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

2012 Annual Report

April 2013

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Lower Colorado River Multi-Species Conservation Program

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

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ACRONYMS AND ABBREVIATIONS

AGFD	Arizona Game and Fish Department
AIC_c	Akaike's Information Criterion
BLM	Bureau of Land Management
BWRNWR	Bill Williams River National Wildlife Refuge
cm	centimeter(s)
eDNA	environmental DNA
GIS	Geographic Information System
LCR	lower Colorado River
LCR MSCP	Lower Colorado River Multi-Species Conservation Program
m	meter(s)
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey

Symbols

%	percent

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ABSTRACT

The purpose of this project was to address data gaps relative to two native Arizona amphibians. The Lowland Leopard Frog (*Rana yavapaiensis*) and the Colorado River Toad (*Bufo alvarius*) are included in the Lower Colorado River Multi-Species Conservation Program's (LCR MSCP) (LCR MSCP 2004) list of evaluation species. According to Work Task D12 of the LCR MSCP, defining the gaps in range and distribution data for these two species is necessary to implement conservation needs. The study area (outlined by the LCR MSCP) encompassed Reaches 3–7 of the lower Colorado River (LCR) extending from Davis Dam south to the International Boundary. In addition to the Colorado River, we also surveyed the Bill Williams River from its confluence with the LCR to Bureau of Land Management land just east of Planet Ranch, including Reaches 7 and 8, because of reports of *B. alvarius* and *R. yavapaiensis* being observed in the area. Beginning in February 2012, we continued surveying according to protocols established for this project during the 2011 field season. Surveys included funnel trapping, dip netting, nocturnal spot lighting, and auditory call response surveys. During the 2012 field season, we surveyed regions of the study area that we had not visited during 2011 and areas with high quality habitat based on the 2011 assessment. We used a logistic regression modeling approach to compare habitat characteristics (i.e., biotic, abiotic, and predator covariates) at 21 sites where *R. yavapaiensis* were detected to random sites at two spatial scales – within Reaches 7 and 8 of the Bill Williams River (27 sites) and throughout the LCR study area (41 sites). We also marked five *B. alvarius* individuals at 14 sites within Reach 8 and evaluated habitat characteristics at capture locations.

INTRODUCTION

Arizona's native ranid frogs are declining throughout their historic ranges (Clarkson and Rorabaugh 1989; Sredl et al. 1997). The Lowland leopard frog (*Rana yavapaiensis*) has been thought to be extirpated from the lower Colorado River (LCR) since 1974, and subsequent surveys have reaffirmed this contention; however, individuals have been observed on the Bill Williams River as recently as 2010 (Vitt and Ohmart 1978; Clarkson and Rorabaugh 1989; Sredl et al. 1997; Kathleen Blair, U.S. Fish and Wildlife Service [USFWS], personal communicati*on*). *R. yavapaiensis* are typically found in pools associated with streams, springs, arroyos, and stock tanks usually near permanent water sources (Stebbins 2003). However, their range may be shrinking toward the most secluded streams and springs due to a suite of threats (e.g., introduced and invasive species, loss of habitat, habitat alteration, toxicants, pathogens, and parasites) (Degenhardt et al. 1996; Hayes and Jennings 1986; Stebbins 2003; Sredl 2005). During 2011, we were unable to locate *R. yavapaiensis* along the LCR or confirm any potential sightings on the Bill Williams River.

The Colorado River Toad (*Bufo alvarius*) is common throughout much of the Sonoran Desert, occupying a variety of habitats including mesquite-creosote flats, grasslands, and the pine oak juniper and deciduous montane communities (Stebbins 2003). There have been a handful of anecdotal reports and sightings of *B. alvarius* along the LCR and within our study area, and we did mark six individuals at one location on Planet Ranch during 2011. However, these individuals were located adjacent to manmade structures and artificial pools created by runoff from sprinkler systems and swimming pools, all of which has recently been destroyed when Planet Ranch changed ownership (Cotten 2011).

The range of *B. alvarius* may overlap that of *R. yavapaiensis;* however, evidence suggests threats to the toad species is primarily from urbanization and hydrological alterations of riparian habitat (Lovich et al. 2009). The lack of information regarding the current distribution of both *R. yavapaiensis* and *B. alvarius* along the LCR confounds employment of conservation measures for recovery. The objectives of this study were to gain a better understanding of the status of the two species, thereby aiding preliminary conservation steps. Specifically, those objectives were:

1. Locate actual and potential habitat for *R. yavapaiensis* and *B. alvarius* along the LCR

2. Determine distribution of *R. yavapaiensis* and *B. alvarius* within our study area

3. Collect genetic samples from *R. yavapaiensis* and *B. alvarius*

4. Determine habitat use for *R. yavapaiensis* and *B. alvarius* along the LCR

STUDY AREA

The study area included Reaches 3–7 of the LCR, extending from Davis Dam to the International Boundary (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 (figure 2). Planet Ranch was not surveyed during the 2012 season due to access issues. While we did perform initial site visits to areas north of the Havasu National Wildlife Refuge, the habitat contained little backwater or side channels with high human traffic. In addition, there have been no voucher specimens collected from this area for either species.

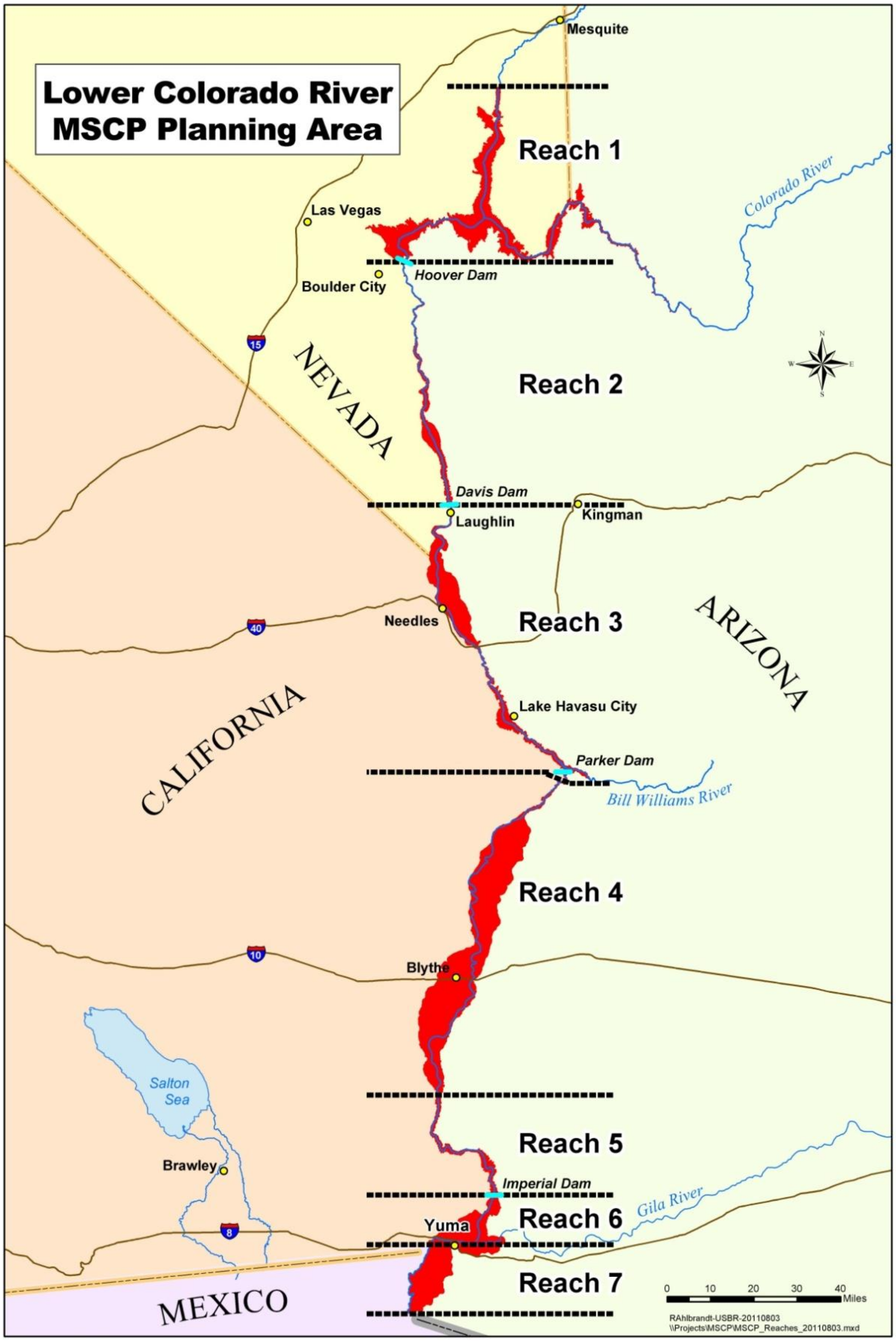

Figure 1.—Map of lower Colorado River with LCR MSCP land highlighted in red. The study area for this project consisted of Reaches 3–7.

