Muleshoe Cooperative Management Area Native Fish Repatriations, One-Year Post-Stocking Monitoring and First Augmentation Stocking September 15-17, 2008

Anthony Robinson
Arizona Game and Fish Department, Research Branch
5000 W. Carefree Hwy.
Phoenix, AZ 85086

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The 2008 native fish monitoring and native fish augmentation stocking in the Muleshoe Cooperative Management Area in the Galiuro Mountains of Arizona was a cooperative undertaking of multiple agencies, organizations, and volunteers. Involved in the design and execution of the work were individuals from The Nature Conservancy (Ken Wiley, Bob Rogers, Mark Haberstich, Paul Hadley, Rob Burton, and Christina Burton), Bureau of Land Management (Heidi Blasius, Jeff Conn, and Joy Price), Arizona Game and Fish Department (Ross Timmons, Codey Carter, Suzanne Ehret, Duane Aubuchon, Amy Bailey, David Ward, and Tony Robinson), U.S. Fish and Wildlife Service (Mary Richardson and Doug Duncan), U.S. Bureau of Reclamation (Rob Clarkson), Arizona State University (Paul Marsh and Abe Karam), and University of Arizona (Peter Reinthal and Lisa McDonough).

INTRODUCTION

On October 4, 2007 four listed-fish species were stocked into waters on the Muleshoe Cooperative Management Area, in the Galiuro Mountains of Arizona. In Redfield Canyon, 210 spikedace *Meda fulfida* and 205 loach minnow *Rhinichthys cobitis* were stocked into the reach upstream of the confluence with Swamp Spring Canyon. In Hot Springs Canyon, 210 spikedace and 205 loach minnow were stocked within an approximately 500-m reach just upstream of the confluence of Wildcat Canyon. In Swamp Springs Canyon, 250 desert pupfish *Cyprinodon macularius* and 250 Gila topminnow *Poeciliopsis occidentalis* were stocked into an approximately 100-m reach located about 1.3 km upstream from the confluence of Swamp Springs Canyon and Redfield Canyon. In Cherry Spring Canyon, 250 desert pupfish and 250 Gila topminnow were stocked into a 50-m reach approximately 3.1 km downstream from the crossing of Forest Service Road 691. In Secret Spring, a pond about 0.5 km northwest of the Muleshoe Ranch Headquarters, 500 desert pupfish and 500 Gila topminnow were stocked.

Fish used in the stockings originated from different localities and were transported via helicopter to the stocking locations mentioned above. Gila topminnow (Bylas Springs lineage) originated from Arizona State University and were transported by Dr. Paul Marsh to The Nature Conservancy's Lower San Pedro River Preserve ponds near Dudleyville. Desert pupfish were collected from the two ponds at the The Nature Conservancy's (TNC) Lower San Pedro River Preserve. Topminnow and pupfish 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. Spikedace and loach minnow were collected directly from the east end of Aravaipa Creek, within TNC's Aravaipa Canyon Preserve, and were transported to stocking locations via helicopter as described above. At the stocking locations, fish were transferred to 5-gallon buckets and walked to actual stocking sites. All fish were tempered, by exchanging approximately ¼ of the water in each bucket with stream water every 10-15 minutes until water tempertures in the bucket were equvalent with the stocking site, whereupon they were released into the stream or pond. Fish behaved normally upon release, and very few mortalities were observed (Secret Spring—four desert pupfish and one Gila topminnow,).

Early monitoring (approximately one-month and six-month post-stocking) of the Gila topmminnow and desert pupfish stocking sites indicated that, for the most part, the species were still present. On November 29, 2007, Heidi Blasius, Bob Rogers, and Ken Wiley hiked to the Swamp Spring Canyon stocking site, and captured several Gila topminnow and two desert pupfish by dip netting. During the six-month post-stocking monitoring of Swamp Springs on April 8, 2008, one Gila topminnow was captured but no desert pupfish were captured or observed; sampling was done by Jesse Bahm and Codey Carter. At Cherry Spring, four Gila topminnow and two desert pupfish were captured on November 5, 2007 by Heidi Blasius and Mary Richardson. During the six-month post-stocking monitoring at Cherry Spring on April 8, 2008, Tony Robinson and Kevin (TNC volunteer) captured one Gila topminnow and five desert pupfish. At Secret Spring, dozens of Gila topminnow were observed and one desert pupfish was captured during one-month post-stocking monitioring on November 5, 2007 conducted by Heidi Blasius and Mary Richardson. During the six-month post-stocking monitoring at Secret Spring done by Tony Robinson, Jesse Bahm, and Codey Carter, many topminnow of multiple size classes were captured, but only two desert pupfish were captured. Loach minnow and spikedace stocked into Hot Springs Canyon and Redfield Canyon were not monitored at one or six-months post stocking; the first monitoring was planned to occur at one-year

post stocking. However, during green sunfish removal efforts in Redfield Canyon on April 8, 2008, one loach minnow was captured.

This report summarizes the results of the one-year post stocking monitoring, and the subsequent augmentation stockings. The goal of the original and subsequent stockings was to establish populations in the systems in which the species were stocked (i.e., to repatriate the species to the systems). A species is considered to have established (a successful repatriation) when it is reproducing to the point where it is self-sustaining. The objectives of monitoring are 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 one monitoring period to the next); 4) determine if species have dispersed outside of stocking area; and 5) report any non-native fish species captured during monitoring. The objectives of the augmentation stocking are to: 1) increase the number of individuals in a system to help them establish; and 2) ensure that genetic composition of stocked populations reflect donor populations and avoid a founder effect.

MONITORING

METHODS

Spikedace and Loach Minnow

Permanent 100-m long sites were established prior to monitoring within Redfield Canyon (four permanent sites) and Hot Springs Canyon (three permanent sites). In addition, sampling crews opportunely selected three to four additional 100-m long sites to sample in each stream. All 100-m long sites were sampled by backpack electrofishing, making a single pass, moving in an upstream direction, throughout each reach. Stunned fish were captured with electrofishing dip nets. Data were recorded after sampling each 100-m reach.

In addition to the 100-m reaches, crews conducted targeted-habitat sampling within and upstream and downstream of the stocking areas, but outside of the 100-m sample reaches. Runs, eddies and pools (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 (loach minnow habitat) were sampled with a combination of kick-seining and electrofishing. The bottom portion of the riffle was blocked off with a seine (held by one or two people), and then beginning approximately 5-m upstream of the seine, the riffle was shocked downstream while sweeping and rolling the substrate material with feet toward the blocking seine. When the electrofisher reached the block net, the people holding the net swept it upwards to capture fish. 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.

All fish captured were held in buckets until each site was surveyed. Aerators were used when necessary. Fish were identified to species, and counted within one of two age classes (20-40 mm = juveniles, > 40 mm = adults) for spikedace, loach minnow, speckled dace, and longfin dace; fish smaller than 20 mm TL were categorized as larvae. After processing, fish were released alive back to the reach from which 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, age class of fish (juvenile or adult), and counts of

individuals within each species-age-class category. Habitat information was collected at the 100-m sites, and some of the targeted-habitat sites. Habitat data recorded included: percentage of site composed of each habitat type (cascade, riffle, run, pool), percentage of site composed of each substrate type (clay, silt, sand, gravel, pebble, cobble, boulder), and if water quality equipment was available, water temperature (°C), dissolved oxygen (mg/L), pH, and conductivity (µS).

Sampling methods were not adequately communicated to the Hot Springs survey crew, and so the methods they used were a variation of those described above. Two of the permanent 100-m sites and two of the opportunely selected 100-m sites were sampled as described above. The one other permanent 100-m site and one other opportunely selected 100-m site were sampled using a variation of the targeted-habitat sampling technique; the bottom portion of the habitat unit was blocked off with a seine, and then the habitat was electrofished downstream towards the seine while dip netting fish, and when the electrofisher reached the block seine, the seine was swept up to capture fish. However, data were not recorded for each habitat unit sampled, but rather all fish and effort was reported for the entire 100-m site. At non-100-m targeted habitat (four pools and one riffle), different methods were used. One pool was both shocked and seined. The other three pools and the riffle were only electrofished.

Gila Topminnow and Desert Pupfish

Sampling was completed throughout the stocking areas, and in Cherry Spring Canyon and Swamp Springs Canyon the stocking area and areas immediately upstream and downstream of the stocking area were sampled. Fish were sampled using seines (3 mm mesh, and typically 2 m long by 1.5 m tall), dip nets (3 mm mesh), and Promar® collapsible minnow traps (0.46 m long x 0.3 m wide, with 2 mm mesh). Collapsible minnow traps were baited with dry dog food or dry fish food. Six or seven traps were set upon arrival at each site, and were pulled two to three hours later. An attempt was made to conduct a minimum of 10 dip net sweeps and 10 seine hauls.

