Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Lowland Leopard Frog and Colorado River Toad Distribution and Habitat Use in the Greater Lower Colorado River Ecosystem

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

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Lower Colorado River Multi-Species Conservation Program

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ACRONYMS AND ABBREVIATIONS

AIC_c	Akaike's information criterion (small sample size)
ANOSIM	analysis of similarity
Bill Williams River NWR	Bill Williams River National Wildlife Refuge
BLM	Bureau of Land Management
Cibola NWR	Cibola National Wildlife Refuge
DFA	discriminant functions analysis
DNA	deoxyribonucleic acid
eDNA	environmental deoxyribonucleic acid
GIS	Geographic Information System
Havasu NWR	Havasu National Wildlife Refuge
Imperial NWR	Imperial National Wildlife Refuge
LCL	lower confidence level
LCR	lower Colorado River
LCR MSCP	Lower Colorado River Multi-Species Conservation Program
MANOVA	multivariate analysis of variance
PCoA	principal coordinates analysis
UCL	upper confidence level
USGS	U.S. Geological Survey

Symbols

Δ	difference
$\geq$	greater than or equal to
$<$	less than
$\leq$	less than or equal to

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Attachments

Attachment

1 List of Species Codes and Scientific Names for Each Amphibian Species Identified in this Report Along with Accepted Common Names and Current Taxonomic Synonyms According to The Center for North American Herpetology

2 List of Notations and Represented Covariates Used in Model Creation and the Gradient Analysis

ABSTRACT

The lowland leopard frog (*Lithobates yavapaiensis*) and the Colorado River toad (*Incilius alvarius*) are included in the Lower Colorado River Multi-Species Conservation Program's (LCR MSCP) list of evaluation species (Bureau of Reclamation 2004). The objective of this project was to address Work Task D12 (Lowland Leopard Frog and Colorado River Toad Surveys) of the LCR MSCP. This task is intended to define the gaps in range and distribution of these two species to help address conservation needs. The study area (outlined by the LCR MSCP) encompassed Reaches 3–7 of the lower Colorado River (LCR) extending from Davis Dam to the Southerly International Boundary with Mexico. In addition to the Colorado River, the Bill Williams River was also surveyed from its confluence with the LCR to Bureau of Land Management land just east of Planet Ranch (private property), including Reaches 7 and 8, due to reports of lowland leopard frogs and Colorado River toads being observed in the area. Beginning in January 2011, habitat assessment surveys were performed throughout the study area based on a Geographic Information System (GIS) analysis of aerial imagery and remotely sensed data specifically identifying backwater systems that may contain suitable habitat. Areas targeted by GIS analysis were then visited by Arizona Game and Fish Department biologists to determine if these locations were occupied by either species. Survey techniques included funnel trapping, dip netting, nocturnal spot lighting, and auditory call response surveys. During the 2013 field season, water samples were also collected to analyze them for suspended deoxyribonucleic acid (DNA) fragments of the target species. To compare habitat characteristics of sites where frogs were detected and where they were not, a logistic regression modeling approach was used (i.e., biotic, abiotic, and predator covariates) at 22 sites where lowland leopard frogs were detected at random sites at two spatial scales – within Reaches 7 and 8 of the Bill Williams River (27 sites) and throughout the LCR study area (42 sites). Additionally, a principle coordinates analysis was performed followed by an analysis of similarity, multivariate analysis of variance, and a discriminate functions analysis to examine relationships between frog presence and habitat features. During the course of this study, 14 Colorado River toad individuals were marked at 5 sites within Reach 8 along the Bill Williams River, and habitat characteristics at capture locations were evaluated.

INTRODUCTION

The lowland leopard frog (*Lithobates yavapaiensis*) and Colorado River toad (*Incilius alvarius*) were once common along the greater lower Colorado River (LCR) ecosystem (Brennan and Holycross 2009). However, due to various anthropogenic influences, both species are thought to be extirpated from the main stem of the Colorado River (Vitt and Ohmart 1978; Clarkson and Rorabaugh 1989; Sredl et al. 1997; K. Blair 2011, personal communication).

Arizona's native ranid frogs are declining throughout their historic ranges due to a suite of threats (e.g., introduced and invasive species, loss of habitat, habitat alteration, toxicants, pathogens, and parasites) (Clarkson and Rorabaugh 1989; Degenhardt et al.1996; Sredl et al. 1997; Stebbins 2003; Sredl 2005). The lowland leopard frog is thought to be extirpated from the LCR as of 1974 (Vitt and Ohmart 1978; Clarkson and Rorabaugh 1989; Sredl et al. 1997). However, occasional observations had occurred on the Bill Williams River National Wildlife Refuge (Bill Williams River NWR) as recently as 2010 (K. Blair 2011, personal communication). This species occurs in permanent and semipermanent water sources through much of central Arizona but has become extirpated from many lowland river systems (Gila, Salt, and Colorado Rivers) (Brennan and Holycross 2009).

The Colorado River toad is common throughout much of the Sonoran Desert, occupying a variety of habitats, including mesquite-creosote flats, grasslands, and pine oak juniper and deciduous montane communities (Stebbins 2003). The range of the Colorado River toad may overlap that of the lowland leopard frog; however, evidence suggests threats to the toad species to be primarily from urbanization and hydrological alterations of riparian habitat (Lovich et al. 2009). There have been anecdotal reports and sightings of the Colorado River toad on private property along the LCR and Bill Williams River, but no confirmed observations existed prior to this study (Cotten 2011; Cotten and Grandmaison 2012).

The lack of information regarding the current distribution of both lowland leopard frogs and the Colorado River toads along the LCR slows the process for conservation. The objectives of this study were to examine the distributional status of the two species along the LCR, thereby aiding preliminary conservation steps. Specifically, those objectives were:

1. Locate potential habitat for lowland leopard frogs and Colorado River toads along the LCR

2. Determine the distribution of lowland leopard frogs and Colorado River toads within the study area

3. Collect genetic samples from lowland leopard frogs and Colorado River toads

4. Determine habitat selection of lowland leopard frogs and Colorado River toads along the lower Colorado and Bill Williams Rivers

STUDY AREA

The study area included Reaches 3–7 of the LCR extending from Davis Dam to the Southerly International Boundary with Mexico (figure 1). The study area also included Reaches 7–12 of the Bill Williams River from its confluence with the LCR to the Bureau of Land Management (BLM) land east of Planet Ranch (private property) (figure 2). Due to landowner access issues, Planet Ranch was surveyed on a few occasions in 2011 but not surveyed during the 2012 or 2013 seasons. Initial site visits to areas north of the Havasu National Wildlife Refuge (Havasu NWR) found that the habitat contained little backwater or had side channels with high human traffic; therefore, this area was excluded from the survey. In addition, there have been no voucher specimens collected from this area for either species.

OBJECTIVE 1 – LOCATE POTENTIAL HABITAT FOR LOWLAND LEOPARD FROGS AND COLORADO RIVER TOADS ALONG THE LOWER COLORADO RIVER

Methods

Potential habitat was first identified through a Geographic Information System (GIS) layer analysis of aerial imagery and remotely sensed data to identify both permanent lentic backwaters and small lotic backwaters along the LCR. Beginning in January 2011, these areas, as well as suitable mid-sized lotic backwaters and desert washes identified or based on historic and anecdotal evidence, were identified and systematically visited. In addition, museum collections with records of amphibians collected from the study area were consulted in order to identify historic population localities. Field surveys were conducted in areas with high concentrations of backwaters, which was determined from the initial site visits. Both diurnal and nocturnal site visits were conducted throughout the field season to identify high ranking habitat locations suitable for amphibians. Backwaters along the main stem LCR that were greater than 5 acres in size were not surveyed due to the high probability of introduced non-native

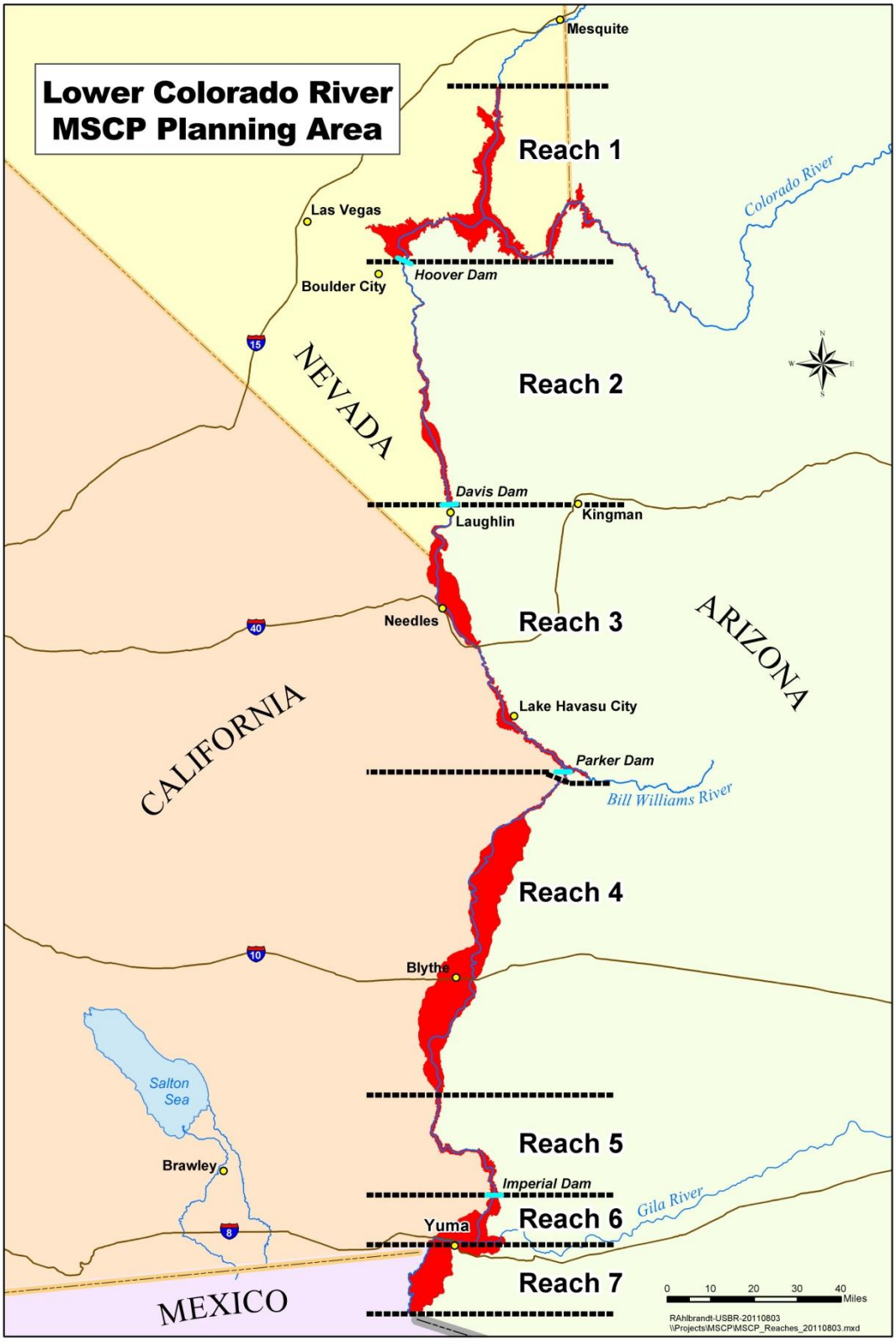

Figure 1.—Map of LCR with LCR MSCP land highlighted in red.
The study area for this project consisted of Reaches 3–7.

