efforts in Thompson Creek in 2021. Included in the top left of each pane is the number of fish captured and measured by pass.

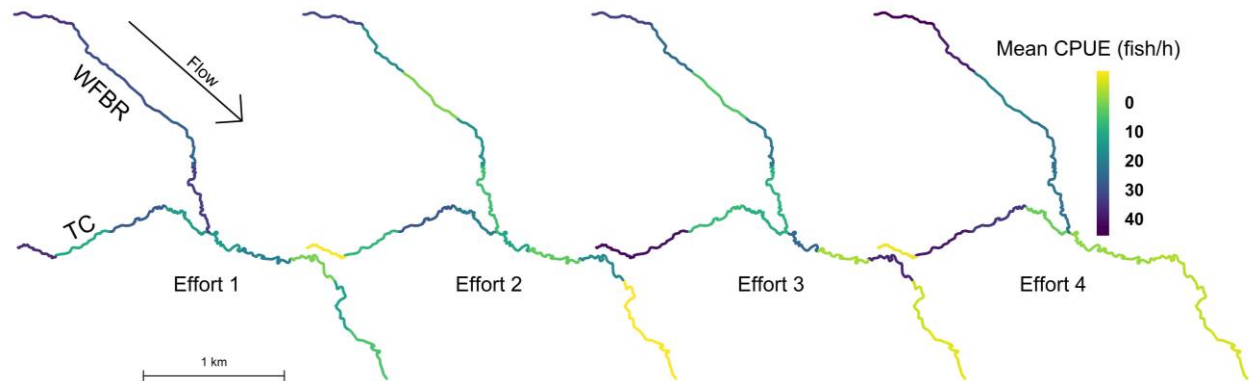


Figure 31.—Mean relative abundance (CPUE, fish/h) of Brook Trout captured by pass in each of the 14, 500 m sub-reaches within the removal reach of West Fork Black River (WFBR) and Thompson Creek (TC) in 2021. Darker colors indicated higher mean CPUE.

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

Recovery Objectives:

- Spikedace recovery objective 8. Plan and conduct investigations on captive holding, propagation and rearing.
- 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 wet lab was constructed in 2000, a well was installed in 2003 to supply water to the facility, and an open-air production and grow-out building was constructed in 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. In late 2018 ARCC staff sent a draft version of the hatchery operation manual with a complete appendix

to Department Research Branch staff for additional edits. A printed version is currently available at ARCC for staff and visitors and has already become a useful reference tool.

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 remove from a given population is a coordinated decision between USFWS and state wildlife agencies, and is usually based on estimated number of fish in the stream derived from the most recent monitoring. Typically fish are removed within a few months of the most recent monitoring. If necessary, new individuals are brought into ARCC every year to maintain the population size and genetic diversity with wild stock. Spikedace and Loach Minnow brought into ARCC to establish and maintain the refuge-broodstock populations are summarized in Table 12.

Counts of Spikedace and Loach Minnow brood stock, fish produced, and fish stocked each year since 2014 are presented in Table 12. 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 facility also holds various other species for research or educational purposes.

During 2018, ARCC staff began testing effects of fish density on propagation success of captive Spikedace and Loach Minnow. After a successful first year of trials, staff planned to conduct a second year of experiments using the exact same design as 2018. Unfortunately, not enough wild Aravaipa lineage fish could be collected for the 2019 season to replace the brood stock lost during the previous year's testing. This resulted in all spawning raceways being setup identical to one another at the lowest successful density identified during 2018 with no preference given to any one lineage. Due to COVID-19 and subsequent restrictions, ARCC staff continued with this raceway setup for the 2020 and 2021 spawning seasons. The number of raceways used for each lineage was dependent on the overall brood stock size and need for larval fish, with each raceway having 32 adult fish and 13 nest sites for Loach Minnow and 34 adults for Spikedace. Loach Minnow were once again given nest sites consisting of medium sized cobbles arranged in 15-cm circles spaced 38 cm from edge of nest to edge of nest on a bed of small chip gravel. For both species, larval fish were manually removed and counted once per week and placed in holding tanks. Algae were carefully removed as needed to minimize the potential effects of high algal biomass on spawning.