After each seine haul, dip net sweep, or trap pull, captured fish were held in buckets 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, age class of fish (juvenile or adult), and counts of individuals within each species-age-class category. Water quality information recorded included water temperature (°C), dissolved oxygen (mg/L), pH, and conductivity (µS).

Physical Environment

A variety of environmental variables were recorded at the monitoring sites. A temperature logger (HOBO© Water Temp Pro v2) was installed into each water where fish were stocked on October 4, 2007, except for Redfield Canyon, where the logger was not installed until April 8, 2008. For the two streams where spikedace and loach minnow were stocked, the percent of habitat types (cascade, riffle, run, pool) and the percent of substrate types (decayed organic matter, clay, silt, sand, gravel, pebble, cobble, boulder) within each 100-m survey reach were visually estimated. At the topminnow and pupfish stocking sites, several water quality

Table 1. Personnel who participated in the one-year post-stocking monitoring of native fishes in Muleshoe Ecosystem waters, September 15-16, 2008. Crew leaders are those in bold type. Affiliation acronyms are Arizona Game and Fish Department (AZGFD), Bureau of Land Management (BLM), Arizona State University (ASU), University of Arizona (UofA), and The Nature Conservancy (TNC).

Site	Sampling type	Crew number	Personnel	Personnel affiliation
Redfield Canyon	Loach minnow and spikedace	1	**Codey Carter**	AZGFD
			Suzanne Ehret	AZGFD
			Abe Karam	ASU
		2	**Heidi Blasius**	BLM
			Jeff Conn	BLM
			Joy Price	BLM
Hot Springs Canyon	Loach minnow and spikedace	1	**Peter Reinthal**	UofA
			Mark Haberstich	TNC
			Amy Bailey	AZGFD
Swamp Spring	Topminnow and pupfish	1	**Tony Robinson**	AZGFD
			Paul (TNC)	TNC
Cherry Spring	Topminnow and pupfish	1	**Ross Timmons**	AZGFD
			Lisa McDonough	UofA
Secret Spring	Topminnow and pupfish	1	**Tony Robinson**	AZGFD
			Amy Bailey	AZGFD

parameters were recorded. Water quality parameters pH, conductivity (µS/cm), salinity (mg/L), total dissolved solids (mg/L), and water temperature (°C), were measured using an EXTECH Instruments Inc., ExStik EC500 meter. Dissolved oxygen (% saturation and mg/L) was measured using an YSI Inc., Model 55 dissolved oxygen meter, or an EXTECH Instruments Inc., Exstik II dissolved oxygen meter.

RESULTS AND DISCUSSION

One-year post-stocking monitoring of fishes was completed on September 15, 2008 for Secret Spring, and on September 16, 2008 for Swamp Springs Canyon, Cherry Spring Canyon, Redfield Canyon, and Hot Springs Canyon. Personnel that participated in the monitoring are shown in Table 1.

Spikedace and Loach Minnow

Seven species of fish were captured in Redfield Canyon (Table 2). Speckled dace was the most abundant species captured, followed by Gila chub, Sonora sucker, longfin dace, spikedace, green sunfish, and loach minnow (Tables 3, 4, and Appendix 1). Both spikedace and loach minnow were captured, so it can be concluded that they have persisted in Redfield Canyon. However, neither species were abundant. In addition, spikedace and loach minnow were only captured within the area (Figure 1) that they were stocked during 2007, so as of yet, we do not have any evidence that they have dispersed outside of the stocking reach. Of the 12 spikedace captured, 10 were adults and two were juveniles; evidence that spikedace reproduced in Redfield Canyon.

Table 2. Numbers of fish of each species captured (all gear types) on September 15 and 16, 2008 in each of five waters on the Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona.

	Water Name				
	Cherry Spring Canyon	Hot Springs Canyon	Redfield Canyon	Secret Spring	Swamp Springs Canyon
Spikedace		4	12		
Loach minnow		12	1		
Speckled dace		206	290		
Longfin dace		306	50		1169
Gila chub		4	173		
Desert sucker		61			
Sonora sucker		3	55		
Desert pupfish				10	14
Gila topminnow	8			2334	69
Green sunfish			6		
TOTAL	8	596	587	2344	1252

Table 3. Backpack electrofishing mean catch-per-unit effort (number fish per minute electrofished) for 100-m long permanent and opportune sites in Hot Springs Canyon and Redfield Canyon, Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona on September 16, 2008.

	Water			
	Hot Springs Canyon N=4		Redfield Canyon N=7	
Species	Number of fish	CPUE mean±SE	Number of fish	CPUE mean±SE
Spikedace	1	0.06±0.06	0	
Loach minnow	1	0.04±0.04	0	
Speckled dace	82	3.98±2.03	265	2.94±1.20
Longfin dace	128	5.98±1.51	50	0.70±0.37
Gila chub	3	0.12±0.12	132	1.25±0.43
Desert sucker	34	1.63±0.49	0	
Sonora sucker	0		51	0.47±0.17
Green sunfish	0		6	0.04±0.02
Total fish	249	11.80±2.97	504	5.39±1.09

Table 4. Fish catch information (# captured and mean catch-per-unit effort plus or minus standard error) for targeted-habitat sampling using various combinations of gear types on September 16, 2008 in Hot Springs Canyon and Redfield Canyon, Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona. Sampling methods were variable in Hot Springs Canyon (see spikedace and loach minnow sampling methods). The two sites in Hot Springs Canyon that were sampled by backpack electrofishing+block seine+dip net were 100-m long encompassing multiple habitat types; each habitat unit was sampled individually, but all fish and seconds shocked were summed for the total 100 m reach. All other sites in Hot Springs Canyon and Redfield Canyon were shorter: riffle sections sampled were 1-3 m long, runs were < 10 m long, and pools < 5 m long. One pool in Hot Springs Canyon was both shocked and seined, but only total number of fish captured were recorded, so effort could not be calculated.

				Fish species							
Stream	Gear	Habitat		Spikedace	Loach minnow	Longfin dace	Speckled dace	Gila chub	Sonora sucker	Desert sucker	Total fish
Hot Springs Canyon	Electrofish+block siene+dip net (N=2)	riffle, run, pool	#	3	11	146	107			21	288
			#/min	0.24	0.88	11.49	8.40			1.65	22.65
				±0.24	±0.88	±3.79	±1.63			±0.42	±6.95
	Electrofish+two seine hauls (N=1)	pool	#			7	7	1		1	16
	Electrofish (N=3)	pool	#			20	3		3	5	31
			#/min			20.20	4.83		1.82	6.04	32.89
						±10.25	±1.52		±1.82	±0.35	±10.62
	Electrofish (N=1)	riffle	#			5	7				12
			#/min			11.54	16.15				27.69
Redfield Canyon	Electrofish +kick seine (N=23)	riffle	#		1		25	2			28
			#/min		0.11		2.16	0.33			2.60
					±0.11		±1.01	±0.22			±1.00
	Seine (N=14)	run	#	12				39	4		55
			#/m	0.17				0.53	0.03		0.73
				±0.10				±0.16	±0.02		±0.20

The one loach minnow captured was an adult, so it is unknown whether or not loach minnow reproduced in Redfield Canyon. Based on the above information, spikedace may be in the early stages of establishing a population in Redfield Canyon, but little can be said of loach minnow except that they have persisted since the 2007 stocking.

