Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Development and Implementation of a Repeatable Monitoring Plan for Lowland Leopard Frogs and Colorado River Toads on the Lower Colorado River

2015 Annual Report

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

December 2015

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Lower Colorado River Multi-Species Conservation Program

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Multi-Species Conservation Program
Bureau of Reclamation
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ACRONYMS AND ABBREVIATIONS

Bill Williams River NWR	Bill Williams River National Wildlife Refuge
DAR	digital automated recorder
eDNA	environmental deoxyribonucleic acid
FTA	funnel trap array
GPS	Global Positioning System
LCR	lower Colorado River
LCR MSCP	Lower Colorado River Multi-Species Conservation Program
m	meter(s)
Reclamation	Bureau of Reclamation
VES	visual encounter survey
VES C/R	visual encounter survey with an audio broadcast call/response component

Symbols

Ψ	occupancy probability
%	percent
p	probability of detection
θ	fine-scale occupancy probability

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ABSTRACT

The purpose of this study was to identify monitoring strategies for two species of amphibians on the lower Colorado River by evaluating the cost efficiency of different methods. Five different survey methods were evaluated for their ability to efficiently detect two study species, the Colorado River toad (*Incilius alvarius*, formerly *Bufo alvarius*) and the lowland leopard frog *(Lithobates yavapaiensis*, formerly *Rana yavapaiensis*). Surveys for Colorado River toads occurred on the Bill Williams River, La Paz and Mohave Counties, Arizona, and surveys for the lowland leopard frogs were conducted in the Bill Williams River National Wildlife Refuge, La Paz and Mohave Counties, and the Big Sandy River, Mohave County, Arizona.

The five survey methods examined for each species were: (1) visual encounter surveys (VESs), (2) VESs with an audio broadcast call/response component (VES C/Rs), (3) funnel trap arrays (FTAs), (4) environmental deoxyribonucleic acid (eDNA) sampling, and (5) digital automated recorder (DAR) recordings. Surveys occurred during the breeding season, during summer monsoons for Colorado River toads and spring for lowland leopard frogs. Two seasons of surveys for Colorado River toads were completed in the summer monsoons of 2014 and 2015. Surveys for lowland leopard frogs were conducted in 2015. A monitoring plan will be established for lowland leopard frogs and Colorado River toads using the results from additional field seasons. The results will be reported in the final 2017 report.

INTRODUCTION

Colorado River Toad

Colorado River toads (*Incilius alvarius*, formerly *Bufo alvarius*) are believed to be extirpated from the main stem of the lower Colorado River (LCR) (Cotten and Leavitt 2014). However, a small population exists on the Bill Williams River (Cotten 2011; Cotten and Leavitt 2014), a tributary to the LCR. The is an evaluation species of the Bureau of Reclamation's (Reclamation) Lower Colorado River Multi-Species Conservation Program (LCR MSCP) and is a species that the Arizona Game and Fish Department believes would benefit from conservation in this portion of its range along the LCR (Reclamation 2004). The breeding behavior of Colorado River toads is dependent on summer monsoon rains, with breeding occurring after a large rain of more than 25 millimeters (Sullivan and Malmos 1994; Sullivan and Fernandez 1999). The unpredictability of these rains makes surveying for this species challenging.

Lowland Leopard Frog

Lowland leopard frogs (*Lithobates yavapaiensis*, formerly *Rana yavapaiensis*) are also believed to be extirpated from the main stem of the LCR (Cotten and Leavitt 2014). They were known from the Bill Williams River in the early 1990s (Sredl et al. 1997) and were observed as recently as 2010 (Cotten and Leavitt 2014). A 3-year study by the Arizona Game and Fish Department from 2011 through 2013 did not find populations of lowland leopard frogs on the LCR or within the Bill Williams River National Wildlife Refuge (Bill Williams River NWR) boundaries until two individuals were found in spring 2013 in the Bill Williams River NWR (Cotten and Leavitt 2014). A breeding population was found east of the wildlife refuge on public land in 2012 (Cotten and Leavitt 2014). This population is usually separated from the Bill Williams River NWR by a large dry stretch of the Bill Williams River bed, except during large monsoon flooding. This suggests that a population of lowland leopard frogs could reestablish itself in the Bill Williams River NWR. Reclamation has placed the lowland leopard frogs on its list of evaluation species (Reclamation 2004) to determine their status in the LCR MSCP area, assess the potential effects of covered activities on the species, and to determine appropriate conservation measures. Lowland leopard frogs typically breed in the spring from March through May but have also been found to breed to a lesser extent in September and October (Sartorius and Rosen 2000).

OBJECTIVE

The objective of this project was to identify monitoring strategies for Colorado River toads and lowland leopard frogs that can be used on the LCR to survey for both species in the future. Five survey methods were evaluated for their ability to detect each species and then analyzed for cost efficiency and cost effectiveness. The results will be reported in the final 2017 report. The five survey methods examined for each species were: (1) visual encounter surveys (VESs), (2) VESs with an audio broadcast call/response component (VES C/Rs), (3) funnel trap arrays (FTAs), (4) environmental deoxyribonucleic acid (eDNA) sampling, and (5) digital automated recorder (DAR) recordings.

STUDY AREA

Colorado River Toad

The Colorado River toad study area is located on the Bill Williams River near Arizona's western border. The study site is located approximately 24 kilometers east of the confluence with the LCR. It begins at the eastern border of Planet Ranch (private property) and proceeds east approximately 7 kilometers along the Bill Williams River and into Bureau of Land Management managed public land and the Swansea Wilderness Area (figure 1).