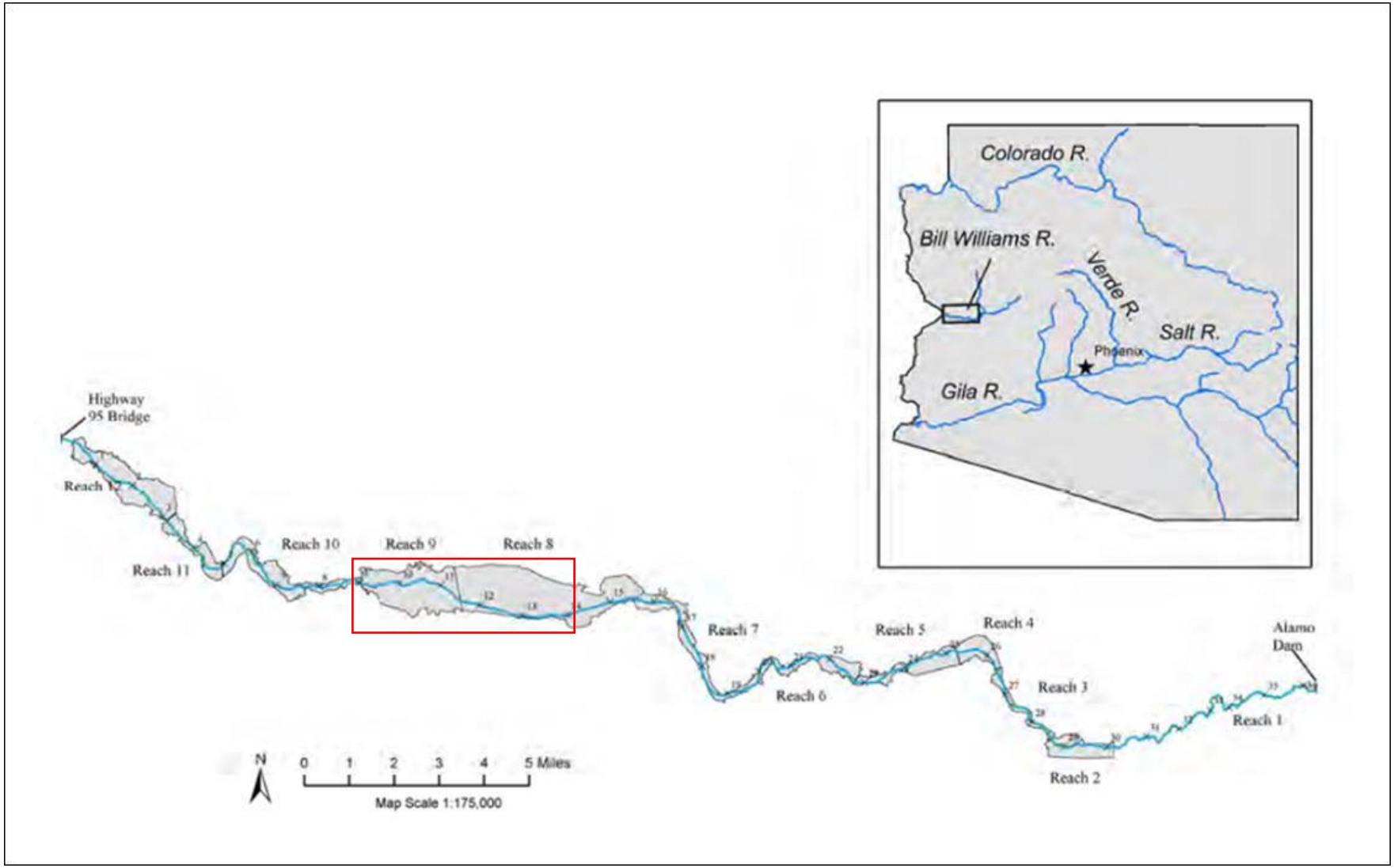

Figure 2.—Map of the Bill Williams River, Arizona, showing the reaches from the SR95 Bridge crossing and the approximate location of Planet Ranch outlined in red (source: Shafroth et al. 2004).

predatory fishes and American bullfrogs (*Lithobates catesbeianus*), which prey upon and compete with native ranids (Lardie 1963; Moyle 1973; Bury and Luckenbach 1976; Vitt and Ohmart 1978; Hammerson 1982; Hayes and Jennings 1986; Kiesecker and Blaustein 1998; Kats and Ferrer 2003). Each site was ranked based on presence of predators, area of potential habitat, water quality and characteristics, and type of site (lentic, lotic, canal, etc.). Sites were ranked from 1–5, with 5 being ideal habitat based on the recorded parameters. Locations ranked 3 or higher were selected as potential locations for presence surveys.

During initial site visits, previously unidentified or unvisited locations with high-quality habitat were recorded to be included in future presence surveys. These high-quality areas contained native cottonwood-willow (*Populus fremontii*, *Salix* spp.) vegetation, shallow backwaters, and a low abundance and richness of introduced predators. Some, but not all sites, were revisited multiple seasons due to reports of frog vocalizations and possible sightings in the area as well as a high likelihood of occupancy given suitable habitat quality (K. Blair 2011, personal communication).

Results

Over 1,740 locations were identified through the GIS analysis. With many overlapping areas identified as potential sites, a total of 139 of these locations were visited during the course of this study. Selected locations were predicted to have a high likelihood of frog presence and, in many cases, were a centralized point for a cluster of high likelihood backwater sites. Of the 139 sites, 69 localities were given a ranking of 3 or better, with no localities receiving a ranking of 5, which indicated ideal habitat. The highest concentrations of quality habitat were located on and adjacent to the Havasu NWR in Reach 3, the Cibola National Wildlife Refuge (Cibola NWR) in Reach 4, the Imperial National Wildlife Refuge (Imperial NWR) in Reach 5, and Mittry Lake/Laguna Dam in Reach 6. The Bill Williams River NWR contained only a few backwater locations, possibly a result of the Bill Williams River having a dynamic flow regime and predominance of lotic habitat. However, the Bill Williams River is still considered a high priority area due to its intact riverine and riparian system and because it is the only region within the study area where any credible observations of lowland leopard frogs have occurred (K. Blair 2011, personal communication). These primary locations and a few isolated sites such as Desilt Wash, the ‘Ahakhav Tribal Preserve, and Yuma East Wetlands with the confluence of the Gila River were deemed the highest priority for subsequent surveys.

Discussion

Areas including and adjacent to the Havasu NWR, the Bill Williams River NWR, the ʻAhakhav Tribal Preserve, the Cibola NWR, the Imperial NWR, Mittry Lake, Desilt Wash, and the Yuma East Wetlands and Gila confluence were deemed by the GIS analysis and site visits to be the most likely places for occupancy of lowland leopard frogs within the study area and where search efforts would be concentrated. While there may be overlap in habitat suitability and habitat use between lowland leopard frogs and Colorado River toads along the LCR, it is expected that Colorado River toads breed and inhabit ephemeral water bodies adjacent to the river during the summer monsoons. This type of habitat was not identified with the spatial analysis techniques employed during this project. Areas observed that had potential for harboring toads during the summer monsoon rains were noted and recorded. Localities where anecdotal reports of toad presence within the past 20 years (Planet Ranch, Parker Golf Course, and Arizona Highway 1 between Ehrenberg and Poston) were also recorded in preparation for return visits after suitable rainfall. BLM holdings to the east of Planet Ranch were not included in the GIS analysis because that stretch of the Bill Williams River was not added to the study area until 2012.

The highest concentration of highly ranked habitat was along the Bill Williams River. The river still contains areas of natural riparian corridor with diverse plant species, a lack of American bullfrogs, and few native weedy plant species such as cattails (*Typha* spp.) and non-native salt cedar (*Tamarix* spp.) (figure 3). In general, outside of the Bill Williams River, most of the habitat along the LCR was very similar. This habitat is best described as occasional monoculture stands of cattails, salt cedar, bulrush (*Schoenoplectus* spp.) and common reed (*Phragmites* spp.) (figure 4).

The Bill Williams River has the greatest potential for occupancy by lowland leopard frogs based on this evaluation of habitat suitability. The Bill Williams River NWR contains areas of natural riparian corridor with native plant diversity and a lack of American bullfrogs. However, the water channel within the Bill Williams River NWR is well established and deep, which allows predatory fishes to become established within the refuge, and this can be detrimental to native amphibians (Rosen et al. 1995; Kiesecker and Blaustein 1998). With few exceptions, there are very limited side channels or shallow backwaters within the refuge to provide habitat for lowland leopard frogs. The locations where isolated shallow backwaters and side channels existed were surveyed several times in 2012 and 2013. Surface water becomes more ephemeral and often percolates into the sand near the Bill Williams River NWR eastern boundary and onto Planet Ranch. This section has fewer predators and an abundance of small ephemeral pools ideal for amphibian breeding. Unfortunately, access to Planet Ranch was not granted after the 2011 season; therefore, this reach was not adequately surveyed. However, good habitat conditions east of Planet Ranch on public

Figure 3.—Example of riparian habitat commonly encountered along the Bill Williams River.

Figure 4.—Example of emergent wetland habitat commonly found along the majority of the LCR.

land managed by BLM were identified. Here, instead of the water re-emerging as it did on Planet Ranch and the Bill Williams River NWR, it spreads and braids across a sandy riverbed. This section of the Bill Williams River is the best amphibian habitat identified within the greater lower Colorado River ecosystem (figure 5). In addition, no American bullfrogs were detected in this section or any surveyed section of the Bill Williams River.

Figure 5.—Example of lowland leopard frog habitat where both adults and tadpoles were found (encountered along the Bill Williams River east of Planet Ranch).

In conclusion, much of the LCR does not support the habitat expected for successful populations of either the lowland leopard frog or the Colorado River toad. The best habitat found within the study region was along the Bill Williams River. Further, the upper reaches of the Bill Williams River to the east of the Bill Williams River NWR supported the best habitat for both amphibians, and this is attributed to low abundance and richness of non-native vegetation and predatory species that may either alter the local environment and/or food web. Restoration efforts throughout the study area have created isolated patches of habitat that may be suitable for lowland leopard frogs and Colorado River toads. However, these areas likely did not support either target species for many years prior to habitat restoration, and introductions may be required for colonization.

OBJECTIVE 2 – DETERMINE THE DISTRIBUTION OF LOWLAND LEOPARD FROGS AND COLORADO RIVER TOADS WITHIN THE STUDY AREA

Methods

Using data collected during inventories of potential lowland leopard frog and Colorado River toad habitat, sites were selected for presence surveys. Selection of sites was weighted by habitat rankings determined during site visits and the spatial analysis (described in objective 1) or by adding locations discovered during field visits that were previously unidentified. Sampling occurred at permanent lentic and lotic locations beginning in January 2011. Following summer monsoon rainfall, dry desert washes, arroyos, or ephemeral pools identified as potential Colorado River toad habitat were also sampled. Surveys were continued in 2012 and 2013 to maintain spring sampling for lowland leopard frogs and summer monsoon sampling for Colorado River toads. In 2013, environmental deoxyribonucleic acid (eDNA) sampling was conducted from September until early October. To detect frogs, four techniques were used during they surveys, including funnel trap arrays, visual surveys, nocturnal manual call surveys, and eDNA sampling. Visual and nocturnal audio surveys were performed at all funnel trap grid locations at least once.

Funnel Trap Arrays

Trap clusters were established at sites identified as containing suitable habitat for lowland leopard frogs. The funnel traps used were conical wire mesh funnel traps (Gee brand). They were wired to emergent or bank vegetation and placed along high traffic corridors for aquatic fauna and submerged so that the entrance was entirely below the water surface but allowed ample breathing area at the top of the trap for adult amphibians or non-target animals (figure 6). The traps were deployed for a minimum of 1 overnight and checked in the morning. All amphibians and non-target animals captured during this effort were identified and data, including date, time, and location, were recorded (Heyer et al. 1994; Olson et al. 1997).

Visual Surveys

Visual surveys were conducted following the techniques described by Heyer et al. (1994). The surveys began by using binoculars to scan the banks and shorelines during the daytime to detect amphibians floating in the water, basking on the banks, or hiding within the aquatic vegetation. D-ring dip nets (Delta Net and Twine) were used to sample the littoral zone for tadpoles. When possible, the entire perimeter of the survey site was surveyed, searching under logs and rocks,

Figure 6.—Funnel trap properly deployed at the Havasu NWR.

as well as in the vegetation, watching for adult amphibians to flush. Large dip nets were also used to search under ledges and within submergent vegetation for hidden adults. Any amphibians encountered were identified and recorded as described earlier.