In Hot Springs Canyon, seven fish species were captured (Table 2). Longfin dace was the most abundant species, followed by speckled dace, desert sucker, loach minnow, spikedace and Gila chub, and Sonora sucker (Tables 3, 4, and Appendix 1). Therefore, there is evidence that stocked spikedace and loach minnow have persisted in Hot Springs Canyon since their stocking

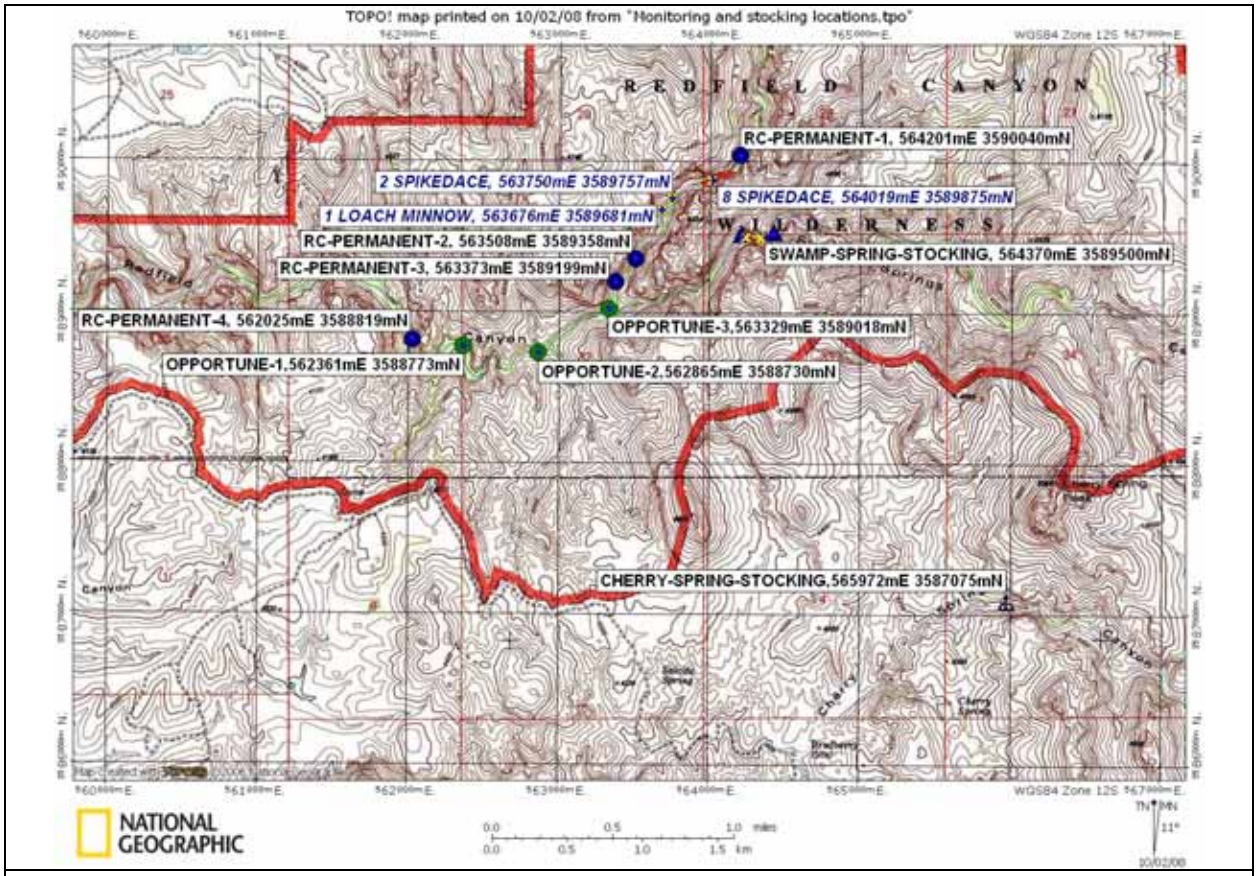

Figure 1. Map of Redfield Canyon, Swamp Spring Canyon, and Cherry Spring Canyon withing the Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona. In Redfield Canyon, the map shows the locations of permanent (blue circles) and opportunely selected (green circles) 100-m long fish monitoring sites that were sampled on September 16, 2008, and locations (yellow x's) where spikedace or loach minnow were captured on September 16 2008, and the reach stocked (red line below RC-Permanent-1) with spikedace and loach minnow on October 4, 2007. Blue triangles in Swamp Springs Canyon are the upstream and downstream boundaries of the reach sampled on September 16, 2008, yellow x's are the upstream and downstream extent of Gila topminnow and desert pupfish captured on the same date, and the red line is the reach that was stocked on October 4, 2007. The blue triangle in Cherry Spring Canyon is the both the location of the October 4, 2007 stocking and the area surveyed on September 16, 2008.

in October 2007. Of the 12 loach minnow captured, 10 were adult and two were juvenile; evidence that loach minnow reproduced in Hot Springs Canyon. The four spikedace captured were all adults, so it is unknown whether, or not spikedace reproduced in Hot Springs Canyon. Three of the four spikedace were captured within the stocking site, but the other was captured approximately 700 m downstream of the stocking site (within site Opportune-2; Figure 2), indicating that some spikedace had dispersed downstream. Eleven of the 12 loach minnow were captured within the stocking area, but one was captured approximately 1.5 km downstream in Permanent Site #2, indicating that some loach minnow had dispersed downstream. Based on the above information, loach minnow may be in the early stages of establishing a population in Hot Springs Canyon, but little can be concluded for spikedace except that they have persisted since the 2007 stocking.

Gila Topminnow and Desert Pupfish

In Swamp Springs Canyon, a reach approximately 210 m long was surveyed, from approximately 160 m downstream (NAD 83, 564205mE and 3589502mN) to 50 m upstream

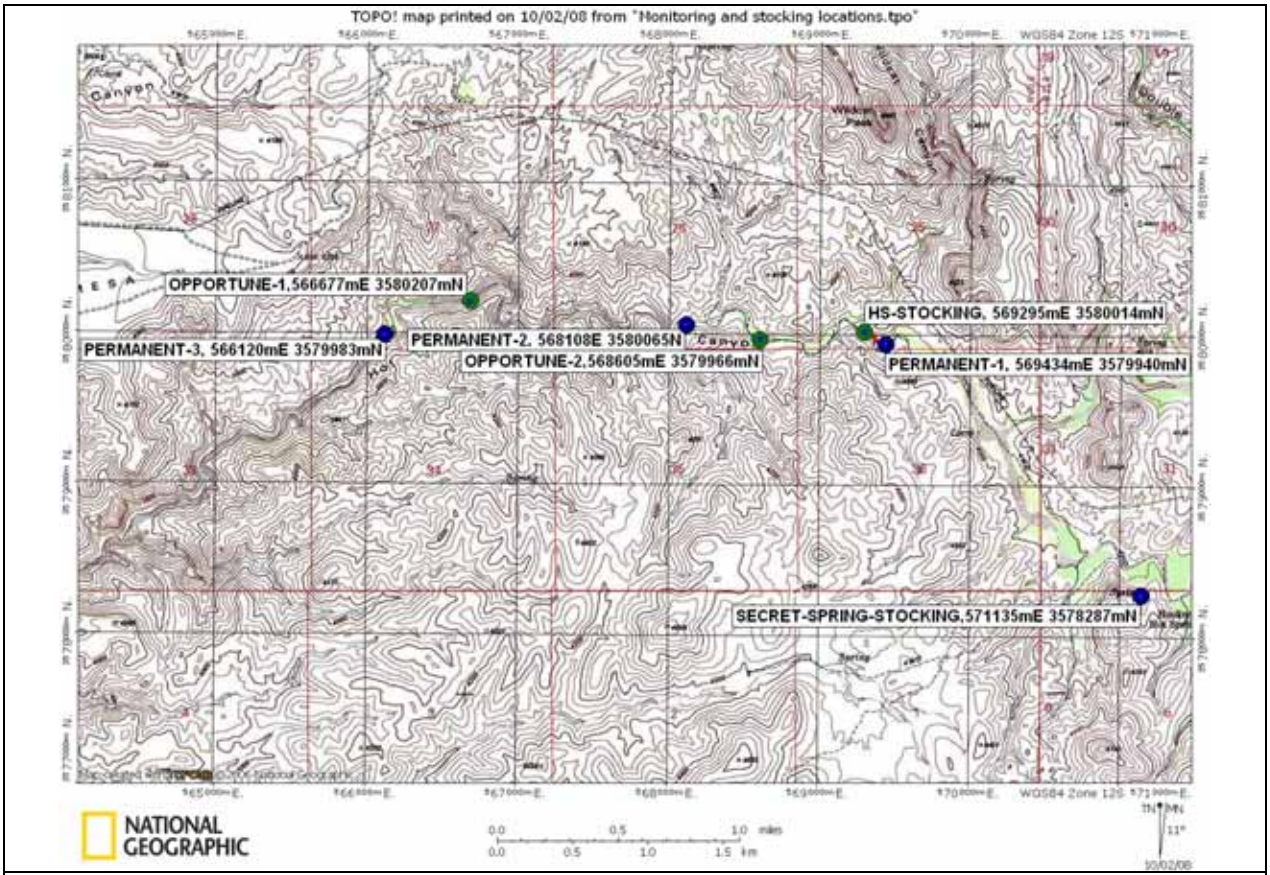

Figure 2. Map of Hot Springs Canyon downstream of the Muleshoe Ranch Headquarters (Hookers Hot Spring), showing locations of permanent (blue circles) and opportunely selected (green circles) 100-m long fish monitoring sites that were sampled on September 16, 2008, and the reach stocked (red line between Permanent-1 and HS-Stocking) with spikedace and loach minnow on October 4, 2007. Secret Spring, where Gila topminnow and desert pupfish were stocked on October 4, 2007 is also shown.