Lowland Leopard Frog

The lowland leopard frog study area is split into two locations. The first location is in the Bill Williams River NWR. The Bill Williams River NWR begins at the confluence of the Bill Williams River and Lake Havasu, which is part of the LCR. The refuge extends east upstream approximately 16 kilometers to the western boundary of Planet Ranch (figure 2). The western boundary of the Bill Williams River NWR study site and the intersection of the Bill Williams River is Plot 4A, located at NAD83 zone 11S 776094 east 3794480 north. The eastern boundary of the Bill Williams River NWR study site is located at the border of Plot 4 at NAD83 zone 12S 226061 east 3794720 north. Plot 4 on the Bill Williams River NWR was split into two locations, Plots 4 and 4A, due to difficulties with access (extensive beaver impoundments and thick tamarisk [*Tamarix* sp.] across the plot). The second site is located on the Big Sandy River approximately 11 kilometers south of Wikieup, Arizona (figure 3). The southernmost boundary of the Big Sandy River and Plot 1 is located at NAD83 zone 12S 263131 east 3831620 north. The northern boundary of the Big Sandy River and Plot 6 is located at NAD83 zone 12S 264223 east 3833473 north.

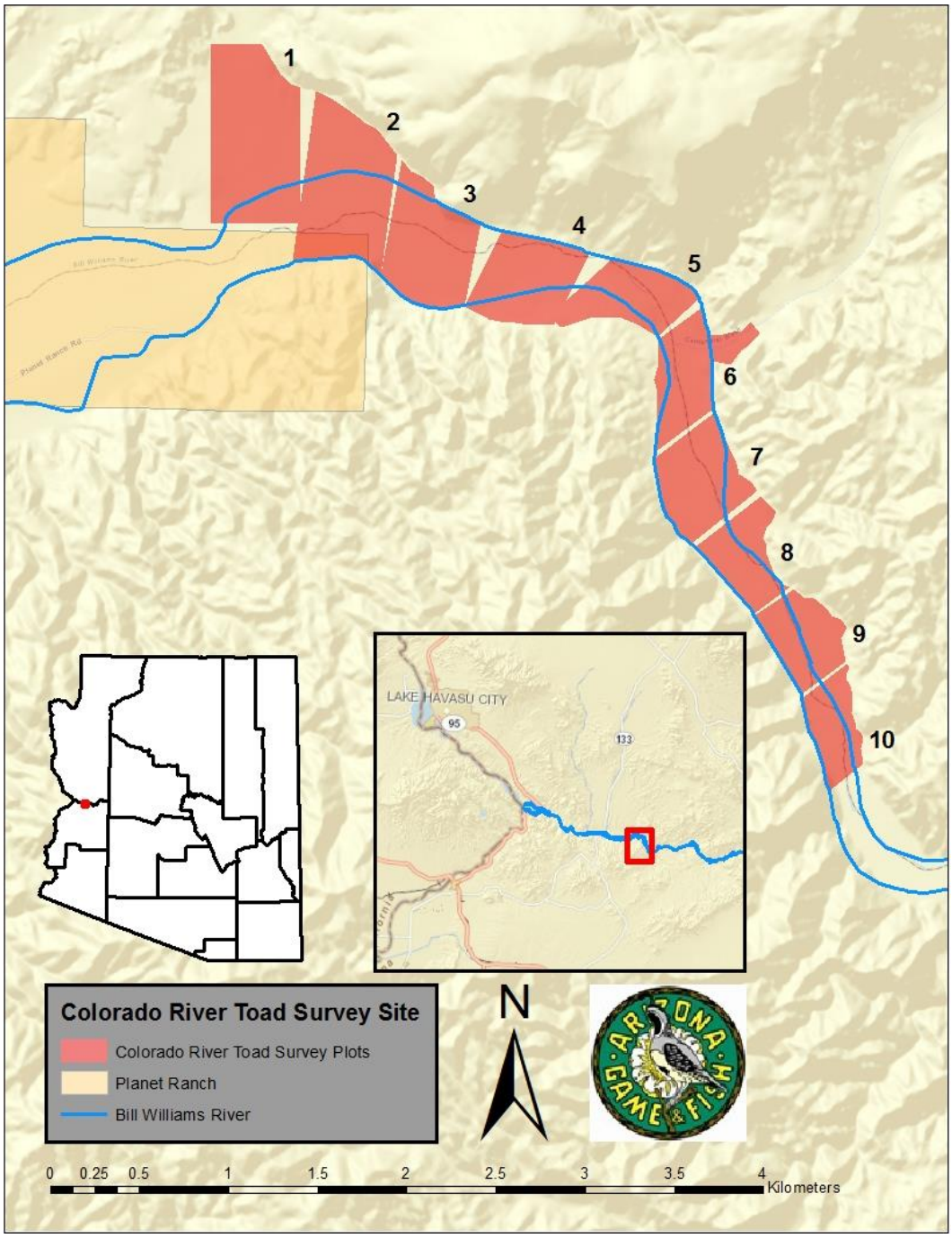

Figure 1.—Colorado River toad survey plots on the Bill Williams River.