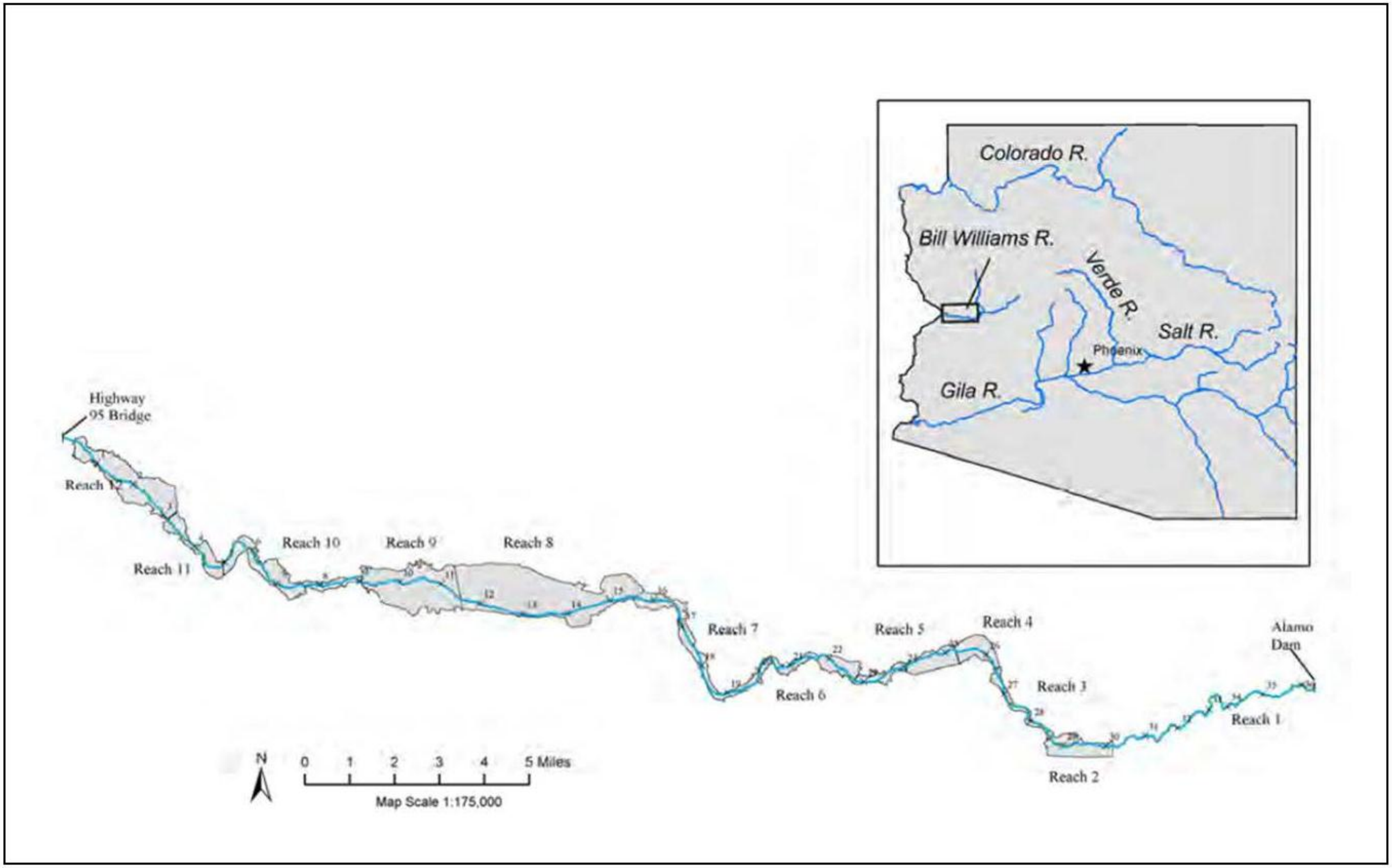

Figure 2.—Map of the Bill Williams River, Arizona, showing the reaches from the SR95 Bridge crossing and the approximate location of Planet Ranch outlined in red.
Source: Shafroth et al. 2004.

OBJECTIVE 1 – LOCATE POTENTIAL HABITAT FOR *RANA YAVAPAIENSIS* AND *BUFO ALVARIUS* 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 dry desert washes identified during field visits or based on historic and anecdotal evidence, were identified and systematically visited by Arizona Game and Fish Department (AGFD) biologists. In addition, we consulted collections and museums that contained specimens or records of amphibians collected from the study area to identify historic population localities. Field surveys were then conducted in areas with high concentrations of backwater. Both diurnal and nocturnal habitat surveys 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 predatory fishes and bullfrogs (*Rana catesbeiana*), 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 that was visited was ranked based on presence of predators, size, water quality and characteristics, and type of site (lentic, lotic, canal, etc.). Sites visited 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 further surveys (Cotten 2011).

During 2012, primary focus was given to sites not surveyed in 2011 and high quality habitat identified during the 2011 habitat analysis (Cotten 2011). These high quality areas contained native cottonwood/willow vegetation, shallow backwaters, and few introduced predators. Some sites that were surveyed in 2011 were revisited in 2012 due to reports of frog vocalizations and possible sightings in the area as well as a high likelihood of occupancy given suitable habitat quality (Kathleen Blair, USFWS, personal communication).

Results

In 2012, we identified 184 new locations throughout the study area that contained suitable habitat for amphibians and sufficient for further surveys. Combined with sites visited in 2011, we have identified 501 locations that were considered adequate for amphibian occupancy (figure 3). There were two major areas of focus in 2012: the Bill Williams River and Topock Gorge. Of the 184 locations, 56 were on BLM land on the Bill Williams River further east than any surveys or site visits performed during 2011. This stretch of the Bill Williams River was surveyed due to a high amount of pristine riparian habitat, its adjacency to Planet Ranch, and the number of amphibians observed in the area. Forty-four new locations were identified within Topock Gorge, south of the Interstate 40 Bridge, within the Havasu National Wildlife Refuge. The other 84 locations were scattered throughout where suitable habitat was identified.

Discussion

We focused 2012 effort on the areas that we had not visited and had the highest probability of supporting amphibians based on vegetation composition and predator abundance (primarily American bullfrog *[R. catesbeiana*]) and on areas that we had previously visited. Topock Gorge was identified as a focal point for 2012 since we had not been able to access it in 2011. Similar to the southern part of the study area around Imperial National Wildlife Refuge, there are large amounts of backwater in the gorge that elevated its potential for occupancy by our target species. Our 2011 GIS analysis revealed a high quantity of backwater within this stretch of river which, when combined with the site's remoteness, created high quality habitat potential for *R. yavapaiensis.* There are a large number of desert washes adjacent to the river in this area, including sections of sand dunes that are suitable for *B. alvarius.* Unfortunately, we were not able to adequately survey those areas at the time of summer monsoon rainfall due to the unpredictable nature of the rains and difficulty in accessing these areas. The desert washes, and specifically the sand dune areas off the water, are an area of interest for future surveys.