Table 5. Mean catch-per-unit effort (±standard error) of fishes for gear types used to survey for Gila topminnow and desert pupfish on September 15 and 16, 2008 in three waters within the Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona. Sample size (N) is the number of traps set, dip-net sweeps, or seine hauls. Catch-per-unit effort for minnow traps is number (#) of fish/hour, for dip nets is number of fish/m^2, and for seines is number of fish/m and number of fish/m^2.

			Water name		
Gear type	Species		Cherry Spring Canyon	Secret Spring	Swamp Springs Canyon
Minnow trap			N=7	N=6	N=7
	Gila topminnow	#	0	1590	26
		#/h		133.45±38.14	1.48±1.36
	Desert pupfish	#	0	10	9
		#/h		0.84±0.46	0.51±0.45
	Longfin dace	#	0	0	908
		#/h			45.35±9.24
	Total Fish	#	0	1600	943
		#/h		134.28±38.34	47.33±9.80
Dip net			N=4+	N=10	N=10
	Gila topminnow	#	0	157	11
		#/m^2		140.72±56.14	3.51±1.89
	Longfin dace	#	0	0	35
		#/m^2			26.01±20.21
	Total Fish	#	0	157	46
		#/m^2		140.72±56.14	29.53±19.79
Seine			N=9	N=10	N=15
	Gila topminnow	#	8	587	32
		#/m	0.11±0.08	22.13±7.33	0.54±0.27
		#/m^2	0.06±0.04	12.24±3.58	0.43±0.18
	Desert pupfish	#	0	0	5
		#/m			0.09±0.06
		#/m^2			0.06±0.04
	Longfin dace	#	0	0	226
		#/m			4.64±0.95
		#/m^2			4.47±0.86
	Total Fish	#	8	587	263
		#/m	0.11±0.08	22.13±7.33	5.27±0.92
		#/m^2	0.06±0.04	12.24±3.58	4.96±0.84

Table 6. Mean catch-per-unit effort (±standard error) of fishes for gear types used to survey for Gila topminnow and desert pupfish on April 8, 2008 in three waters within the Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona. Sample size (N) is the number of traps set, dip-net sweeps, or seine hauls. Catch-per-unit effort for minnow traps is number (#) of fish/hour, for dip nets is number of fish/m^2, and for seines is number of fish/m and number of fish/m^2. Lengths of dip net sweeps were not recorded in Swamp Springs Canyon, so catch-per-unit effort was not calculated.

			Water name		
Gear type	Species		Cherry Spring Canyon	Secret Spring	Swamp Springs Canyon
Minnow trap			N=2		N=2
	Longfin dace	#	0		111
		#/h			34.47±6.84
Dip net			N=8		N=53
	Longfin dace	#	0		21
		#/m^2			--
Seine			N=18	N=20	N=12
	Gila topminnow	#	1	117	1
		#/m	0.06±0.06	5.95±3.27	0.17±0.17
		#/m^2	0.06±0.06	4.07±2.12	0.11±0.11
	Desert pupfish	#	5	2	0
		#/m	0.22±0.13	0.10±0.07	
		#/m^2	0.22±0.13	0.10±0.07	
	Longfin dace	#	0	0	288
		#/m			28.59±13.85
		#/m^2			19.06±9.23
	Total Fish	#	6	119	289
		#/m	0.28±0.14	6.05±3.27	28.76±13.82
		#/m^2	0.28±0.14	4.17±2.12	19.17±9.21

(564418mE and 3589521mN) of the location of the temperature logger, which was the upstream end of the stocking area (Figure 1). Small waterfalls marked the upstream and downstream bounds of the area sampled, and except for a small plunge pool immediately below the downstream waterfall, water was not present downstream. Gila topminnow and desert pupfish were only found within an approximately 140-m reach, downstream of the upper end of the stocking area, indicating that they have not spread outside of the stocking area. Sixty-nine Gila topminnow and 14 desert pupfish were captured, indicating that both species have persisted in Swamp Springs Canyon since they were stocked on October 4, 2007 (Table 2). Gila topminnow seining catch rates appear greater in September (Table 5) when 32 fish were captured than April 2008 (Table 6) when only one individual was captured, but the difference was not statistically

significant (Mann Whitney U = 67.5, P = 0.13), so all that can be safely concluded is that abundance remains low. Likewise, catch rates of desert pupfish remained low in September, but none were captured during April 2008. Fifty-nine percent (41 of 69) of the Gila topminnow captured were juveniles, indicating that Gila topminnow reproduced and are recruiting in Swamp Spring Canyon. All of the desert pupfish captured in September were categorized as adults, so it is unknown whether or not they reproduced in Swamp Springs Canyon. Longfin dace, which occur naturally in the stream (i.e., were not stocked) was the most abundant species (Table 5), and 10% (122 of 1047) of the longfin dace were categorized as juveniles.

In Cherry Spring Canyon on September 16, 2008, the two stocking pools were surveyed, as was the short watered reach (~30-50 m) immediately downstream. The only species of fish captured was Gila topminnow (Table 2 and 5), all eight of which were captured by seining. Catch information indicate that Gila topminnow have persisted in Cherry Spring Canyon since they were stocked last year. Catch rates in September remained low compared to April 2008 (Table 6; Mann-Whitney U = 60.0, P = 0.08). The topminnow were all captured in the lower stocking pool, indicating that they have not dispersed beyond the stocking area. Eighty-eight percent (7 of 8) of the topminnow were juveniles, indicating that Gila topminnow reproduced in Cherry Spring Canyon. It is unknown whether or not desert pupfish persist in Cherry Spring Canyon, but if they do, numbers are extremely low.

In Secret Spring, both topminnow and pupfish were captured on September 15, 2008, indicating that both species have persisted in the pond since the original stocking. Approximately 2,334 Gila topminnow were captured, 1,590 in minnow traps, indicating that abundance of topminnow has increased from the 500 that were stocked on October 4, 2007 (Tables 2 and 5); note minnow traps were not used in April 2008 monitoring. Gila topminnow seine catch rates (# fish/m2) increased from April 2008 to September (Mann-Whitney U = 28.5, P = 0.02), also indicating abundance of topminnow has increased from April to September 2008. Of the 744 Gila topminnow that were categorized to age-class during September, 53% were considered juveniles. Therefore, Gila topminnow reproduced in Secret Spring. Only 10 desert pupfish were captured in Secret Spring (Tables 2 and 5) during September, all in collapsible minnow traps, and unfortunately the age-class was not recorded. However, most if not all of the pupfish were larger individuals, so they were probably all adults. Therefore, it is unknown if desert pupfish reproduced in Secret Spring, but since the species typically only lives one year, they may have reproduced. Because this is a pond environment, dispersal out of the pond was not assessed (in-flow to the pond is from a short (~3 m), high gradient stream section, and outflow is shallow, and flows downhill onto flats, where the water percolates into the ground).

Physical Environment

Habitat in Redfield Canyon was comprised mostly of pools with intervening riffles, whereas that in Hot Springs Canyon was comprised of mostly runs (Figure 3). Substrate types were reflective of the habitat types in the two streams, as Redfield Canyon had predominantly (~65%) pebble-sized and larger sized substrates, whereas Hot Springs Canyon was dominated by gravel-sized and smaller-sized substrates (Figure 3). Four pools were surveyed in Cherry Spring Canyon, where substrates were: 100% decayed organic matter in the upper stocking pool, 60% gravel and 40% decayed organic matter in the lower stocking pool, 60% gravel and 40% bedrock in the next

