Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Ecology and Habitat Requirements of Lowland Leopard Frogs and Colorado River Toads

2015 Annual Report

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

April 2017

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Lower Colorado River Multi-Species Conservation Program

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2015 Annual Report

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ACRONYMS AND ABBREVIATIONS

CI	confidence interval
cm	centimeter(s)
F	probability distribution for testing difference among two or more group means (*e.g.*, in a multivariate analysis of variance)
ha	hectare(s)
LCR	lower Colorado River
LCR MSCP	Lower Colorado River Multi-Species Conservation Program
m	meter(s)
m^2	square meter(s)
n	sample size
NMDS	non-metric multi-dimensional scaling
p	calculated from a statistical test (i.e., *t* or *F*), the probability of the observed difference between groups if the null hypothesis of no difference is true
pH	scale of acidity from 0 to 14
R^2	proportion or percentage of explained variation
Reclamation	Bureau of Reclamation
t	probability distribution for testing difference between two sample means with a *t*-test or calculating confidence intervals

Symbols

<	less than
%	percent
±	plus or minus

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INTRODUCTION

In order for the Lower Colorado River Multi-Species Conservation Program (LCR MSCP) to determine the feasibility of establishing two once-extirpated species from the lower Colorado River (LCR), they must first gain an understanding of the factors essential to both species' biology. This report evaluates the ecology and habitat requirements of the lowland leopard frog (*Rana yavapaiensis* [also known as *Lithobates yavapaiensis* and *R.* [*Lithobates*] *yavapaiensis*) and the Colorado River toad (*Bufo alvarius* [also known as *Incilius alvarius* and *Bufo* = *Incilius alvarius*) in Arizona.

Both lowland leopard frogs and Colorado River toads are believed to be extirpated from the LCR (Vitt and Ohmart 1978; Clarkson and Rorabaugh 1989; Cotten and Leavitt 2016). However, both species persist approximately 11 miles from the LCR along the Bill Williams River, a tributary that enters Lake Havasu from the east. These two species are evaluation species under the LCR MSCP and are being evaluated to determine the need to mitigate impacts for the LCR MSCP covered activities in their former range within the LCR flood plain and a portion of the Bill Williams River below Alamo Dam (Bureau of Reclamation [Reclamation] 2004). Specific measures established in the Habitat Conservation Plan (CRTO1 and LLFR1) include conducting research to better define the distribution, habitat requirements, and factors that limit the distribution of lowland leopard frogs and Colorado River toads (CRTO2 and LLFR2), protect existing unprotected occupied lowland leopard frog and Colorado River toad habitat, and (CRTO3 and LLFR3) conduct research to determine the feasibility of establishing lowland leopard frogs and Colorado River toads in unoccupied habitat (Reclamation 2004).

Lowland leopard frogs and Colorado River toads are ecologically unique within the amphibian community of the LCR MSCP area, but they are being studied together at the request of the LCR MSCP (Cotten and Leavitt 2016).

Lowland leopard frogs are medium-sized leopard frogs known from lower and middle elevation streams in the Sonoran and Chihuahuan Deserts of Arizona, California, and New Mexico (Sredl 2005). Within this distribution they occupy a variety of habitats that include wide stream channels with low stream gradients and large pools (Wallace et al. 2010). Most breeding for lowland leopard frogs occurs between January and mid-May (Sredl 2005).

Colorado River toads were historically found in both the Sonoran and Chihuahuan Deserts and occupied a variety of habitats, including mesquite (*Prosopis* sp.) and creosote bush (*Larrea tridentata*) lowlands, arid grasslands, and oak-pine (*Quercus* sp. – *Pinus* sp.) woodlands, typically within close proximity to permanent or predictable water (Brennan and Holycross 2006). The breeding season and general terrestrial behavior of Colorado River toads is dictated by

monsoon activity (July – September), with breeding occurring in ephemeral pools and swollen oxbows following large rain events (Sullivan and Malmos 1994; Sullivan and Fernandez 1999).

This report examines the ecology and habitat requirements of both lowland leopard frogs and Colorado River toads at three spatial scales: (1) specific *oviposition sites* (egg mass laying locations) at the smallest scale, (2) *oviposition clusters* (locations of high egg mass concentration) at the middle scale, as this would be representative of the conditions needed locally for successful oviposition (approximately 1 ha), and (3) the *population center* as compared with other possible regions within the study area (approximately 10 km). Because amphibian egg masses indicate the first level of habitat selection (Egan and Patton 2004), this aspect of their biology was the focus. Their oviposition ecology was evaluated along the Bill Williams River and within the Agua Fria and Verde River watersheds of central Arizona, which allowed for observations of a range of potentially occupied habitat within the Sonoran Desert. Surveys were conducted throughout these drainages for breeding lowland leopard frogs or Colorado River toads or their habitat. Oviposition sites, oviposition clusters (locations of high egg mass concentration), and the drainages within a landscape context were analyzed at the three spatial scales.