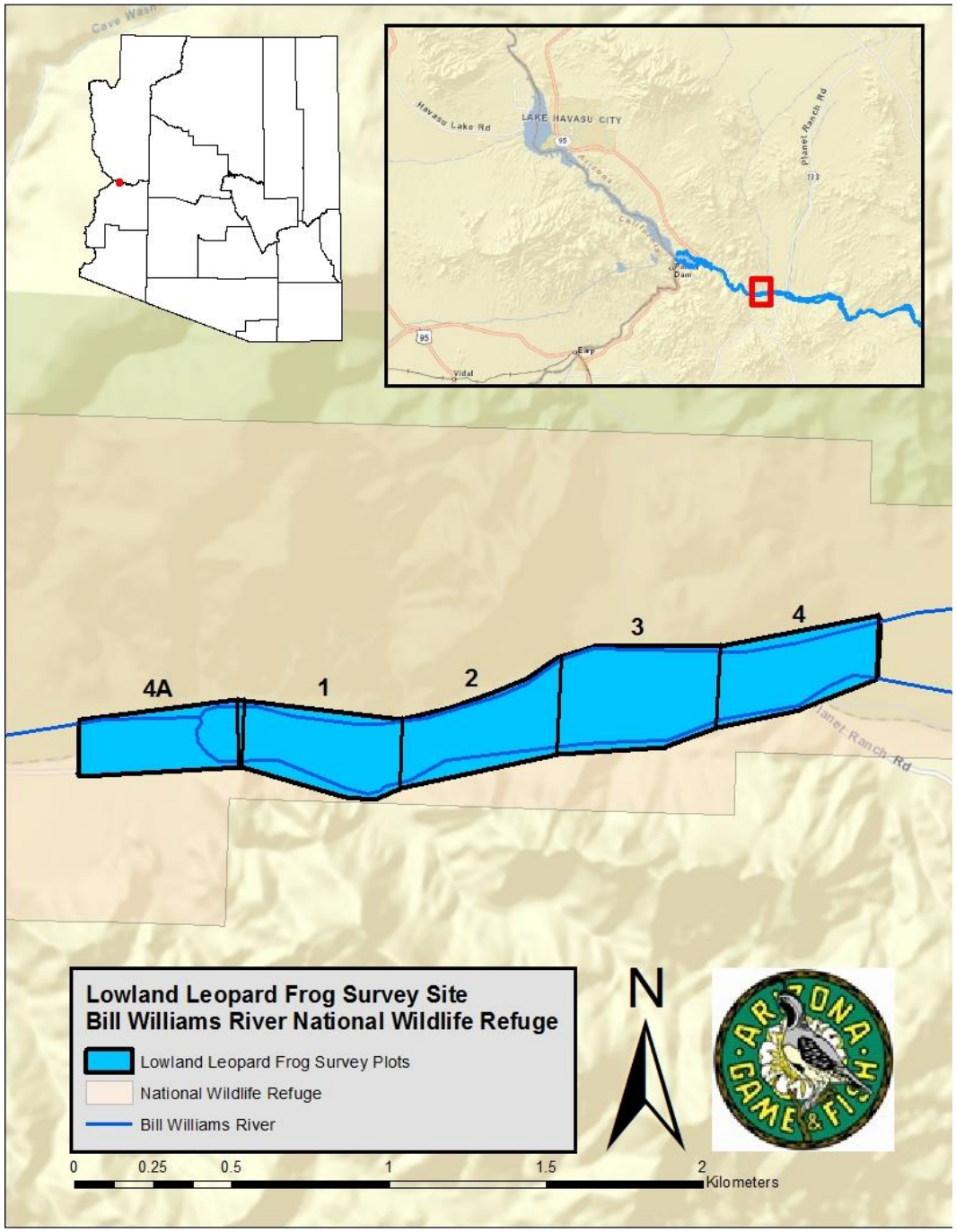

Figure 2.—Lowland leopard frog survey plots on the Bill Williams River.