Nocturnal visual surveys were also conducted. Beginning approximately 30 minutes after sunset, survey sites were scanned using flashlights, primarily searching for the eye shine of adult amphibians on the bank, but also observing the littoral zone for feeding amphibian larvae and breeding adult amphibians.

Nocturnal Manual Call Surveys

Beginning approximately 30 minutes after sunset, in concurrence with nocturnal visual surveys, amphibian vocalizations were broadcasted and listened for at each survey site using an aural survey methodology following the U.S. Geological Survey (USGS) North American Amphibian Monitoring Program protocol (http://www.pwrc.usgs.gov/naamp/index.cfm). This method begins with a passive listening period of 10 minutes. A portable audio system was then used to broadcast either a lowland leopard frog or Colorado River toad male advertisement breeding call for a minimum of 30 seconds to elicit male responses. All vocalizing amphibian species were identified, and an estimate of the numbers of individuals was recorded. Environmental conditions recorded included wind speed, air temperature, water temperature, pH, conductivity, cloud cover, and the presence of non-target noise, all of which may potentially affect amphibian vocalizations and breeding behavior.

Environmental DNA

This technique provided an additional survey tool that does not rely on direct observation of the target organism (Ficetola et al. 2008). At the end of the final year, additional funding was received to evaluate eDNA as a possible tool for surveys. In September and October 2013, samples of filtered water were collected from locations within and adjacent to the study area. Fifty samples were collected within the Bill Williams River NWR, 30 samples were collected from the best habitat areas on the main stem LCR as identified by GIS analysis and site visits (see objective 1, above), and 20 samples were collected from upstream of the study area on the Bill Williams River (figure 7). The upstream samples will be used to establish whether the technique is a viable option and to establish a baseline on sampling the Bill Williams River system due to large numbers of lowland leopard frogs. Samples were collected based on the protocol developed by Pilliod et al. (2012) and sent to the University of Idaho for analyses (C. Goldberg 2013, point of contact).

Results

A total of 2,560 funnel traps were deployed at 260 sites for over 45,000 trap-hours, and 432 visual and manual call surveys were performed. Ten species of amphibians were captured or observed during these surveys (table 1). The Bill Williams River held the most native amphibian diversity of all sites surveyed, whereas on the LCR, non-native amphibians, fishes, and crawfish (table 1) were frequently encountered.

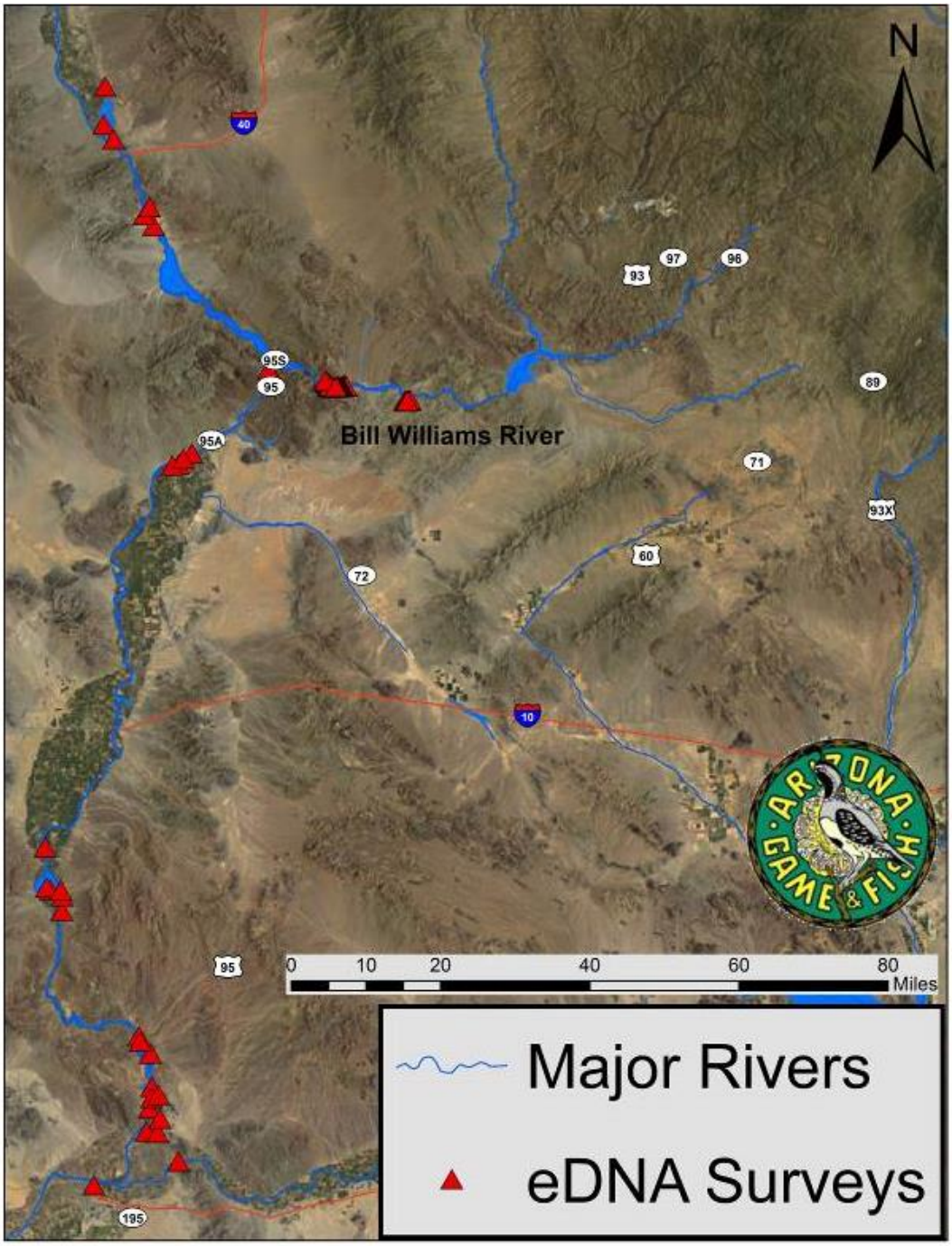

Figure 7.—Environmental DNA sampling on the lower Colorado and Bill Williams Rivers.
Red triangles indicate sample locations.

Table 1.—Summary of findings from funnel trap captures, manual call surveys, and dip net visual encounter surveys.

(Species codes: BUAL [Colorado River toad], RAYA [lowland leopard frog], RABE [Rio Grande leopard frog], BUWO [Woodhouse's toad; *Anaxyrus woodhousii*], BUCO [Great Plains toad; *Anaxyrus cognatus*], BUPU [red-spotted toad; *Anaxyrus punctatus*], BUMI [Arizona toad; *Anaxyrus microscaphus*], SCCO [Couch's spadefoot toad*; Scaphiopus couchii*), HYRE [Pacific treefrog; Hyla = *Psuedacris regilla*], and RACA [American bullfrog]).

Site	BUAL	RAYA	RABE	BUWO	BUCO	BUPU	BUMI	SCCO	HYRE	RACA	Bass	Crawfish
Bill Williams River NWR		X			X	X	X				X	X
Havasu NWR				X	X				X	X	X	X
Cibola NWR				X	X					X	X	X
'Ahakhav Tribal Preserve				X	X					X	X	X
Mittry/Imperial NWR			X							X	X	X
Gila River			X	X						X	X	X
Planet Ranch	X				X	X	X	X				
Bill Williams River	X	X				X	X				X	X

In 2012, a population of lowland leopard frogs was detected along the Bill Williams River on public land managed by the BLM east of the Planet Ranch boundary (figures 8 and 9). Twelve adults were marked, but many more individuals were observed at 18 different locations. Three instances of amplexus (i.e., breeding behavior) and three individual egg masses were also observed throughout this section of the study area. Beginning in June, lowland leopard frog tadpoles were observed along this stretch of river, indicating a successful breeding population. In 2013, two individual male lowland leopard frogs were observed/ marked on the Bill Williams River NWR: one downstream from the Mineral Wash access point and the other a few meters downstream from the eastern refuge boundary (figure 8).

Two adult male and one adult female Colorado River toad was captured on June 29, 2011, on Planet Ranch. Additionally, one female and two male Colorado River toads were captured on August 14, 2011, at the same location. Each individual was marked and released successfully. Three individuals were re-observed on subsequent trips to this location in 2011.

In 2012, five adult Colorado River toads were found and marked from the sandy flats adjacent to the stretch of the Bill Williams River where the lowland leopard frog population was observed, with seven additional toads observed and not marked. Two toads were identified in the monsoon-swollen waters of the Bill Williams River before it lost surface flow downstream. While no Colorado River toad tadpoles were observed and no male vocalizations were heard, one male Colorado River toad was observed moving several meters away from the water with freshly deposited eggs stranded on its back, suggesting breeding was taking place. In total, 11 Colorado River toad individuals from both the BLM locations and the 1 location on Planet Ranch were marked. Researchers were unable to verify if the site on Planet Ranch was still occupied due to access issues with the landowners. In addition, the locations of adult leopard frogs that were vocalizing near the border between Planet Ranch and the Bill Williams River NWR could not be confirmed. No additional locations for Colorado River toads were found in 2013.

The 2013 eDNA sampling evaluation resulted in 15 positive samples from the Bill Williams River NWR and upstream on the Bill Williams River, with no positive samples from the main stem LCR (figure 10). Two of the 50 total sites sampled on the Bill Williams River NWR were positive for lowland leopard frogs. Of the 20 samples collected upstream of the Bill Williams River NWR, 13 were positive for lowland leopard frogs. At 6 of the 15 positive samples, frogs were not observed during visual surveys, but there was a positive eDNA result. Frogs were observed within 10 meters of sample collection sites and produced a positive eDNA result at nine locations, and at three sites, frogs were observed, but the water sample was not positive for lowland leopard frogs (table 2). Of the total sites that were positive for lowland leopard frogs, 25% were negative and 75% were positive for eDNA. Of the total sites that were negative for lowland leopard