Objective

The objectives were to examine the ecology of the lowland leopard frog and Colorado River toad and to determine the habitats these species were selecting for oviposition at the three spatial scales. Logic follows that if these two species are selecting certain habitat factors, the presence of these factors along the LCR could be evaluated as well as the feasibility of re-establishing the species within the portion of their historic range. The objective was to determine the breeding habitat requirements of lowland leopard frogs and Colorado River toads within the Bill Williams, Agua Fria, and Verde River watersheds.

Study Area

The study area occupies three different river drainages in western and central Arizona (figure 1). For surveys of lowland leopard frogs, the focus was on sites at the Bill Williams (n = 4) and Big Sandy Rivers (n = 6) within the Bill Williams River watershed. In addition, breeding habitat surveys were conducted at 82 historic locations along the Agua Fria (n = 45) and Verde Rivers (n = 37). For Colorado River toads, surveys were concentrated on the area east of Planet Ranch on the Bill Williams River and at 53 locations (historic and potential) in the Bill Williams, Agua Fria, and Verde Rivers.

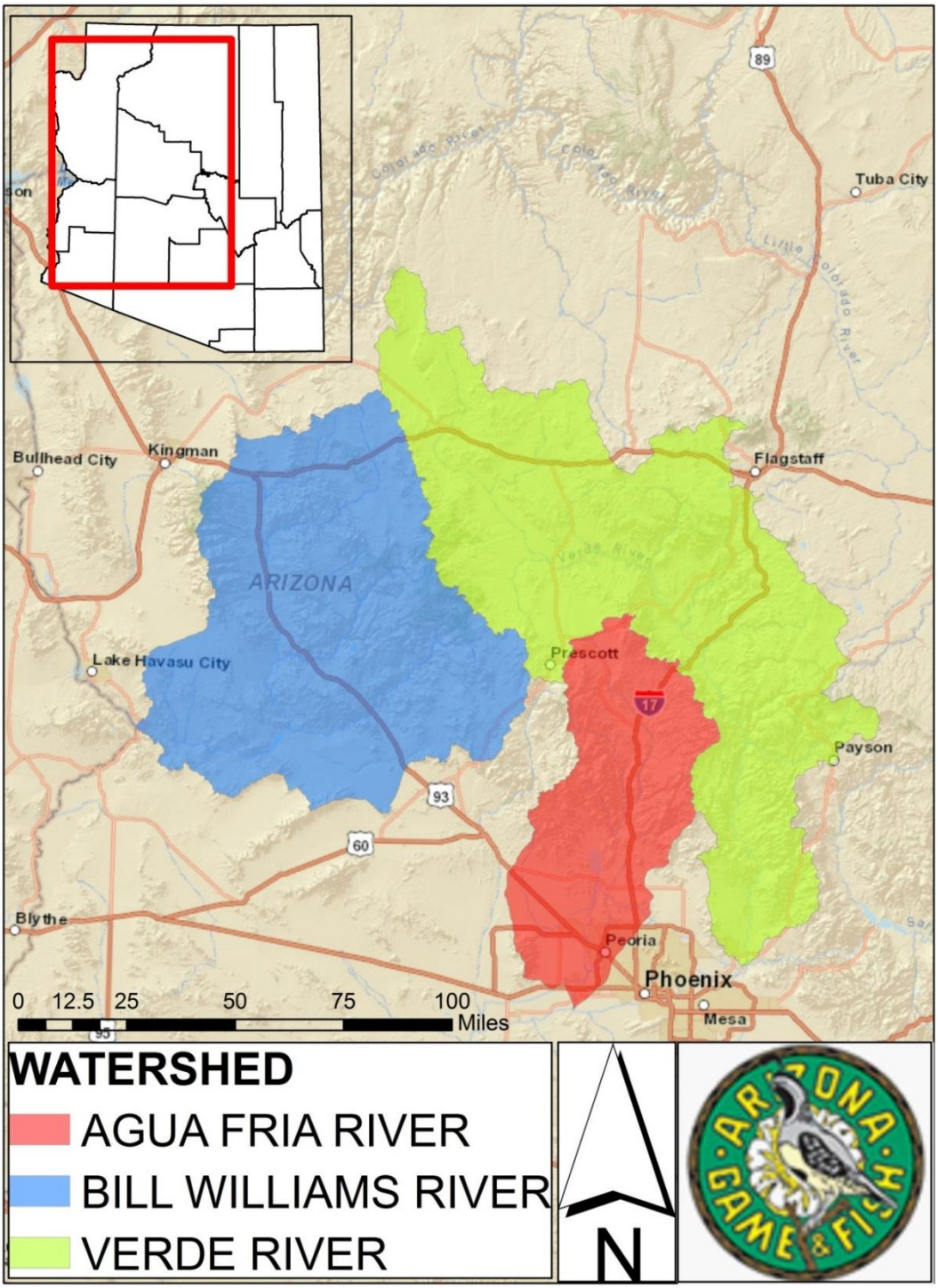

Figure 1.—Map of study areas within the Bill Williams, Verde, and Agua Fria River watersheds.