Results:
ARCC O&M

The Department continued to operate ARCC in 2021. The ARCC maintains refuge populations of three lineages of Spikedace (Aravaipa Creek, upper Gila River, and Gila River Forks) and five lineages of Loach Minnow (Blue River, Aravaipa Creek, San Francisco River, Gila River Forks, and Bear Creek).

In 2021, ARCC produced 1,293 Aravaipa Creek Spikedace, 914 upper Gila River Spikedace, 203 Gila River Forks Spikedace, 919 Blue River Loach Minnow, 504 Aravaipa Creek Loach Minnow, 541 San Francisco River Loach Minnow, 196 Bear Creek Loach Minnow and 0 Gila River Forks Loach Minnow (Table 12).

No new large scale physical improvements to ARCC were completed in 2021.

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

On March 3, 2021, Department staff received 52 Spikedace (Gila Forks) and 102 Loach Minnow (Gila Forks) from NMDGF staff.

On July 2, 2021, Department staff collected 60 Longfin Dace from the west side of Aravaipa Creek by backpack electrofishing. The fish were transported to Department headquarters for a fish health assessment. No pathogens or parasites of concern were detected in the subsequent fish health assessment.

Following the University of Arizona's annual fall monitoring in Aravaipa Creek, it was determined that populations of both Spikedace and Loach Minnow were not abundant enough to warrant collection of fish to augment the broodstock at ARCC.

On August 24, 2021, Department staff attempted to collect Speckled Dace from the lower Blue River at Juan Miller Crossing, but were unable to capture any target species.

On September 29, 2021, Department staff captured 30 Speckled Dace from the middle Blue River near The Box. The fish were transported to Department headquarters for a fish health assessment. No pathogens or parasites of concern were detected in the subsequent fish health assessment.

On November 8, 2021, Department staff collected 130 Loach Minnow from the Blue River in the vicinity of the Jackson Canyon confluence. The fish were held overnight and transferred to ARCC staff the following morning for transport to ARCC. There were no mortalities during collection, holding and transport.

Recommendations: For 2022, ARCC staff will focus on running all raceways at the lowest density identified in 2018 with testing being conducted on Loach Minnow nest spacing using the 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.

Recommendations for acquiring wild fish in 2022 include continuing to collect Spikedace and Loach Minnow from remnant populations, with goals to minimize impacts on remnant population while also acquiring the number of fish necessary to maintain a refuge population of at least 500 adults. More Loach Minnow should be collected from the Blue River and brought into ARCC to attain or exceed 500 broodstock. More Aravaipa Spikedace and Loach Minnow should be brought into ARCC to maintain the broodstocks. Aquatic Research and Conservation Center staff should coordinate with NMDGF regarding acquiring more stock of the New Mexico lineages.

Tables and Figures:

Table 12.—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 2014 through 2021. Data for years prior to 2014 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		2014	2015	2016	2017	2018	2019	2020	2021
Spikedace	upper Gila River	#B	380	392	531	267	159	254	219	176
		#P	1000	296	0	384	352	2404	408	914
		#S	0	296	0	327	0	0	0	0
		#A	0	0	0	0	0	0	0	0
Spikedace	Gila River Forks	#B	250	204	138	122	83	71	76	151
		#P	300	0	0	1183	195	1132	833	203
		#S	0	0	0	1000	0	0	0	0
		#A	NA	0	0	0	1	0	0	52
Spikedace	Aravaipa Creek	#B	480	412	262	382	331	523	529	379
		#P	221	35	120	1347	3214	4250	2182	1032
		#S	0	221	67	0	2234	0	2897	106
		#A	26	150	80	160	0	322	49	0
Loach Minnow	Bear Creek	#B								112
		#P								196
		#S								0
		#A								0
Loach Minnow	Gila River Forks	#B	57	81	96	128	97	169	121	0
		#P	250	0	220	7	1207	665	15	0
		#S	0	0	0	159	0	0	0	0
		#A	61	0	0	110	145	0	0	102
Loach Minnow	San Francisco R.	#B	27	119	215	314	318	231	208	173
		#P	500	0	26	177	1627	601	3	541
		#S	0	0	0	243	0	0	0	0
		#A	0	0	0	0	0	0	0	0
Loach Minnow	Blue River	#B	180	245	214	156	117	290	266	364