Similar to 2011, the Bill Williams River has the greatest potential for occupancy by *R. yavapaiensis* based on habitat suitability. The Bill Williams River National Wildlife Refuge (BWRNWR) still contains areas of natural riparian corridor with plant diversity and a lack of *R. catesbeiana*, but the water channel is well established and deep, allowing for predatory fish to establish throughout most of the refuge, which is detrimental to occupancy of native amphibians (Rosen et al. 1995; Kiesecker and Blaustein 1998). With the exception of a handful of locations, there are very few side channels or shallow backwaters within the refuge to provide habitat for *R. yavapaiensis*. The few locations that did have isolated shallow backwaters and side channels were re-surveyed several times in 2012 because they had the highest probability of *R. yavapaiensis* occupancy on the refuge. The water becomes more ephemeral and often percolates into the sand on Planet Ranch on the refuge's eastern boundary. This section had fewer predators and an abundance of small ephemeral pools ideal for amphibian

Figure 3.—Survey locations throughout the entire survey area from 2011 and 2012. Yellow triangles indicate where a visual or auditory survey was conducted or a funnel trap grid deployed.

breeding. Unfortunately, we were not granted access to Planet Ranch during the 2012 survey effort and were unable to survey farther along this reach. However, we did identify similar habitat conditions east of Planet Ranch on BLM land.

Here, instead of the water re-emerging as it did on Planet Ranch and the BWRNWR, it spreads and braids across the sandy riverbed before going underground near the eastern boundary of Planet Ranch. This section of the Bill Williams River is the best amphibian habitat we have identified within the study area (figure 4). The braided shallow channels found here create suitable amphibian breeding habitat while limiting predator numbers. High beaver (*Castor canadensis*) activity and periodic flooding maintain fluctuating water levels and pathways, which has limited colonization of salt cedar (*Tamarix* spp.) and promoted growth of native wetland vegetation. In addition, no *R. catesbeinana* were detected in this section or any surveyed section of the Bill Williams River.

Figure 4.—Example of *R. yavapaiensis* habitat where both adults and tadpoles were found (encountered along the Bill Williams River east of Planet Ranch).

OBJECTIVE 2 – DETERMINE DISTRIBUTION OF *RANA YAVAPAIENSIS* AND *BUFO ALVARIUS* WITHIN OUR STUDY AREA

Methods

We initiated amphibian surveys on February 14, 2012, in conjunction with the anticipated start of *R. yavapaiensis* breeding behavior (Brennan and Holycross 2006). Surveys continued through August 14, 2012, to overlap with the summer monsoon season triggering *B. alvarius* movements and breeding (Brennan and Holycross 2006). Initial sampling efforts began at permanent lentic and lotic locations primarily for the presence of *R. yavapaiensis*, but beginning with summer monsoon activity, broadened to include dry desert washes, arroyos, or other areas identified as potential *B. alvarius* habitat. We used three techniques in our surveys: funnel trap arrays, visual surveys, and nocturnal aural surveys. Visual and nocturnal audio surveys were performed at least once at all funnel trap grid locations.

Funnel Trap Arrays

Six grids of up to 10 inverted conical wire mesh funnel traps were deployed at sites identified as containing suitable potential habitat for *R. yavapaiensis*. Individual traps were wired to emergent or bank vegetation and placed along high traffic corridors for aquatic fauna. The traps were submerged so that the entrance of the funnel 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 5). Traps were deployed for a minimum of 24 hours and checked at least once within the 24-hour period. All amphibians and non-target animals captured during this effort were identified to species and data including the date, time, and location were recorded (Heyer et al. 1994; Olson et al. 1997).

Visual Surveys

Visual surveys were conducted based on techniques outlined by Heyer et al. (1994). We began by scanning the banks and shorelines with binoculars during the daytime to detect amphibians floating in the water, basking on the banks, or hiding within the aquatic vegetation. D-ring dip nets were used to sample the littoral zone for amphibian larvae. When possible, we surveyed along the entire perimeter of the survey site, searching under logs and rocks, as well as in the vegetation, watching for adult amphibians to flush. We also used large dip nets to search under ledges and within submergent vegetation for hidden adults. Any amphibians encountered were identified to species and recorded as described earlier.

Figure 5.—Funnel trap properly deployed at Havasu National Wildlife Refuge.

We also conducted nocturnal surveys at each site to improve detection rates. 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 Aural Surveys

Beginning approximately 30 minutes after sunset, we began listening and recording male amphibian vocalizations at each survey site using an aural survey methodology based on the U.S. Geological Survey (USGS) North American Amphibian Monitoring Protocol (http://www.pwrc.usgs.gov/naamp/index.cfm). This approach started with a passive listening period of 10 minutes. We then used a portable audio system to broadcast either a *R. yavapaiensis* or *B. alvarius* male advertisement breeding calls for a minimum of 30 seconds to elicit male responses. All vocalizing amphibians were identified to species, and an estimate of numbers of individuals calling and observed was recorded. Covariates including 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, were recorded. Any target species, or potential target species, was captured for proper identification.

Results

We deployed funnel traps at 93 different sites for a total of 22,320 trap hours. Combined with the 2011 effort, we have trapped at 194 locations and accumulated over 77,000 trap hours. Visual and dip net surveys were conducted at 93 locations, and approximately 55 hours of nocturnal call back surveys were completed. Combined with the 2011 effort, we have performed over 180 hours of nocturnal call back surveys and over 600 hours of visual encounter and dip net surveys. A total of 10 species of amphibians were captured or observed during the surveys (table 1). A population of *R. yavapaiensis* was detected along the Bill Williams River on property managed by BLM east of the Planet Ranch boundary (figures 6–7). We marked 12 individuals from 10 different locations, but many more individuals were observed. We also observed three instances of amplexus (i.e., breeding behavior) and three individual egg masses throughout this section of the study area. Beginning in June, *R. yavapaiensis* tadpoles were observed along this stretch of river, indicating a successful breeding population.

Five adult *B. alvarius* were found and marked from the sandy flats adjacent to the stretch of the Bill Williams River where the *R. yavapaiensis* population was observed, with seven additional toads observed and not marked. We also identified two individual toads in the monsoon-swollen waters of the Bill Williams River before it lost surface flow downstream. While no *B. alvarius* tadpoles were observed and no male vocalizations were heard, we did observe one male *B. alvarius* moving several meters away from the water with freshly deposited eggs stranded on its back, suggesting breeding behavior was taking place. Combined with the 2011 toads, we have marked 11 *B. alvarius* individuals from both the BLM locations and the one location on Planet Ranch. We were unable to verify if the site on Planet Ranch was still occupied due to access issues

Table 1.—Summary of 2011 and 2012 findings from funnel trap captures, auditory surveys, and dip net visual encounter surveys
Species codes: BUAL (*Bufo alvarius*), RAYA (*Rana yavapaiensis*), *RABE* (*Rana berlandieri*), BUWO (*Bufo woodhousii*), BUCO (*Bufo cognatus*), BUPU (*Bufo punctatus*), BUMI (*Bufo microscaphus*), SCCO (*Scaphiopus couchii*), HYRE (*Hyla regilla*), RACA (*Rana catesbeiana*)[1]

Site	BUAL	RAYA	RABE	BUWO	BUCO	BUPU	BUMI	SCCO	HYRE	RACA	Bass	Crawfish
BWRNWR					X	X	X				X	X
Havasu National Wildlife Refuge				X	X				X	X	X	X
Cibola National Wildlife Refuge				X	X					X	X	X
‘Ahakhav Tribal Preserve				X	X					X	X	X
Mittry/Imperial National Wildlife Refuge			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

[1] Attachment 1 lists all species codes, common and scientific names, and synonyms.