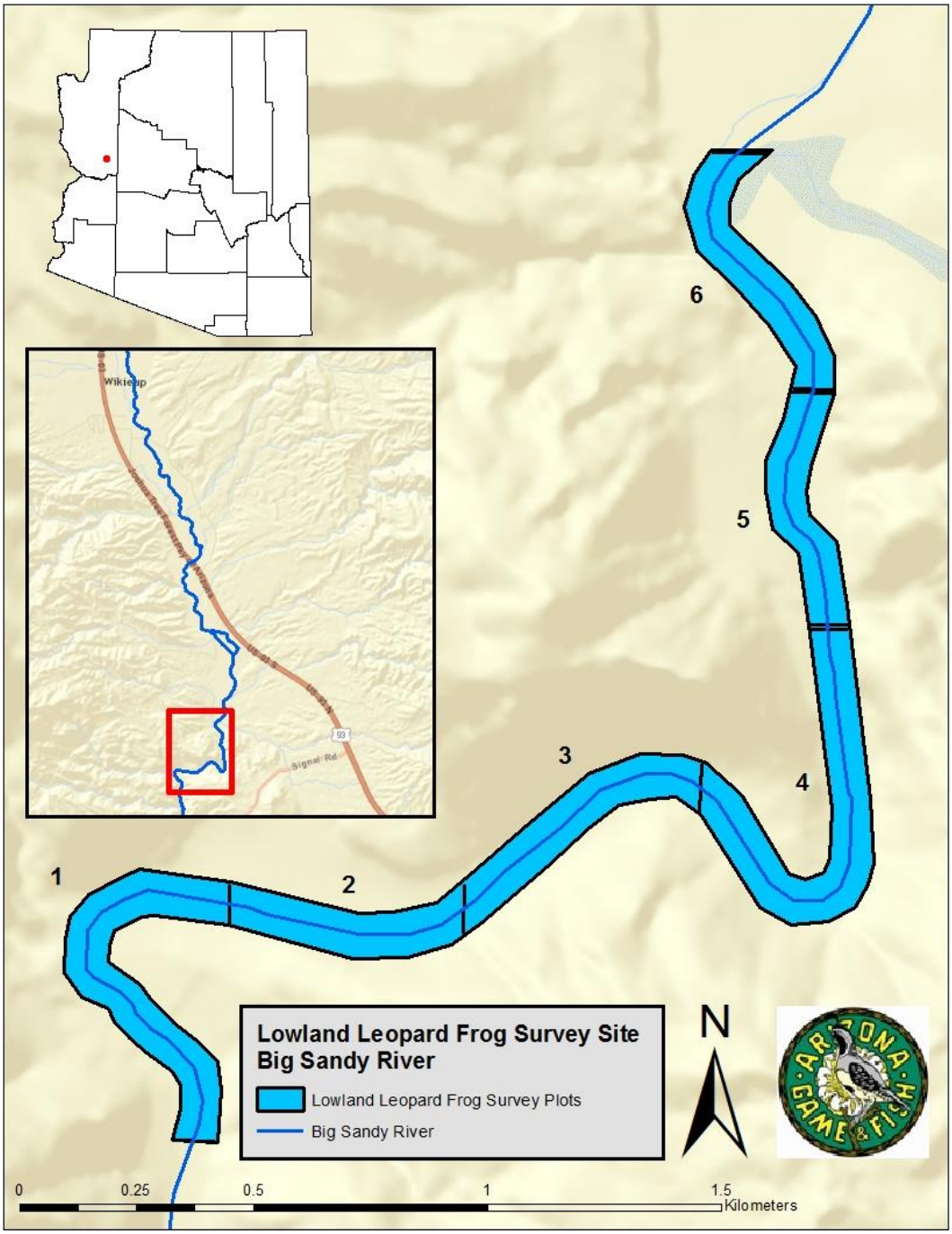

Figure 3.—Lowland leopard frog survey plots on the Big Sandy River.

METHODS

Colorado River Toad

Ten study plots, each 500 × 1,000 meters (m) (5 hectares), were set up along the Bill Williams River on Bureau of Land Management land east of Planet Ranch where a known population of Colorado River toads exists (Cotten and Grandmaison 2012) (see figure 1). The study plot size was determined by the approximate width of the flood plain (1,000 m) and the effective range of audio recorders (2,000 square meters) (Saenz et al. 2006). The number of plots surveyed was limited by the budget available for purchase of DARs. The plots were initially mapped with a Geographic Information System unit. Only suitable habitat was surveyed within each plot, and all areas deemed unsuitable within a plot were ignored. Habitat (*sensu* Hall et al. 1997) is "the resources and conditions present in an area that produce occupancy-including survival and reproduction by a given organism." Based on that definition, within all of the study sites, large portions of the plots did not contain habitat for Colorado River toads. The preferred habitat of Colorado River toads is Sonoran desert scrub and, more specifically, the LCR desert scrub subdivision (Brennan and Holycross 2006). This biotic community consists of low elevation, flat, dry and sandy features dominated by creosote bush (*Larrea tridentata*), white bursage (*Ambrosia dumosa*), and desert salt bush (*Atriplex polycarpa*) (Brennan and Holycross 2006). The only portion of the study plots within this type of biotic community is the river and its historic flood plain represented by the survey area. With the exception of Plots 1 and 2, all the other plots had a significant amount of steep mountainous terrain. Plots 3–10 had between 20–60% of their surface area covered with mountainous terrain. These plots were not surveyed because of a lack of suitable habitat for Colorado River toads.

From the eastern border of Planet Ranch, plots were placed east (or upstream) consecutively along the river. Surveys were conducted during the summer monsoon season, which begins in July and continues through mid-September (Adams and Comrie 1997; Adang and Gall 1989). Typically, Colorado River toads breed in monsoonal ephemeral pools (Brennan and Holycross 2006). Surveys began on July 8, 2014, and continued until October 4, 2014, and began again on July 7, 2015, through October 6, 2015, which encompasses the monsoon and Colorado River toad breeding season.

Visual Encounter Surveys

Each VES was 2 hours in duration. Surveys consisted of two biologists walking a plot with flashlights to detect adults, egg masses, or tadpoles. The location, life stage, and sex, if known, for each individual Colorado River toad captured was recorded. All captured Colorado River toads were marked with a passive

integrated transponder tag for individual identification. The location and count of all amphibian or reptile species seen on a plot during a survey were also recorded.

Audio broadcast call/responses (VES C/Rs) were included in each VES. Each VES C/R used a FOXPRO, Inc.,© broadcast caller to play a Colorado River toad advertisement call. The call was played for two cycles, each approximately 30 seconds long, followed by a break to listen for responses. This was repeated a minimum of four times by systematically calling every 30 minutes throughout each VES. Amphibians heard calling before broadcast or more than 3 minutes after broadcast were counted as being detected by VES whether they were seen or not. Amphibians typically responded immediately to broadcasted calls; therefore, it was assumed for purposes of this study that any responses more than 3 minutes after the end of broadcast (i.e. during VES) were not due to delayed responses to VES C/R and were attributed to VES alone. Broadcast points were spaced out across the study area to ensure optimal coverage of the area: when visual detection was not hindered by vegetation, we walked the perimeter of plots; when vegetation blocked our view, we crossed through the plots to ensure complete visual coverage. Calling by any amphibian species during a call survey was recorded. Finally, any suitable breeding sites detected were noted in order to test other survey methods.

In 2014, due to logistical constraints, a minimum of four surveys were performed at each plot, with a maximum of seven surveys. Surveys were set on a predetermined schedule starting with Plots 1 and 2 for 2 nights, then Plots 3 and 4 for 2 nights, and so on until all plots were completed. Each VES started 1 hour prior to sunset and continued throughout the night.