		#P	288	0	426	47	6	713	16	919
		#S	288	0	390	0	0	0	500	400
		#A	0	0	12	0	223	80	269	130
Loach Minnow	Aravaipa Creek	#B	340	316	297	490	439	354	337	261
		#P	0	0	265	305	1848	1398	57	504
		#S	0	0	0	0	0	0	300	0
		#A	48	50	200	100	0	57	82	0
Roundtail Chub	Eagle Creek	#B	85	85	101	99	99	99	98	84
		#P	1500	2000	0	57	0	0	0	0
		#S	0	876	1194	0	0	0	0	0
		#A	0	0	0	0	0	0	0	0

PROJECTS REMOVED FROM PRIORITY LIST IN 2021

Acquire Spikedace, Loach Minnow and rare populations of other native fish (Task AZ-2003-2). Merged into Aquatic Research and Conservation Center O&M (Task HA-2006-2).

Assess potential repatriation waters (Task AZ-2008-1). Merged into Gila Topminnow stockings (AZ-2002-1).

Boyce Thompson Ayer Lake native fish restoration (Task AZ-2000-1). Merged into Gila Topminnow stockings (AZ-2002-1).

Eagle Creek repatriations (Task AZ-2018-1). Dropped from priority list due to fish barrier construction delays.

Sweetwater dam nonnative removal (Task AZ-2020-1). Project completed in 2020.

LITERATURE CITED

- Arizona Game and Fish Department. 2011. Fish Collection, transport, and stocking protocol: best management practice (BMP #4). Arizona Game and Fish Department, Phoenix.
- Armstrong, D. P., and P. J. Seddon. 2007. Directions in reintroduction biology. *Trends in Ecology and Evolution* 23:20-25.
- Bright, P. W., and T. J. Smithson. 2001. Biological invasions provide framework for reintroductions: selecting areas in England for pine martin releases. *Biodiversity and Conservation* 10:1247-1265.
- Carle, F. L. and M. R. Strub. 1978. A new method for estimating population size from removal data. *Biometrics* 34:621-360.
- Ehret, S., and B. Dickens. 2009. Sabino Canyon survey trip report, June 3-4, 2009. Arizona Game and Fish Department, Region V, Tucson.
- Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. *Science* 245:477-480.
- Hickerson, B. T., and A. T. Robinson. 2019. Red Tank Draw native fish restoration project: nonnative fish removal plan; draft. Arizona Game and Fish Department, Phoenix.
- Hickerson, B. T., E. R. Grube, and A. T. Robinson. 2020. Harden Cienega Creek Fish Restoration Project: Nonnative Fish Removal Plan. Draft.
- Hickerson, B. T., E. R. Grube, K. T. Mosher, A. T. Robinson. 2021a. Successful Restoration of a Native Fish Assemblage in the Blue River, Arizona. *North American Journal of Fisheries Management* 41(3):746-756.
- Hickerson, B. T., J. Walters and A. T. Robinson. 2021b. Gila River Basin Native Fishes Conservation Program: Arizona Game and Fish Department's native fish conservation efforts during 2020. An Arizona Game and Fish Department Annual Report for Cooperative Agreement No. R16AC00077 submitted to U.S. Bureau of Reclamation, Phoenix Area Office. Arizona Game and Fish Department, Aquatic Wildlife Branch, Phoenix.
- Love-Chezem, T. S., A. T. Robinson, and C. D. Crowder. 2015. Muleshoe Cooperative Management Area native fish restoration: 2014 activities. A Gila River Basin Native Fishes Conservation Program Progress Report for Task 3-75f; U.S. Fish and Wildlife Service