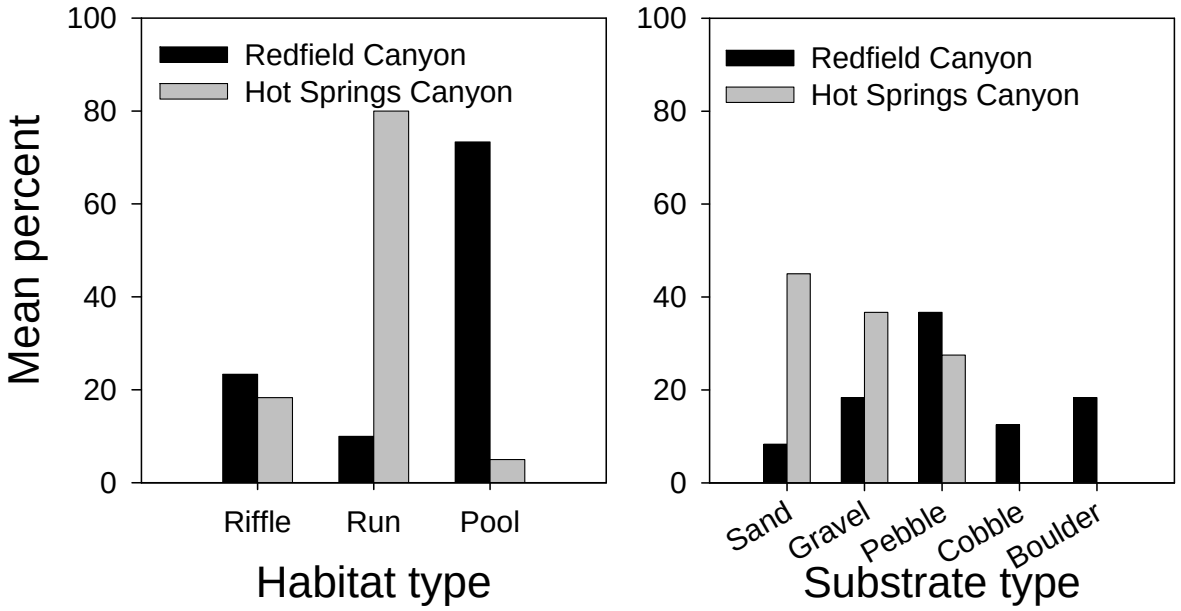

Figure 3. Percent habitat types and substrate types in 100-m fish survey reaches in Redfield Canyon (N=3) and Hot Springs Canyon (N=3), Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona on September 16, 2008.

Table 7. Physico-chemical characteristics measured in three waters stocked with Gila topminnow and desert pupfish within the Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona, during monitoring of stocked fish populations on September 15 and 16, 2008. Measurements were made on September 15 for Secret Spring and September 16 for the other two sites (except for dissolved oxygen at Cherry Spring Canyon which was measured on September 17 at 9:15 am).

	Water		
Physico-chemical characteristic	Secret Spring	Cherry Spring Canyon	Swamp Springs Canyon
Time of day (military)	1410	1115	1215
Elevation (m)	1,249	1,296	1,281
Water temperature (ºC)	23.6	18.3	19.5
pH	9.16	7.4	7.73
Conductivity (µS)	256	339	493
Salinity (ppm)	127		240
TDS (mg/L)	175		346
Dissolved oxygen (mg/L)	8.33	1.98	4.5

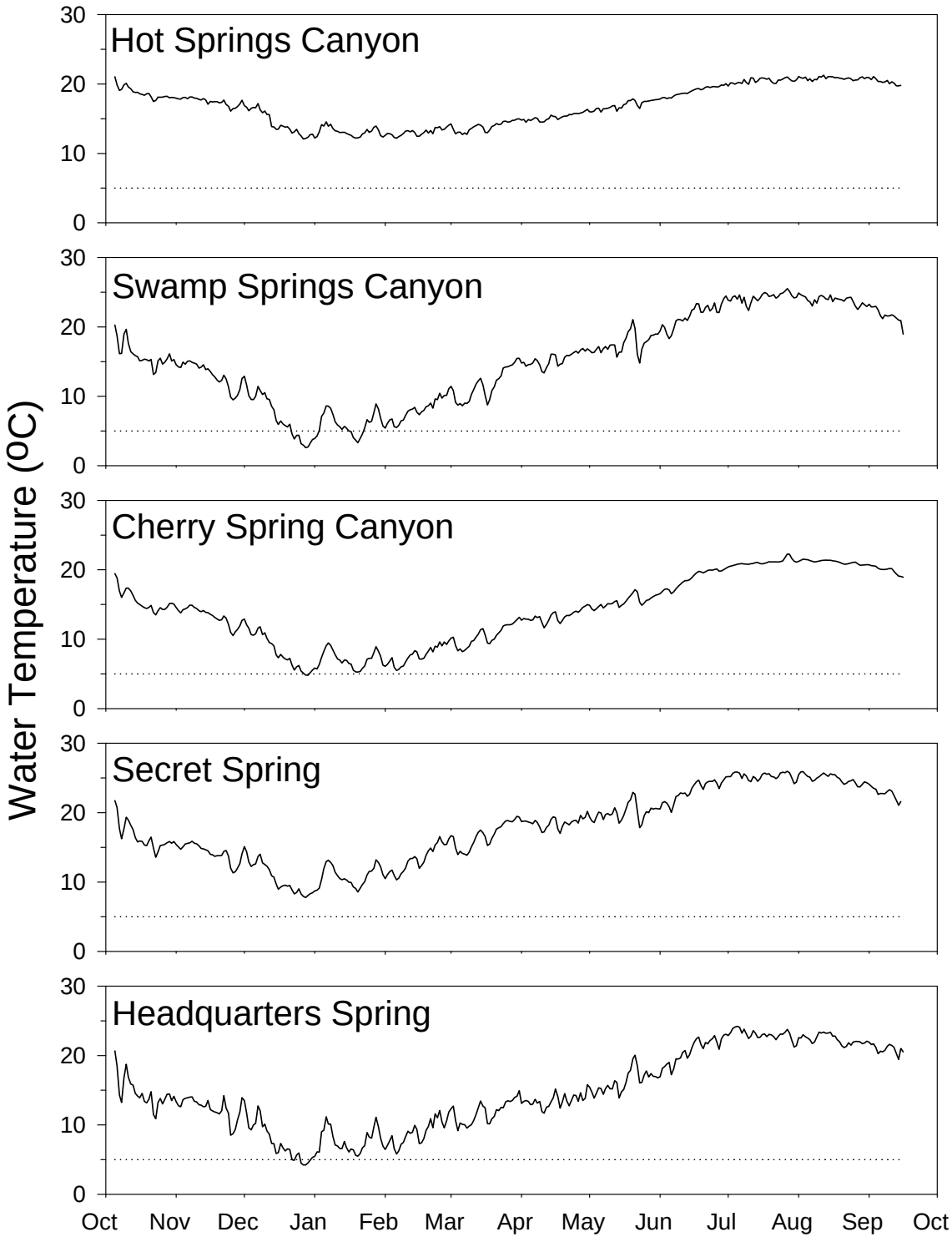

Figure 4. Daily mean water temperature (°C) October 2007 through September 2008 in waters monitored for native fish stocking, Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona. The dotted line in each graph indicates 5°C.

downstream pool, and 10% sand and 90% decayed organic matter in the furthest downstream pool. Riparian tree canopy cover was estimated to be 80% at the Cherry Spring stocking sites, which likely accounted for the high amount of decayed organic matter in the pools. Habitat and substrate types were not quantified in Swamp Springs Canyon or Secret Spring, however general observations were recorded. At the Swamp Springs Canyon stocking site, riparian cover was fairly dense (~75% of the ground was shaded) at the upper end, but thinned out at the lower end (~15% shaded) of the watered reach. Habitat in Swamp Springs Canyon was composed of pools and shallow runs, sand predominated, but cobble and pebble were also present. Secret Spring empties into a constructed pond, which is where the topminnow and pupfish were stocked in 2007. The pond is surrounded by trees, is highly shaded (~90% cover), and the substrate is 100% decayed organic matter (up to ~0.5 m thick).

Water quality was similar at the Cherry Spring Canyon and Swamp Spring Canyon stocking sites (Table 7), although conductivity and dissolved oxygen appear to be lower at Cherry Spring Canyon than at Swamp Springs Canyon. Dissolved oxygen concentrations were also low at Cherry Spring Canyon during the April 8, 2008 monitoring (2.55 mg/L and 11.7°C at 0920h in the upper pool and 4.10 mg/L and 13.1°C at 1045h in the lower stocking pool). Secret Spring appeared to have a greater pH and dissolved oxygen concentration and a lower conductivity than either Cherry Spring or Swamp Spring (Table 7). Daily water temperatures in Swamp Springs Canyon and Cherry Spring Canyon decreased to ≤5°C in December 2007 and January 2008 (Figure 4). Daily water temperatures over the course of the year were less variable in Hot Springs Canyon than in the other sites, probably because the location of the temperature logger in Hot Springs Canyon was in a flowing portion of the stream, whereas the other sites are in locations with very little flow. Daily water temperatures are not reported for Redfield Canyon because the logger was not installed until April 2008, and has not yet been downloaded. Daily water temperatures for Headquarters Spring, which was stocked on September 17, 2008, were fairly similar to Swamp Springs Canyon, Cherry Spring Canyon, and Secret Spring.