METHODS

Lowland Leopard Frogs

To locate breeding habitat for lowland leopard frogs, visual encounter surveys were conducted using a series of historic sites (n = 82) from the Agua Fria and Verde Rivers (figure 2). Up to four surveys were conducted per site between February 10 and April 27, 2015, which coincides with their breeding season (Sredl 2005). Sites with adult frogs and easy access were returned to for followup visits. In addition, any egg masses observed during the concurrent project (LCR MSCP Work Task D12 – Lowland Leopard Frog and Colorado River Toad Surveys) on the Bill Williams and Big Sandy Rivers were recorded.

Colorado River Toads

To locate breeding habitat for Colorado River toads, visual encounter surveys were conducted in 2015 using as series of potential and historic sites (n = 53) in the Agua Fria, Verde, and Bill Williams Rivers. Up to four surveys were conducted per site between June 23 and September 3, 2015. In addition, 83 surveys were conducted at 8 sites in the Agua Fria and Verde River drainages, respectively, between July 8 and October 4, 2014. Any egg masses observed on the Bill Williams River during concurrent monitoring projects were recorded.

Habitat Selection

Survey Methods

Visual encounter surveys were conducted using an out-and-back survey of the sites whereby the streambed was walked in parallel transects upstream and downstream by a pair of observers. The surveys involved looking for different cues for each target species. For lowland leopard frogs, surveys were conducted during daylight and at night looking for adult frogs or listening for calling frogs. For Colorado River toads, surveys did not occur until after the summer monsoonal rainfall began. Habitat was checked for calling toads at night and standing water; if either of these were observed, the survey area was examined for egg masses. Since egg mass shapes are very different for the two species (figure 3), the biologists had to train themselves to visually search for two distinct patterns in the water.

Data Collection

A series of environmental variables at a site and at a randomly selected nearby “non-site” were recorded when an egg mass was observed. Non-sites were

