Muleshoe Cooperative Management Area Native Fish Restoration Monitoring and Stocking During 2010

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The 2010 native fish monitoring and native stocking activities within the Muleshoe Cooperative Management Area in the Galiuro Mountains of Arizona were cooperative undertakings of multiple agencies, organizations, and volunteers. Involved in the execution of the 2010 work were individuals from The Nature Conservancy (Bob Rogers, Larry Young, Lori Young, Ken O'Brien, Charlie Anderson, Cliff Todd, Molly Hanson, and Nicole Vasquez), Bureau of Land Management (Heidi Blasius, Jeff Conn, Richard England, Greg Brittain, Jony Cockman, and Rachel Priddis), Arizona Game and Fish Department (Clayton Crowder, David Boyarski, Steve Prager, Abigal Medina, Duane Aubuchon, David Ward, Amberle Vasey, Nick Berndt, Kent Mosher, and Tony Robinson), U.S. Fish and Wildlife Service (Mary Richardson), U.S. Bureau of Reclamation (Rob Clarkson), and Americorps volunteers Brian Dugan, Tanya Wallin, Halley Bagley and Selena Pao.

INTRODUCTION

During 2007 through 2009 four listed-fish species were stocked into seven waters within the Muleshoe Cooperative Management Area (CMA), near the Galiuro Mountains of Arizona (Appendix 1, Figures 1 and 2; Robinson 2008, Robinson et al. 2010). Spikedace *Meda fulfida* and loach minnow *Rhinichthys cobitis* were stocked into Redfield Canyon within the reach upstream of the confluence with Swamp Spring Canyon. Both species were stocked into Hot Springs Canyon within an approximate 500-m reach just upstream of the confluence of Wildcat Canyon. Lineages of both species originated from Aravaipa Creek, directly so in 2007, but in 2008 and 2009 from stock propagated at Bubbling Ponds Native Fish Conservation Facility (BPNFCF). Gila topminnow *Poeciliopsis occidentalis* and desert pupfish *Cyprinodon macularius* were stocked into Swamp Springs Canyon, Cherry Spring Canyon, Secret Spring, and Headquarters Spring, and desert pupfish were stocked into Larry&Charlie Tank (referred to as Headquarters Hill Spring in Robinson et al. 2010). Gila topminnow were of Bylas Springs lineage, and originated from Arizona State University in 2007 and from The Nature Conservancy's (TNC) Lower San Pedro River Preserve (Dudleyville) ponds near Dudleyville during 2008. Desert pupfish stocked in 2007 and 2008 originated from TNC's Dudleyville ponds, whereas those stocked in 2009 were acquired from BPNFCF but originated from Dexter National Fish Hatchery.

The first post-stocking annual monitoring was conducted on September 15-16, 2008 (Robinson 2008). Gila topminnow were present in each of the three sites where they were stocked the previous year. Desert pupfish were present in Secret Springs and Swamp Springs Canyon, but none were found in Cherry Spring Canyon. Both loach minnow and spikedace were present in Redfield Canyon and Hot Springs Canyon. Additional fish of all species were stocked into the same waters on September 17, 2008, except that no additional Gila topminnow or desert pupfish were stocked into Secret Spring. Also, an additional site, Headquarters Spring, was stocked with desert pupfish and Gila topminnow. The second post-annual monitoring was conducted on September 14-15, 2009; augmentation stockings were completed a month later, on October 28, 2009. Gila topminnow were captured in all of the sites where they had been stocked. Desert pupfish were only captured in Secret Spring and Cherry Spring. Both loach minnow and spikedace were present in Redfield Canyon and Hot Springs Canyon. Not all locations were augmented with more fish during the October 2009 stocking. Gila topminnow were considered to have established populations in all of the locations where they had been stocked, so no additional fish were stocked in 2009. Source stocks of desert pupfish were low in abundance, so only one site, Larry&Charlie Tank, was stocked in 2009. Stocks of Aravaipa lineage spikedace and loach minnow at Bubbling Ponds Native Fish Conservation Facility (BPNFCF) produced relatively few offspring, so all were stocked into Hot Springs Canyon, which was thought to have the best and most habitat for the two species.

This report summarizes the results of the native fish monitoring during March and September 2010 and subsequent stockings during October 2010. The goal of the stocking program is to establish populations within the systems where the species are stocked (i.e., to repatriate the species to the systems). A population is considered to have established (a successful repatriation) when it is reproducing to the point where it is self-sustaining (Griffith et al. 1989, Bright and Smithson 2001, Armstrong and Seddon 2007). The objectives of monitoring were to: 1) verify persistence of fish species since stocking; 2) detect recruitment of young (and hence reproduction) into the population; 3) evaluate if relative abundance (measured as catch-per-unit effort) increases over time (i.e., from the starting point of zero); 4) determine if species have dispersed outside of the

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stocking area; and 5) report any non-native fish species captured during monitoring. The objective of the supplemental stockings is to increase the number of individuals to help a population establish.

STUDY AREA

The Muleshoe CMA is located on the southwestern edge of the Galiuro Mountains in southern Arizona. The Muleshoe CMA is jointly managed by U. S. Bureau of Land Management (BLM), U. S. Forest Service, and TNC. The 57,500 acre CMA contains major portions of the Redfield Canyon, Cherry Springs Canyon, and Hot Springs Canyon watersheds, all of which are tributaries to the San Pedro River. The Nature Conservancy's Muleshoe Ranch CMA headquarters is located along Hot Springs Canyon at a location previously known as Hookers Hot Springs.

Before fish stockings in 2007, fish species reported from Redfield Canyon included Sonora sucker *Catostomus insignis*, desert sucker *Catostomus clarki*, longfin dace *Agosia chrysogaster*, speckled dace *Rhinichthys osculus*, Gila chub *Gila intermedia*, and green sunfish *Lepomis cyanellus* (Griffith and Tiersch 1989, Bureau of Land Management 1998; AGFD Native Fish Database). Gila topminnow were stocked into Redfield Canyon in 1977 (Minckley and Brooks 1985) but did not persist. In Swamp Springs Canyon, longfin (SONFISHES and AGFD Native Fish databases) and speckled dace (Bob Rogers, The Nature Conservancy, personal communication) were present. Fish species reported from the Hot Springs Canyon drainage include the same five native fishes found in Redfield Canyon (SONFISHES and AGFD Native Fish databases; Bureau of Land Management 1998). No fish were present in Headquarters Spring, Secret Spring, or Larry&Charlie Tank before stockings in 2007-2009.

Fish monitoring and stockings described in this report occurred within the following waters: Redfield Canyon and its tributary Swamps Springs Canyon, Cherry Spring Canyon, Hot Springs Canyon, and three isolated and unnamed springs (referred to as Secret Spring, Headquarters Spring, and Larry&Charlie Tank) within the Hot Springs Canyon drainage. Length of perennial water in the streams was previously estimated to be 7.5 km in Redfield Canyon, 2.6 km in Swamp Springs Canyon, 5.1 km in Hot Springs Canyon, and 0.7 km in Cherry Spring Canyon, but during dry periods is likely much less and interrupted (Bureau of Land Management 1998). Secret Spring is an earthen pond below a hillside spring and is located about 600 m southwest of the Muleshoe Ranch headquarters. Headquarters Spring is a pooled area at the edge of but within the Hot Springs Canyon stream bed and is located about 25 m east of and down the hill from the Muleshoe Ranch headquarters dormitory building; springs originating near the dormitory drain downhill into the pooled area. During 2009, two small tanks (Larry and Charlie Tanks) about 1 m apart were dug below a spring located about 25 m west and up the hill from the casitas at the Muleshoe Ranch headquarters; a shallow (~3 mm) film of water flowed over the ridge between the two tanks. During May 2010 a trench was dug to join the two tanks, which then became known as Larry&Charlie Tank.