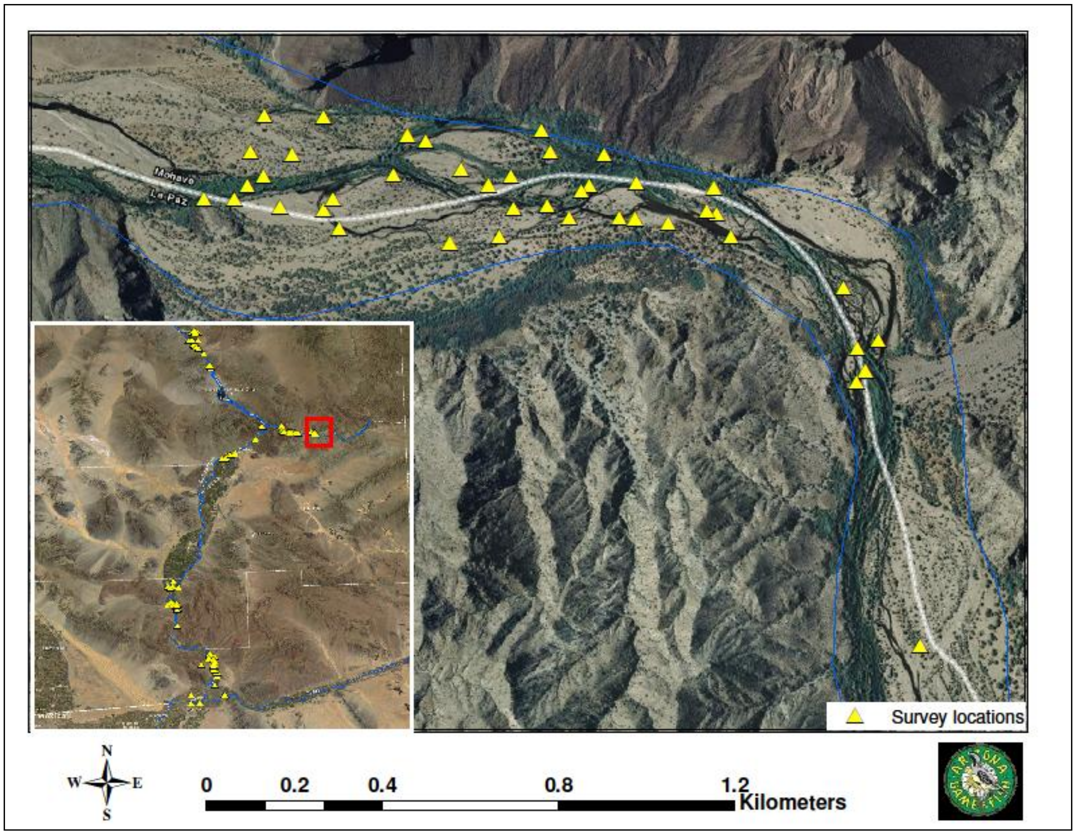

Figure 6.—Survey locations on the Bill Williams River upstream of Planet Ranch. Yellow triangles indicate survey locations.

with the landowners. In addition, we were unable to confirm or record the locations of adult leopard frogs that were observed near the border between Planet Ranch and the BWRNWR.

Discussion

We identified the Bill Williams River as the best location to find *R. yavapaiensis* within our study area. The population that was found east of Planet Ranch appeared to be a large and viable population due to a high abundance of adults and tadpoles observed during surveys. We terminated surveys near the Reach 7 boundary of the river due to access issues, but our survey results suggest that frogs will be just as abundant further upstream. There is an approximately 5-kilometer gap in surface water between the section of BLM land where we have found frogs and the BWRNWR downstream where we have not found *R. yavapaiensis* over the course of 2 survey seasons. However, with the periodic

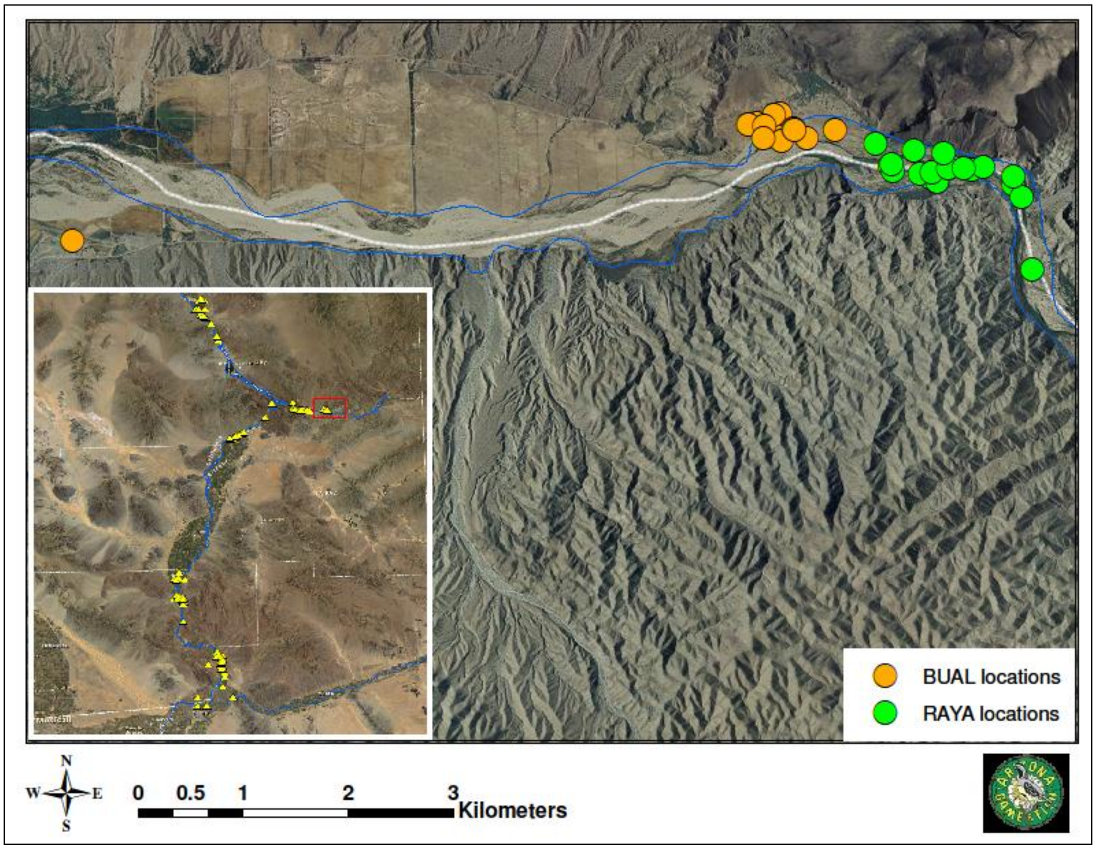

Figure 7.—Locations on the Bill Williams River where *Bufo alvarius* (BUAL) and *Rana yavapaiensis* (RAYA) were observed during the 2011 and 2012 field seasons. Green circles indicate *R. yavapaiensis* locations, while orange circles identify *B. alvarius* locations.

flooding of Alamo Dam, such as in 2010 (USGS Surface-Water Daily Data for Arizona), this dry corridor could support adequate water for dispersing individuals to make it to the lower sections of the Bill Williams River, which could account for the periodic sightings on the refuge. In addition, the only other area we have observed within the study area that looks analogous to the area supporting *R. yavapaiensis* is where the surface water reappears on Planet Ranch, just east of the BWRNWR boundary. We did search this area in 2011, but after the Lowland leopard frog breeding season, and we did not have an opportunity to trap the area in 2011. Adult Lowland leopard frogs were observed along the Planet Ranch border with the BWRNWR. However, due to access issues in 2012, we were unable to confirm the frogs or measure their habitat use, nor were we able to deploy funnel traps or perform surveys.

The sandy desert flats around the Bill Williams River upstream of Planet Ranch supported scattered *B. alvarius* adults. It is reasonable to assume that the individuals that were marked on Planet Ranch in 2011 were part of this same population, and further surveys could result in identifying even greater numbers of individuals occupying the sandy riverbed around the Bill Williams River. Even though we did not observe breeding behavior with this species, numbers suggest breeding is occurring, most likely during heavy summer rain events.

Within the BWRNWR 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 in both 2011 and 2012, and there were no detections of either target species. These isolated ponds on wide sandy sections of riverbed are still the best locations away from Planet Ranch that we have identified that could support additional populations of both *R. yavapaiensis* and *B. alvarius* within the study area.

The summer monsoon in 2012 produced a large amount of rain in sections of the study area and resulted in isolated flooding in certain washes along the LCR. We kept track of these rain events and tried to survey as many locations as we could in which there appeared to be adequate rainfall to support breeding behavior. We did not observe *B. alvarius* during any of these flood events even though amphibians were breeding in these swollen washes after the rainfall occurred. In particular, high numbers of couch's spadefood (*Scaphiopus couchii)* were observed using these temporary water sources.

OBJECTIVE 3 – COLLECT GENETIC SAMPLES FROM *RANA YAVAPAIENSIS* AND *BUFO ALVARIUS*

Methods

We followed the AGFD non-destructive protocol for collecting genetic samples from amphibians. 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-percent (%) ethanol solution. The wounds were disinfected, and all animals were successfully released after being monitored for several minutes. Using 95% ethanol, all equipment was sterilized after each use. Samples will be stored in the ethanol-filled vials for future analysis.