- Cooperative Agreement No. F09AC00084. Arizona Game and Fish Department, Nongame Wildlife Branch, Phoenix.
- Reclamation (U.S.D.I. Bureau of Reclamation). 2010. Final environmental assessment, Blue River native fish restoration project, Apache-Sitgreaves National Forests, Greenlee and Apache Counties, Arizona. U. S. Department of the Interior, Bureau of Reclamation, Phoenix Area Office.
- Robinson, A. T., C. Crowder, and D. Boyarski. 2010. Mechanical removal of nonnative fishes from the Blue River during June 1-3, 2009. Nongame Branch, Arizona Game and Fish Department, Phoenix, AZ.
- Robinson, A. T., Y. M. Paroz, M. J. Clement, T. W. Franklin, J. C. Dysthe, M. K. Young, K. S. McKlevey and K. J. Carim. 2019. Environmental DNA sampling of small-bodied minnows: performance relative to location, species, and traditional sampling.
- U.S. Fish and Wildlife Service. 1983. Gila and Yaqui Topminnow Recovery Plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico. 56 pp.
- U.S. Fish and Wildlife Service. 1991a. Spikedace Recovery Plan. Albuquerque, New Mexico. 38 pp.
- U.S. Fish and Wildlife Service. 1991b. Loach Minnow Recovery Plan. Albuquerque, New Mexico. 38 pp.
- U. S. Fish and Wildlife Service. 1998. Razorback sucker (*Xyrauchen texanus*) recovery plan. U. S. Fish and Wildlife Service, Denver, CO. 81 pp.
- U.S. Fish and Wildlife Service. 2002. Razorback sucker (*Xyrauchen texanus*) Recovery Goals: amendment and supplement to the Razorback Sucker Recovery Plan. U.S. Fish and Wildlife Service, Mountain-Prairie Region (6), Denver, Colorado.
- U.S. Fish and Wildlife Service. 2015. Gila chub (*Gila intermedia*) Draft Recovery Plan. U.S. Fish and Wildlife Service, Southwest Region, Albuquerque, New Mexico. 118 pp. + Appendices A-C.
- Weedman, D. A. 1999. Gila topminnow, *Poeciliopsis occidentalis*, revised recovery plan. Draft. August 1999. U.S. Fish and Wildlife Service, Phoenix, AZ.

Weedman, D. A. and K. L. Young. 1995. Gila topminnow extirpation sites in Arizona: 1994-95 field season results. Nongame and Endangered Wildlife Program Technical Report 80. Arizona Game and Fish Department, Phoenix, Arizona.

APPENDICES

Appendix 1.—Summary of native fish stocked in Arizona during 2021 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	Site Name	Easting	Northing	Date	Lineage	# Stocked	# Mortalities
Gila Topminnow	Maternity Well Wildlife Pond	Maternity Well Wildlife Pond	531700	3514065	4/21/2021	Cienega Creek	248	2
Gila Topminnow	Telegraph Canyon	0.75 km upstream of confluence with Arnett Creek	486991	3679922	5/20/2021	Redrock Canyon	389	5
Gila Topminnow	Harden Cienega	Below Barrier	674768	3674598	6/23/2021	Bylas Spring	469	47
Roundtail Chub ¹	Rarick Canyon	F20	439788	3844482	10/21/2021	Red Tank Draw	53	0
Roundtail Chub ¹	Rarick Canyon	F18	440148	3844833	10/21/2021	Red Tank Draw	46	0
Roundtail Chub ¹	Rarick Canyon	F17	436311	3841758	10/21/2021	Red Tank Draw	51	0

¹ Roundtail Chub stocked at this location previously classified as Gila Chub.

Appendix 2.—Summary of monitoring results during 2021 for the five priority species and other target native fish species that were previously stocked into various waters in the Gila River Basin Arizona. Included is the number of sites sampled, number of individuals captured at a particular location (#Ind), the proportion of young of year individuals captured (%YOY), Mean relative abundance (CPUE) and standard error of the mean relative abundance (SE).

Taxa	Location	Date	Gear Type	Sample Size	Statistics	2021
Desert Pupfish	Black Canyon City Heritage Pond	6/29/2021	Minnow Trap	10	#Ind	235
					%YOY	27
					Mean CPUE	10.81
					SE	3.74
Desert Pupfish	Black Canyon City Heritage Pond	6/29/2021	Dip Net	7	#Ind	16
					%YOY	88
					Mean CPUE	3.75
					SE	1.14
Gila Topminnow	Arnett Creek	10/28/2021	Dip Net	3	#Ind	21
					%YOY	90
					Mean CPUE	14.41
					SE	6.69
Gila Topminnow	Bass Canyon	8/30/2021	Minnow Trap	11	#Ind	27
					%YOY	30
					Mean CPUE	4.73
					SE	3.12
Gila Topminnow	Bass Canyon	8/30/2021	Dip Net	7	#Ind	3
					%YOY	0
					Mean CPUE	1.8
					SE	1.52
Gila Topminnow	Black Canyon City Heritage Pond	6/29/2021	Minnow Trap	10	#Ind	479
					%YOY	68
					Mean CPUE	18.63
					SE	3.1
Gila Topminnow	Black Canyon City Heritage Pond	6/29/2021	Dip Net	7	#Ind	19
					%YOY	63