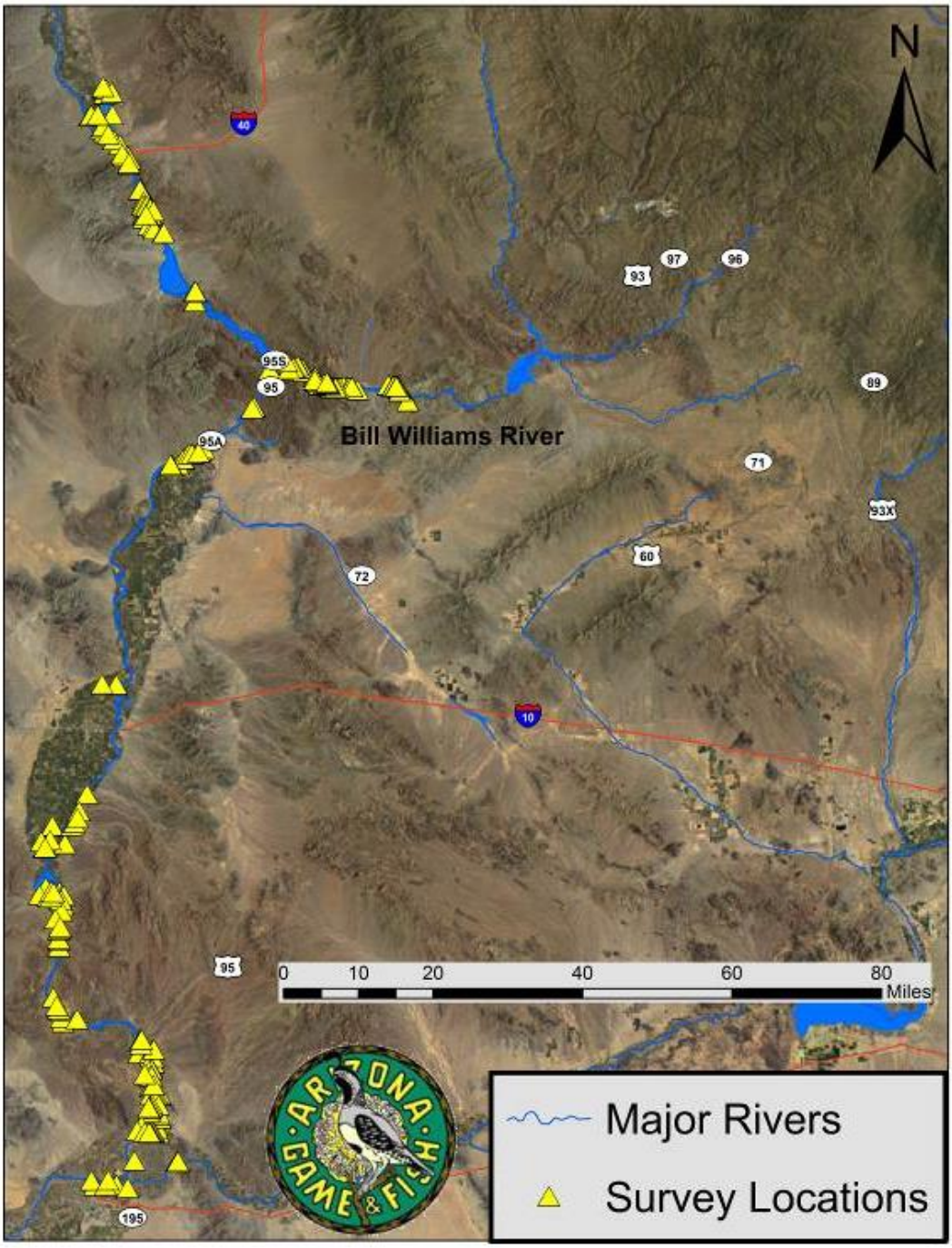

Figure 8.—Survey locations on the lower Colorado and Bill Williams Rivers.
Yellow triangles indicate survey locations.

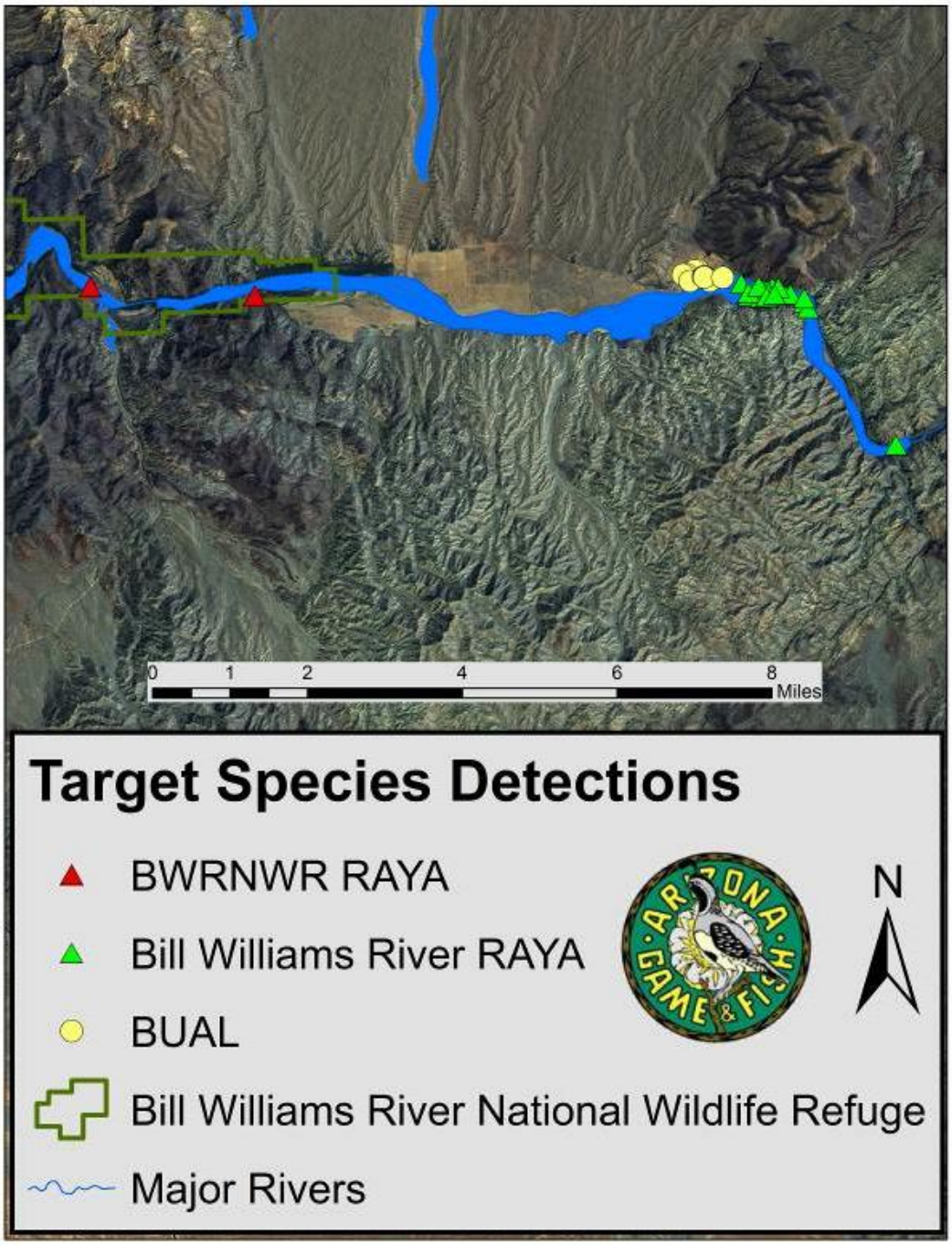

Figure 9.—Locations on the Bill Williams River where lowland leopard frogs (RAYA) and Colorado River toads (BUAL) were observed.
Green triangles indicate lowland leopard frog locations on BLM property, red triangles indicate lowland leopard frog locations on the Bill Williams River NWR, and yellow circles identify Colorado River toad locations.

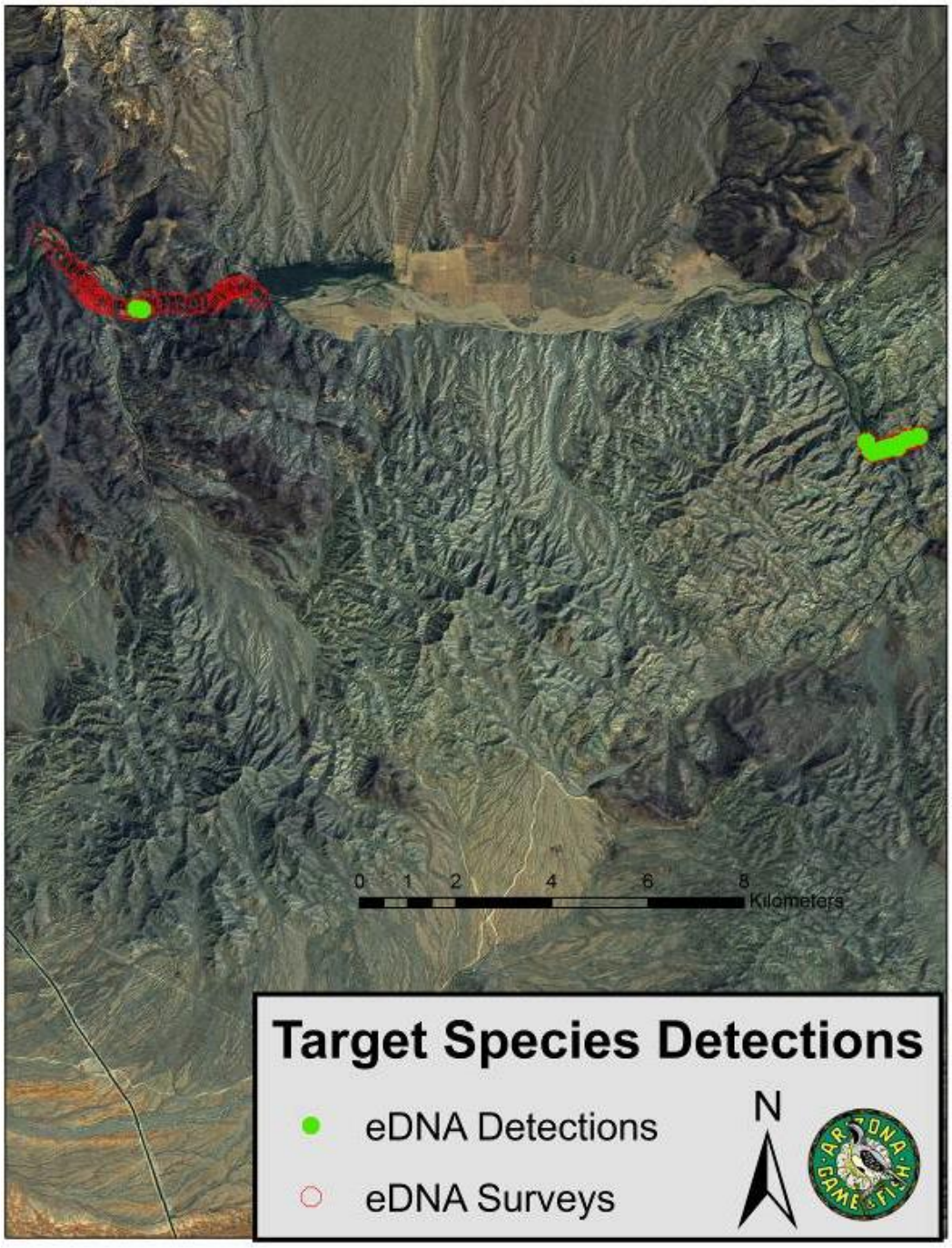

Figure 10.—Locations on the Bill Williams River where eDNA samples were collected.
Hollow red circles indicate sample collection sites, and red circles with green centers indicate sample locations positive for lowland leopard frogs (RAYA).

Table 2.—Summary of findings for positive and negative detections of eDNA where RAYA (lowland leopard frog) detections were observed

	RAYA -	RAYA+
eDNA +	6	9
eDNA -	82	3

frogs, 7% were positive and 93% were negative for eDNA. One thousand milliliters of water were filtered for all samples collected from upstream of the Bill Williams River NWR, and 1,500 and 2,000 milliliters were filtered from two positive locations on the Bill Williams River NWR.

Discussion

The Bill Williams River was identified as the only location with extant populations of both target species within the study area. The population of lowland leopard frogs that was identified east of Planet Ranch appears to be a large and viable population due to a high abundance of adults, tadpoles, and eggs observed during surveys in each year. Surveys near the Reach 7 boundary of the river were terminated due to access issues, but the survey results suggest that the frogs are equally abundant further upstream. There was an approximate 5- to 8-kilometer gap in surface water between the section of public land managed by BLM where frogs have been found and the Bill Williams River NWR downstream, depending on water discharge from Alamo Dam. However, with the periodic flooding of Alamo Dam, such as in 2010 (http://waterdata.usgs.gov/az/nwis/dv/), this dry corridor could support sufficient water for dispersing individuals to make it to the lower sections of the Bill Williams River, which could create a dispersal corridor.

The only other location within the study area that appears to have habitat for lowland leopard frogs is where the surface water reappears on Planet Ranch, just east of the Bill Williams River NWR boundary. This area was searched in 2011; however, this was after the breeding season for lowland leopard frogs. In 2012, while unable to confirm their presence due to access issues with Planet Ranch, calling male leopard frogs were heard coming from east of the Bill Williams River NWR/Planet Ranch boundary, but no individuals were observed on the refuge. The individuals that were captured on the Bill Williams River NWR in 2013 are likely to be dispersers from the upstream population.

The sandy desert flats around the Bill Williams River east of Planet Ranch supported a few highly dispersed Colorado River toad adults. It is possible that the individuals marked on Planet Ranch in 2011 were part of this same

population. Future surveys on the Planet Ranch property could result in identifying even greater numbers of individuals occupying the sandy riverbed around the Bill Williams River. While breeding behavior was not observed with this species, the number of individuals observed suggests breeding is occurring, most likely during heavy summer rain events.