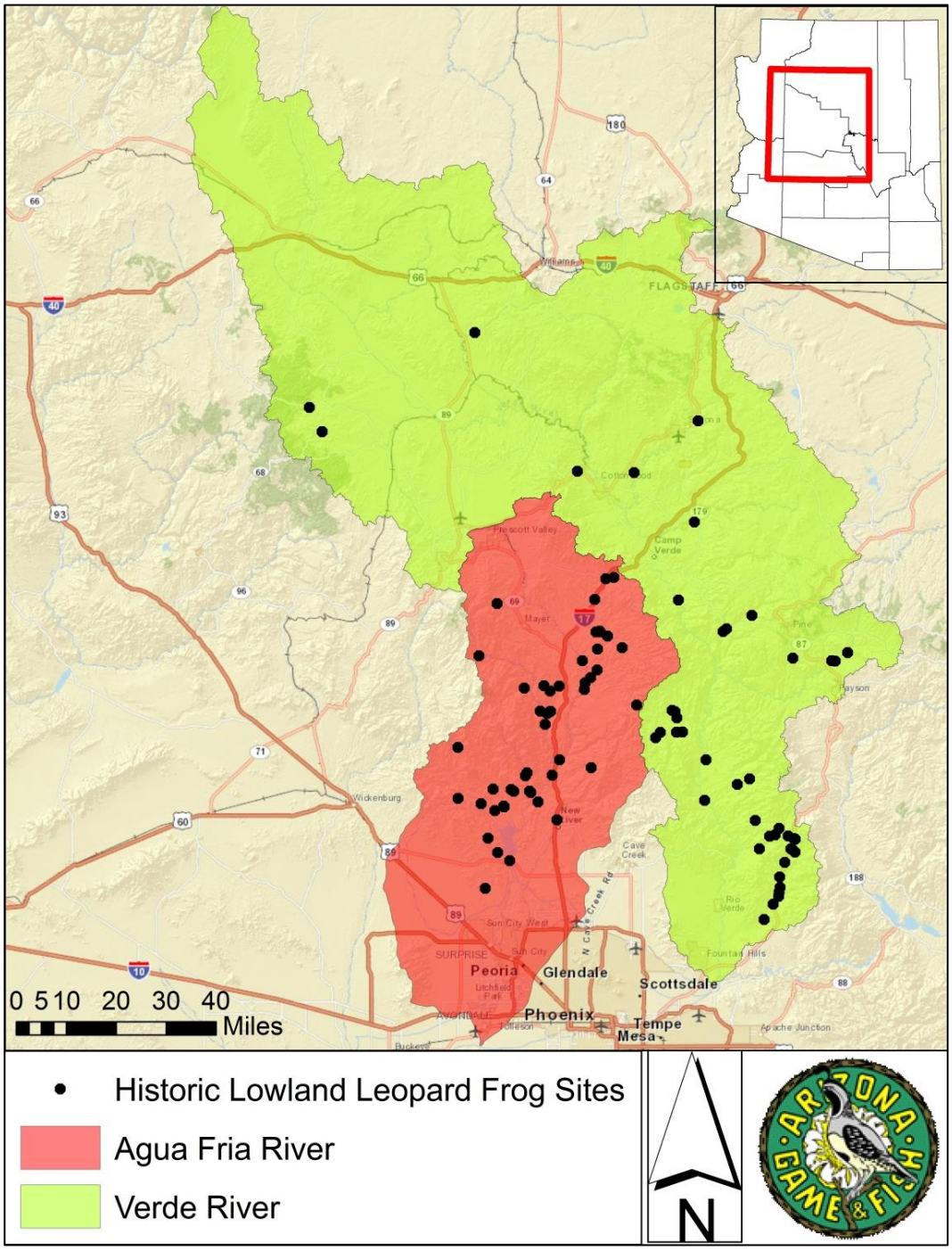

Figure 2.—Historic locations for and current survey areas of lowland leopard frogs in 2015.

Figure 3.—Egg mass of a lowland leopard frog (left) and Colorado River toad (right).

selected by walking in a randomly selected direction (as determined by a random number generator) for a randomly selected distance (< 50 meters [m]; also determined by a random number generator). All random locations had to be in the riparian zone; no random sites were established in desert scrub habitat. If more than one egg mass was observed within a 100 m^2 area, it was considered to be a cluster, and another batch of environmental data was collected at that location and at a nearby randomly selected "non-cluster" location. Random non-clusters were selected in the same way as the non-sites.

Oviposition Site Environmental Data

If individual oviposition sites were attached to vegetation, the species of plants were identified using available taxonomic keys. In addition, the following variables were measured:

- Dimensions (height, length, and width) of the egg mass (millimeters)
- Depth of the egg mass from the surface of the water to the top of the mass (centimeters [cm])
- Distance from the bottom of the water to the underside of the egg mass (cm)
- Percent canopy cover for each plant species directly over the egg mass
- Distance to dense cover (cm)

- Distance to crawl[1] (cm)
- Substrate at the bottom of the water column directly below the egg mass
- Water flow (recorded with a "yes" or "no" depending on the continuity of the flow)
- Water temperature (degrees Celsius)
- Water pH (recorded using a digital pH meter)
- Depth of the water column near the egg mass, measuring from the surface to the substrate at the bottom (cm)

Habitat variables measured at a randomly selected non-site location included:

- Distance to dense cover
- Distance to crawl[1]
- Substrate at the bottom of the water column
- Water flow
- Temperature
- Water pH
- Depth of the water column

Oviposition Cluster Site Environmental Data

For each oviposition cluster, the habitat in which it was found was evaluated by establishing a 0.01-hectare (ha) plot following a modified design of Peet et al. (1998). Four 1 x 1 m quadrats were randomly selected within or adjacent to the 0.01-ha plot to quantify environmental variables. A random non-cluster site with the same variables recorded was included for each cluster site. Quadrats were marked using a 1 x 1 m polyvinyl chloride pipe square that was placed around a center point. Center points were chosen randomly from the center of the 0.01-ha plot.

Four oviposition cluster quadrats were established by cardinal position within the plot, and all data recorded were taken from within the 1x1 m square quadrat:

- Total cover, percent dry, and percent wet were visually estimated within each quadrat.

[1] Distance to crawl is defined as the distance required to reach an area from which an individual could escape out of water).

- At each cluster site, measurements were taken on environmental variables within the 0.01-ha plot.
- The percent of space occupied by the egg masses within the plot was visually estimated.
- The average, minimum, and maximum depths of the water column were measured in centimeters.
- The percent of the entire plot inundated was estimated as a visual observation of the total amount of water on plot.
- The three most abundant substrate types within the plot were visually estimated.
- Any potential amphibian predators at any stage of development were recorded if observed.
- The presence of discernible flow and any coarse woody debris within the plot were recorded.
- Non-sites were randomly selected (at least 100 m from an oviposition cluster) with the stipulation that some water source be present within the boundary of the 0.01-ha plot.

Population Center Environmental Data

This analysis has not yet been conducted.

Data Analysis

Multivariate assessments of the data at the oviposition site scale were conducted to examine the breeding habitat requirements of lowland leopard frogs and Colorado River toads (data analyses on the oviposition cluster and population center scales has not yet been conducted). A *z-transform* was conducted on continuous variables (Gotelli and Ellison 2004) to prepare the data for multivariate analyses. The environmental characteristics were evaluated for the sites and non-sites using non-metric multidimensional scaling (NMDS). NMDS is a non-parametric ordination technique that preserves the rank order of sample units in the original dissimilarity matrix to accurately represent the pairwise similarity among samples in as few dimensions as possible. It can be used with any dissimilarity measure. This technique can be applied to better ascertain which variables contribute to differentiation among sample units within

multivariate datasets. In this case, comparing environmental parameters of known breeding sites to nearby random sites allows for making inferences regarding oviposition site selection.