Each of the major stream courses was divided into sections (reaches) to facilitate a stratified random sampling design or to encompass stocking locations (Appendix 1, Figures 1 and 2). Reaches in Hot Springs Canyon were: Reach 1 = from Bass Canyon down 1.4 km to Wildcat Canyon, Reach 2 = from Wildcat Canyon down 1.7 km to the second unnamed tributary from the

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south, and Reach 3 = from the lower end of Reach 2 down 3.7 km to the first unnamed tributary from the south. Only the lower half of Reach 3 was surveyed because the upper half was mostly canyon bound and pool habitat, and thus likely provides less habitat for spikedace or loach minnow. Reaches in Redfield Canyon were: Reach 1 = from the Swamp Springs Canyon confluence upstream about 1.5 km in Redfield Canyon to the first tributary from the east (the approximate location of the waterfall at UTM coordinates 564212mE, 3590025mN; NAD 83), and Reach 2 = from Swamp Springs Canyon confluence downstream about 2.9 km to an unnamed tributary from the north (561433mE and 3589266mN; NAD 83). Most of Reach 2 is intermittent (Bob Rogers, The Nature Conservancy, personal communication). The two reaches designated in Swamp Springs Canyon were: Lower = an approximately 300 m long section beginning approximately 1.3 km upstream of the mouth, and Upper = an approximately 1 km section beginning about 4.2 km upstream from the mouth, in the vicinity of the southern unnamed tributary that drains Cherry Spring Peak. The one reach in Cherry Spring Canyon begins at the unnamed tributary containing Cherry Spring and extends upstream for about 2,100 m. The reach is dry except for a small, ~30 m long tinaja about 690 m upstream of the bottom, and the uppermost 100 m, the latter of which is where topminnow and pupfish were stocked.

METHODS

MONITORING

Spikedace and Loach Minnow

Annual monitoring of spikedace and loach minnow was completed on September 13 and 14 and October 7, 2010 in Hot Springs Canyon and on September 14, 2010 in Redfield Canyon. Fixed and randomly selected 100-m long sites (transects) were sampled in Redfield Canyon (Figure 1) and Hot Springs Canyon (Figure 2). The three fixed sites in Redfield Canyon and three fixed sites in Hot Springs Canyon were also sampled during 2008 (Robinson 2008) and 2009 (Robinson et al. 2010). A fourth fixed site in Redfield Canyon, located about 2.1 km downstream of the Swamp Springs confluence, was not sampled because it was dry. Three additional random sites in each stream were selected in the office by mapping the stream courses (National Geographic TOPO! software), dividing the lengths into 100-m-long segments, and using a random number table to select sites within an identified reach. Six sites in each stream were what could be feasibly sampled by two crews of three people in one day.

Fish sampling within each 100-m transect was dependent upon habitat type. Runs and pools were sampled using a backpack electrofisher (Smith Root model LR24) moving upstream in a single pass, and stunned fish were netted with 3 mm-mesh dip nets. Cobble riffles were sampled by a combination of electrofishing and kick seining; the lower end was blocked with a 3 mm-mesh seine and then beginning about 2-5 m upstream, the segment was shocked downstream while sweeping and rolling the substrate material with feet toward the seine. When the electrofisher reached the seine, the person(s) holding the net swept it upwards to capture fish. Fish were processed and data were recorded after each segment was sampled.

In addition to and outside of the 100-m transects, crews conducted targeted-habitat sampling within each reach if they determined they had time to do so. Runs, eddies and pools (presumed spikedace habitat) were sampled either by electrofishing or by seining. Seining was done in a downstream direction, moving faster than the current. Cobble-bottom riffles (presumed loach minnow habitat) were sampled with a combination of kick-seining and electrofishing as

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described above. Each seine haul or electrofishing-kick-seining was a separate event and was considered a separate site. Data were recorded after each seine haul or kick-seining event.

Captured fish were identified to species and counted. All spikedace and loach minnow were measured (mm total length, TL). Other species were counted within two size classes: 20-40 mm and > 40 mm for speckled dace and longfin dace, ≤10 and >10 mm for desert pupfish and Gila topminnow, and 20-100 mm and >100 mm for suckers and Gila chub. Except for topminnow and pupfish, we categorized fish <20 mm TL as larvae. After processing, fish were released alive just downstream from where they were captured. Data recorded for each sampling effort included: site name, site location (GPS coordinates), length of site, date, time, participants, gear type, gear settings, gear dimensions, effort (seconds shocked or length and width of seining or dip netting), species of fish captured, size class of fish, and counts of individuals within each species-size-class category. Habitat information was sometimes collected at the 100-m sites. Habitat data recorded included: percentage of site composed of each habitat type (cascade, riffle, run, pool), and percentage of site composed of each substrate type (clay, silt, sand, gravel, pebble, cobble, boulder). Water quality was measured using EXTECH Instruments Inc. meters and included water temperature (°C), pH, and conductivity (µS) using an ExStik EC500 meter and dissolved oxygen (mg/L) using an ExStik II dissolved oxygen meter.

Gila Topminnow and Desert Pupfish

Monitoring for Gila topminnow and desert pupfish was conducted on September 12 within Secret Spring and Headquarters Spring, on September 13 in Cherry Spring, and on September 13 and October 8, 2010 in Swamp Springs Canyon. Monitoring for desert pupfish in Larry and Charlie tanks occurred on April 19 and in Larry&Charlie Tank on September 12, 2010. Sampling was completed throughout the Secret Spring pond, Larry&Charlie Tank, and Headquarters Spring pool. Within Cherry Spring Canyon and Swamp Springs Canyon the stocking locations and areas immediately upstream and downstream of the stocking locations were sampled. In upper Swamp Springs Canyon, sampling on September 13 occurred in the reach beginning at the upper stocking site and extending downstream about 200 m. In lower Swamp Spring Canyon, sampling on October 8 occurred in an approximately 30-m reach encompassing the stocking locations. Fish were sampled using Promar® collapsible minnow traps (0.46 m long x 0.3 m wide, with 2 mm mesh) and dip nets (3 mm mesh, 1.5 m long pole), and seines (3 mm mesh, and typically 2 m long by 1.5 m tall). Collapsible minnow traps were baited with dry dog food. Six to ten traps were set upon arrival at each site, and were pulled 2-3 h later. An attempt was made to conduct a minimum of 10 dip net sweeps and or 10 seine hauls at each site.

After each seine haul, dip net sweep, or trap pull, captured fish were held in buckets or the nets in the water until they could be identified, counted and data recorded. After processing, fish were released alive back to the area from which they were captured. Data recorded for each sampling effort included: site name, site location (GPS coordinates), date, time, participants, gear type, gear dimensions, effort (length and width of seining or dip netting, and duration of trap set in minutes), species of fish captured, size class of fish (≤10 mm or >10 mm), and counts of individuals within each species-size-class category. Water quality information sometimes recorded included water temperature (°C), dissolved oxygen (mg/L), pH, and conductivity (µS).