Within the Bill Williams River NWR there are a handful of locations where the water has seeped up into small ponds away from the main river channel. These areas were thoroughly surveyed each year, without producing detections of either target species. Both lowland leopard frog detections were on the main channel of the Bill Williams River. These isolated ponds on wide sandy sections of riverbed are still locations identified that could support additional populations of both lowland leopard frogs and Colorado River toads within the study area.

The summer monsoon in 2012 and 2013 produced large amounts of rain in sections of the study area and resulted in isolated flooding in certain washes along the LCR. These rain events were tracked, and surveys were conducted at as many locations as possible in order to observe breeding behavior. Colorado River toads were not observed during any of these flood events even though amphibians were breeding in these swollen washes after rainfall. In particular, high numbers of Couch's spadefoot toads and red-spotted toads were observed using these temporary water sources.

OBJECTIVE 3 – COLLECT GENETIC SAMPLES FROM LOWLAND LEOPARD FROGS AND COLORADO RIVER TOADS

Methods

The Arizona Game and Fish Department non-destructive protocol for collecting genetic samples from amphibians was followed. This protocol incorporates safeguards to prevent the transmission of pathogens. Briefly, each captured anuran was first rinsed with fresh water to remove any mud or debris. Then, using sterilized scissors, toes were clipped between the first and second phalange and collected in 1.5-milliliter vials with a 95% ethanol solution. The wounds were disinfected, and all animals were successfully released after being monitored for several minutes. All equipment was sterilized after each use using 95% ethanol. Samples were stored in the ethanol-filled vials for future genetic analyses.

Results

Tissue samples from 11 Colorado River toad adults and 12 lowland leopard frog adults were successfully collected. Samples were collected from a single digit, and each adult was monitored and released the same night. The number of tissues collected does not accurately estimate the large number of individual anurans actually observed during the surveys. Tissue collection was discontinued once an adequate sample size was collected.

Objective 4 – Determine Habitat Selection of Lowland Leopard Frogs and Colorado River Toads Along the Lower Colorado and Bill Williams Rivers

Methods

Lowland leopard frog habitat selection was evaluated by comparing habitat characteristics within three habitat covariate categories (i.e., biotic, abiotic, and predator covariates) at sites with lowland leopard frogs present to random sites within the lower Colorado and Bill Williams Rivers where they were not detected. In 2012, 103 plots were measured, including all the non-sites in which target species were not found. Two additional locations with lowland leopard frogs present were found in 2013. However, the habitat was only quantified at one location due to a loss of water at the second site prior to habitat assessment. Habitat selection analyses for lowland leopard frogs were conducted at two spatial scales to identify important habitat components at the local scale (i.e., within a reach) and the regional scale (i.e., the lower Colorado and Bill Williams Rivers).

A 10-meter radius plot was used as the sample unit. To evaluate habitat, researchers returned to the sites within 3 days of detection and randomly selected at least one site within a 300-meter radius where the target species were not encountered. At the local scale, 27 non-sites were identified to correspond with the 22 locations containing lowland leopard frogs (figure 11). Non-sites were identified by randomly selecting an azimuth and distance within suitable habitat. Each non-site was also surveyed and trapped to ensure leopard frogs were not utilizing the site. For regional-scale non-sites, 42 locations from the entire study area were randomly selected from where surveys were previously performed without finding the target species, and the habitat at these locations was quantified.

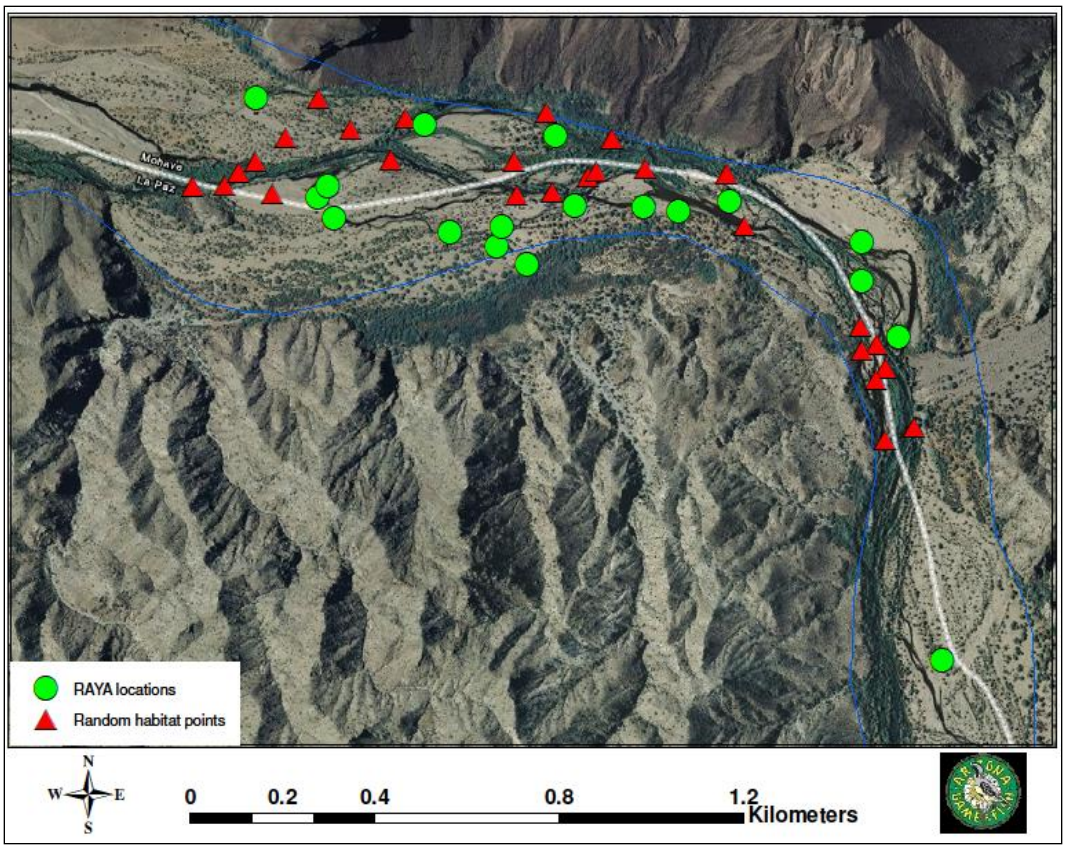

Figure 11.—Locations on BLM land where habitat variables were measured. Green circles indicate lowland leopard frog locations, while red triangles identify locations where lowland leopard frogs were not observed.

For aquatic habitats, minimum and maximum water depth (meters), substrate type (e.g., gravel, sand, etc.), and stream discharge (cubic feet per second; lotic habitats only) within the 10-meter radius plot were recorded. Vegetation composition and density were measured using the line-intercept method (Canfield 1941). Terrestrial plants were categorized as grasses, forbs, shrubs, or trees, while aquatic plants were categorized as trees, emergent vegetation, submergent vegetation, or floating vegetation. Any coarse woody debris that was ≥ 3 meters in length and ≥ 10 centimeters in diameter was recorded. The distance to the nearest water source was also recorded as well as the type of water source (e.g., pond, stream, etc.). The same data were collected for the 13 locations where Colorado River toads were observed; however, due to the small sample size and uniform nature of the microhabitat the toads were found in, non-sites were not measured as was done for lowland leopard frogs. Instead, a descriptive account of the habitat within which Colorado River toads were detected was provided.

Analysis: Logistic Regression

Correlations among continuous covariates were first evaluated using Pearson correlation coefficients (Pearson 1920; Zar 1999). Covariates in which correlation coefficient values of $|r| \geq 0.50$ ($P < 0.05$) were not included in the same statistical model in order to avoid multicollinearity (Glanz and Slinker 1990; Graham 2003). Habitat characteristics were compared using logistic regression models in which the binomial response represented detection of the target species or non-detection. Logistic regression models were constructed to describe hypotheses regarding habitat selection by lowland leopard frogs (Hosmer and Lemeshow 2000). This process was performed individually for both the local- and regional-scale habitat data.

The analysis followed a three-phased approach in which habitat models were created for each of the three covariate categories individually at each scale (see below). Models were compared within each covariate category under a model-selection framework (Burnham and Anderson 2002). The small sample correction for the marginal Akaike's information criterion (AIC_c) (Akaike 1973; Hurvich and Tsai 1989; Burnham and Anderson 2001; Vaida and Blanchard 2005) was used to rank candidate models within a covariate category. AIC_c difference (ΔAIC_c) and Akaike weight (Buckland et al. 1997) were calculated for each model to assess model uncertainty and the likelihood of each model given the data. Models with $\Delta AIC_c \leq 2$ were considered to be well supported by the data, and models with Akaike weights of > 0.1 were included for model averaging (Burnham and Anderson 2002; Richards et al. 2010). A Pearson χ^2 goodness of fit test was performed against the saturated model. This process was repeated for each of the three covariate categories, with the most well-supported models in each category being combined to identify the most well-supported model covariates from all three categories.

Model selection uncertainty was accounted for by calculating unconditional parameter and variance estimates for each parameter across the set of supported models and then determining the 95% confidence intervals and odds ratio for each parameter (Burnham and Anderson 2002). Program R (R Development Core Team 2008) was used to evaluate correlations as well as to construct and compare logistic regression models.

Analysis: Gradient Analysis

In addition to the logistic regression, a gradient analysis was conducted on the environmental data where the dataset was separated into two categories (detected and not detected) to evaluate the similarities and differences between the categories. First, the environmental covariates for collinearity were examined, and one variable was removed from any of the collinear pairs (Zuur et al. 2010). Next, the data were standardized to fit them to a proportional scale of 0–100 (Krebs 1999). Environmental variables that were either extremely skewed or

categorical were excluded. Thus, the final environmental covariate dataset included 13 variables (attachment 2). With this final covariate set, a principal coordinates analysis (PCoA), which represents objects in multidimensional space while preserving the distances ("differences") between the observed points, was conducted. For this analysis, the Morisita's distance metric (Morisita 1962) was selected because it is often independent of sample size (Krebs 1999), which is important with the comparison of detected and non-detected sites. To examine similarity between the two categories of sites, an analysis of similarity (ANOSIM) (Clark 1993) was conducted. Finally, a discriminant functions analysis (DFA) was performed to determine how well these environmental variables categorized plots as to whether lowland leopard frogs were detected. The significance of this relationship with a multivariate analysis of variance (MANOVA) was evaluated. There were no concerns regarding outliers or homogeneity of variances. The program PAST (Hammer et al. 2001) was used to calculate PCoA, ANOSIM, MANOVA, and DFA.

Results

Local-Scale Habitat Selection: Logistic Regression

Fifteen models were developed and compared using the abiotic covariates (table 3). The best fitting model for the abiotic covariates included two covariates, maximum and minimum water depth within the 10-meter plot. This model had an Akaike weight of 0.96, and no other model had a $\Delta AIC_c \leq 2$. The second most well-supported model was the saturated model containing all covariates with a ΔAIC_c of 7.63. The goodness of fit test on the saturated model was not significant for a lack of fit (p-value = 0.58, 37 residual degrees of freedom).

Table 3.—Local abiotic candidate models used to evaluate habitat use by lowland leopard frogs within Reaches 7 and 8 of the Bill Williams River
(K = Number of parameters.)

Model number	Model	AIC_c	ΔAIC_c	Log (L)	AIC_c weight	K
3	Maximum.Depth + Minimum.Depth	40.76	0	-17.38	0.96	3
15	Sand + Mud.Silt + Gravel + Cobble + H2O.Class + H2O.Type + Discharge + Maximum.Depth + Minimum.Depth	48.39	7.63	-13.20	0.02	10

Twenty-three models were developed and compared using the biotic covariates (table 4). The saturated model containing all 23 covariates resulted in the lowest AIC value, with no other models producing a $\Delta AIC_c \leq 2$. The model with the highest Akaike weight (0.55) was the global model. The second most

Table 4.—Local biotic candidate models used to evaluate habitat use by lowland leopard frogs within Reaches 7 and 8 of the Bill Williams River
(K = Number of parameters.)