FISH STOCKINGS

Fish used in the stockings originated from two locations. Spikedace and loach minnow (F1 generations of Aravaipa Creek lineages) were transported from Bubbling Ponds Research Facility to The Nature Conservancy's Lower San Pedro River Preserve in Dudleyville early in the morning on September 17, 2008. Desert pupfish (El Doctor Marsh lineage) and Gila topminnow (Bylas lineage) were collected from the eastern, smaller, pond on the TNC Lower San Pedro River Preserve on September 16 and 17. Fish were transported by helicopter from TNC Lower San Pedro River Preserve to five sites within the Muleshoe Cooperative Management Area on September 17, 2008 (five flights; Table 8); equipment and methods used were as described in the introduction. The crew at the receiving site quickly transferred water and fish into 5-gal buckets, and carried the buckets to the stocking sites. At the stocking sites, fish were tempered to the stocking site water temperature and chemistry (Table 9) by exchanging a quarter of the water in each 5-gallon bucket with stream water every 10-15 minutes, making 2-3 exchanges until water temperatures in buckets were within 1°C of stream temperatures, after which fish were released into the stocking site. Fish were observed during the tempering process and after release, and behaviors (stressed or normal) noted. Stockings at the four stream systems were augmentations (Table 9), whereas this was the first stocking of fish at Headquarters Spring.

During July 2008, *Lenaea cyprinacea*, a parasitic copepod was detected on desert pupfish in the larger pond on TNC Lower San Pedro River Preserve. This raised concerns about stocking potentially infected fish into Muleshoe Cooperative Management Area waters (or any other waters), where *Lernaea* have not been reported. However, *Lernaea* were not detected in the smaller pond, which contained both Gila topminnow and desert pupfish. After a consultation among biologists (Tony Robinson, Ross Timmons, Rob Clarkson, Heidi Blasius), it was decided to capture Gila topminnow and desert pupfish from the smaller pond and examine them for presence of *Lernaea*. If *Lernaea* were found, the stockings of Gila topminnow and desert pupfish from the TNC Lower San Pedro River Preserve ponds into Muleshoe waters would be aborted. If *Lernaea* were not found, then the stockings would proceed as planned. Rob Burton, Christina Burton, Rob Clarkson, and Paul Marsh captured fish the day before the planned stocking, and did not observe any *Lernaea*, therefore fish were transported and stocked as planned.

HEADQUARTERS SPRING

The helicopter arrived and dropped off the barrel of fish at 0850 h. A tag attached to the net in the barrel indicated that the barrel contained 275 Gila topminnow and 290 desert pupfish. Water temperature was 24°C in the barrel, but only 18°C (Table 9) in the stocking site. Water and fish from the barrel were transferred to three 5-gallon buckets and walked to the stocking site. Approximately 25% of the water in each bucket was exchanged with Headquarters Spring water at 0909, 0920, and 0935 h. Fish were released from 0945-0955 h into a pool (Figure 5) approximately 15 m upstream of the wooden plank walkway and lower hot tub. No mortalities were observed during tempering or upon release. Fish stayed in release area for 30-60 seconds then moved off and dispersed into the pond. The temperature logger that was installed last year on October 4, 2007 was retrieved and data were downloaded onto a computer.

HOT SPRINGS CANYON

The helicopter arrived and dropped off the barrel of fish (500 spikedace and 1,000 loach minnow) at 1300 h. Water in the barrel was 22.0°C, nearly identical to the temperature of the stream (Table 9). Water and fish were transferred into four 5-gal buckets outfitted with bubblers and walked to the stocking sites (Figure 5); approximately 375 fish (not sorted to species) were placed into each bucket. Approximately 25% of the water in each bucket was exchanged with stream water twice at 15 minute intervals. Fish were stocked into the reach immediately upstream of the reach that was stocked in October 2007, to slightly increase the range of the species because a small waterfall likely prevents upstream migration out of the October 2007 stocking reach during low flows (but not flood flows), and because monitoring results indicated that both species had persisted within the 2007 stocking reach and had dispersed downstream. Fish were stocked at approximately 1343 h at site 1, 1338 h at site 2, 1340 h at site 3, and 1340 h at site 4. We observed only one mortality; a loach minnow at site 2. Fish behaved normally during tempering and upon release. The helicopter arrived to retrieve the barrel at about 1400 h. The temperature logger set last year was retrieved (was floating at water surface) and downloaded, and a new one installed deeper at the same site (bedrock and large sycamore tree plunge pool, left side of pool looking downstream, attached with zip tie to a root).

Table 8. Summary of helicopter flights to transport native fish into the Muleshoe Cooperative Management Area on September 17, 2008 for augmentation stockings at the receiving locations; times are approximate. Note, the barrel dropped at Swamp Spring was loaded into a truck and driven out, and a sixth flight was used to pick up the Hot Spring Canyon barrel.

Flight No.	Species	No. of fish	From	Source Crew	To	Time	Receiving Crew	Distance (miles)
1	Gila topminnow and desert pupfish	275 290	Dudleyville 32°55.451'N 110°44.468'W	Rob Clarkson (USBR) Paul Marsh (ASU) Rob Burton (TNC) Christina Burton (TNC)	Headquarters Spring 32°20.196'N 110°14.272'W	0850	Tony Robinson (AZGFD) Joy Price (BLM) Amy Bailey (AZGFD)	50
2	Gila topminnow and desert pupfish	275 275	Dudleyville 32°55.451'N 110°44.468'W	Rob Clarkson (USBR) Paul Marsh (ASU) Rob Burton (TNC) Christina Burton (TNC)	Cherry Spring 32°25.165'N 110°18.038'W	1000	Ross Timmons (AZGFD) Mark Haberstich (TNC)	43
3	Gila topminnow and desert pupfish	275 275	Dudleyville 32°55.451'N 110°44.468'W	Rob Clarkson (USBR) Paul Marsh (ASU) Rob Burton (TNC) Christina Burton (TNC)	Swamp Spring 32°25.920'N 110°16.220'W	1100	Heidi Blasius (BLM) Bob Rogers (TNC) Jeff Conn (BLM) Ken Wiley (TNC)	44
4	Loach minnow and spikedace	1000 500	Dudleyville 32°55.451'N 110°44.468'W	Rob Clarkson (USBR) Paul Marsh (ASU) Rob Burton (TNC) Christina Burton (TNC) David Ward (AZGFD)	Redfield Canyon 32°26.448'N 110°19.345'W then pick up Cherry Spring barrel	1200	Codey Carter (AZGFD) Suzanne Ehret (AZGFD) Duane Aubuchon (AZGFD) Abe Karam (ASU)	41
5	Loach minnow and spikedace	1000 500	Dudleyville 32°55.451'N 110°44.468'W	Rob Clarkson (USBR) Paul Marsh (ASU) Rob Burton (TNC) Christina Burton (TNC) David Ward (AZGFD)	Hot Springs Canyon 32°21.273'N 110°15.624'W then pick up Redfield barrel	1300	Tony Robinson (AZGFD) Joy Price (BLM) Amy Bailey (AZGFD)	48

Table 9. Physico-chemical characteristics measured in waters within the Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona, within 1 hour before stocking native fish on September 17, 2008.

	Water				
Physico-chemical characteristic	Headquarters Spring	Cherry Spring Canyon	Redfield Canyon	Swamp Springs Canyon	Hot Springs Canyon
Time of day (military)	0830	0915	1200	1225	1332
Elevation (m)	1,244	1,296	1,145	1,281	1,196
Water temperature (ºC)	18.0	18.9	19.0	24.0	21.4
pH	8.05	7.45	8.1		7.55
Conductivity (µS)	352				468
Salinity (ppm)	178				231
TDS (mg/L)	245				326
Dissolved oxygen (mg/L)	2.13	1.98			5.10
Substrate	Silt-sand	Sand-silt			

CHERRY SPRING CANYON

The helicopter arrived and dropped off the barrel of fish (275 Gila topminnow and 275 desert pupfish) at 1005 h. Water in the barrel had a pH of 7.9, conductivity of 1,464 µS and temperature of 28.2°C; water in the barrel was about 10°C warmer than in the stream (Table 9). Water and fish from the barrel were transferred to two 5-gallon buckets and walked to the stocking site. Approximately 25% of the water in each bucket was exchanged with stream water at 1010-1020, 1030-1040, 1040-1055, and 1055-1105 h. Fish were released from 1105-1120 h into the lower pool of the two pools that were stocked in 2007 (Figure 6). No mortalities were observed during tempering or upon release. Fish appeared acclimated upon release and most schooled with their own species. After ten minutes, desert pupfish displayed much greater startle response (dashing away from stimulus) than Gila topminnow. Fish appeared to move randomly about the pool, with Gila topminnow in mid-water and surface zones, and desert pupfish near the substrate. Behavior appeared normal. The temperature logger that was installed last year on October 4, 2007 was retrieved and data were downloaded onto a computer.