Results

During the 2011 and 2012 field seasons, we successfully collected tissue samples from 12 *R. yavapaiensis* adults and 11 *B. alvarius* adults. Samples were collected from a single digit corresponding with the individual's identification number (i.e., TC1). Each adult was monitored and released the same night. The numbers of tissues collected do not accurately estimate the large number of individual anurans actually observed during surveys; we discontinued tissue collection once an adequate sample size was collected.

OBJECTIVE 4 – DETERMINE HABITAT SELECTION FOR *RANA YAVAPAIENSIS* AND *BUFO ALVARIUS* ALONG THE LOWER COLORADO RIVER

Methods

We evaluated *R. yavapaiensis* habitat selection by comparing habitat characteristics within three habitat covariate categories (i.e., biotic, abiotic, and predator covariates; table 2) at used sites to random sites within the LCR and Bill Williams Rivers. Habitat selection analyses for *R. yavapaiensis* 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 LCR and Bill Williams River) (see attachment 2).

We used a 10-meter (m) radius plot as our sample unit. We returned to the sites within 3 days of detecting either target species and quantified habitat characteristics at the used site and at least one randomly selected available site where target species were not encountered within a 300-m radius and within the riparian corridor. Locally, 27 non-sites were identified to correspond with the 21 locations containing Lowland leopard frogs (figure 8). Non-sites were identified by randomly selecting an azimuth and distance within suitable habitat. Each non-site was also surveyed and trapped to ensure Lowland leopard frogs were not utilizing the site. Additionally, we randomly selected 42 locations from the entire study area where we had previously performed surveys without finding target species and quantified those locations using the following methodology.

For aquatic habitats, minimum and maximum water depth, substrate type (e.g., gravel, sand), water temperature, pH, turbidity, and stream discharge (lotic habitats only) within the 10-m radius plot were recorded. We measured vegetation composition and density 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. We recorded any coarse woody debris that was ≥ 3 m in length and ≥ 10 centimeters (cm) in diameter. We also recorded the distance to the nearest water source and the type of water source (e.g., pond, stream). The same data were collected for the 13 locations where we observed *B. alvarius*; however, due to the small sample size and uniform nature of the microhabitat the toads were found in, we did not measure any non-sites as we did with *R. yavapaiensis*. Instead, we provided a descriptive account of the habitat within which *B. alvarius* were detected.

Table 2.—Local abiotic candidate models used to evaluate habitat use by *R. yavapaiensis* within Reaches 7 and 8 of the Bill Williams River

Model number	Model	AIC_c	ΔAIC_c	Log(L)	AIC_c weight	K
3	Maximum depth + Minimum depth	40.65	0	-17.32	0.95	3
15	Sand + Mud/silt + Gravel + Cobble + H_2O class + H_2O type + Discharge + Maximum depth + Minimum depth	47.64	6.99	-12.82	0.02	10
2	Minimum depth	49.8	9.15	-22.9	0.01	2
5	Discharge + Maximum depth	50.36	9.71	-22.18	0.01	3
1	Maximum depth	50.58	9.93	-23.29	0.01	2
6	Discharge + H_2O class	59.85	19.2	-26.93	> 0.01	3
4	Discharge	62.89	22.24	-29.44	> 0.01	2
10	Sand	66.37	25.72	-31.19	> 0.01	2
7	Discharge + H_2O type	66.72	26.07	-31.36	> 0.01	3
12	Gravel	68.16	27.51	-31.08	> 0.01	2
9	H_2O class	69.12	28.47	-32.55	> 0.01	2
8	H_2O type	69.68	29.03	-32.84	> 0.01	2
11	Cobble	69.76	29.11	-32.88	> 0.01	2
13	Sand + Cobble	71.1	30.45	-32.53	> 0.01	3
14	Sand + Mud/silt	71.67	31.02	-32.84	> 0.01	3

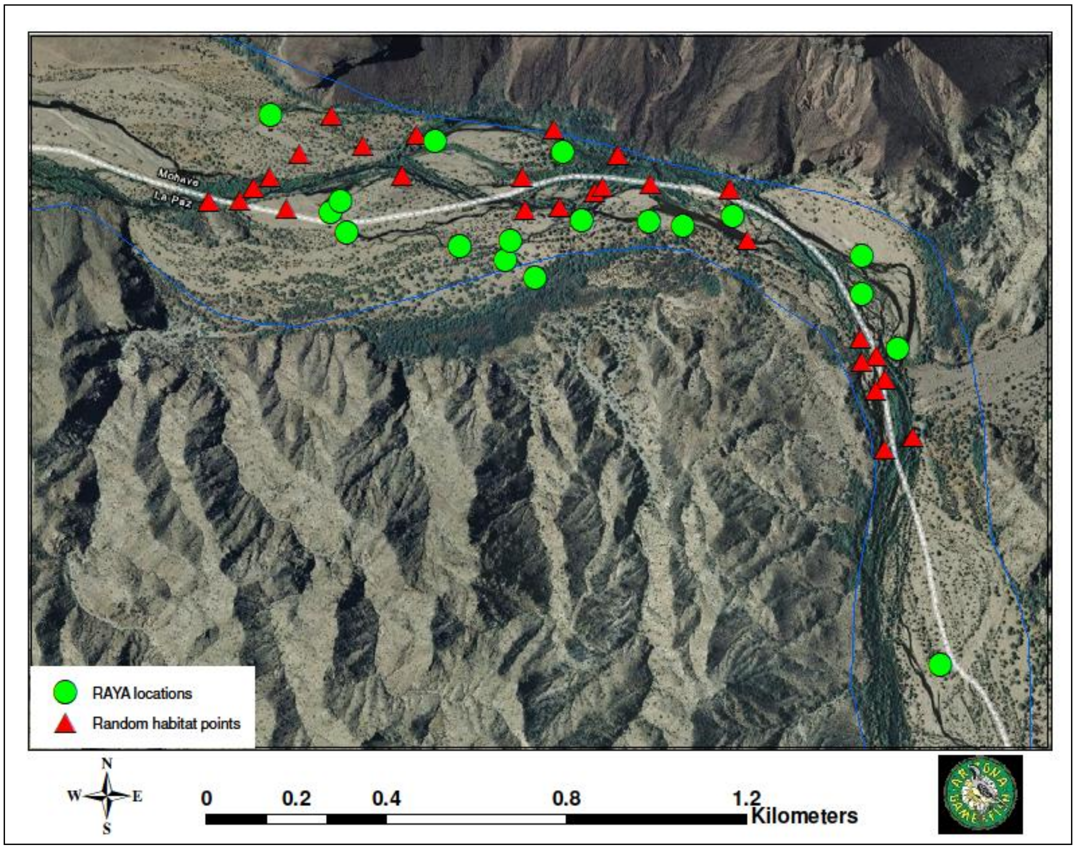

Figure 8.—Locations on the Bill Williams River where habitat variables were measured.
Green circles indicate *R. yavapaiensis* locations while red triangles identify locations where *R. yavapaiensis* were not observed.

Analysis

We first evaluated correlations among continuous covariates using Pearson correlation coefficients (Pearson 1920; Zar 1999). We did not include covariates in which correlation coefficient values $|r| \geq 0.50$ ($P < 0.05$) were in the same statistical model to avoid multicollinearity (Glanz and Slinker 1990; Graham 2003). We compared used resources to available resources using logistic regression models in which the binomial response represented detection of the target species or non-detection. We constructed logistic regression models to describe hypotheses regarding habitat selection by *R. yavapaiensis*. This process was performed individually for both the local and regional scale habitat data.

Our analysis followed a three-phased approach in which habitat models were created for each of the three covariate categories individually (see table 2). We compared models within each covariate category under a model selection framework (Burnham and Anderson 2002). We used 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) to rank candidate models within a covariate category. AIC_c difference (ΔAIC_c) and Akaike weight (w_i;) (Buckland et al. 1997) were calculated for each model to assess model uncertainty and the likelihood of each model given the data. We considered models with $\Delta AIC_c \leq 2$ to be well supported by the data (Burnham and Anderson 2002). 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 combining covariates from all three categories.