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Analysis
Numbers of fish in each size class captured in each water and catch-per-unit effort were calculated. We use catch-per-unit effort (catch rates) as an index of abundance, and not the absolute number of fish captured. Catch rates adjust the number of fish captured by the amount of effort, and so are more correlated with abundance than the total number of fish captured. Catch rates depend on the number of fish present, the number of fish captured, and the amount of and effectiveness of the sampling effort, among other things. Length frequency diagrams for spikedace and loach minnow and length category frequencies for Gila topminnow and desert pupfish were examined to determine if reproduction and recruitment had occurred. Distance between capture location and the stocking reach within Hot Springs Canyon and Redfield Canyon was used as a measure of dispersal distance for spikedace and loach minnow.

FISH STOCKINGS
Presence, abundance, and size class structure of repatriated populations derived from monitoring in 2008, 2009 and 2010 were used to determine if augmentation stockings were needed. Availability of stock of the four species was also used to determine stocking priorities. Aravaipa lineage spikedace and loach minnow held at BPNFCF produced thousands of offspring in 2010. Therefore, a multi-agency team recommended that the fish be divided amongst the three available repatriation streams (Fossil Creek, Redfield Canyon, and Hot Springs Canyon) that were scheduled for augmentations. For spikedace, the plan was to stock 1,000 into Redfield Canyon, 1,000 into Hot Springs Canyon, and the remaining 3,900 into Fossil Creek. For loach minnow the plan was to stock 400 into Redfield Canyon, 400 into Hot Springs Canyon, and 500 into Fossil Creek.

Based on monitoring information, Gila topminnow were present and abundant at each of the sites where they had been stocked. Therefore the multi-agency team recommended that no augmentation stockings were needed for 2010. Gila topminnow were not stocked into any sites on the Muleshoe CMA during 2010.

Based on trapping, seining, and visual observations, desert pupfish were considered to be abundant enough within TNC's Dudleyville pond for approximately four repatriation locations. Of the five sites on the Muleshoe CMA where desert pupfish had been stocked, Secret Spring, Larry&Charlie Tank, and Headquarters Spring were deemed to have the best habitat for desert pupfish. Examination of monitoring information indicated that pupfish were abundant in Larry&Charlie tank. In addition, two sites outside of the Muleshoe CMA, Bonita Creek and a private pond near Pima, were scheduled to be stocked with desert pupfish from TNC's Dudleyville pond. Therefore the multi-agency team recommended augmentation stockings of desert pupfish into Secret Spring and Headquarters Spring within the Muleshoe CMA.

The Nature Conservancy's Lower San Pedro River Preserve was used as a helicopter staging area for transport of spikedace, loach minnow, and desert pupfish stocked into Muleshoe CMA waters during 2010. Spikedace and loach minnow were transported in a 100-gal fish transport tank in the bed of a truck from BPNFCF to TNC Dudleyville early in the morning of October 28, 2010. Water in the fish transport tank originated from the well at BPNFCF and was treated with salt and AMQUEL® and was aerated using oxygen tanks to minimize stress to the fish during

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transport. Desert pupfish were collected from the smaller-eastern pond at TNC Dudleyville using collapsible minnow traps which were baited and set overnight, and using seines on the morning of October 28, 2010. Fish and water were loaded into 55-gallon barrels, outfitted with plastic liners and aeration devices, and then transported by helicopter long-line to the designated stocking locations.

After the helicopter pilot set a barrel down near the stocking location, fish were transferred to 5-gal buckets that had battery powered aerators attached to their lids and hand-carried to the stocking site. To minimize stress to the fish, at the stocking site water was tempered to stream conditions by exchanging approximately 25% of the bucket water with stream water every 10-15 minutes, making 2-4 exchanges until the water in the bucket is within 1.5˚C of the stream temperature. After the tempering process, the fish were removed from the bucket with a dip net, identified to species, counted and released to the stream. Fish were observed after release and stress behaviors (e.g., loss of equilibrium, rapid respiration, etc.) noted.

RESULTS AND DISCUSSION

MONITORING

Spikedace and Loach Minnow

Seven species of fish were captured in Redfield Canyon during September 2010 (Table 1). Speckled dace was the most abundant species captured, followed by Gila chub, Sonora sucker, longfin dace, Gila topminnow, loach minnow and green sunfish (Table 2). Only one loach minnow was captured, so it can be concluded that they continue to persist in Redfield Canyon. No spikedace were captured, so it is unclear whether or not spikedace persist. The one loach minnow captured was 39 mm TL, and hence was likely a young-of-year fish, and so is evidence of reproduction, particularly since loach minnow and spikedace were last stocked in Redfield Canyon in 2009. We did not sample the two transects in reach 2 because we had insufficient time and personnel to sample transect Random-4, about 70% of which was wetted, and transect Permanent-4 was dry (Figure 1). Therefore all transects sampled were within the stocking reach (upstream of Swamp Springs Canyon), so we did not collect any data that would allow us to evaluate whether or not loach minnow had dispersed outside of the stocking reach. Fewer individuals of both spikedace and loach minnow were captured in September 2010 than in the previous two years (Robinson 2008, Robinson et al. 2010). The decrease in catch may indicate that the populations, if they ever actually established, are decreasing in abundance. We surveyed about 40% of reach 1, so do not think that the decrease in catch was a result of insufficient sampling effort.

We captured Gila topminnow in Redfield Canyon within reaches 1 and 2. Capture of Gila topminnow in transect Permanent-3, the downstream end of which was about 80 m upstream of the confluence with Swamp Springs Canyon, indicates that Gila topminnow moved upstream from their point of origin in Redfield Canyon (Gila topminnow were not stocked into Redfield Canyon, but were stocked into Swamp Springs Canyon in 2007 and 2008). Similar to September 2009 (Robinson et al. 2010), we captured and observed Gila topminnow a few hundred meters downstream of the confluence, down to the end of the watered reach.

Results of the 2010 fish survey in Hot Springs Canyon were similar to the September 2009 survey. Longfin dace and speckled dace were by far the most abundant species followed by

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loach minnow, desert sucker, Gila chub, spikedace, and Sonora sucker (Table 2). Catch rates for each species are summarized in Table 2. In reach 1, the backpack electrofishing unit malfunctioned on September 13, such that only the first 35 m of Permanent-1 was electrofished; the remaining 65 m and all of transect Random-1 was sampled via seining or kick seining. Therefore, to keep sampling methods consistent throughout the stream, the two transects in reach 1 were re-sampled using backpack electrofishing gear: Permanent 1 was re-sampled on September 14, and Random-1 was re-sampled on October 7. Electrofishing catch rates for reach 1 presented in Table 2 are for these later two dates; electrofishing catch rates for the September 13 sampling of transect Permanent-1 are not presented, because they are questionable. Seining catch rates on September 13 for reach 1 are also not presented in Table 1 because length and width of seine hauls or kick seine efforts was not recorded.

Both spikedace and loach minnow have persisted and the numbers captured were greater than during September 2008 (4 spikedace and 12 loach minnow; Robinson 2008) or September 2009 (24 spikedace and 23 loach minnow; Robinson et al. 2010). The increase could have been simply because three consecutive years (2007-2009) of stocking into the stream, or reproduction may have occurred and the population actually increased, or may represent different sampling methods or sample variation. If populations of both species actually increased, there is an increased likelihood that they will continue to increase in abundance to the point where they can persist after stochastic events such as floods or drought. Continued monitoring will be used to detect such changes in abundance.