To conduct the NMDS, a similarity metric was selected that preserved the lowest stress value (a measure of how well the data fit the ordination; smaller values reflect a better fit) and maintained the highest coefficient of determination, R^2 (McCune and Grace 2002). The Gower's similarity metric is technically the most appropriate due to the combination of continuous and categorical variables in this dataset (Hammer et al. 2001) [2]. A solution was chosen for three dimensions (axes). Each NMDS run was a sequence of 11 trials (1 based on Principal Coordinates Analysis for the initial position of sample scores, the others on random starts) from which the 1 with the smallest stress value was selected. Following the NMDS, a discriminant functions analysis was conducted to determine whether the pre-determined groupings (site versus non-site) were correctly classified by the environmental variables measured in each plot (Gotelli and Ellison 2004). Following this analysis, a multivariate analysis of variance was conducted to determine if the groupings were significantly different from one another. Finally, univariate tests of means (Student's t-test) were conducted on any variable that was identified in the multivariate analysis (reported mean ± standard error). All analyses were conducted in program PAST 3.01 (Hammer et al. 2001).

RESULTS

Lowland Leopard Frogs

In 2015, 146 surveys were conducted in the Agua Fria and Verde River drainages to find lowland leopard frog egg masses. A total of 62 egg masses were found

[2] Based on an independent literature review of the NMDS ordination method used in this report, Reclamation believes the ordination for lowland leopard frogs may not be reliable due to the use of the Morisita's index, which is only appropriate for abundance data. The Gower's index is the suitable choice for the mix of continuous and categorical variables used in the analysis (Hammer 2016 – the PAST manual, version 3.14). The ordination for Colorado River toad uses the Gower's index, so it should be reliable, though its value is limited due to the very small sample size.

The choice of a similarity index should be based on the type of data collected and the goal of the analysis, not on the NMDS stress test. The goal of NMDS ordination is to rank order sample sites based on their similarity according to several environmental variables, and it assumes the location of samples in the ordination reflects meaningful ecological gradients. The NMDS stress test reflects how well the ordination fits the data, but it does not measure how well an ordination reflects unknown ecological gradients. It is possible to get a low-stress solution for an ordination that does not reflect biological reality if an inappropriate similarity index is used.

during the surveys. The majority of the egg masses was found on the Big Sandy River (n = 39), but there were additional egg masses found at the Bill Williams (n = 6), Agua Fria (n = 9), Verde Rivers (n = 8). Environmental characteristic data were collected for the sites on all occasions, and matching non-site data were collected on 58 occasions (table 1). Additionally, a total of 11 egg mass clusters were identified, and random non-cluster data were collected on 9 occasions. Egg masses (measured in millimeters) were 55.8 ± 3.5 (height) 55.8 ± 2.9 (width), and 75.3 ± 3.0 (length), which resulted in an average volume of 982 milliliters (33.2 ounces) using the geometric calculation for a prolate spheroid.

Table 1.—Environmental variables predicted to influence lowland leopard frog breeding habitat selection in Arizona, 2015

Variable	Units	Mean or tally (n = 62)
Attachment species	Species (categorical)	Unknown (25); rush (*Juncus* sp. and *Typha* sp.) (15); tamarisk (*Tamarix* sp.) (9); willow (*Salix* sp.) (9); twig (2); cottonwood (*Populus* sp.) (1); leaf (1)
Egg mass depth from surface	cm (continuous)	3.27 ± 0.56
Distance from bottom to egg mass	cm (continuous)	5.85 ± 0.90
Percent canopy cover	% (continuous)	15.63 ± 3.11
Species canopy cover	Species (categorical)	None (33); unknown (15); willow (12); cottonwood (1); tamarisk (1)
Distance to dense cover	cm (continuous)	85.49 ± 10.69
Distance to a location to crawl out of water	cm (continuous)	51.32 ± 8.78
Substrate type	type (categorical)	Sand (41); mud/silt (15); gravel (2); boulder (2); cobble (1); bedrock (1)

Colorado River Toads

In 2015, 56 surveys were conducted in the Bill Williams, Agua Fria, and Verde River drainages to find Colorado River toad egg masses. No Colorado River toad egg masses were found during surveys in 2015; therefore, no additional environmental data were collected (table 2). A total of 14 Colorado River toad egg masses were found and collected at 5 random points in 2014 in the Bill Williams and Agua Fria Rivers (Cotten and Leavitt 2016).