Model number	Model	AIC_c	ΔAIC_c	Log (L)	AIC_c weight	K
39	Wet + Dry + Open.Aqu. + Open.Terr. + Total.Open. + Em + Sub + Shrub + Tree + Grass + Forb + Floating	55.89	0	-16.95	0.55	13
24	Sub	58.27	2.38	-27.14	0.17	2
19	Total.Open	60.39	4.5	-28.12	0.06	2
17	Open.Terr	60.79	4.90	-28.39	0.05	2
26	Open.Terr + Em	60.82	4.92	-27.13	0.05	3
33	Em + Grass	60.89	5.00	-27.45	0.05	3

well-supported model included only the covariate submergent vegetation with an ΔAIC_c of 2.38. The goodness of fit test was significant for a lack of fit (p-value = 0.03, 46 residual degrees of freedom).

Five models were developed and compared using the predator covariates (table 5). Four models had an $\Delta AIC_c \leq 2$. The four best fitting models for the biotic covariates included the presence of Cambarids (crayfish), Centrarchids (sunfish), and Centrarchids with Cambarids, as well as the global model containing *Micropterus* (largemouth bass), Cambarids, and Centrarchids. The model with the highest Akaike weight was Centrarchids and Cambarids, with an Akaike weight of 0.30. The goodness of fit test was significant for a lack of fit (p-value = 0.04, 45 residual degrees of freedom).

Table 5.—Local predator candidate models used to evaluate habitat use by lowland leopard frogs within Reaches 7 and 8 of the Bill Williams River
(K = Number of parameters.)

Model number	Model	AIC_c	ΔAIC_c	Log (L)	AIC_c weight	K
43	Centrarchids + Cambarids	68.18	0	-31.09	0.30	3
44	Cambarids	68.19	0.01	-32.09	0.30	2
42	Centrarchids	68.96	0.78	-32.48	0.20	2
45	Micropterus + Centrarchids + Cambarids	69.12	0.94	-30.96	0.13	4

Twenty models were developed and compared using the best fit models from the three covariate categories within the local habitat criteria (table 6). Five models had an $\Delta AIC_c \leq 2$. The five best fitting models for the local habitat scale included maximum water depth, minimum water depth, total water depth, and Cambarids; maximum water depth, minimum water depth, total water depth, and Centrarchids; maximum water depth, minimum water depth, total water depth, emergent cover, grass cover, and Cambarids; maximum water depth, minimum water depth, total water depth, Centrarchids and Cambarids. The highest Akaike weight was 0.25. The absolute values for unconditional parameter estimates were larger than the unconditional standard error for each covariate. The approximate 95% confidence intervals for odds ratio show only maximum water depth and Cambarids strongly affected the probability of frog occurrence, for the negative (table 7). Centrarchids were dropped from model averages due to high estimates and standard errors as a result of low occurrences. The goodness of fit test was not used for regional abiotic parameters due to a lack of power in the dataset as a result of low sample size per parameter measured (Hosmer and Lemeshow 2000).

Table 6.—Local combined candidate models used to evaluate habitat use by lowland leopard frogs within Reaches 7 and 8 of the Bill Williams River

(K = Number of parameters.)

Model number	Model	AIC_c	ΔAIC_c	Log (L)	AIC_c weight	K
66	Maximum.Depth + Minimum.Depth +Total.Open + Cambarids	32.81	0	-11.41	0.25	5
67	Maximum.Depth + Minimum.Depth + Total.Open + Centrarchids	33.20	0.51	-11.60	0.20	5
54	Maximum.Depth + Minimum.Depth + Em + Grass + Cambarids	34.54	1.73	-11.27	0.11	4
68	Maximum.Depth + Minimum.Depth + Total.Open + Centrarchids + Cambarids	34.71	1.9	-11.36	0.10	6
55	Maximum.Depth + Minimum. Depth + Em + Grass + Centrarchids	34.73	1.92	-12.06	0.10	6
63	Maximum.Depth + Minimum.Depth + Sub + Centrarchids	36.44	3.63	-10.26	0.04	5
56	Maximum.Depth + Minimum.Depth + Em + Grass + Centrarchids + Cambarids	36.56	3.75	-11.16	0.04	7
57	Maximmum.Depth + Minimum.Depth + Em + Grass + Centrarchids + Cambarids + Micropterus	38.16	5.35	-10.86	0.02	8
64	Maximum.Depth + Minimum.Depth + Sub + Centrarchids + Cambarids	38.41	5.6	-10.17	0.02	6
65	Maximum.Depth + Minimum.Depth + Sub + Centrarchids + Cambarids + Micropterus	39.51	6.7	-10.10	0.01	7

Table 7.—Model averaged estimates and standard errors for parameters included in logistic regression combined local models
(Values are considered ‘‘unconditional’’ because they incorporate sampling variability and variability caused by model selection uncertainty [Burnham and Anderson 2002]. Back transformed values [odds ratio] are also given along with 95% confidence intervals – lower confidence level [LCL] and upper confidence level [UCL].)

Parameter	Unconditional parameter estimate	Unconditional standard error	Odds ratio	95% LCL	95% UCL
Maximum.Depth	-0.3646	0.2236	0.69	0.448	1.076
Minimum.Depth	-1.5050	0.4704	0.22	0.088	0.558
Em	-0.0754	0.0192	0.93	0.893	0.963
Grass	-0.0536	0.0239	0.95	0.904	0.993
Cambarids	-2.8202	0.7450	0.06	0.014	0.257
Total.Open	0.2534	0.1375	1.29	0.984	1.687

Regional-Scale Habitat Selection: Logistic Regression

Sixteen models were developed and compared using the abiotic covariates (table 8). Three models had a $\Delta AIC_c \leq 2$. The three best fitting models for the abiotic covariates included maximum water depth; discharge and maximum water depth, and maximum water depth with minimum water depth within the 10-meter plot. This best fitting model had an Akaike weight of 0.35. The goodness of fit test was not used for regional abiotic parameters due to a lack of power in the dataset as a result of low sample size per parameter measured (Hosmer and Lemeshow 2000).

Table 8.—Regional abiotic candidate models used to evaluate habitat use by lowland leopard frogs within Reaches 7 and 8 of the Bill Williams River
(K = Number of parameters.)

Model number	Model	AIC_c	ΔAIC_c	Log (L)	AIC_c weight	K
5	Discharge + Maximum.Depth	34.77	0	-14.39	0.35	3
2	Maximum.Depth	34.82	0.05	-15.41	0.34	2
3	Maximum.Depth + Minimum.Depth	35.86	1.08	-14.93	0.20	3

Twenty-four models were developed and compared using the biotic covariates (table 9). The best fitting model for the biotic covariates included two covariates: percent cover of emergent vegetation and shrubs. This model had an Akaike weight of 0.75, and no other model had a $\Delta AIC_c \leq 2$. The second most well-supported model had an ΔAIC_c of 5.15. The goodness of fit test on the saturated model for regional biotic parameters was not significant for a lack of fit (p-value = 0.55, 53 residual degrees of freedom).

Table 9.—Regional biotic candidate models used to evaluate habitat use by lowland leopard frogs within Reaches 7 and 8 of the Bill Williams River
(K = Number of parameters.)

Model number	Model	AIC_c	ΔAIC_c	Log (L)	AIC_c weight	K
36	Em + Shrub	65.14	0	-29.57	0.75	3
37	Forb + Open.Terr.	70.29	5.15	-32.15	0.08	3
21	Forb	70.70	5.57	-33.35	0.06	2

Seven models were developed and compared using the predator covariates (table 10). Two models had an $\Delta AIC_c \leq 2$. The two best fitting models for the biotic covariates included the presence of American bullfrogs (RACA) and the global model, including RACA with *Micropterus*, Cambarids, and Centrarchids. The model with the highest Akaike weight was the global model, with an Akaike weight of 0.57. The goodness of fit test on the saturated model was not significant for a lack of fit (p-value = 0.75, 59 residual degrees of freedom).

Table 10.—Regional predator candidate models used to evaluate habitat use by lowland leopard frogs within Reaches 7 and 8 of the Bill Williams River
(K = Number of parameters.)

Model number	Model	AIC_c	ΔAIC_c	Log (L)	AIC_c weight	K
47	RACA + Micropterus + Cambarids + Centrarchids	61.87	0	-25.93	0.57	5
44	RACA	63.59	1.8	-29.79	0.24	2
45	RACA + Micropterus	65.38	3.51	-29.51	0.1	3

Six models were developed and compared using the best fit models from the three covariate categories for the regional scale (table 11). The best fitting model for the biotic covariates included the covariates water discharge in cubic feet, maximum depth, percent cover by forbs, and dry, unvegetated area within the 10-meter plot. The highest model Akaike weight was 0.77. The second most well-supported model had a ΔAIC_c of 2.99. The goodness of fit test was not used for regional combined candidate models due to a lack of power in the dataset (Hosmer and Lemeshow 2000).

Table 11.—Regional combined candidate models used to evaluate habitat use by lowland leopard frogs within Reaches 7 and 8 of the Bill Williams River
(K = Number of parameters.)

Model number	Model	AIC_c	ΔAIC_c	Log (L)	AIC_c weight	K
50	Discharge + Maximum.Depth + Forb + Open.Terr.	32.07	0	-11.04	0.77	5
49	Maximum.Depth + Forb + Open.Terr	35.14	2.99	-13.48	0.17	4

Gradient Analysis

The gradient analysis with PCoA suggested the top three axes (gradients) were related to open areas with and without water, canopy cover, and water depth gradients (figures 12–13). These gradients accounted for 68% of the variation in the dataset (table 12). Overall, there was overlap between the groups' habitat characteristics. Habitat characteristics of presence sites were nested within those of the absence sites' characteristics (ANOSIM; $R = 0.19$, $p < 0.01$). The gradient selected by the DFA identified a significant difference among these groups (MANOVA: Wilks' λ: $F = 2.08$, $p < 0.01$) to be related to maximum water depth and vegetation (table 13; figure 14).

Due to low sample size and uniformity of the habitat where toads were found, logistic regression and gradient analysis procedures were not performed for Colorado River toads. Habitat was measured at 15 locations where Colorado River toads were observed during the 2011 and 2012 field seasons. Only one site, located on the Bill Williams River, was aquatic habitat. Water discharge at this location was 0.10 cubic foot per second. All toad detections occurred at locations where the maximum water depth was 13 centimeters and the minimum was 1 centimeter. The substrate consisted of sand, gravel, and mud/silt. Colorado River toad habitat was comprised of both dry and inundated portions of flood plain, with emergent vegetation, shrub, and forb vegetation classes present as well as 47% unvegetated area (table 13). The other 14 locations where Colorado River toads were detected were situated away from permanent water sources, with a mean distance to water of 218 meters, ranging from 140 to 353 meters. All toad detections in 2011 were near small puddles, but these water sources were created primarily from water use by the surrounding buildings and facilities and not natural, seasonal, or permanent. Habitat measured in which toads were observed contained an average of 2.5% emergent vegetation, 8.52% shrub cover, 0.1% forb vegetation cover, 0.07% tree cover, and 7.95% cover by grass, with an average of 81.46% unvegetated area within the 10 x 10 meter plot (table 14).

Figure 12.—Scatter plot of locations within the greater lower Colorado River ecosystem where habitat variables were measured.
Red squares indicate lowland leopard frog locations, and blue diamonds identify locations where lowland leopard frogs were not observed. The X-axis describes the amount of inundation at each plot, and the Y-axis describes the amount of combined species vegetative cover at each plot.