During the 2015 season, five survey sessions were completed on each plot. Each session consisted of two surveys per plot. Plots 1 and 2 would be surveyed the first night in order, and then the following night the order would be reversed. This enabled each plot to have one early sunset survey and one late night survey for each session. Surveys began at sunset and continued throughout the night, with most surveys ending at or before midnight.

Funnel Trap Arrays

If water was found during a VES, inverted conical wire mesh funnel traps (Gee minnow traps) were used to capture Colorado River toads (Heyer et al. 1994). Four clusters of FTAs, each consisting of 10 traps, were set for 2 nights and checked once after the first night and again upon removal. Funnel traps were arranged such that all 10 traps remained within a 10 × 10 m area in suitable habitat, and thus 0–3 m from a trap to its nearest neighbor. They were typically set along banks. No bait was placed in the traps. They were set in water only to the height of the funnel, leaving space at the top so any amphibian or reptile species caught had access to air. If target species were captured, they were marked using the same techniques mentioned above and released. All non-target species were noted and released (attachment 1).

Digital Automated Recorders

DARs were placed in each plot in order to record calling from male Colorado River toads. A Wildlife Acoustics Song Meter SM2+ automated recording device was set in each study unit for a total of 89 nights in 2014 and 92 nights in 2015. They were set along the river channel as close to the center of each plot as possible. Each recorder was set to record 1 minute of every hour on the hour from 21:00 through 02:00 hours each night from the first day to the final day of the study season (figure 4). Recordings were downloaded in the field and evaluated later. Any amphibian species heard during the recordings was noted along with the time and estimated number of individuals calling.

Environmental DNA

Water samples were filtered at sites where an FTA was set to conduct eDNA sampling using the protocol developed by the U.S. Geological Survey Snake River Field Station, Forest and Rangeland Ecosystem Science Center (U.S. Geological Survey 2012). A maximum of four samples were filtered at each plot during each trapping event. The samples were filtered after the first trap night. Between 1,000 and 2,000 milliliters of water from each site were filtered through a 0.45-micron filter. The samples were filtered onsite if time permitted or collected in disinfected 1-liter Nalgene bottles and processed at the field lab at a later time. All supplies were disinfected using a 50% bleach and water solution. The filters were placed in a vial of 95% ethanol solution, labeled, and stored for later analyses. No predetermined amount of samples were expected to be taken due to the unreliability of surface water throughout each season. The filters were sent to Reclamation's Environmental Applications and Research Group in Denver, Colorado, for analyses.

Lowland Leopard Frog

Ten study plots, each 500 × 1,000 m (5 hectares), were split up between two study sites. The study plot size was determined by the approximate width of the flood plain (1,000 m) and the effective range of audio recorders (2,000 square meters) (Saenz et al. 2006). The number of plots surveyed was limited by the budget available for purchase of DARs. The plots were initially mapped with a Geographic Information System unit. Within those plots, only suitable habitat was surveyed. Lowland leopard frogs are greatly dependent on aquatic habitat (Sredl 2005). We used the plot size to establish the survey area, but we concentrated only on the river itself and any pools, channels, or backwaters within the plot.

Four plots were placed in the Bill Williams River NWR (see figure 2) and six in the Big Sandy River (see figure 3). As mentioned above, Plot 4 on the