Except for one loach minnow, all spikedace and loach minnow captured in Hot Springs Canyon were categorized into two size classes: <41 mm and > 40 mm TL. Some, but not all of the fish were measured because we failed to bring measuring boards, but two crews had rulers. Of the 29 spikedace captured, 62% (18 of 29) were ≤ 40 mm TL, the rest were > 40 mm TL. For loach minnow, 41% (26 of 64) were ≤ 40 mm TL, the rest were > 40 mm TL Forty three of the loach minnow were measured for length, and the length-frequency diagram of these fish was slightly bi-modal (Figure 3). Only five of the spikedace were measured, with lengths of: 30, 52, 53, 63 and 63 mm TL Therefore, assuming that all fish stocked during September 2009 or that were naturally produced during 2009 grew to be larger than 40 mm TL by September 2010, the data indicate that both spikedace and loach minnow reproduced in Hot Springs Canyon during 2010. Four unidentified larval fish were also captured, three in Reach 1 and 1 in Reach 2, indicating that one of the five native fish species had spawned during the summer.

Our catch information indicates that both species have dispersed downstream of the stocking reach (reach 1) in Hot Springs Canyon. Spikedace were more dispersed than in previous years. We captured two spikedace in reach 3, downstream of the narrows: one in transect Random-3 and the other in transect Permanent-3, approximately 3.9 km and 4.6 km downstream from reach 1 (stocking reach) respectively. The segment downstream of the narrows appeared to have habitat suitable for loach minnow, but none were captured in the two transects. The furthest downstream location where loach minnow were captured was transect Permanent-2, approximately 1.4 km m downstream of reach 1 (Figure 2).

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Gila Topminnow and Desert Pupfish

Desert pupfish were captured in Larry&Charlie Tank during both the six-month (April 19, 2010) and annual (September 12, 2010) post-stocking surveys. On April 19, 2010, 85 desert pupfish were captured in six collapsible minnow traps set in Larry Tank; mean #/h = 7.0±2.31 SE. Nine lowland leopard frog tadpoles were also captured. Six traps were also set in Charlie Tank, but only 17 lowland leopard frog tadpoles were captured.

During September 2010, Gila topminnow were captured in all, and desert pupfish in some, of the sites where they had been stocked in 2007 and 2008 (Figures 1 and 2; Table 3). At Larry&Charlie Tank where only desert pupfish were stocked, 142 desert pupfish were captured; mean #/h = 5.4±1.65 SE. Therefore it can be concluded that desert pupfish are persisting in Larry&Charlie Tank.

At Secret Spring, over 4,000 Gila topminnow but only 12 desert pupfish were captured (Table 3). More Gila topminnow were captured than in previous September annual surveys but minnow trap catch rates were relatively similar: 133.5±38.14 in 2008, 78.7±11.82 in 2009, and 92.5±17.97 in 2010. Of 1,165 Gila topminnow that were categorized into length classes, 17.5% were less than 10 mm TL. Therefore, based on abundance of Gila topminnow during the three years of annual monitoring and the evidence of reproduction, it can be concluded that Gila topminnow are established at Secret Spring. Similar to previous years, few desert pupfish were captured, and minnow trap catch rates remain low: 0.8±0.46 in 2008, 0.1±0.07 in 2009, and 0.3±0.23 in 2010. All of the desert pupfish captured in 2010 were categorized as larger than 10 mm TL. Therefore, based on the evidence reported, and the fact that desert pupfish were only stocked in October 2007, it seems likely that they have established a small population, but whether or not the population will continue to persist is unknown.

At Headquarters Spring, similar to September 2009, Gila topminnow, but no desert pupfish were captured. Few Gila topminnow were captured (Table 3) and minnow trap catch rates (1.0±0.28 fish/h) were lower than reported for 2009 (8.4±5.48 fish/h; Robinson et al. 2010). All Gila topminnow captured were categorized as adults. Nonetheless, because Gila topminnow were only stocked once, on September 17, 2008, and based on the evidence presented, we conclude that Gila topminnow likely have established a population, albeit a seemingly small one, in Headquarters Spring. Because no desert pupfish were captured in September 2010 or September 2009, it seems unlikely that they established a population in Headquarters Spring, but more years of monitoring are necessary to confirm absence.

In Cherry Spring Canyon, 33 Gila topminnow and 1 desert pupfish were captured. Fewer topminnow were captured in 2010 than 2009, and minnow trap catch rates were also lower in 2010 (1.5±1.13) than in 2009 (5.5±3.46); no topminnow or desert pupfish were captured in minnow traps September 2008. However, 42% of the Gila topminnow captured in September 2010 were <10 mm TL. The one desert pupfish captured was >10 mm TL. Gila topminnow and desert pupfish were stocked into Cherry Spring Canyon in October 2007 and again in September 2008. Therefore, it seems likely that Gila topminnow have established a small population in Cherry Spring Canyon. We can conclude little regarding desert pupfish, except that at least one individual continues to persist.

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In Swamp Springs Canyon, similar to September 2009, Gila topminnow, but no desert pupfish were captured. In the upper reach, fewer Gila topminnow were captured in 2010 (204 fish) than in 2009 (952), and minnow trap catch rates were correspondingly lower in 2010 (3.6±1.30) than in 2009 (16.8±3.26). Only 1.5% of the Gila topminnow captured in the upper reach during 2010 were <10 mm TL. In the lower reach, more Gila topminnow were captured in 2010 (113) than in 2009 (83), or 2008 (63), but different gear types were used in the three years; minnow traps and dip nets were used in 2010, seines and dip nets in 2009, and minnow traps, seines and dip nets in 2008. Minnow trap catch rates in 2010 (3.3±1.51) were somewhat greater than in 2008 (1.5±1.36). About 50% of the Gila topminnow captured in the lower reach in 2010 were <10 mm TL. Gila topminnow and desert pupfish were only stocked once into each reach: October 4, 2007 in the lower reach and September 17, 2008 in the upper reach. Therefore, we conclude that Gila topminnow have established a population in lower Swamp Springs Canyon, and likely have established a population in upper Swamp Springs Canyon. Desert pupfish have not been captured in the lower reach since 2008 and in the upper reach since April 2009. Therefore it seems likely that desert pupfish failed to establish populations in Swamp Springs Canyon, but more years of monitoring are necessary to confirm this conclusion.

FISH STOCKINGS

Spikedace, loach minnow, and desert pupfish were stocked into various waters on the Muleshoe CMA on October 28, 2010 (Appendix 1). In Hot Springs Canyon, 514 loach minnow and 750 spikedace were stocked; no mortalities occurred during the transportation or tempering process. Upon arrival, water temperature was 19-20°C in the stream and 20-21°C in the buckets, so only two water exchanges were completed before fish were released. Both species were transported in each bucket so they were sorted to species and counted upon release. Fish were released into a 280-m segment of stream between Bass Canyon and Wildcat Canyon, dispersed over six locations, three upstream of and three downstream of a small bedrock waterfall. Fish behaved normally upon release.

The number of spikedace and loach minnow stocked into Redfield Canyon was similar to what was stocked into Hot Springs Canyon. However, we do not have the total numbers of each species stocked because one of the volunteers stocked fish without distinguishing the species. Number of fish stocked was: 730 spikedace, 273 loach minnow, and 279 other fish that were either spikedace or loach minnow. Nine additional spikedace and one loach minnow died during the transportation and tempering process. Most of the unidentified fish were likely loach minnow, as based on counts at BPNFCF in August 2010 we planned to stock 1,000 spikedace and 400 loach minnow into each stream; mortality in the spikedace holding tanks reduced the number available to be stocked. Water temperature was 16.4°C in the stream and 21.8°C in the barrel, so three water exchanges were completed before fish were released. Both species were transported in each bucket so they were sorted to species and counted upon release, except as noted above. Fish were released into six locations within a 130-m segment of stream near the upper end of Reach 1. Fish behaved normally upon release.