SWAMP SPRINGS CANYON

The helicopter arrived and dropped off the barrel of fish (275 Gila topminnow and 275 desert pupfish) at 1115 h on FS road where it crosses Swamp Springs Canyon wash. One pupfish was found dead in the barrel. Water and fish from the barrel were transferred to three 5-gallon buckets outfitted with bubblers and hiked approximately 2.5 km downstream to the stocking site. Upon arrival at the stocking site at 1225 h, water in the buckets was 27.0°C, three degrees warmer than in the stream (Table 9). Approximately 25% of the water in each bucket was exchanged with stream water at ten minute intervals, making three exchanges. Approximately 225 desert pupfish and 225 Gila topminnow were released at stocking site 1 (Figure 6) at 1255 and 1300 h. Approximately 48 desert pupfish and 50 Gila topminnow were released into a

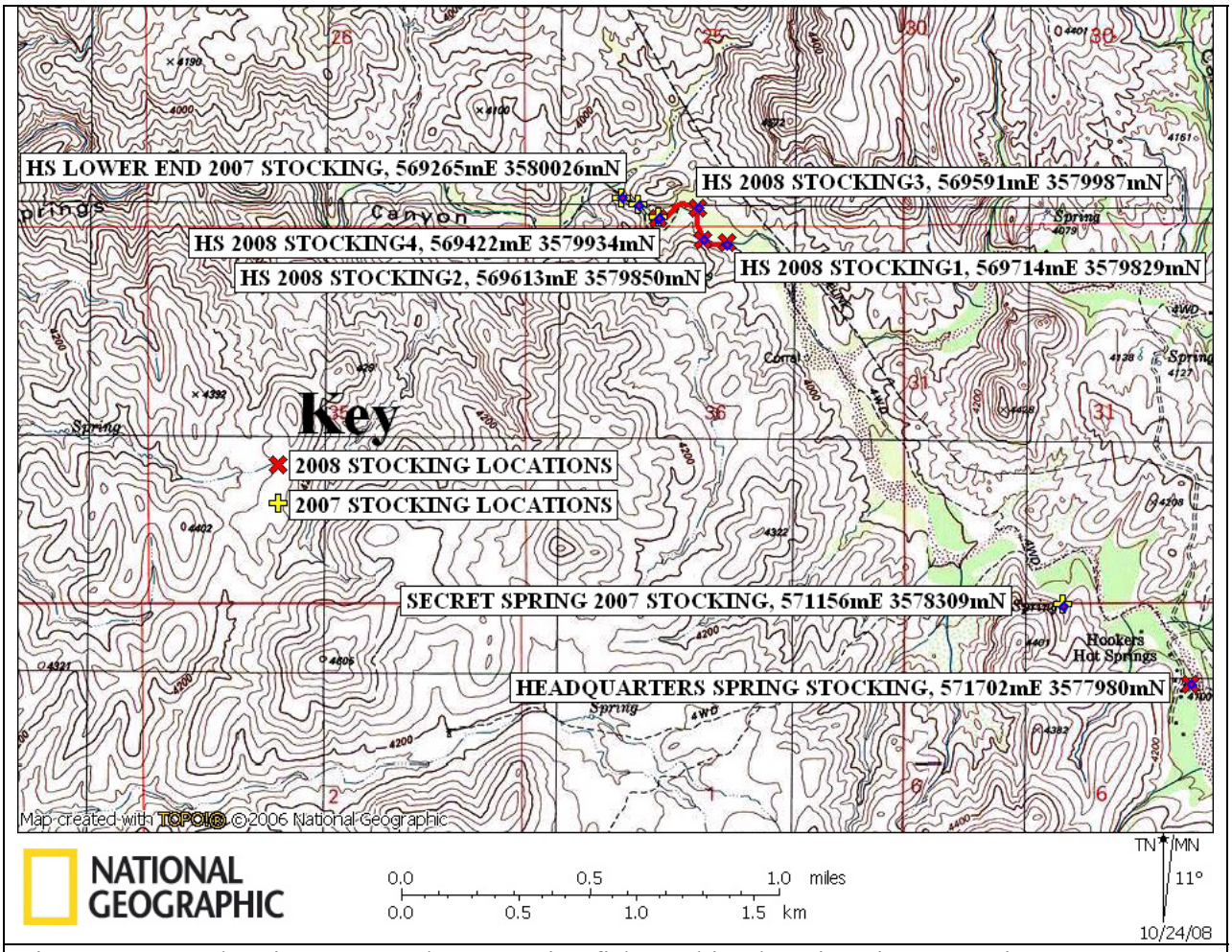

Figure 5. Map showing 2007 and 2008 native fish stocking locations in Hot Springs Canyon, Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona.

second site (Stocking2, Figure 6) about 30 m upstream at 1315h. One pupfish died during transport. After release, fish were observed schooling and all appeared well.

REDFIELD CANYON

The helicopter arrived and dropped off the barrel of fish (500 spikedace and 1,000 loach minnow) at about 1200 h. Water was transferred into six 5-gal buckets, and fish were sorted and placed into separate buckets for each species. Water in the buckets was 22.0°C with a pH of 7.8 before tempering; three degrees warmer and more neutral than the stream water (Table 8). Approximately 25% of the water in each bucket was exchanged with stream water twice at ten minute intervals. Buckets were outfitted with bubblers and hiked upstream to the stocking sites (Figure 6). Approximately 200 loach minnow were released at stocking sites 1 and 2. The remaining spikedace and loach minnow were divided up approximately evenly and were released at stocking sites 3, 4, 5, and 6. Fish were spread out further into suitable habitat upstream of the Swamp Springs confluence than during the October 2007 stocking event, because more fish were available.

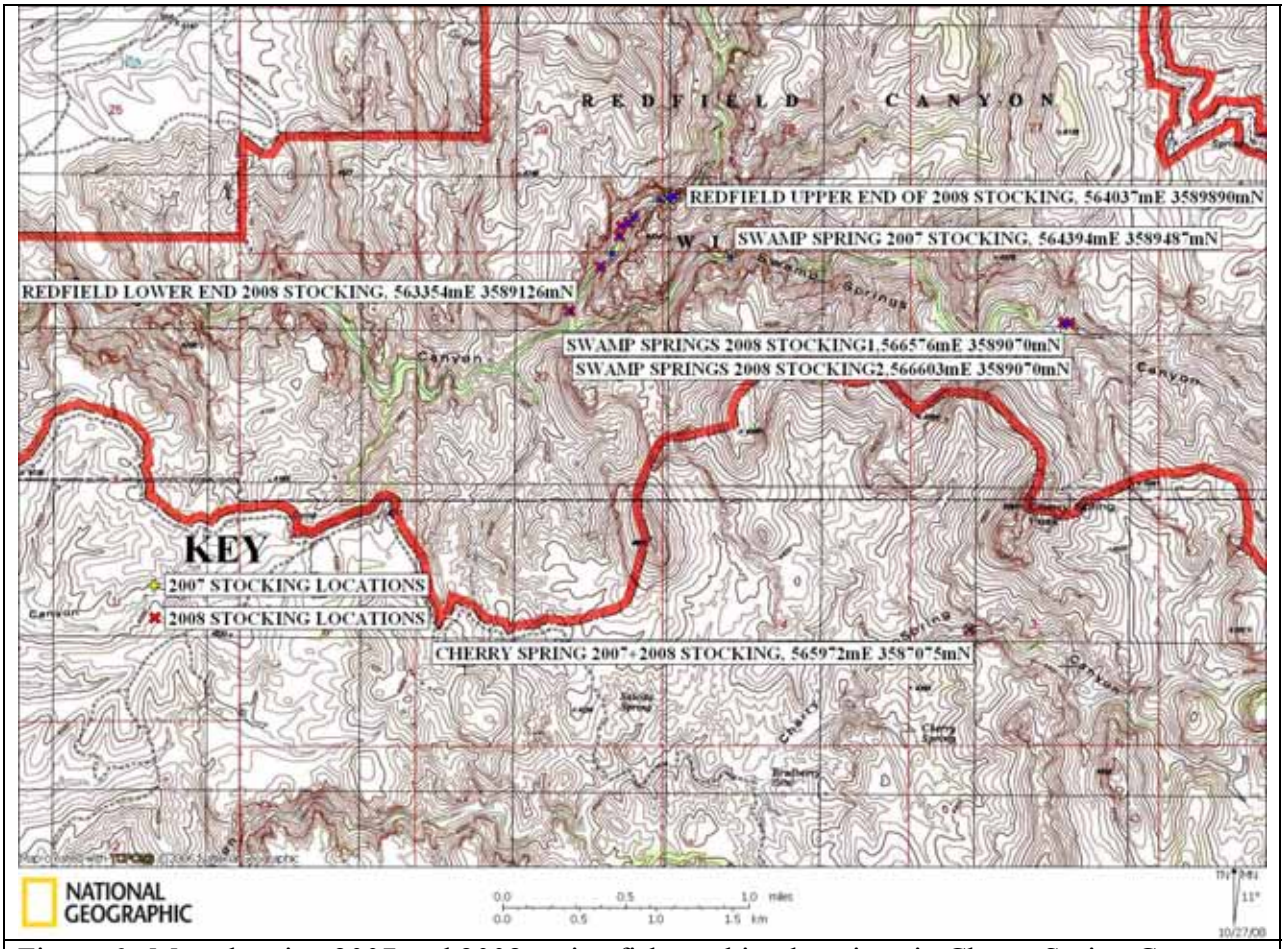

Figure 6. Map showing 2007 and 2008 native fish stocking locations in Cherry Spring Canyon, Swamp Springs Canyon, and Redfield Canyon, Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona.