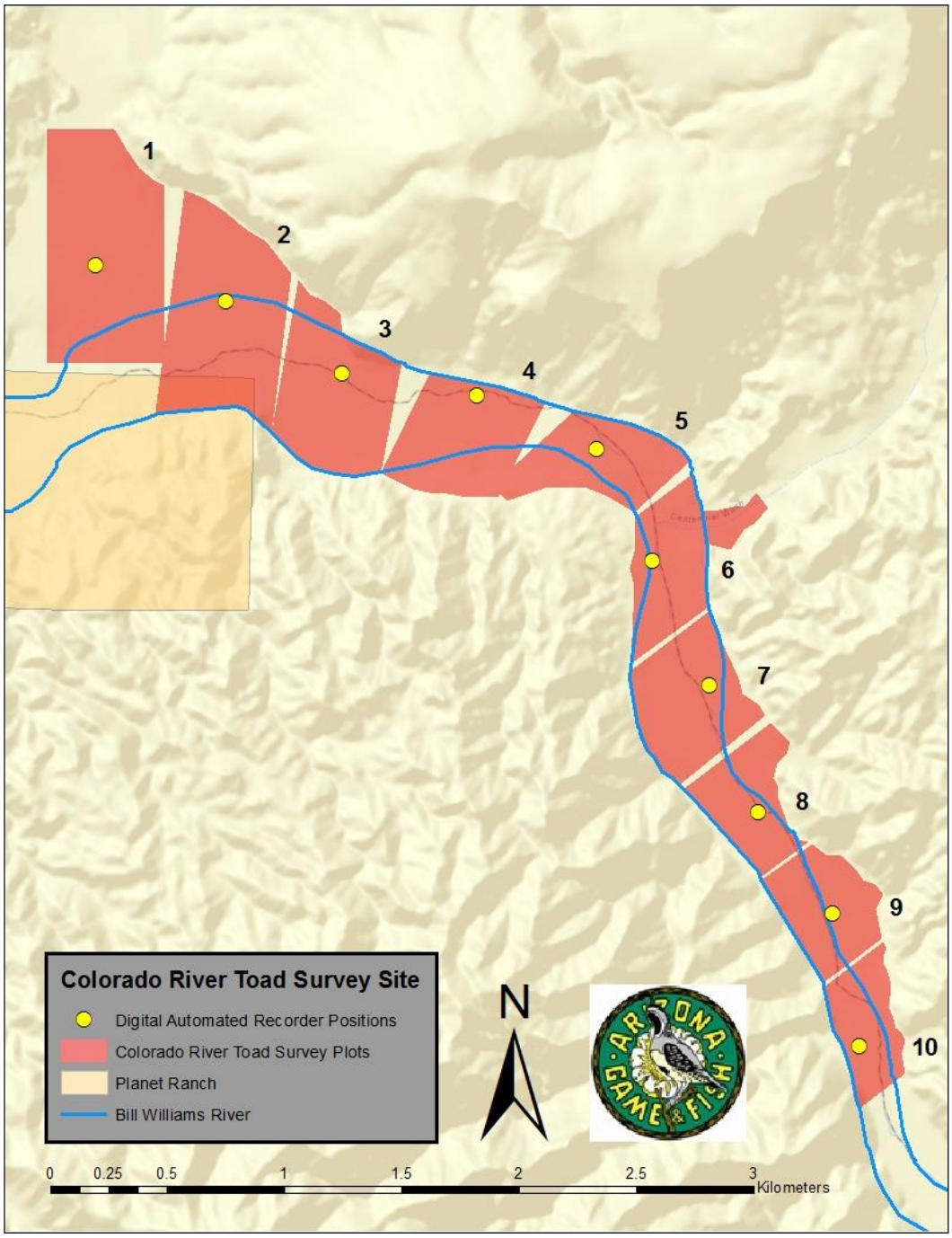

Figure 4.—Colorado River toad DAR locations on the Bill Williams River.

Bill Williams River NWR was first split into two locations, Plots 4 and 4A, due to access issues. After the first trapping session, sampling at Plot 4 was discontinued entirely, and the sampling effort continued at Plot 4A only, located downstream at the end of the Bill Williams River sampling area (see figure 2). On the Big Sandy River, six plots were placed to the south of Wikieup, Arizona. Lowland leopard frogs have two breeding seasons each year: the first occurs roughly March through May and the second roughly during September and October. The spring season is considered the more significant of the two seasons (Sartorius and Rosen 2000). Surveys began on February 3, 2015, and ended on May 2, 2015, to encompass the spring breeding season for lowland leopard frogs.

Visual Encounter Surveys

One VES was completed each night for lowland leopard frogs. VESs were conducted in the same manner as Colorado River toads for 2015. Effort was concentrated only on the immediate riparian area of each river because lowland leopard frogs require aquatic conditions (Sredl 2005). Individuals caught during a VES were marked with the same batch marking for the survey session. One toe was clipped, marking an individual to a specific session or time period. Once they were marked and given a brief health evaluation they were released.

Funnel Trap Arrays

Four clusters of 10 funnel traps were set in each plot. Two plots were surveyed at a time over a 2-night period. A total of 80 traps, 40 per plot, were set in streamflow, small channels, or side pools adjacent to the Bill Williams River. Traps were set throughout each plot in an effort to cover the river with traps over the course of the season. The traps were checked twice, once after the first night and again upon removal from the plot. All target species caught were recorded, batch marked, and released. All by-catch was also recorded and released. When moving traps between study sites, they were disinfected to reduce the risk of spreading chytridiomycosis by immersion in a 1:640 dilution of quaternary ammonium chloride and water (Johnson et al. 2003).

Digital Automated Recorders

One DAR was placed in the center of each plot within the riparian area of the Bill Williams River NWR (figure 5). Recordings were similar to Colorado River toads: 1 minute every hour from 21:00 to 02:00 hours each night from the first day to the final day of the study season. Data storage cards were collected from each unit and processed throughout the season. All calling amphibian species heard were recorded and counted to the best of our ability.