In Headquarters Spring, 374 desert pupfish were stocked; an additional three died during the transportation and tempering process. Water temperature at the stocking location was 23.7°C compared to 19.9°C in the buckets, so four water exchanges were completed before fish were

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released. Fish were released into the pool closest to the walkway and hot tubs. Fish behaved normally upon release.

In Secret Spring, 311 desert pupfish were stocked; an additional five died during the transportation and tempering process. Water temperature at the stocking location was 19.0°C compared to 19.9°C in the buckets, so two water exchanges were completed before fish were released. Fish behaved normally upon release. Fish behaved normally upon release.

RECOMMENDATIONS

The environmental assessment for the establishment of aquatic vertebrate species into Muleshoe CMA waters (Bureau of Land Management 2004) indicated that stockings of the federally threatened and endangered fish species would occur every year for five years, at which time success (establishment of a self-sustaining population) would be evaluated. The assessment also indicated that monitoring of the populations would occur during the five years of augmentation to determine the success of the stockings, and continue for at least five years after stocking ceased. The T&E fish species were stocked during 2007, 2008, 2009, and 2010 so if the plan outlined in the environmental assessment is followed and fish are available, stockings should continue until 2011 and monitoring until at least 2016. However, for the reasons given in the results and discussion, during 2009 and 2010 we did not stock all of the waters with all of the species where they had been stocked during 2007 and 2008. Partners (AZGFD, BLM, BOR, FWS, and TNC) that comprise the Muleshoe Native Fishes Team should meet to discuss results so far and decide which stream to stock based on results to date and availability of fish stocks. If sufficient numbers of spikedace and loach minnow are available in 2010 and 2011, then more could be stocked into both Redfield Canyon and Hot Springs Canyon. However, if 2011 monitoring information indicates that spikedace and loach minnow in Hot Springs Canyon are even more abundant and widespread than in previous years, then perhaps it is unnecessary to stock more fish in this stream during 2011. If fewer fish are available, then it may not be feasible to stock both streams. Similarly, for desert pupfish, partners need to discuss results to date and whether or not some of the sites stocked are suitable, and which waters to stock based on numbers of available fish. Gila topminnow appear to be established in Secret Spring and Swamp Springs Canyon, and are likely established in Headquarters Spring and Cherry Spring Canyon, so supplemental stockings are probably not necessary because the goal of the stockings (to establish self-sustaining populations) for this species seems to have been achieved. The Muleshoe Native Fishes Team will meet in 2012 (5 years after the initiation of stocking) to discuss results and determine future actions.

The study design and methods appear to be sufficient to address objectives 1 (verifying persistence), 2 (detecting recruitment), and 4 (detecting dispersal) for all species, and also appeared sufficient to address objective 3 (change in abundance) for Gila topminnow and possibly desert pupfish. Techniques used to sample for Gila topminnow and desert pupfish have been pretty consistent, and the catch rates for the trapping data can be validly compared among years. However, it is unclear whether the current design and methods are sufficient to detect a change in abundance of loach minnow or spikedace. We used multiple sampling techniques in each water to increase the probability of capturing rare species (George et al. 2009). In Hot Springs Canyon and Redfield Canyon the field crews decided which technique to use in a given habitat within a transect. As a result, comparison of catch rate results from year to year has been

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problematic because the amount of sampling effort for a given technique has not been consistent among years, and comparisons among years need to be restricted to a given technique to be statistically valid; i.e., cannot lump all gear types. For example, in Hot Springs Canyon, reach 3 was sampled only by single-pass electrofishing in 2008 and 2009, but using both single-pass electrofishing and combination of electrofishing and kick seining in 2010. In addition, because the species are relatively rare, they are not always captured in transects even when only a single sampling technique is used. For example, only single-pass electrofishing was used within the Redfield Canyon transects during 2008, 2009 and 2010, but the only spikedace and loach minnow captured in Redfield Canyon during 2008 were captured outside of the transects using a combination of backpack electrofishing and kick seining or seining.

Based on data from 2008 through 2010, electrofishing in combination with kick seining appears to be a more effective technique to capture spikedace and loach minnow than single-pass electrofishing. For a fixed number of seconds electrofished, more loach minnow and spikedace can be captured using a combination of backpack electrofishing and kick seining than with single-pass electrofishing. However, the combination of backpack electrofishing and kick seining is only effective in the relatively high current velocities found in riffles and some runs, whereas backpack electrofishing is effective in virtually all habitats except large deep pools, which are relatively rare in these systems. In addition, because more time is spent doing single-pass electrofishing than kick seining, similar numbers of fish are captured using the two techniques. Single-pass electrofishing is simple, relatively unbiased, can be applied to the entire length of each transect regardless of habitat type, and the resulting catch rate data can be validly compared among years. The two down sides are that fewer spikedace and loach minnow may be captured using only single-pass electrofishing, and the resultant sample sizes would be low (six in Redfield Canyon and six in Hot Springs Canyon) so temporal change in abundance might not be very detectable. We recommend that partners meet to discuss monitoring study design and implementation.

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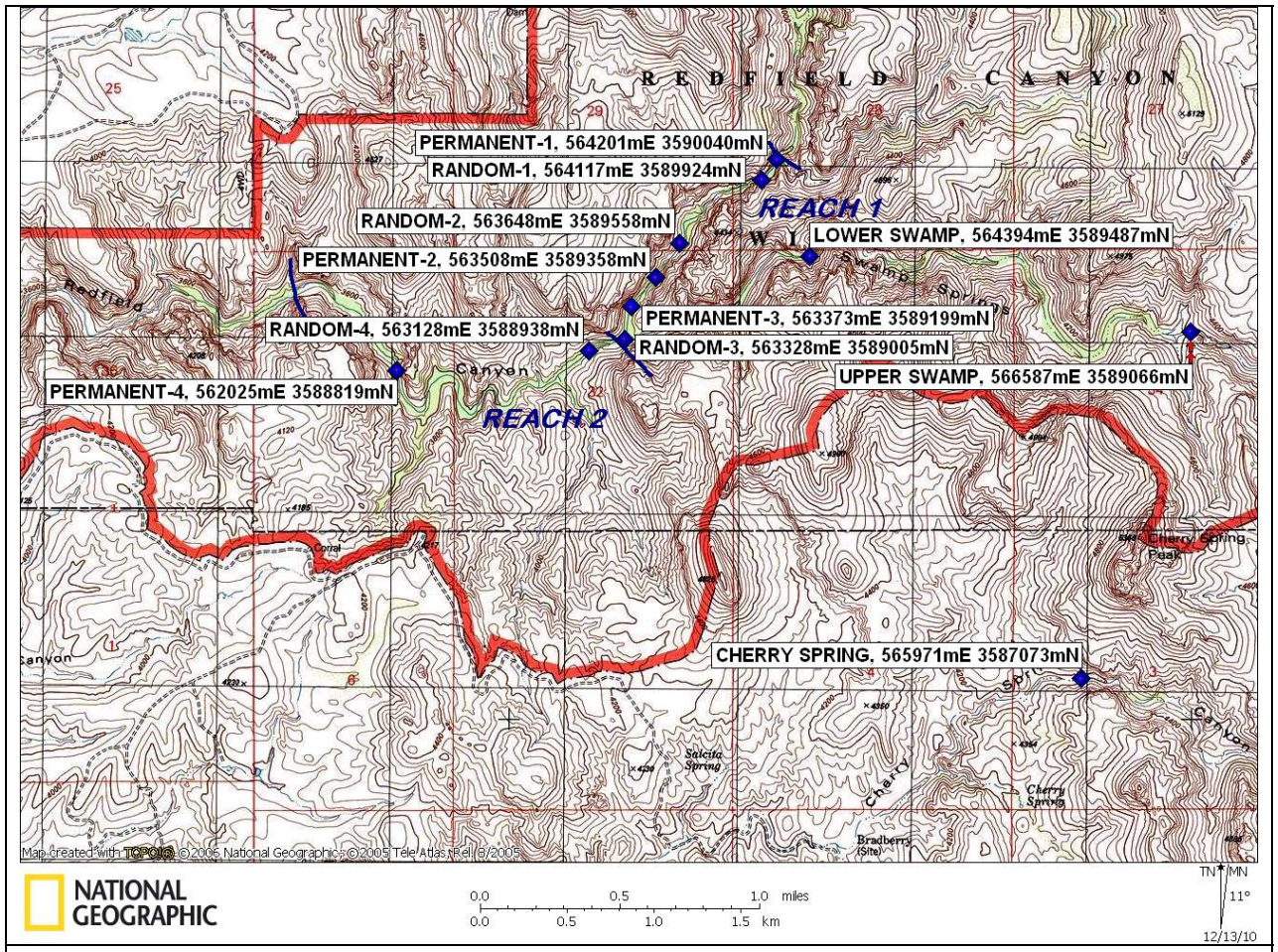