Table 2.—Environmental variables predicted to influence Colorado River toad breeding habitat selection in Arizona, 2015

Variable	Units	Mean or tally (*n* = 14)
Attachment species	Species (categorical)	Unknown (10); twig (3); mesquite (1)
Egg mass depth from surface	cm (continuous)	1.07 ± 0.85
Distance from bottom to egg mass	cm (continuous)	15.64 ± 15.26
Percent canopy cover	% (continuous)	0.00 ± 0.00
Species canopy cover	Species (categorical)	None (14)
Distance to dense cover	cm (continuous)	1434.5 ± 498.29
Distance to a location to crawl out of water	cm (continuous)	323.00 ± 223.07
Substrate type	type (categorical)	Mud/silt (11); cobble (3)

Habitat Selection

Lowland Leopard Frogs

Similarity metrics, the associated stress values, and an associative R^2 value for the data interpreted for lowland leopard frog oviposition sites were compared (table 3). For the NMDS[3] conducted on oviposition sites, three habitat variables (axes) described the majority of variation in the oviposition dataset and were best explained by canopy cover, distance to cover, and distance to crawl (the results showing axis 1 and axis 3 are depicted on figure 4). In general, the majority of sites selected by lowland leopard frogs for oviposition had higher canopy than the randomly selected sites and were relatively close to cover and sites where they could crawl out of the water (figure 4). The discriminant functions analysis suggested a difference between the groups (site and non-site) that was supported by the multivariate analysis of variance ($F = 5.14$; $p < 0.01$) and was weighed heavily on values for distance to cover and water depth. Univariate tests of means demonstrated varying levels of statistical support for differences in water depth (10.93 ± 1.91; 20.11 ± 4.7; $t = 1.81$, $p = 0.07$), distance to cover

[3] Based on an independent literature review for the NMDS ordination method used in this report, Reclamation believes the ordination for lowland leopard frogs may not be reliable due to the use of the Morisita's index, which is only appropriate for abundance data. The Gower's index is the suitable choice for the mix of continuous and categorical variables used in the analysis (Hammer 2016 – the PAST manual, version 3.14).

The choice of a similarity index should be based on the type of data collected and the goal of the analysis, not on the NMDS stress test. The goal of NMDS ordination is to rank order sample sites based on their similarity according to several environmental variables, and it assumes the location of samples in the ordination reflects meaningful ecological gradients. The NMDS stress test reflects how well the ordination fits the data, but it does not measure how well an ordination reflects unknown ecological gradients. It is possible to get a low-stress solution for an ordination that does not reflect biological reality if an inappropriate similarity index is used.

Table 3.—Comparison of similarity metrics, the associated stress values, and an associative R^2 value for the data interpreted for lowland leopard frog oviposition sites

Similarity metric	Stress	R^2
Gower	0.15	0.51
Morisita	0.13	0.56
Horn	0.18	0.50
Malhanobis	0.19	0.59
Jaccard	0.33	0.71
Kulczynski	0.33	0.74
Raup-Crick	0.41	0.47
Euclidean	0.22	0.62