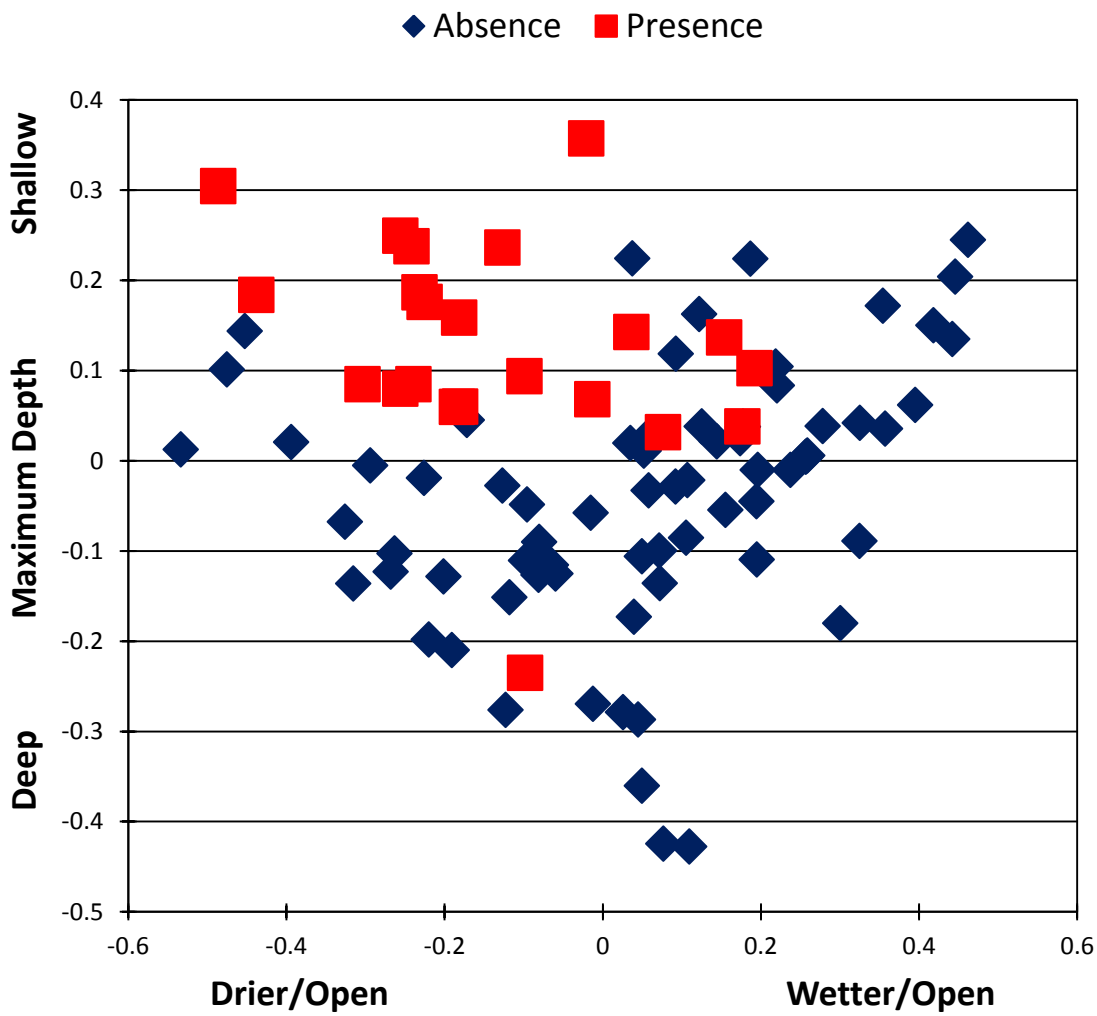

Figure 13.—Scatter plot of locations within the greater lower Colorado River ecosystem where habitat variables were measured.
Red squares indicate lowland leopard frog locations, and blue diamonds identify locations where lowland leopard frogs were not observed. The X-axis describes the amount of inundation at each plot, and the Y-axis describes the maximum water depth within each plot.

Table 12.—Principal coordinate axes created on environmental covariates for presence and absence of lowland leopard frogs along the lower Colorado and Bill Williams Rivers

PCoA axis	Eigenvalue	Percent	Cumulative	Interpretation – gradient
1	4.92	33.97	33.97	Open areas with and without water
2	2.68	18.46	52.44	Canopy cover (open – closed)
3	2.23	15.35	67.79	Water depth (minimum to maximum)
4	1.65	11.36	79.14	Vegetation cover (tree – shrub)
5	1.02	7.01	86.15	Vegetation cover (percent grass)
6	0.65	4.45	90.59	Vegetation cover (percent forb)
7	0.45	3.1	93.69	Vegetation cover (percent forb)
8	0.37	2.53	96.22	Discharge
9	0.22	1.55	97.77	Water depth (minimum)
10	0.19	1.34	99.11	N/A
11	0.1	0.67	99.78	N/A
12	0.03	0.22	100	N/A

Table 13.—Discriminant functions scores created on environmental covariates for presence and absence of lowland leopard frogs along the lower Colorado and Bill Williams Rivers

Environmental variable	Discriminant score
Minimum water depth	0.02
Maximum water depth	0.08
Total discharge	0.01
Percent cover wet	-0.02
Open terrestrial	-0.01
Open aquatic	0.01
Total open	0.00
Emergent vegetation	0.05
Shrub vegetation	0.01
Forb vegetation	-0.02
Tree vegetation	0.03
Grass vegetation	0.02
Submerged vegetation	-0.03

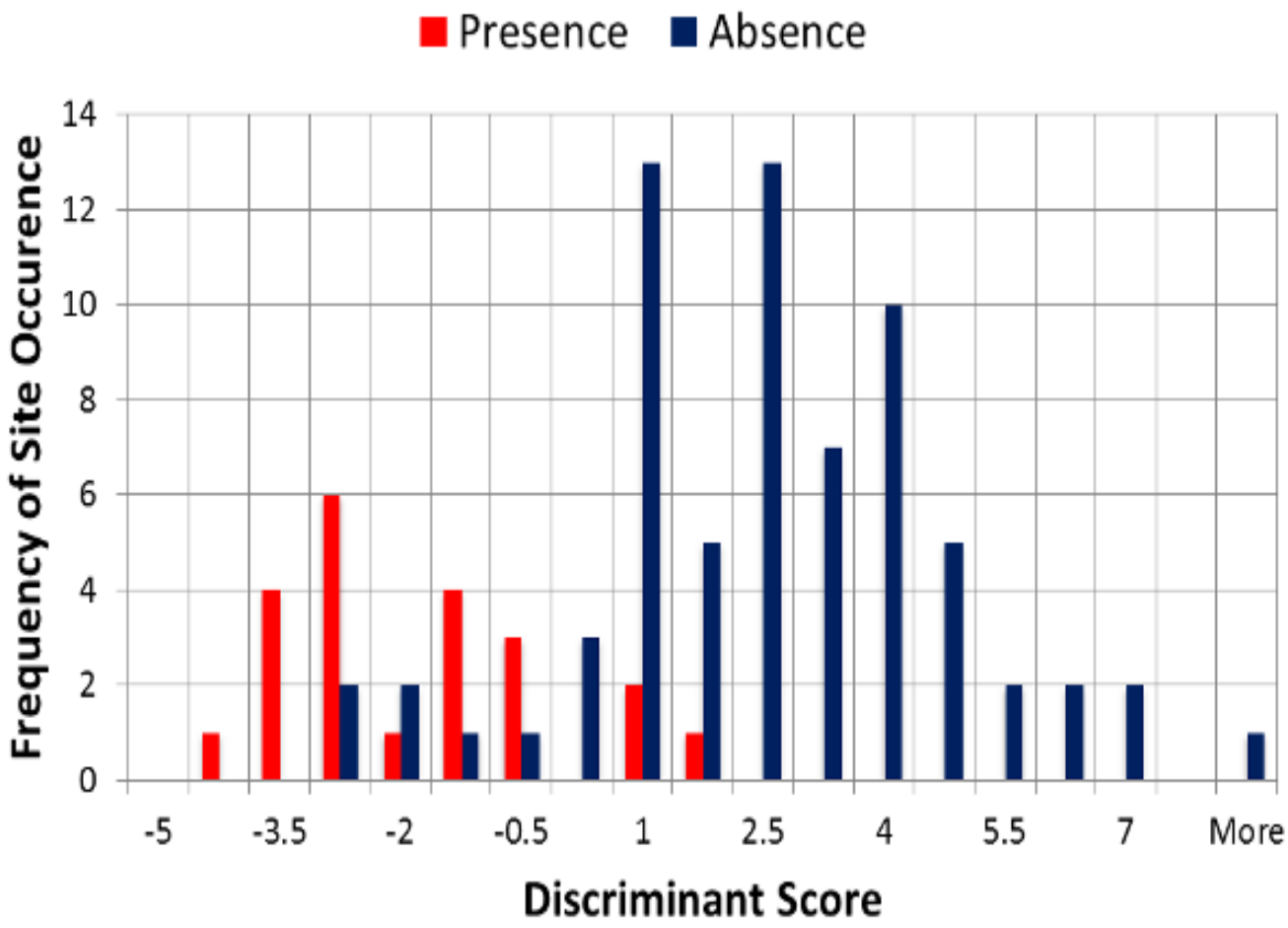

Figure 14.—Discriminant functions analysis frequency of occurrence for each site for locations within the greater lower Colorado River ecosystem where habitat variables were measured.
Red bars indicate lowland leopard frog locations, and blue bars identify locations where lowland leopard frogs were not observed. The X-axis describes the singular gradient that separates these two datasets, and the Y-axis describes the frequency of occurrence for each level of the newly created discriminant function.

Table 14.—Habitat characteristics from locations where Colorado River toads were observed

Site	Minimum depth	Maximum depth	Sub-strate 1	Sub-strate 2	Sub-strate 3	Discharge	Distance to water	Wet	Dry	Open Terrestrial	Open Aquatic	Total Open	Emergent	Shrub	Forb	Tree	Grass
BLM1			Sand	Gravel	Cobble		240		100	98.82		98.82	0	0	0	0	1.18
BLM10			Sand	Sand	Sand		257		100	81.92		81.92	0	17.92	0.03	0	0.13
BLM11			Sand	Sand	Sand		256		100	80.17		80.17	0	19.83	0	0	0
BLM12			Sand	Sand	Sand		287		100	89.29		89.29	0	10.52	0.02	0	0.17
BLM13			Gravel	Cobble	Sand		222		100	75.62		75.62	19	0	0	0.93	6.38
BLM2			Sand	Sand	Sand		353		100	99.65		99.65	0	0	0	0	0.35
BLM3	1	13	Sand	Mud/silt	Gravel	0.104	0	50	50	6.88	40.15	47.03	17	42.45	0.65	0	0
BLM4			Sand	Sand	Sand		214		100	89.2		89.2	0	10.82	0.03	0	0.12
BLM5			Sand	Sand	Sand		181		100	98.98		98.98	0	0	0.55	0	0.47
BLM6			Sand	Sand	Sand		161		100	90.6		90.6	0	9.17	0	0	0.23
BLM7			Sand	Sand	Sand		167		100	94.25		94.25	0	5.28	0	0	0.5
BLM8			Sand	Sand	Sand		161		100	98.63		98.63	0	0	0	0	1.37
BLM9			Sand	Sand	Sand		140		100	96.23		96.23	0	3.32	0.1	0	0.35
PR1			Sand	Mud/silt			0		100	0		0	0	0	0	0	100
Means	1	13				0.1	219.92	50	96	78.59	40.15	81.45	2.5	8.522	0.1	0.07	7.946
Standard deviation							16.71		3.57	8.75		7.29	1.75	3.19	0.06	0.07	7.09

Discussion

The fit and interpretation of logistic regression habitat models was handicapped due to the small sample size of habitat points and large number of habitat parameters measured (Hosmer and Lemeshow 2000). In future studies, an attempt should be made to increase the number of samples. However, in dealing with rare species, this may not be practical; in such situations, a gradient analysis may be a more appropriate statistical tool.

The models with the least loss of data for the local-scale habitat analysis included the parameters of maximum depth, minimum depth, percent cover of emergent vegetation, percent cover of grass, percent unvegetated area, Cambarid presence, and Centrarchid presence. Within these models, the covariant Centrarchid presence should be considered with some skepticism. The variance of estimates was high due to a lack of occurrences in the dataset. Centrarchids were only present at two habitat points quantified.