Figure 1. Map showing locations (blue diamonds) of fish monitoring sites in Redfield Canyon, Swamp Springs Canyon, and Cherry Spring Canyon within the Muleshoe Cooperative Management Area, west of the Galiuro Mountains, Arizona during 2010. In Redfield Canyon, the map shows the locations of permanent and randomly selected 100-m long sites within two reaches (delineated with blue lines); spikedace and loach minnow were stocked into Reach 1 during 2007 and 2008. In Swamp Springs Canyon desert pupfish and Gila topminnow were stocked into the lower monitoring site in 2007 and the upper site during 2008. The monitoring location shown in Cherry Spring Canyon was stocked with Gila topminnow and desert pupfish during 2007 and 2008. The thick red line is the boundary of Redfield Canyon Wilderness.

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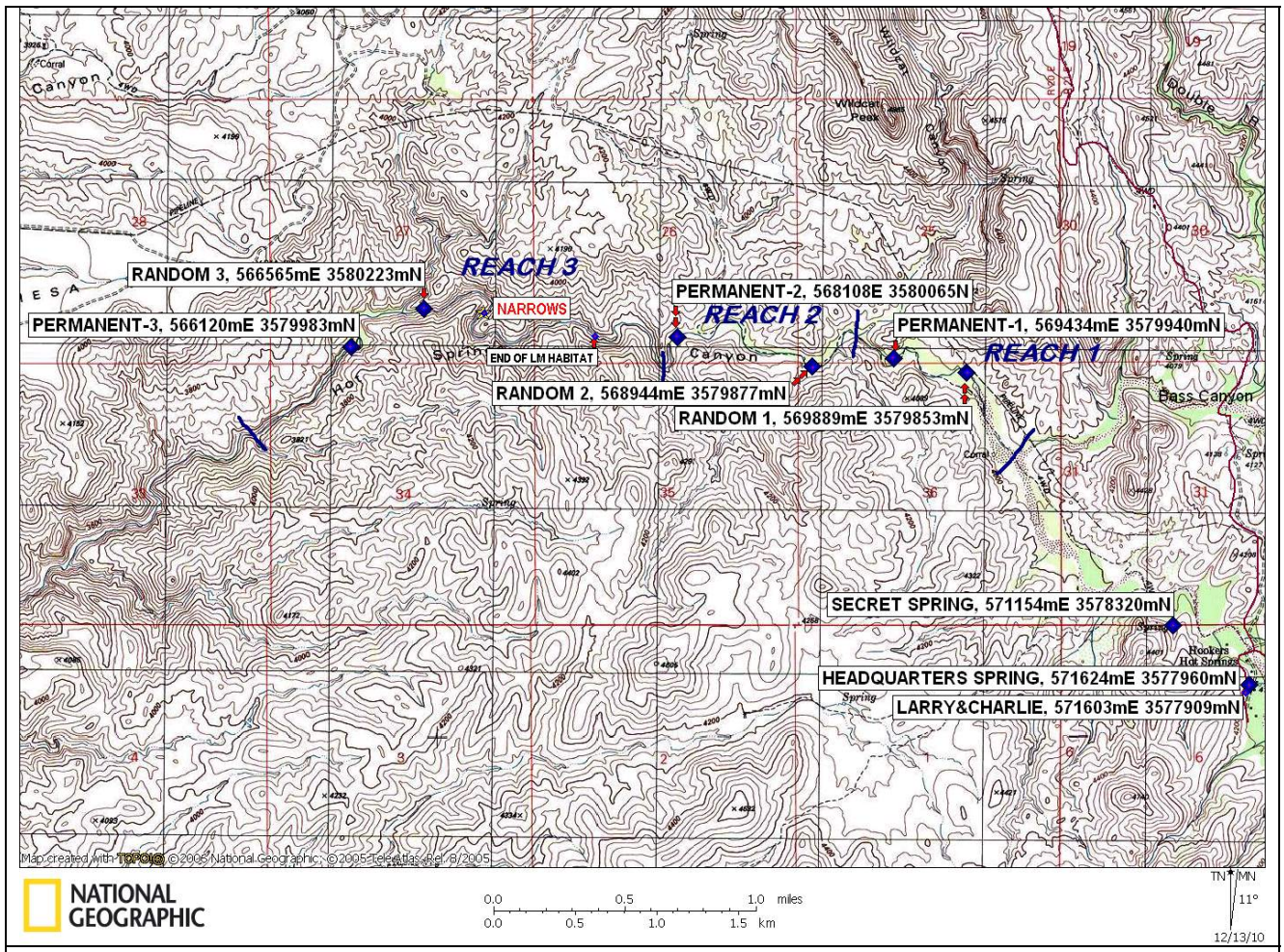

Figure 2. Map showing locations (blue diamonds) of fish monitoring sites in Hot Springs Canyon and in the vicinity of the Muleshoe Ranch Headquarters (Hookers Hot Springs) within the Muleshoe Cooperative Management Area, west of the Winchester Mountains, Arizona during 2010. In Hot Springs Canyon downstream (north and west) of Bass Canyon, the map shows the locations of permanent and randomly selected 100-m long sites within three reaches (delineated with blue lines); spikedace and loach minnow were stocked into Reach 1 during 2007, 2008, and 2009. Also shown are Secret Spring where Gila topminnow and desert pupfish were stocked during 2007, Headquarters Spring where the same two species were stocked in 2008, and Larry&Charlie Tank where desert pupfish were stocked in 2009.