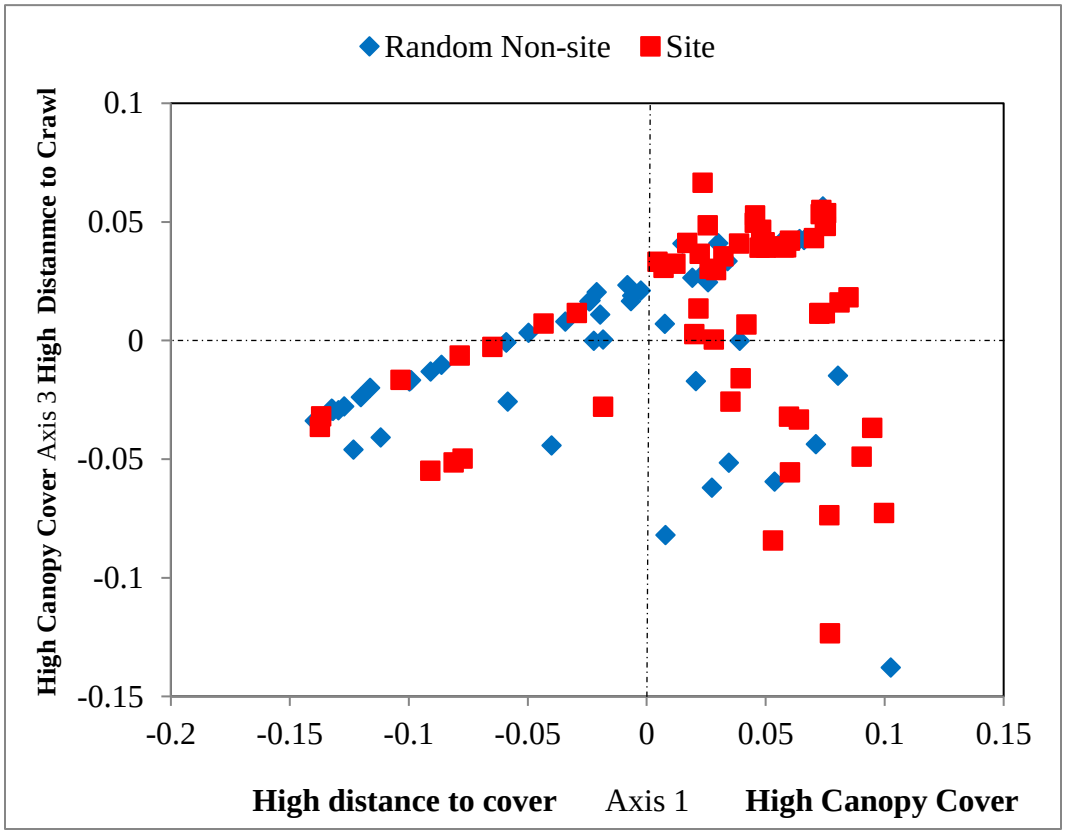

Figure 4.—Non-metric multidimensional scaling of oviposition sites for lowland leopard frogs in Arizona, 2015.

(172.16 ± 24.68; 42.82 ± 10.84; $t = 4.79$, $p < 0.01$), and distance to crawl (88.07 ± 15.29; 52.00 ± 8.90; $t = 2.04$; $p = 0.04$), but no difference in canopy cover (12.06 ± 3.05; 15.87 ± 3.15; $t = 0.87$, $p = 0.39$) was observed between the non-site and oviposition sites, respectively.

Colorado River Toads

Similarity metrics, the associated stress values, and an associative R^2 value for the data interpreted for Colorado River toad oviposition sites were compared (table 4). For the NMDS[4] conducted on oviposition sites, three habitat variables (axes) described the majority of variation in the oviposition dataset and were best explained by water depth, distance to cover, and egg mass depth (figure 5). Note that this analysis is based on data collected in 2014 because no egg masses were found in 2015, and it will be the only data for the final analysis. Variation of environmental characteristics within sites selected for oviposition provide some understanding of Colorado River toad breeding (see table 2). No comparisons were conducted between the site and non-site data due to low sample size.

Table 4.—Comparison of similarity metrics, the associated stress values, and an associative R^2 value for the data interpreted for Colorado River toad oviposition sites

Similarity metric	Stress	R^2
Gower	0.06	0.80
Morisita	0.16	0.64
Horn	0.43	0.49
Malhanobis	0.13	0.45
Jaccard	0.35	0.21
Kulczynski	0.35	0.59
Raup-Crick	0.15	0.80
Euclidean	0.08	0.45

[4] Based on an independent literature review for the NMDS ordination method used in this report, Reclamation believes the ordination for Colorado River toads should be reliable, as it uses the Gower's index, though its value is limited due to the very small sample size. The Gower's index is the suitable choice for the mix of continuous and categorical variables used in the analysis (Hammer 2016 – the PAST manual, version 3.14).

The choice of a similarity index should be based on the type of data collected and the goal of the analysis, not on the NMDS stress test. The goal of NMDS ordination is to rank order sample sites based on their similarity according to several environmental variables, and it assumes the location of samples in the ordination reflects meaningful ecological gradients. The NMDS stress test reflects how well the ordination fits the data, but it does not measure how well an ordination reflects unknown ecological gradients. It is possible to get a low-stress solution for an ordination that does not reflect biological reality if an inappropriate similarity index is used.