					Mean CPUE	5.96
					SE	2.08
Gila Topminnow	Double R Canyon	8/30/2021	Minnow Trap	10	#Ind	1
					%YOY	0
					Mean CPUE	0.05
					SE	0.05
Gila Topminnow	Double R Canyon	8/30/2021	Seine	2	#Ind	291
					%YOY	73
					Mean CPUE	10.68
					SE	1.69
Gila Topminnow	Las Cienegas - Bill's Wildlife Pond	8/2/2021	Minnow Trap	10	#Ind	3457
					%YOY	20
					Mean CPUE	95.23
					SE	17.67
Gila Topminnow	Las Cienegas - Maternity Wildlife Pond	8/2/2021	Minnow Trap	10	#Ind	1554
					%YOY	23
					Mean CPUE	63.64
					SE	13.46
Gila Topminnow	Mud Spring-Coronado National Forest	8/4/2021	Minnow Trap	10	#Ind	815
					%YOY	53
					Mean CPUE	36.88
					SE	3.94
Gila Topminnow	Peterson Ranch Pond	8/3/2021	Minnow Trap	10	#Ind	613
					%YOY	77
					Mean CPUE	28.22
					SE	5.34
Gila Topminnow	Telegraph Canyon	10/28/2021	Minnow Trap	13	#Ind	398
					%YOY	15
					Mean CPUE	16.45
					SE	4.37
Gila Topminnow	Telegraph Canyon	10/28/2021	Dip Net	28	#Ind	165

						% YOY	78
						Mean CPUE	17.72
						SE	2.87
Gila Topminnow	Tortilla Creek	10/26/2021	Minnow Trap	10	#Ind	2245	
					% YOY	43	
					Mean CPUE	98.41	
					SE	11.81	
Gila Topminnow	Tortilla Creek	10/26/2021	Dip Net	14	#Ind	145	
					% YOY	61	
					Mean CPUE	37.82	
					SE	10.06	
Loach Minnow	Upper Blue River	9/20/2021	Backpack Electrofisher	15	#Ind	128	
					% YOY	8	
					Mean CPUE	148.66	
					SE	17.88	
Loach Minnow	Middle Blue River	9/27/2021	Backpack Electrofisher	12	#Ind	86	
					% YOY	1	
					Mean CPUE	36.80	
					SE	2.28	
Roundtail Chub	Upper Blue River	9/21/2021	Backpack Electrofisher	12	#Ind	1	
					% YOY	0	
					Mean CPUE	0.22	
					SE	0.22	
Roundtail Chub	Middle Blue River	9/27/2021	Backpack Electrofisher	15	#Ind	80	
					% YOY	1	
					Mean CPUE	52.34	
					SE	4.44	
Roundtail Chub	Rarick Canyon	10/12/2021	Minnow Trap	12	#Ind	2	
					% YOY	50	
					Mean CPUE	0.04	
					SE	0.03	

Roundtail Chub	Rarick Canyon	10/12/2021	Mini-Hoop Net	15	#Ind	3
					%YOY	0
					Mean CPUE	0.08
					SE	0.05
Roundtail Chub	Sabino Canyon	4/20/2021	Mini-Hoop Net	10	#Ind	10
					%YOY	0
					Mean CPUE	1.77
					SE	0.43
Roundtail Chub	Sabino Canyon	4/20/2021	Seine	4	#Ind	8
					%YOY	0
					Mean CPUE	0.58
					SE	0.11
Spikedace	Upper Blue River	9/21/2021	Backpack Electrofisher	15	#Ind	4
					%YOY	0
					Mean CPUE	1.55
					SE	0.81
Spikedace	Middle Blue River	9/27/2021	Backpack Electrofisher	12	#Ind	42
					%YOY	0
					Mean CPUE	25.70
					SE	3.49
Spikedace	Spring Creek	9/9/2021	Backpack Electrofisher	3	#Ind	5
					%YOY	0
					Mean CPUE	4.33
					SE	1.73