RECOMMENDATIONS

Locations where fish were stocked during 2007 and 2008 should be surveyed during 2009 to assess the status of the fish populations. The stocking reach in Hot Springs Canyon, including the 2007 and 2008 stocking locations, should now be considered to extend from the confluence of Wildcat Canyon to approximately 700 m upstream. In Redfield Canyon, fish stockings were more spread out in 2008 than in 2007, so the stocking reach should now be considered the portion of stream from the confluence with Swamp Springs Canyon to about 1.2 km upstream, terminating at the waterfall. In Swamp Springs Canyon the 2008 stocking sites were about 3 km upstream from the 2007 stocking site, but the stream is partly dry (interrupted) between the two stocking sites. The 210-m reach encompassing the 2007 stocking site should be surveyed again. The 2008 stocking sites should also be surveyed, as should any water immediately upstream (the stream was essentially dry upstream of the upper stocking site) and downstream at least to the

first tributary entering from the south (approximately 300-m). In Cherry Springs Canyon, fish were stocked into the same locations during 2007 and 2008, so the stocking reach has not changed. Headquarters Spring was stocked in 2008, so the stocking location and all perennial water (approximately 50 m) in the immediate vicinity should be surveyed.

Targeted-habitat sampling appears to be a more effective means to capture spikedace and loach minnow than single-pass electrofishing through a 100-m reach. All of the spikedace and loach minnow captured in Redfield Canyon were captured during targeted-habitat sampling, and all loach minnow and three of the four spikedace captured in Hot Springs Canyon were captured during targeted-habitat sampling. Only one spikedace in Hot Springs Canyon was captured during single-pass electrofishing through a 100-m reach. Therefore, it is recommended that targeted-habitat sampling be used to survey for spikedace and loach minnow. If it is desirable to continue to survey the permanent 100-m reaches, then targeted habitat sampling could be conducted within those reaches, rather than single-pass electrofishing. If this recommendation is implemented, it is important that data be recorded after each seine haul or kick-seining event (i.e., do not wait until the whole 100-m reach is sampled to count fish and record electrofishing seconds and area seined). A greater sample size for abundance estimates could be achieved with targeted-habitat sampling than with the single pass through a 100-m reach, with approximately the same amount of labor. A greater sample size is needed to detect statistically significant changes in abundance estimates from one year to next. Preliminary power analysis indicates that if the same four 100-m sites in Hot Springs Canyon were sampled again next year, the power to detect a 50% increase in loach minnow abundance would only be 16%. Even with the electrofishing and kick seining in Redfield Canyon which had a sample size of 23, the power to detect a 50% change in the mean CPUE for loach minnow would be about 9%, and for speckled dace the power would be about 49%. A power of 80% is desirable, and that could be achieved for speckled dace in Redfield Canyon by increasing the yearly sample size to 47, but for loach minnow a yearly sample size of 63 would be necessary to achieve 80% power. For seining in Redfield Canyon, if 14 sites are again sampled in 2009, power would be 25% to detect a 50% change; increasing power to 80% could be achieved by increasing the sample size to 64. Further power analyses should be conducted so that sufficient numbers of sites are sampled in future years.

Some modifications to stocking sites might improve the chances of establishing desert pupfish and Gila topminnow populations. Secret Spring is highly shaded by trees and has a thick layer of decayed organic matter on the pond bottom, both of which might make the system more allochthonously driven than autochthonously driven, and result in lower phytoplankton and hence zooplankton and invertebrate densities (foods for the Gila topminnow and desert pupfish) than it might have with less shading and more sunlight. Removing some of the trees around Secret Spring would allow for more direct sunlight to hit the pond, and hence increase productivity, and possibly increase food supply for Gila topminnow and desert pupfish. The Cherry Spring Canyon stocking site is also highly shaded, with low dissolved oxygen levels in the pools. Removing some of the trees in the immediate area of the stocking site would allow more direct sunlight to hit the pools, which might increase productivity, and hence dissolved oxygen levels and food supply for both Gila topminnow and desert pupfish. Headquarters Spring is also highly shaded, but it might be better to monitor the fish for a year to see if they establish, before deciding to remove any trees.

If second year monitoring indicates that topminnow or pupfish have not established in Cherry Spring Canyon, or at the 2007 stocking location in Swamp Spring Canyon, then abandoning further stockings of these species into these waters should be considered. Other waters within Muleshoe Cooperative Management Area should be evaluated for repatriations of native fish. Wildcat Canyon was evaluated by Tony Robinson on September 17, 2008, and was assessed to have good habitat for both Gila topminnow and desert pupfish (several large and deep pools in a 400-m long interrupted-water reach, with water temperature = 23.6ºC, pH = 7.53, conductivity = 395µS, and dissolved oxygen = 3.05 mg/L). No fish are present above a small waterfall about 300-m upstream of the pipeline road (Bob Rogers, personal communication, and Tony Robinson's observation). Therefore, it is recommended that Wildcat Canyon be further evaluated and if it is deemed suitable, stocked with Gila topminnow and desert pupfish as soon as feasible.

Subsequent to the September 17, 2008 fish stockings into the Muleshoe Cooperative Management Area, Gila topminnow and desert pupfish from the smaller pond at TNC Lower San Pedro River Preserve were stocked into Oak Grove Spring, a tributary to Aravaipa Creek. During the Oak Grove Spring stocking process, *Lernaea* was observed on one of the desert pupfish. Therefore, it is possible that *Lernaea* was transported into Swamp Spring Canyon, Cherry Spring Canyon, and Headquarters Spring. Fish from these three waters, as well as Redfield Canyon and Hot Springs Canyon, should be examined for *Lernaea* during all future monitoring.

Appendix

Appendix 1. Location (GPS coordinates of downstream end of site) and fish catch information including total seconds electrofished, total number of each species captured, and catch-per-unit effort (in parentheses) for permanent 100-m sites in Hot Springs Canyon and Redfield Canyon, Muleshoe Cooperative Management Area, Galiuro Mountains, Arizona during September 16, 2008 monitoring. All sites were sampled by single-pass electrofishing and dip netting, except for Hot Springs Canyon permanent site #1. At Hot Springs Canyon permanent site #1, a seine was used to block off the lower end of each of 11 habitat units within the 100-m site, and then each unit was electrofished downstream into the seine, which was then swept upward to catch fish; fish were also dip netted during the electrofishing. Because of the sampling method differences, catch per unit effort was not calculated for Hot Springs Canyon site #1.

	Hot Springs Canyon			Redfield Canyon			
	1	2	3	1	2	3	4
NAD 83 Northing	3579940	3580065	3579983	3590040	3589358	3589199	3588819
NAD 83 Easting	569434	568108	566120	564201	563508	563373	562025
Seconds shocked	390 into 11 seines	374	295	649	1497	1319	867
Fish Species							
Loach minnow	0	1 (0.003)	0	0	0	0	0
Speckled dace	44	41 (6.58)	2 (0.41)	4 (0.37)	75 (3.01)	72 (3.28)	1 (0.07)
Longfin dace	50	59 (9.47)	21 (4.27)	0	0	0	28 (1.94)
Gila chub	0	3 (0.48)	0	37 (3.42)	42 (1.68)	35 (1.59)	1 (0.07)
Desert sucker	8	15 (2.41)	1 (0.20)	0	0	0	0
Sonora sucker	0	0	0	12 (1.11)	12 (0.48)	21 (0.96)	0
Green sunfish	0	0	0	0	4 (0.16)	1 (0.05)	1 (0.07)
Total	102	118 (18.93)	24 (4.88)	53 (4.90)	133 (5.33)	129 (5.87)	31 (2.15)