We assessed the fit of the global model (the model containing all of the supported covariate combinations) using the Hosmer and Lemeshow (2000) goodness-of-fit test. Overdispersion of the global model was examined using the variance inflation factor ($\hat{c}$), which is calculated by dividing the Hosmer and Lemeshow goodness-of-fit statistic by its degrees of freedom (Burnham and Anderson 2002). We accounted for model selection uncertainty by calculating unconditional parameter and variance estimates for each parameter across the set of supported models and then determined the 95% confidence intervals and odds ratio for each parameter (Burnham and Anderson 2002).

Results

Local-Scale Habitat Selection

We developed and compared 15 models using the abiotic covariates (see table 2). The best fitting model for the abiotic covariates included two covariates: the maximum and minimum water depth within the 10-m plot. This model had an Akaike weight of 0.95, and no other model had a $\Delta AIC_c \leq 2$. The second most well-supported model had a ΔAIC_c of 6.99.

We developed and compared 23 models using the biotic covariates (table 3). Four models had a $\Delta AIC_c \leq 2$. The four best fitting models for the biotic covariates included emergent and grass cover, substrate cover, and total unvegetated area within the 10-m plot as well as the global model containing all covariates measured. The model with the highest Akaike weight was the global model, with an Akaike weight of 0.24.

We developed and compared five models using the predator covariates (table 4). Four models had a $\Delta AIC_c \leq 2$. The four best fitting models for the biotic covariates included the presence of Cambarids, Centrarchids, and Centrarchids with Cambarids as well as the global model containing Micropterus, Cambarids, and Centrarchids. The model with the highest Akaike weight was Centrarchids and Cambarids, with an Akaike weight of 0.267.

Table 3.—Local biotic candidate models used to evaluate habitat use by *R. yavapaiensis* within Reaches 7 and 8 of the Bill Williams River

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	0.24	13
24	Sub	56.12	0.23	-26.06	0.21	2
32	Em + Grass	57.02	1.13	-25.51	0.14	3
19	Total open	57.28	1.39	-26.64	0.12	2
26	Open terr. + Em	57.9	2.01	-25.95	0.09	3
17	Open terr.	58.37	2.48	-27.18	0.07	2
35	Forb + Open terr.	58.97	3.08	-26.49	0.05	3
20	Em	60.43	3.15	-28.22	0.02	2
34	Em + Shrub	61.17	5.28	-27.58	0.02	3
31	Em + Forb	61.26	5.37	-27.63	0.02	3
27	Open aqu. + Em	62.31	6.42	-28.16	> 0.01	3
28	Dry	64.76	8.87	-30.38	> 0.01	2
22	Tree	65.02	9.13	-30.51	> 0.01	2
37	Forb + Tree	65.95	10.06	-29.98	> 0.01	3
21	Forb	67.49	11.6	-31.74	> 0.01	2
23	Grass	67.67	11.78	-31.83	> 0.01	2
30	Forb + Grass	67.74	11.85	-30.87	> 0.01	3
29	Wet	67.76	11.87	-30.38	> 0.01	2
25	Floating	68.12	12.23	-32.05	> 0.01	2
36	Forb + Open aqu.	68.24	12.35	-31.12	> 0.01	3
18	Open aqu.	68.58	12.69	-32.29	> 0.01	2
38	Forb + Shrub	69.05	13.16	-31.52	> 0.01	3
33	Shrub	69.42	13.53	-32.71	> 0.01	2

Table 4.—Local predator candidate models used to evaluate habitat use by *R. yavapaiensis* within Reaches 7 and 8 of the Bill Williams River

Model number	Model	AIC_c	ΔAIC_c	Log(L)	AIC_c weight	K
43	Centrarchids	67.42	0	-31.71	0.27	2
44	Centrarchids + Cambarids	67.42	0	-30.71	0.27	3
42	Cambarids	67.43	0.01	-31.71	0.27	2
45	Micropterus + Centrarchids + Cambarids	69.16	1.74	-30.58	0.11	4
41	Micropterus	69.62	2.2	-32.81	0.09	2

We developed and compared 20 models using the best fit models from the three covariate categories within the local habitat criteria (table 5). Five models had a $\Delta AIC_c \leq 2$. The five best fitting models for the local habitat scale included: (1) Maximum depth + Minimum depth + Emergent cover + Grass cover + Cambarids, (2) Maximum depth + Minimum depth + Emergent cover + Grass cover + Centrarchids, (3) Maximum depth + Minimum depth + Unvegetated open area + Cambarids, (4) Maximum depth + Minimum depth + Unvegetated open area + Centrarchids, and (5) Maximum depth + Minimum depth + Unvegetated open area + Centrarchids + Cambarids. The highest Akaike weight was 0.27. The approximate 95% confidence intervals for odds ratio showed only maximum depth and emergent vegetation cover affect the probability of frog occurrence. Both decrease the probability of finding a Lowland leopard frog (table 6).

Regional-Scale Habitat Selection

We developed and compared 17 models using the abiotic covariates (table 7). Four models had a $\Delta AIC_c \leq 2$. The four best fitting models for the abiotic covariates included: (1) Maximum depth, (2) Maximum depth + Minimum depth, (3) Discharge + Maximum depth, and (4) the global model within the 10-m plot. This best fitting model had an Akaike weight of 0.32.

We developed and compared 24 models using the biotic covariates (table 8). The best fitting model for the biotic covariates included two covariates, the percentage of cover by forbs and the percent of dry ground without vegetation within the 10-m plot. This model had an Akaike weight of 0.83, and no other model had a $\Delta AIC_c \leq 2$. The second most well-supported model had a ΔAIC_c of 6.34. The absolute value of the parameter estimates was lower than the standard error for all covariates except Open terr.

Table 5.—Local combined candidate models used to evaluate habitat use by *R. yavapaiensis* within Reaches 7 and 8 of the Bill Williams River

Model number	Model	AIC_c	ΔAIC_c	Log(L)	AIC_c weight	K	Did not converge
46	Maximum depth + Minimum depth + Wet + Dry + Open aqu. + Open terr. + Total open + Em + Sub + Shrub + Tree + Grass + Forb + Floating + Cambarids	NA	NA	NA	NA	16	X
47	Maximum depth + Minimum Depth + Wet + Dry + Open aqu. + Open terr. + Total open + Em + Sub + Shrub + Tree + Grass + Forb + Floating + Centrarchids	NA	NA	NA	NA	16	X
48	Maximum depth + Minimum depth + Wet + Dry + Open aqu. + Open terr. + Total open + Em + Sub + Shrub + Tree + Grass + Forb + Floating + Cambarids + Centrarchids	NA	NA	NA	NA	17	X
49	Maximum depth + Minimum depth + Wet + Dry + Open aqu. + Open terr. + Total open + Em + Sub + Shrub + Tree + Grass + Forb + Floating + Cambarids + Micropterus	NA	NA	NA	NA	17	X
58	Maximum depth + Minimum depth + Sub + Cambarids	NA	NA	NA	NA	5	X
62	Maximum depth + Minimum depth + Total open + Cambarids	32.37	0	-11.19	0.27	5	
63	Maximum depth + Minimum depth + Total open + Centrarchids	32.57	0.2	-11.28	0.25	4	
50	Maximum depth + Minimum depth + Em + Grass + Cambarids	34.07	1.7	-11.04	0.12	6	
64	Maximum depth + Minimum depth + Total open + Centrarchids + Cambarids	34.27	1.9	-11.14	0.12	6	
51	Maximum depth + Minimum depth + Em + Grass + Centrarchids	34.28	1.91	-11.14	0.11	6	
59	Maximum depth + Minimum depth + Sub + Centrarchids	35.94	3.57	-12.97	0.05	5	
52	Maximum depth + Minimum depth + Em + Grass + Centrarchids + Cambarids	36.06	3.69	-11.03	0.04	7	
53	Maximum depth + Minimum depth + Em + Grass + Centrarchids + Cambarids + Micropterus	37.66	5.29	-10.83	0.02	8	
60	Maximum depth + Minimum depth + Sub + Centrarchids + Cambarids	37.91	5.54	-12.95	0.02	6	
61	Maximum depth + Minimum depth + Sub + Centrarchids + Cambarids + Micropterus	39.01	6.64	-12.51	> 0.01	6	
55	Maximum depth + Minimum depth + Open terr. + Centrarchids	40.51	8.14	-15.25	> 0.01	5	
54	Maximum depth + Minimum depth + Open terr. + Cambarids	40.78	8.41	-15.34	> 0.01	5	
56	Maximum depth + Minimum depth + Open terr. + Centrarchids + Cambarids	42.29	9.92	-15.14	> 0.01	6	
57	Maximum depth + Minimum depth + Open terr. + Centrarchids + Cambarids + Micropterus	43.66	11.29	-14.83	> 0.01	6	

Table 6.—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.