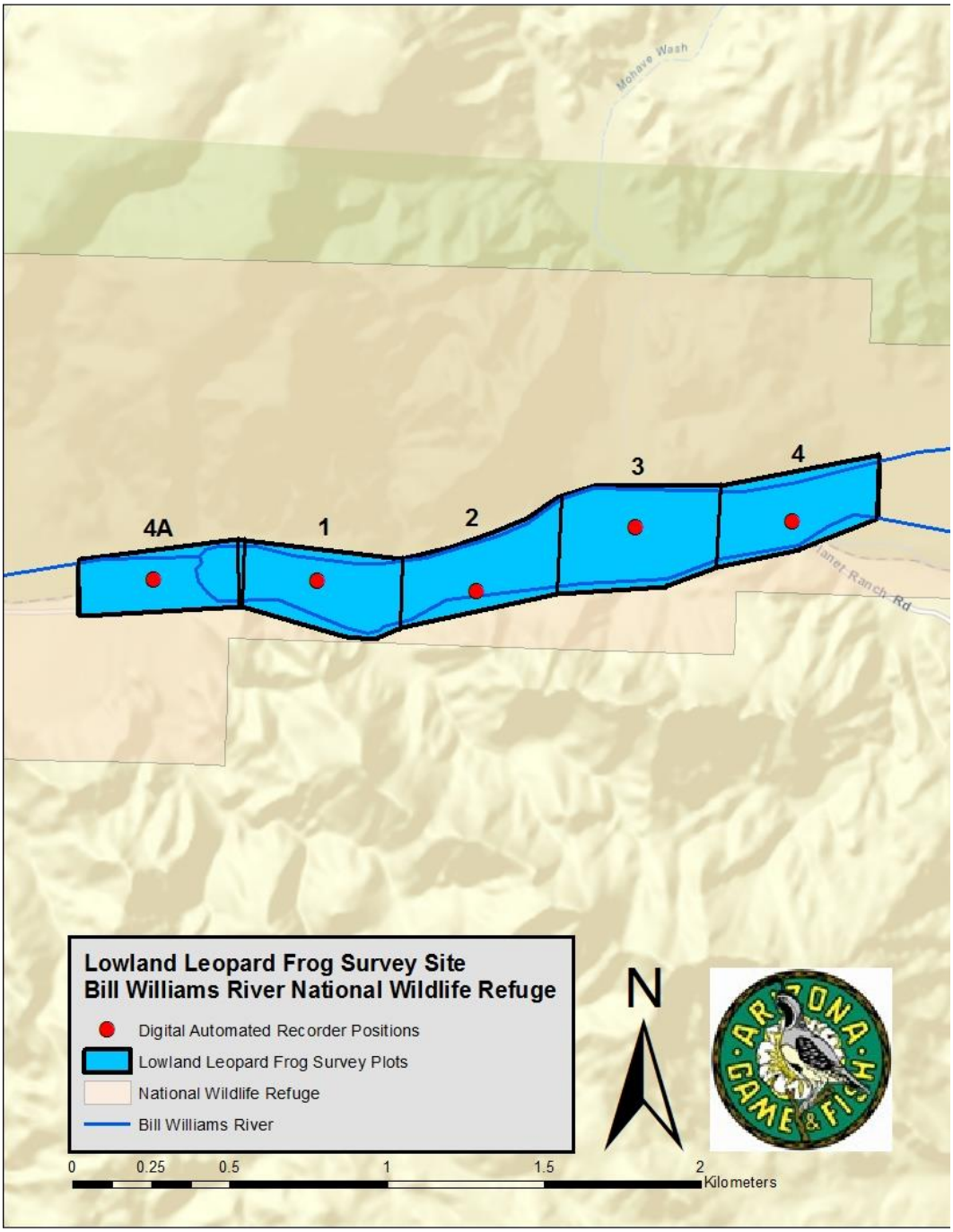

Figure 5.—DAR positions in the Bill Williams River NWR.

Figure 6.—DAR positions in the Big Sandy River.

Environmental DNA

One sample was collected at each cluster of traps that was deployed using similar methods as for Colorado River toads. A total of four samples were collected and filtered per plot for each of the four trapping sessions. Sixteen samples were collected from each plot, resulting in a total of 160 samples for the entire season. These samples were also sent to Reclamation's Environmental Applications and Research Group in Denver, Colorado, for analyses (results pending).

Data Collection and Curation

All data were collected digitally with Trimble® Nomad Global Positioning System (GPS) units and Terrasync® software. Mobile electronic field forms were created by Reclamation for each survey method to standardize data collection. Paper data sheets were also utilized as a backup for digital data and for quality assurance and quality control. Data files created in the field were differentially corrected to obtain the most accurate location data possible and then subjected to quality assurance and quality control before being exported into a Microsoft Access® database through the program GPS Pathfinder®.

Data Analysis and Processing

To examine the differences among the methods of detection, three separate modeling exercises were developed. Multi-method occupancy models were developed in Program PRESENCE version 9.8 (Hines 2006) for Colorado River toads in 2014 and 2015 and lowland leopard frogs in 2015. The modeling exercise left occupancy probability (Ψ) for a site as a constant parameter, the probability that an individual can be detected (θ) was allowed to vary by survey session and method, and the probability of detection (p) was evaluated by survey session, method, and for an interaction between survey session and method. A survey session was defined as 1 week of field work for each method (two surveys per plot for VESs and VES CRs, 1 week of DAR recordings, 1 week of FTAs, or four samples for eDNA). Thus, although DARS were used throughout the field season, for analyses, only recordings from the nights on which VESs were conducted were used. This resulted in 4 survey sessions for lowland leopard frogs and 4–10 for Colorado River toads. The models were compared using the Akaike's information criterion and corrected for small sample size. The small number of sites available (10) precluded using a goodness of fit test, as the results would have had low statistical power. Because the plots were conterminous, it is likely that the detection probabilities may be spatially autocorrelated. This may have resulted in estimated standard errors for detection that were lower than they would have been if plots were truly independent.

A cost-corrected detection probability was created to compare each survey method in terms of probability of detection and overall cost. This was calculated by dividing the estimated detection probability of each method (from the PRESENCE models, above) by its total cost and then multiplying by a scaling factor of 10,000. The total cost was calculated as the sum of equipment and personnel costs dedicated specifically to that method. Although this coarse approach does not account for the difference between startup and maintenance costs for a method, nor does it consider logistical difficulties or personnel needs associated with a method beyond the dollar amount spent, it does provide a quantitative measure from which to begin discussions of method selection.

RESULTS

The results comparing all methods will be included in the 2016 and 2017 annual reports.

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LITERATURE CITED

Adams, D.K. and A.C. Comrie. 1997. The North American monsoon. Bulletin of the American Meteorological Society 78:2197–2213.

Adang, T.C. and R.L. Gall. 1989. Structure and dynamics of the Arizona monsoon boundary. American Meteorological Society 117:1423–1438.

Brennan, T.C. and A.T. Holycross. 2006. A Field Guide to Amphibians and Reptiles in Arizona. Arizona Game and Fish Department Publication, Phoenix, Arizona.

Bureau of Reclamation. 2004. Lower Colorado River Multi-Species Conservation Program, Volume II: Habitat Conservation Plan, Final. Sacramento, California.

Cotten, T.B. 2011. Lowland Leopard Frog *(Rana yavapaiensis)* and Colorado River Toad (*Bufo alvarius*) Distribution and Habitat Use in the Greater Lower Colorado River Ecosystem: 2011 Annual Report. Submitted to the Bureau of Reclamation by the Arizona Game and Fish Department, Research Branch, Phoenix, Arizona.