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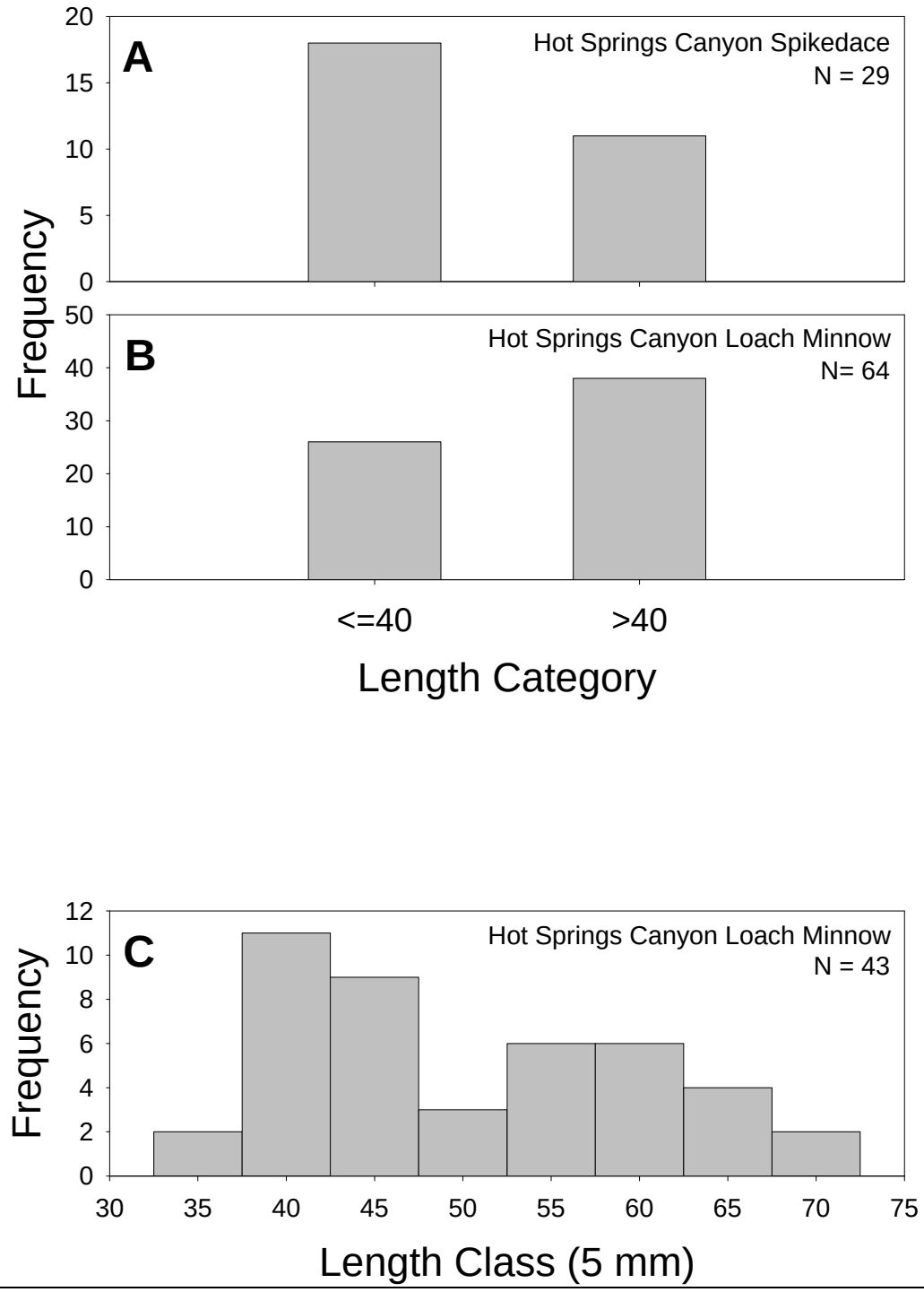

Figure 3. Length frequency distributions for spikedace and loach minnow in Hot Springs Canyon during September through October, 2010, showing frequencies of spikedace (A) and loach minnow (B) in two length categories, and loach minnow frequencies (C) within 5-mm length classes. Sample sizes (N) in B and C are not equal because only 43 of the loach minnow in B were measured, the rest were visually categorized.

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Table 1. Numbers of fish of each species captured (all gear types) within 100-m transects in Hot Springs Canyon and Redfield Canyon, Muleshoe Cooperative Management Area, Arizona during September through October, 2010.

Water	Reach	Transect	Spikedace	Loach minnow	Speckled dace	Longfin dace	Gila chub	Desert sucker	Sonora sucker	Gila topminnow	Unknown larvae	Green sunfish	Total
Hot Springs Canyon	1	Permanent-1	2	44	59	148	10	39	4	0	0	0	306
		Random-1	1	0	48	168	10	4	0	0	3	0	234
		All	3	44	107	316	20	43	4	0	3	0	540
	2	Permanent-2	10	5	46	25	9	1	0	0	1	0	97
		Random-2	14	16	262	60	0	7	0	0	0	0	359
		All	24	21	308	85	9	8	0	0	1	0	456
	3	Permanent-3	1	0	46	101	8	1	0	0	0	0	157
		Random-3	1	0	61	94	12	6	2	0	0	0	176
		All	2	0	107	195	20	7	2	0	0	0	333
		Total	29	65	522	596	49	58	6	0	4	0	1329
Redfield Canyon	1	Permanent-1	0	0	36	0	16	0	4	0	0	0	56
		Random-1	0	0	64	0	33	0	22	0	0	0	119
		Permanent-2	0	1	64	4	28	0	12	0	0	1	110
		Random-2	0	0	53	0	37	0	13	0	0	0	103
		Permanent-3	0	0	46	9	44	0	27	1	0	0	127
		Random-3	0	0	24	30	92	0	46	8	0	0	200
		All	0	1	287	43	250	0	124	9	0	1	715

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Table 2. Number of fish captured (#) and mean and standard error (SE) catch rates for backpack electrofishing (#/min) and combination backpack electrofishing plus kick seining (#/min) and seining (#/m^2) in three reaches of Hot Springs Canyon and one reach of Redfield Canyon, September-October, 2010. Reach definitions are given in the methods.

Water	Reach	Gear method	Statistic	Spikedace	Loach minnow	Speckled dace	Longfin dace	Gila chub	Desert sucker	Sonora sucker	Gila topminnow	Unknown	Green sunfish	Total
Hot Springs Canyon	1	BP electrofish+kick seine (N = 4)	#	0	18	20	33	2	0	0	0	0	0	73
			Mean #/min		6.8	8.7	12.0	0.7						28.2
			SE		(4.86)	(3.00)	(1.93)	(.45)						(7.99)
		Backpack electrofish (N=12)	#	0	24	86	231	18	43	3	0	0	0	405
			Mean #/min		0.4	4.8	11.2	1.3	1.1	0.1				19.6
			SE		(.25)	(1.64)	(5.36)	(.70)	(.48)	(.05)				(5.86)
	2	BP electrofish+kick seine (N = 8)	#	6	12	71	11	2	2	0	0	0	0	104
			Mean #/min	1.67	4.1	20.8	2.3	0.3	0.7					29.9
			SE	(1.67)	(2.05)	(7.73)	(1.93)	(.28)	(.47)					(10.60)
		Backpack electrofish (N = 9)	#	3	6	42	31	3	6	0	0	1	0	92
			Mean #/min	0.32	0.4	3.3	2.6	0.3	0.3			0.14		7.4
			SE	(.17)	(.22)	(.87)	(.57)	(.29)	(.27)			(.14)		(1.48)
		Seine (N = 5)	#	15	3	195	43	4	0	0	0	0	0	260
			Mean #/m2	0.27	0.03	3.0	0.8	0.1						4.1
			SE	(.11)	(.02)	(.93)	(.27)	(.09)						(1.06)
	3	BP electrofish+kick seine (N = 6)	#	0	0	29	35	3	1	0	0	0	0	68
			Mean #/min			9.6	10.8	0.7	0.3					21.3
			SE			(2.26)	(2.75)	(.45)	(.26)					(4.05)
		Backpack electrofish (N = 6)	#	2	0	78	160	17	6	2	0	0	0	265
			Mean #/min	0.08		3.0	7.0	0.6	0.2	0.1				11.0
			SE	(.05)		(1.03)	(1.09)	(.27)	(.08)	(.05)				(.96)
Redfield Canyon	1	Backpack electrofish (N = 23)	#	0	1	287	43	250	0	124	9	0	1	715
			Mean #/min		0.0	4.2	0.1	2.4		1.2	0.02		0.0	8.0
			SE		(.00)	(.92)	(.08)	(.40)		(.26)	(.02)		(.00)	(1.17)

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Table 3. Number of individuals captured (#), mean catch-per-unit effort (#/h for traps, #/N for dip nets and #/m for dip nets), and standard error (SE) of the mean for three different gear types used to survey for Gila topminnow and desert pupfish during September through October 2010 in five waters within the Muleshoe Cooperative Management Area, Arizona. Sample size (N) is the number of collapsible minnow traps set, dip-net sweeps, or seine hauls.