Parameter	Unconditional parameter estimate	Unconditional standard error	Odds ratio	95% LCL	95%UCL
Maximum depth	-0.0978	0.0415	0.91	0.836	0.984
Minimum depth	-0.3492	0.2337	0.71	0.446	1.115
Em	-0.0776	0.0337	0.93	0.866	0.989
Grass	-0.059	0.0526	0.94	0.85	1.045
Cambarids	-0.7232	1.5464	0.49	0.023	10.052

We developed and compared seven models using the predator covariates (table 9). Two models had a $\Delta AIC_c \leq 2$. The two best fitting models for the biotic covariates included the presence of (1) RACA and (2) RACA + Micropterus + Cambarids + Centrarchids. The model with the highest Akaike weight was RACA + Micropterus + Cambarids + Centrarchids with an Akaike weight of 0.57.

We developed and compared six models using the best fit models from the three covariate categories within the local habitat criteria (table 10). The best fitting model for the biotic covariates included the covariates Discharge + Maximum depth + Forb + Open terr. within the 10-m plot. The highest model Akaike weight was 0.76. The second most well- supported model had a ΔAIC_c of 2.99. The absolute value of the parameter estimates was larger for every covariant except for Open terr. The approximate 95% confidence intervals for the odds ratio indicate only maximum depth affects the probability of frog occurrence. Maximum depth decreases the probability of finding a Lowland leopard frog (table 11).

Due to low sample size and uniformity of the habitat where the toads were found, logistic regression and model selection procedures were not performed for *B. alvarius*. Habitat was measured at 15 locations where *B. alvarius* 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 cm and the minimum was 1 cm. The substrate consisted of sand, gravel, and mud/silt. *B. alvarius* 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. The other 14 locations where we detected *B. alvarius* were situated away from permanent water sources with a mean distance to water of 218 m, ranging from 140 to 353 m. All toad detections in 2011 were near small puddles, but these water sources were created primarily from water use by the surrounding buildings and

Table 7.—Regional abiotic candidate models used to evaluate habitat use by *R. yavapaiensis* within Reaches 7 and 8 of the Bill Williams River

Model number	Model	AIC_c	ΔAIC_c	Log(L)	AIC_c weight	K
2	Maximum depth	34.56	0	-15.28	0.32	2
5	Discharge + Maximum depth	34.58	0.02	-14.29	0.33	3
3	Maximum depth + Minimum depth	35.64	1.08	-14.82	0.19	3
17	Sand + Mud/silt + Gravel + Cobble + H_2O class + H_2O type	36.5	1.94	-5.23	0.13	10
13	Mud/silt	41.29	6.73	-18.64	0.02	2
16	Sand + Mud/silt	42.79	8.23	-18.39	> 0.01	3
1	Minimum depth	57.18	22.62	-26.59	> 0.01	2
8	H_2O type	76.14	41.58	-33.07	> 0.01	2
7	Discharge + H_2O type	76.44	41.88	-32.22	> 0.01	3
12	Gravel	82.16	47.6	-39.08	> 0.01	2
4	Discharge	83.14	48.58	-39.57	> 0.01	2
9	H_2O class	83.15	48.59	-39.58	> 0.01	2
6	Discharge + H_2O class	83.58	49.02	-38.79	> 0.01	3
11	Cobble	83.58	49.02	-39.79	> 0.01	2
14	Sand + Gravel	84.15	49.59	-39.08	> 0.01	3
10	Sand	84.2	49.64	-40.1	> 0.01	2
15	Sand + Cobble	85.57	51.01	-39.78	> 0.01	3

Table 8.—Regional biotic candidate models used to evaluate habitat use by *R. yavapaiensis* within Reaches 7 and 8 of the Bill Williams River

Model number	Model	AIC_c	ΔAIC_c	Log(L)	AIC_c weight	K
37	Forb + Open terr.	61.17	0	-27.59	0.83	3
23	Forb	67.51	6.34	-31.75	0.03	2
39	Forb + Tree	67.56	6.39	-30.78	0.03	3
38	Forb + Open aqu.	67.91	6.74	-30.95	0.02	3
33	Em + Forb	68.82	7.65	-31.41	0.02	3
40	Forb + Shrub	69.4	8.23	-31.7	0.01	3
32	Forb + Grass	69.48	8.31	-31.74	0.01	3
19	Open terr.	69.83	8.66	-32.91	0.01	2
41	Wet + Dry + Open aqu. + Open terr. + Total open + Em + Sub + Shrub + Tree	71.12	9.95	-24.56	> 0.01	13
28	Open terr. + Em	71.68	10.51	-32.84	> 0.02	3
30	Dry	77.4	16.23	-36.7	> 0.00	2
31	Wet	77.4	16.23	-36.7	> 0.01	2
29	Open aqu. + Em	78.32	17.15	-36.16	> 0.01	3
20	Open aqu.	80.54	19.37	-38.27	> 0.01	2
24	Tree	81.35	20.18	-38.67	> 0.01	2
21	Total open	81.45	20.28	-38.72	> 0.01	2
22	Em	81.88	20.71	-38.93	> 0.01	2
36	Em + Shrub	83.36	22.19	-38.68	> 0.01	3
27	Floating	83.56	22.39	-39.78	> 0.01	2
34	Em + Grass	83.56	22.39	-38.93	> 0.01	3
26	Sub	83.6	22.43	-39.8	> 0.01	2
25	Grass	84.04	22.87	-40.02	> 0.01	2
35	Shrub	84.2	23.03	-40.1	> 0.01	2

Table 9.—Regional predator candidate models used to evaluate habitat use by *R. yavapaiensis* within Reaches 7 and 8 of the Bill Williams River

Model number	Model	AIC_c	ΔAIC_c	Log(L)	AIC_c weight	K
48	RACA + Micropterus + Cambarids + Centrarchids	60.42	0	-25.21	0.57	2
45	RACA	62.22	1.8	-29.11	0.23	2
46	RACA + Micropterus	63.84	3.42	-28.92	0.13	2
47	RACA + Cambarids	64.11	3.69	-29.06	0.09	2
44	Centrarchids	68.62	8.2	-32.31	> 0.01	2
43	Cambarids	82.96	22.54	-39.48	> 0.01	2
42	Micropterus	84.2	23.78	-40.1	> 0.01	2

Table 10.—Regional combined candidate models used to evaluate habitat use by *R. yavapaiensis* within Reaches 7 and 8 of the Bill Williams River

Mod. number	Model	AIC_c	ΔAIC_c	Log(L)	AIC_c weight	K	Did not converge
51	Discharge + Maximum depth + Forb + Open terr.	31.53	0	-10.77	0.76	5	
49	Maximum depth + Forb + Open terr.	34.52	2.99	-13.26	0.17	4	
50	Maximum depth + Minimum depth + Forb + open terr.	36.4	4.87	-13.2	0.07	5	
48	RACA + Micropterus + Cambarids + Centrarchids	60.42	28.89	-25.21	> 0.01	5	
45	RACA	62.22	30.69	-29.11	> 0.01	2	
52	Sand + Mud/silt + Gravel + Cobble + H_2O class + H_2O type + Discharge + Maximum depth + Minimum depth + Forb + Open terr.	NA	NA	NA	NA	11	X

Table 11.—Parameter estimates, standard error, odds ratio, and 95% confidence intervals for the most well-supported regional combined model

Model #50					
Parameter	**Estimate**	**Standard error**	**Odds ratio**	**95% LCL**	**95% UCL**
Maximum depth	-0.1041	0.04025	0.90	0.833	0.975
Discharge	-1.08458	0.64609	0.34	0.095	1.199
Forb	0.71265	0.41603	2.04	0.902	4.609
Open terr.	0.02346	0.03084	1.02	0.964	1.088

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 m plot (table 12).

Discussion

The models with the least loss of data for the local scale habitat analysis included the parameters 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 and estimates are high due to a lack of data. Of the 48 locations quantified, Centrarchids were only present at two (see table 4).