Cotten, T.B. and D. Grandmaison. 2012. Lowland Leopard Frog (*Rana yavapainsis*) and Colorado River Toad (*Bufo alvarius*) Distribution and Habitat Use in the Greater Lower Colorado River Ecosystem. Arizona Game and Fish Department, Contracts Branch, Flagstaff, Arizona.

Cotten, T.B. and D.J. Leavitt. 2014. Lowland Leopard Frog and Colorado River Toad Distribution and Habitat Use in the Greater Lower Colorado River Ecosystem. Arizona Game and Fish Department, Contracts Branch, Phoenix, Arizona.

Hall, L.S., P.R. Krausman, and M.L. Morrison. 1997. The habitat concept and a plea for standard terminology. Wildlife Society Bulletin 25:173–182.

Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.C. Hayer, and M.S. Foster (editors). 1994. Measuring and Monitoring Biological Diversity. Standards and Methods for Amphibians. Smithsonian Institute Press, Washington, D.C. 364 p.

Hines, J.E. 2006. Program PRESENCE. https://www.mbr-pwrc.usgs.gov/software/presence.html

Johnson, M.L., L. Berger, L. Philips, and R. Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium* dendrobatidis. Diseases of Aquatic Organisms 57:255–260.

Saenz, D., L.A. Fitzgerald, K.A. Baum, and R.N. Conner. 2006. Abiotic correlates of anuran calling phenology: the importance of rain temperature and season. Herpetological Monographs 20:64–82.

Sartorius, S.S. and P.C. Rosen. 2000. Breeding phenology of the lowland leopard frog (*Rana yavapaiensis*): implications for conservation and ecology. The Southwestern Naturalist 45:267–273.

Sredl, M.J. 2005. *Rana yavapaiensis* (Platz and Frost 1984) lowland leopard frogs *in* M.J. Lannoo (editor). Amphibian Declines: The Conservation Status of United States Species, pp. 596–599. University of California Press, Berkeley.

Sredl, M.J., J.M. Howland, J.E. Wallace, and L.S. Saylor. 1997. Status and distribution of Arizona's native ranid frogs. Pages 37–89 *in* M.J. Sredl (editor). Ranid Frog Conservation and Management. Nongame and Endangered Wildlife Program Technical Report 121. Arizona Game and Fish Department, Phoenix, Arizona.

Sullivan, B.K. and K.B. Mallmos. 1994. Call variation in the Colorado River toad (*Bufo alvarius*): behavioral and phylogenetic implications. Herpetologica 50:146–156.

Sullivan, B.K. and P.J. Fernandez. 1999. Breeding activity, estimated age-structure, and growth in Sonoran Desert anurans. Herpetologica 55:334–343.

U.S. Geological Survey. 2012. Snake River Field Station, Forest and Rangeland Ecosystem Science Center. eDNA Protocol—Filtering Water to Capture DNA from Aquatic Organisms. Boise, Idaho.

ATTACHMENT 1

Non-Target Herpetofauna Captures for Each Method
Across All Study Sites and Years

Species (common name)[2]	Big Sandy River 2015				Bill Williams River National Wildlife Refuge 2015				Swansea[1], 2014				Swansea, 2015			
	VES[3]	VES/CR[4]	FTA[5]	DAR[6]	VES	VES/CR	FTA	DAR	VES	VES/CR	FTA	DAR	VES	VES/CR	FTA	DAR
Arizona toad	X	X	X	X	X	X	X	X	X			X	X			
Red-spotted toad	X			X				X	X	X	X	X	X			X
Canyon treefrog	X	X		X												
Couch's spadefoot									X	X		X	X			X
Sonora mud turtle	X		X													
Western banded gecko													X			
Greater earless lizard													X			
Common lesser earless lizard													X			
Ornate tree lizard													X			
Desert spiny lizard	X															
Gila monster									X							
Glossy snake									X				X			
Western shovel-nosed snake													X			
Western diamond-backed rattlesnake									X				X			
Speckled rattlesnake													X			
Common kingsnake									X				X			
Western threadsnake									X				X			
Spotted leaf-nosed snake													X			
Mexican garter snake	X		X						X		X		X			

[1] The Swansea Wilderness Area is the location of the Colorado River toad survey plots on figure 1. It is located east of Planet Ranch and the Bill Williams River NWR.
[2] Scientific names can be found on the next page.
[3] VES = visual encounter survey.
[4] VES/CR= VES with an audio broadcast call/response component.
[5] FTA = funnel trap array.
[6] DAR = digital automated recorder.

Common name	Scientific name
Arizona toad	*Anaxyrus microscaphus*
Red-spotted toad	*Anaxyrus punctatus*
Canyon treefrog	*Hyla arenicolor*
Couch's spadefoot	*Scaphiopus couchii*
Sonora mud turtle	*Kinosternon sonoriense*
Western banded gecko	*Coleonyx variegatus*
Greater earless lizard	*Cophosaurus texanus*
Common lesser earless lizard	*Holbrookia maculata*
Ornate tree lizard	*Urosaurus ornatus*
Desert spiny lizard	*Sceloporus magister*
Gila monster	*Heloderma suspectum*
Glossy snake	*Arizona elegans*
Western shovel-nosed snake	*Chionactis occipitalis*
Western diamond-backed rattlesnake	*Crotalus atrox*
Speckled rattlesnake	*Crotalus mitchellii pyrrhus*
Common kingsnake (also known as eastern kingsnake or chain kingsnake)	*Lampropeltis getula*
Western threadsnake	*Leptotyphlops humilis*
Spotted leaf-nosed snake	*Phyllorhynchus decurtatus* (Cope, 1868)
Mexican garter snake	*Thamnophis eques megalops*