Water	Gear and number of efforts	Statistics	Gila topminnow	Desert pupfish	Longfin dace	Total
Larry&Charlie Tank	Minnow trap (N = 10)	#	0	142	0	142
		Mean #/h		5.4		5.4
		SE		(1.65)		(1.65)
	Dip net (N = 10)	#	0	6	0	6
		Mean #/N		0.6		0.6
		SE		(0.34)		(.34)
Headquarters Spring	Minnow trap (N = 10)	#	29	0	0	29
		Mean #/h	1.0			1.0
		SE	(0.28)			(.28)
	Dip net (N = 10)	#	4	0	0	4
		Mean #/N	0.4			0.4
		SE	(0.22)			(.22)
Secret Spring	Minnow trap (N = 10)	#	4135	12	0	4147
		Mean #/h	92.5	0.3		92.8
		SE	(17.97)	(0.23)		(18.01)
	Dip net (N = 10)	#	134	0	0	134
		Mean #/N	13.4			13.4
		SE	(3.63)			(3.63)
Swamp Springs-upper	Minnow trap (N = 10)	#	84	0	60	144
		Mean #/h	3.6		2.6	6.2
		SE	(1.30)		(1.20)	(1.50)
	Dip net (N = 22)	#	40	0	16	56
		Mean #/N	1.8		0.7	2.6
		SE	(0.77)		(0.37)	(.78)
	Seine (N = 10)	#	80	0	179	259
		Mean #/m	2.2		6.2	8.5
		SE	(0.82)		(2.49)	(2.33)
Swamp Springs-lower	Minnow trap (N = 10)	#	81	0	217	298
		Mean #/h	3.3		9.6	12.9
		SE	(1.51)		(3.35)	(4.17)
	Dip net (N = 13)	#	32	0	7	39
		Mean #/N	2.5		0.5	3.0
		SE	(0.96)		(0.29)	(1.03)
Cherry Spring Canyon	Minnow trap (N = 10)	#	30	1	0	31
		Mean #/h	1.5	0.1		1.5
		SE	(1.13)	(0.05)		(1.17)
	Dip net (N = 10)	#	3	0	0	3
		Mean #/N	0.3			0.3
		SE	(0.21)			(0.21)

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Appendix 1. Summary of species stocked and stocking location information for seven waters within the Muleshoe Cooperative Management Area, Arizona during 2007-2009. Reach definitions are given in the Study Area section.

Water	Reach	Species	Date	Site name	Meters from bottom of reach	Easting (NAD 83)	Northing (NAD 83)	Number Stocked
Redfield Canyon	1	Loach minnow	10/4/2007		613	563625	3589507	77
			10/4/2007		904	563760	3589433	44
			10/4/2007		1134	563932	3589858	92
			9/17/2008		1212	564011	3589842	200
			9/17/2008		1258	564013	3589918	200
			9/17/2008		924	563768	3589918	120
			9/17/2008		881	563732	3589744	120
			9/17/2008		813	563688	3589683	120
			9/17/2008		746	563674	3589617	120
			9/17/2008		501	563674	3589414	120
			10/28/2010		825	563707	3589690	273
		Loach minnow or spikedace	10/28/2010		825	563707	3589690	279
		Spikedace	10/4/2007		1180	564027	3589881	192
			9/17/2008		924	563768	3589918	100
			9/17/2008		881	563732	3589744	100
			9/17/2008		813	563688	3589683	100
			9/17/2008		746	563674	3589617	100
			9/17/2008		134	563354	3589126	100
			10/28/2010		960	563803	3589781	346
			10/28/2010		930	563793	3589743	261
			10/28/2010		915	563784	3589737	123
Hot Springs Canyon	1	Loach minnow	10/4/2007		160	569319	3579964	205
			9/17/2008		717	569706	3579826	250
			9/17/2008		609	569620	3579844	249
			9/17/2008		476	569592	3579986	250
			9/17/2008		255	569422	3579933	250

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Water	Reach	Species	Date	Site name	Meters from bottom of reach	Easting (NAD 83)	Northing (NAD 83)	Number Stocked
			10/28/2009		160	569334	3579986	87
			10/28/2009		609	569624	3579847	69
			10/28/2010		465	569590	3579935	130
			10/28/2010		420	569580	3579983	45
			10/28/2010		350	569501	3579998	88
			10/28/2010		215	569378	3579948	63
			10/28/2010		200	569366	3579961	130
			10/28/2010		195	569359	3579961	58
		Spikedace	10/4/2007		68	569265	3580026	105
			10/4/2007		255	569424	3579941	105
			9/17/2008		717	569706	3579826	125
			9/17/2008		609	569620	3579844	125
			9/17/2008		476	569592	3579986	125
			9/17/2008		255	569422	3579933	125
			10/28/2009		68	569277	3580018	212
			10/28/2009		609	569624	3579847	174
			10/28/2010		465	569590	3579935	73
			10/28/2010		420	569580	3579983	171
			10/28/2010		350	569501	3579998	91
			10/28/2010		215	569378	3579948	197
			10/28/2010		200	569366	3579961	114
			10/28/2010		195	569359	3579961	104
Swamp Springs Canyon	Lower	Gila topminnow	10/4/2007		1275	564394	3589487	249
		Desert pupfish	10/4/2007		1275	564394	3589487	248
	Upper	Gila topminnow	9/17/2008		4245	566576	3589070	225
			9/17/2008		4267	566603	3589070	50

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Water	Reach	Species	Date	Site name	Meters from bottom of reach	Easting (NAD 83)	Northing (NAD 83)	Number Stocked
		Desert pupfish	9/17/2008		4245	566576	3589070	225
			9/17/2008		4267	566603	3589070	48
Cherry Spring Canyon	1	Gila topminnow	10/4/2007	Lower pool	1310	565977	3587082	130
			10/4/2007	Upper pool	1320	565981	3587090	134
			9/17/2008	Lower pool	1310	565977	3587082	275
		Desert pupfish	10/4/2007	Lower pool	1310	565977	3587082	148
			10/4/2007	Upper pool	1320	565981	3587090	98
			9/17/2008	Lower pool	1310	565977	3587082	275
Secret Spring		Gila topminnow	10/4/2007			571100	3578303	499
		Desert pupfish	10/4/2007			571100	3578303	496
			10/28/2010			571100	3578303	311
Headquarters Spring		Gila topminnow	9/17/2008			571624	3577960	275
		Desert pupfish	9/17/2008			571624	3577960	290
			10/28/2010			571624	3577960	374
Larry&Charlie Tank		Desert pupfish	10/28/2009			571603	3577909	196

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