There is very little research on the habitat requirements for *R. yavapaiensis* and virtually no peer-reviewed data on the species in a riverine system, but based on the estimates and standard error (see table 6), these models describe a shallow water habitat with a high percentage of open space and low percentage of vegetation cover. Deep water allows for the presence of large predatory fishes to invade amphibian habitat which, in addition to crayfish, have been shown to be detrimental to amphibians (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). While both Cambarids and Centrarchids were captured in plots with amphibians, there was a higher incidence of crayfish or amphibians being observed separately then their co-occurrence, in confluence with the earlier studies.

The study area for the local habitat analysis supported a high density of locations where Lowland leopard frogs were observed (see figure 8); with regular flooding and the influence of *Castor* sp., locations in which we did not locate frogs during this study could become suitable in the future. This section of the Bill Williams River is without a doubt the best habitat we have found in the study area. The abundance of frog locations identified is testament to the high quality habitat. In addition to the presence of *R. yavapaiensis* and *B. alvarius* we captured 10 Northern Mexican garter snakes (*Thamnophis eques*) in funnel traps. This species is in decline in Arizona and a candidate species for listing under the Endangered Species Act (USFWS). A large part of *T. eques'* diet consists of amphibians, and this location is one of the only places the snake exists along with *R. yavapaiensis* (Brennan and Holycross 2006). The presence of both target species and *T. eques* underlines the quality of habitat found here.

Table 12.—Habitat characteristics from locations where *B. alvarius* were observed during the 2011 and 2012 field season

Site	Min. depth	Max. depth	Sub.1	Sub.2	Sub.3	Discharge	Dist. to water	Wet	Dry	Open terr.	Open aqu.	Tot. open	Em	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				0.10	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

The best fitting regional model for the presence of *R. yavapaiensis* included the covariates 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. In line with the low water depth, *R. yavapaiensis* was 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 the habitat described by the most supported models. Surprisingly, *R. catesbeiana* was not supported in the combined model even though bullfrogs were never found in locations with Lowland leopard frogs. However, *R. catesbeiana* was correlated with many of the other variables measured that were associated with the mainstream LCR and was subsequently relegated to a model on its own. This model did not describe the majority of the habitat located along the mainstream LCR. The LCR is categorized by high stream discharge, deep water, and thick *Typha* spp. growth. Outside of the stretch of the Bill Williams River where we located *R. yavapaiensis,* only Planet Ranch and a handful of isolated locations on the BWRNWR are analogous to the habitat described by this model. While our habitat data support shallow side channels as conducive to the presence of *R. yavapaiensis,* a study quantifying *R. yavapaiensis* 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 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 bullfrogs correlated with permanent lakes and wetlands. In addition, the southern leopard frog (*Rana sphenocephala*) 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 we have found throughout our study area. Lowland leopard frogs are associated with more open canopy and herbaceous vegetation, and the bullfrogs have inundated the permanent water bodies. Due to *R. yavapaiensis'* ability to live in diverse habitats, future studies should focus on the specific metapopulations found in particular habitat types. By understanding the habitat requirements for the species in each habitat class, management and conservation strategies can be tailored specifically for each. 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.

B. alvarius, with the exception of the site on Planet Ranch, were located primarily in open sandy desert habitat with little vegetation. Shrub was the largest vegetation layer measured from the habitat points, as the toads were often observed sitting and foraging under creosote bushes on desert flats. Other then creosote, most of the vegetation measured in each plot was sporadic small clumps of grass and forb, 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 1964).

Future Plans

Additional surveys should be conducted to obtain additional samples for a more quantitative analysis of *B. alvarius* habitat selection along the Bill Williams River streambed and in the sandy stretches on the river's perimeter. Also, inland washes and sandy areas around Topock Gorge during and after monsoon rain events need to be surveyed due to the presence of habitat similar to sites where *B. alvarius* have been detected elsewhere in the study area. As with previous seasons, any large rain event should continue to be investigated for toad breeding behavior in desert washes and ephemeral pools. Targeted surveys should be conducted in sandy areas across the southern part of their range near the international border. Surveys for *R. yavapaiensis* should continue in 2013 within high quality habitats where frogs have yet to be detected (e.g., near the BWRNWR boundary with Planet Ranch and in sandy stretches along the riverbed with standing water). Two large areas that will require additional survey effort on the mainstem LCR are near Imperial Refuge and dam and the southern end of Topock Gorge.

Reaches 7 and 8 of the Bill Williams River should be revisited to provide more detailed vegetation data using an adaptation of the Peet et al. (1998) method of vegetation sampling. This technique will include nested subquadrates, which will evaluate species' richness and density at different orders of magnitude as well as identify variation in vegetation composition in addition to our estimates of percent cover. This method will provide a more detailed understanding of the vegetation assemblage used by amphibians in the study area.

Emerging techniques for surveying species that are rare or found in low densities may provide an additional tool for delineating the distribution of *R. yavapaiensis* and *B. alvarius* on the LCR and Bill Williams River. We recommend collecting water samples for environmental DNA (eDNA) analysis. Sampling with eDNA is a relatively new technique in which the presence of mitochondrial DNA from target species dispersed in the water column is determined through small water samples and polymerase chain reaction amplification (Ficetola et al. 2008). The technique will allow us to sample large areas of habitat for amphibians by analyzing the water sample for amphibian DNA released into the water column. If successful, we will be able to quickly and easily search large areas for *R. yavapaiensis* and *B. alvarius* without having to directly observe or capture the animals.

Surveys need to be reinitiated on Planet Ranch. The habitat on the ranch is conducive to occupancy by both *R. yavapaiensis* and *B. alvarius*, and the latter has already been observed on the property. Additional surveys would help confirm the presence of frogs near the border of the ranch and allow for the quantification of habitat used on Planet Ranch. Based on survey work in 2011, we identified that the segment of the Bill Williams River located on the ranch contains habitat features similar to other areas where target species have been observed.

Preliminary Recommendations

Expand Habitat Use Study

The Bill Williams River amphibian populations of *R. yavapaiensis and B. alvarius* can be used to further understand their habitat use by building on data collected in 2012 and 2013. These studies are the first to investigate the two species in a riverine habitat, similar to what is found throughout the entire study area. More research should be conducted along the Bill Williams River to better understand this system. Recommendations for future research include:

- 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

- Radio telemetry of individuals to determine microhabitat use as well as dispersal and post breeding habitat use

- Hibernacula and breeding habitat selection of *B. alvarius* where they are found elsewhere in the species' range

Until now, this study focused on the presence of amphibians during the breeding season, but we know virtually nothing 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 as how the animals colonize new habitats. These data would be vital for use in habitat improvement and reintroductions within the LCR.

Impacts of Flooding

Another unknown is the effect of flooding on the two species. *R. yavapaiensis* appears to have adapted better with periodic droughts and floods than 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 *B. alvarius* (Brennan and

Holycross 2006). This is the only known population of *R. yavapaiensis* and *B. alvairus* 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 events 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 BWRNWR. Flooding could also play a role in the dispersion of the species in desert environments as well as controlling invasive plant species.

Habitat Translocation and Improvement

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

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

- Demonstration refugia at locations where habitat is experimentally 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 Code and Scientific Name for Each Amphibian Species Identified in This Report Along With Accepted Common Name and Current Taxonomic Synonym According to the Center for North American Herpetology

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

ATTACHMENT 2

List of Notations and Represented Covariates Used in Model Creation

Category	Covariate	Description
Abiotic	Minimum depth	Minimum depth of plot
Abiotic	Maximum depth	Maximum depth of plot
Abiotic	Discharge	Stream discharge measured in feet per second
Abiotic	H_2O class	Either a lentic or lotic site
Abiotic	H_2O 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.	Percent of plot that is without vegetation or water
Biotic	Open aqu.	Percent of plot that is without vegetation but inundated
Biotic	Total open	Total percent of plot without vegetation
Biotic	Wet	Percent of plot that is inundated
Biotic	Dry	Percent of plot out of water
Biotic	Em	Percent of plot covered by emergent vegetation
Biotic	Forb	Percent of plot covered by herbaceous vegetation other than grasses, rushes, and sedges
Biotic	Sub	Percent of plot covered by submerged vegetation
Biotic	Tree	Percent of plot covered by tree canopy
Biotic	Grass	Percent of plot covered by grass
Biotic	Floating	Percent of plot covered by floating vegetation
Biotic	Shrub	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 *R. catesbeiana*