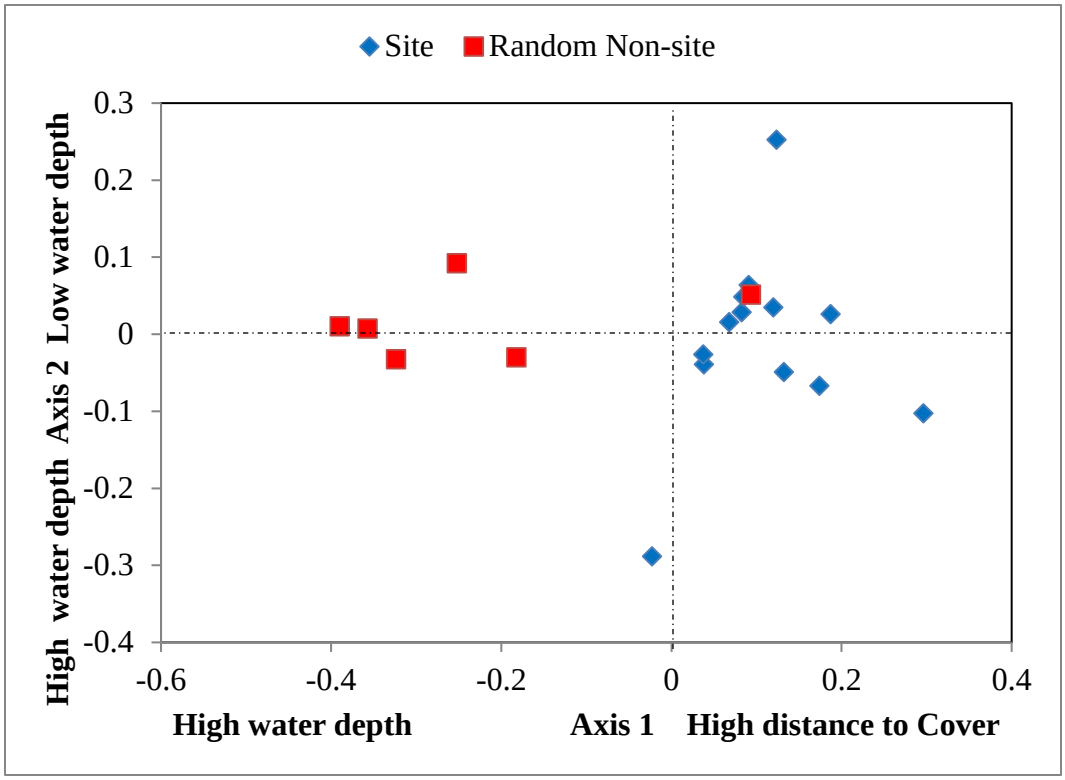

Figure 5.—Non-metric multidimensional scaling of oviposition sites for Colorado River toads in Arizona, 2014.

DISCUSSION

Surveys were conducted at the Agua Fria, Verde, Bill Williams, and Big Sandy Rivers to learn more about lowland leopard frog and Colorado River toad breeding habitat and to inform potential reintroduction efforts for these species on the main stem of the LCR. After 1 season of field study, a total of 62 lowland leopard frog egg mass oviposition sites were found. However, after 2 seasons of field study, the number of egg mass oviposition sites detected for Colorado River toads numbered only 14. As outlined earlier, the ecologies of these two species are highly distinct from one another. Below is a discussion of the characteristics of the breeding habitat of these two species as observed thus far.

Lowland Leopard Frogs

Lowland leopard frog breeding habitat was most likely to occur in sites with higher canopy cover and a moderate distance to crawl out of the water (see figure 4). Canopy cover for oviposition sites averaged 15.63% (95% confidence interval [CI] 9.5%, 22.2%), which can often be found near streambanks. Similar to the findings of Sartorius and Rosen (2000), egg masses were near the surface

(above or below) and an average of 3.27 cm below the surface (95% CI 2.2 cm, 4.5 cm). Not only were egg masses near the surface, the average egg mass distance from the bottom was 5.85 cm, suggesting that shallow water was preferred over deep water for oviposition.

Shallow water, near a potential location to crawl out (e.g., a streambank or downed tree), under a portion of canopy (i.e., willow trees [*Salix* sp.]), would be an ideal location for egg masses based on the data collected. The average distance for most egg masses to dense vegetative cover was 42.82 cm (95% CI 21.1 cm, 64.5 cm). Substrate was not an important factor within the areas where oviposition habitat was examined, and this does not differ from the findings of Wallace et al. (2010). The overall site characteristics that were common for successful breeding of lowland leopard frogs were flowing, shallow water near vegetative cover (figure 6).

Figure 6.—Field crew conducting habitat assessment at the location of a lowland leopard frog oviposition site with typical characteristics of shallow, flowing water with vegetative cover.

Colorado River Toads

Breeding for Colorado River toads is strongly correlated with the summer monsoon rain events (Sullivan and Fernandez 1999). While much of the

Agua Fria and Verde River sites were saturated and adequately inundated following a single week of rain during the 2014 field season, the 2015 field season was exceptionally dry in these drainages. As a result, the only data that exist on egg mass oviposition site selection for Colorado River toads come from the 2014 field effort. Colorado River toads lay long strings of eggs that can cover a very large area (see figure 3). The data analysis suggests that Colorado River toads select shallow water with no canopy cover for oviposition.

Average egg mass oviposition sites were 711.3 cm wide (95% CI 269 cm, 1,382 cm) and 571 cm long (95% CI 58 cm, 1,000 cm). Site descriptions from 2014 suggest that very little selection occurs at the site level; rather, it is at a much larger scale that Colorado River toads are selecting breeding habitat (Cotten and Leavitt 2016). They breed in open canopy water sources that are ephemeral in nature; therefore, any data related to water column depth or pH should be considered as transient variables that do not represent the moment of oviposition.

Restoration of Populations

Habitats immediately adjacent to the confluence of the Bill Williams River with the LCR are critical to the presence of both lowland leopard frogs and Colorado River toads. At this time, the Arizona Game and Fish Department does not recommend any translocations of Colorado River toads or lowland leopard frogs to Planet Ranch, as they are present on adjacent properties. The Arizona Game and Fish Department believes that management and restoration activities on Planet Ranch may benefit these species.

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