Based on the estimates and standard error (see table 7), these models describe a shallow water habitat with a high percentage of open space and relatively low percentage of vegetation cover. This same trend is observed in the scatter plots developed following the gradient analysis (see figures 12–13). These general trends reflect strongly on observations that were made in the field. Deep water allows for the persistence of large predators that, in addition to smaller predators such as crayfish, have been shown to be detrimental to amphibians in prior studies (Hayes and Jennings 1986; Clarkson and Rorabaugh 1989; Rosen et al. 1995; Axelsson et al. 1997; Gherardi et al. 2001; Kats and Ferrer 2003; Cruz et al. 2006; Ficetola et al. 2011). Much of the main stem LCR has been modified to discourage these shallow open side channels and braids. Outside of the Bill Williams River NWR, Planet Ranch, and upstream sections of the Bill Williams River, habitat with dynamic shallow back waters has not been seen. The other refuges on the main stem LCR have the capacity to support restoration areas analogous to the habitat described in this analysis, but intensive anti-predator management techniques will be required to knock exotic species out of established habitats. The phenomenon of American bullfrogs not surviving on the Bill Williams River plays a key role in the persistence of lowland leopard frogs.

The Bill Williams River east of Planet Ranch supported a high density of leopard frogs (see figure 9; however, the study area from 2012 was void of water and amphibians when surveys were conducted in 2013. Surface water had receded approximately 3–5 kilometers upstream from a decrease in water being released from Alamo Dam. A trip upstream confirmed the presence and breeding of lowland leopard frogs. Lowland leopard frogs were able to adapt to the newly reclaimed habitat downstream during 2012, which is likely consistent with the historic flood and drought patterns of the river. Summer heat and lack of rain influence frog presence at the Bill Williams River NWR as well. Surface water

retreats away from the Colorado River confluence beginning as early as May. This dynamic nature may contribute to the lack of American bullfrogs in this section of the river.

The presence of Colorado River toads is also likely due to periodic flooding of the Bill Williams River. Unlike lowland leopard frogs, Colorado River toads are not likely to follow the tongue of the river upstream. Instead, Colorado River toads likely persist near the dry riverbed and breed in the deep pools and ephemeral streams during high water pulses and monsoon rains. Unfortunately, the area was not surveyed for Colorado River toads during their breeding season due to access issues with Planet Ranch.

The Bill Williams River is the best habitat found in the study area. In addition to the presence of lowland leopard frogs and Colorado River toads, 13 northern Mexican garter snakes (*Thamnophis eques*) were captured in funnel traps (Cotten et al. 2013). This species is in decline in Arizona and a candidate species for listing under the Endangered Species Act (U.S. Fish and Wildlife Service 2006). A large part of the northern Mexican garter snake's diet consists of amphibians, and this location is one of the only places the snake exists along with lowland leopard frogs (Brennan and Holycross, personal observation). The presence of both target species and northern Mexican garter snakes underlines the quality of habitat found here.

The best fitting regional model for the presence of lowland leopard frogs included the covariates of discharge, maximum depth, percent cover by forbs, and percent open dry ground. Just as in the local model, low water depth was important for the presence of amphibians and is supported by the gradient analysis. In line with the low water depth, lowland leopard frogs were associated with low stream discharge in lotic habitats. In general, the areas throughout the study area that had high discharge were associated with large, wide stretches of river and strong current, which does not coincide with habitat described by the most supported models. While the presence of American bullfrogs was not a highly supported covariate in the analysis, the target species was never found co-occurring with American bullfrogs. The threat from American bullfrogs is well supported in the literature and has been observed to directly prey upon lowland leopard frogs (Hayes and Jennings 1986; Clarkson and Rorabaugh 1989; Rosen et al., personal observation). American bullfrogs were correlated with many of the other variables measured that were associated with the main stem LCR and were subsequently relegated to a model on their own. This model did not describe the majority of the habitat located along the main stem LCR. The LCR is categorized by high stream discharge, deep water, and thick *Typha* spp. growth. Open habitat is described as a highly influential variable in the analysis and is of particular importance for future restoration efforts on the main stem LCR. Much of the habitat from the LCR that was quantified is choked with exotic and/or invasive emergent species. There is a lack of crawl out and basking locations for

amphibians. Restoration project manager's efforts should consider altering water levels and other management techniques to control invasive plant encroachment.

While the habitat data support shallow side channels as conducive to lowland leopard frog presence, a study quantifying lowland leopard frog habitat in desert canyons of southeastern Arizona found large bodies of water supported more frogs (Wallace et al. 2010). The canyons of southeastern Arizona are under constant threat of drought and are frequently cut off from perennial water sources. Consequently, these arroyos and canyon pools may remain relatively free of predatory fishes and American bullfrogs, which are less adapted to periods of drought and flood (Sartorius and Rosen 2000). Lichtenburg et al. 2006 compared species richness and composition of anurans across different habitat types and found American bullfrogs correlated with permanent lakes and wetlands. In addition, the southern leopard frog (*Lithobates sphenocephalus*) was associated with herbaceous plant litter and low canopy cover, similar to the results of this study (Lichtenburg et al. 2006). These findings were similar to what was found throughout the study area: leopard frogs are associated with more open canopy and herbaceous vegetation, and American bullfrogs have invaded the permanent water bodies. Due to the lowland leopard frog's ability to live in diverse habitats, future studies should focus on the specific metapopulations found in particular habitat types. In addition, the pattern of boom and bust streamflow on the Bill Williams River supports landscape conservation measures for lowland leopard frogs. Frog population fluctuations may be a natural occurrence on the lower stretches of the Bill Williams River, and a larger drainage-wide assessment, monitoring, and conservation effort should be undertaken. The lack of research on lowland leopard frogs in riverine environments stresses the importance of the study and the need for future research along stretches of river that still support frog populations.

Colorado River toads, with the exception of the site on Planet Ranch, were located primarily in open, sandy desert habitat with little vegetation. Shrubs were the largest vegetation layers measured from the habitat points; this was the case due to toads often being observed sitting and foraging under creosote (*Larrea tridentate*) bushes on desert flats. Other than creosote, most of the vegetation measured in each plot was sporadic small clumps of grasses and forbs, with the area predominantly open sand. Sand has been the substrate variable in all toad locations, likely due to toads using mammal burrows in the sand as refuges during the day and cooler months (Lowe, personal observation).

RECOMMENDATIONS

Continue Habitat Use Study

More research should be conducted along the Bill Williams River to better understand this system, including further upstream. The populations of

amphibians on the Bill Williams River should be viewed as one large population instead of several small metapopulations associated with property boundaries. This study focused on amphibian breeding habitat, but virtually nothing is known about dispersal, post-breeding movements, or where they reside during drier months. A study related to these points would provide more information on yearly habitat use of the species as well how the animals colonize new habitats; these data would be vital for use in habitat improvement and reintroductions within the LCR.

Recommendations for future research include:

- Radio telemetry of individuals to determine microhabitat use as well as dispersal and post-breeding habitat use
- Population and distribution of amphibians throughout the Bill Williams River drainage
- Continued habitat quantification and monitoring
- Hibernacula and breeding habitat selection of Colorado River toads where they are found elsewhere in the species' range
- Habitat quantification at similar riverine/riparian areas where they are found elsewhere in the species' range to broaden our understanding of habitat characteristics these anurans are using
- Restoration of locations just downstream of known lowland leopard frog habitat to assist in their recovery from the main stem LCR

Impacts of Flooding

Another unknown is the effect of flooding on the two species. Lowland leopard frogs appear to have adapted better, with periodic droughts and floods then some introduced anurans (Sartorius and Rosen 2000). However, there have been no studies to determine how artificial flooding of the Bill Williams River from Alamo Dam management affects these species. Natural flooding created by monsoon rains may create breeding habitat for Colorado River toads (Brennan and Holycross 2006). This is the only known population of lowland leopard frogs and Colorado River toads within the greater lower Colorado River ecosystem, and water released from Alamo Dam could have significant impacts on their populations and distribution. Recommendations for future research include:

- Distribution of amphibians in months/years directly following floods on the Bill Williams River

- Radio telemetry of individuals before and after floods to determine movement and dispersal patterns

- A habitat study to compare habitat use post-flood to evaluate if the flooding increases the amount of suitable habitat

Periodic flooding from Alamo Dam could allow for dispersal of individuals to downstream locations on the Bill Williams River NWR. Flooding could also play a role in dispersion of the species in desert environments, as well as controlling invasive plant species.

Habitat Improvement and Monitoring

In expectation of future reintroductions for both species on the LCR, studies should be designed to investigate the methodology of translocation and habitat improvement, ensuring the highest probability of success. Projects aimed at better understanding these methods should include:

- Genetic analyses of amphibian populations to determine potential source populations for translocation

- GIS and field site visits to identify the best locations for restoration

- Experimental refugia at locations where habitat has been enhanced or restored

- Design and test various predator exclusion devices that will be used on the LCR to increase the success rate of translocations

- Monitoring techniques for evaluating the success of new populations

Information gained from these studies will improve the success rate of any major translocation or habitat improvement project on the LCR.

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ATTACHMENT 1

List of Species Codes and Scientific Names for Each Amphibian Species Identified in this Report Along with Accepted Common Names and Current Taxonomic Synonyms According to The Center for North American Herpetology

Species code	Scientific name	Common name	Synonym
BUAL	*Incilius alvarius*	Colorado River toad	*Bufo alvarius*
RAYA	*Lithobates yavapaiensis*	Lowland leopard frog	*Rana yavapaiensis*
RABE	*Lithobates berlandieri*	Rio Grande leopard frog	*Rana berlandieri*
RACA	*Lithobates catesbeianus*	American bullfrog	*Rana catesbeiana*
BUMI	*Anaxyrus microscaphus*	Arizona toad	*Bufo microscaphus*
BUPU	*Anaxyrus punctatus*	Red-spotted toad	*Bufo punctatus*
BUCO	*Anaxyrus cognatus*	Great Plains toad	*Bufo cognatus*
BUWO	*Anaxyrus woodhousii*	Woodhouse's toad	*Bufo woodhousii*
SCCO	*Scaphiopus couchii*	Couch's spadefoot toad	N/A
HYRE	*Hyla regilla*	Pacific treefrog	*Pseudacris regilla*

ATTACHMENT 2

List of Notations and Represented Covariates Used in Model Creation and the Gradient Analysis

Category	Covariate	Included in PCoA	Description
Abiotic	Minimum.Depth	X	Minimum depth of plot
Abiotic	Maximum.Depth	X	Maximum depth of plot
Abiotic	Discharge		Stream discharge measured in feet per second
Abiotic	H2O.Class		Either a Lentic or Lotic site
Abiotic	H2O.Type		Habitat type selected from marsh/wetland, riverine, lake/pond, or canal
Abiotic	Sand		If sand is present in substrate
Abiotic	Gravel		If gravel is present in substrate
Abiotic	Cobble		If cobble is present in substrate
Abiotic	Mud.Silt		If mud or silt is present in substrate
Biotic	Open.Terr.	X	Percent of plot that is without vegetation or water
Biotic	Open.Aqu.	X	Percent of plot that is without vegetation but inundated
Biotic	Total.Open	X	Total percent of plot without vegetation
Biotic	Wet	X	Percent of plot that is inundated
Biotic	Dry		Percent of plot out of water
Biotic	Em	X	Percent of plot covered by emergent vegetation
Biotic	Forb	X	Percent of plot covered by herbaceous vegetation other than grasses, rushes, and sedges
Biotic	Sub	X	Percent of plot covered by submerged vegetation
Biotic	Tree	X	Percent of plot covered by tree canopy
Biotic	Grass	X	Percent of plot covered by grass
Biotic	Floating	X	Percent of plot covered by floating vegetation
Biotic	Shrub	X	Percent of plot covered by shrub vegetation
Predators	Cambarids		Presence of crayfish
Predators	Micropterus		Presence of largemouth bass
Predators	Centrarchids		Presence of sunfish
Predators	RACA		Presence of *Lithobates catesbeianus*