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

2016 Annual Report

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

April 2017

Lower Colorado River Multi-Species Conservation Program Steering Committee Members

Federal Participant Group

Bureau of Reclamation
U.S. Fish and Wildlife Service
National Park Service
Bureau of Land Management
Bureau of Indian Affairs
Western Area Power Administration

Arizona Participant Group

Arizona Department of Water Resources
Arizona Electric Power Cooperative, Inc.
Arizona Game and Fish Department
Arizona Power Authority
Central Arizona Water Conservation District
Cibola Valley Irrigation and Drainage District
City of Bullhead City
City of Lake Havasu City
City of Mesa
City of Somerton
City of Yuma
Electrical District No. 3, Pinal County, Arizona
Golden Shores Water Conservation District
Mohave County Water Authority
Mohave Valley Irrigation and Drainage District
Mohave Water Conservation District
North Gila Valley Irrigation and Drainage District
Town of Fredonia
Town of Thatcher
Town of Wickenburg
Salt River Project Agricultural Improvement and Power District
Unit "B" Irrigation and Drainage District
Wellton-Mohawk Irrigation and Drainage District
Yuma County Water Users' Association
Yuma Irrigation District
Yuma Mesa Irrigation and Drainage District

Other Interested Parties Participant Group

QuadState Local Governments Authority
Desert Wildlife Unlimited

California Participant Group

California Department of Fish and Wildlife
City of Needles
Coachella Valley Water District
Colorado River Board of California
Bard Water District
Imperial Irrigation District
Los Angeles Department of Water and Power
Palo Verde Irrigation District
San Diego County Water Authority
Southern California Edison Company
Southern California Public Power Authority
The Metropolitan Water District of Southern California

Nevada Participant Group

Colorado River Commission of Nevada
Nevada Department of Wildlife
Southern Nevada Water Authority
Colorado River Commission Power Users
Basic Water Company

Native American Participant Group

Hualapai Tribe
Colorado River Indian Tribes
Chemehuevi Indian Tribe

Conservation Participant Group

Ducks Unlimited
Lower Colorado River RC&D Area, Inc.
The Nature Conservancy

Lower Colorado River Multi-Species Conservation Program

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

2016 Annual Report

Prepared by:

O'Donnell, R.P. and D.J. Leavitt

Arizona Game and Fish Department

Lower Colorado River
Multi-Species Conservation Program
Bureau of Reclamation
Lower Colorado Region
Boulder City, Nevada
http://www.lcrmscp.gov

April 2017

O'Donnell, R.P. and D.J. Leavitt. 2017. Development and implementation of a Repeatable Monitoring Plan for Lowland Leopard Frogs and Colorado River toads on the Lower Colorado River, 2016 Annual Report. Submitted to the Bureau of Reclamation, Boulder City, Nevada, by the Arizona Game and Fish Department, Phoenix, Arizona.

ACRONYMS AND ABBREVIATIONS

AIC	Akaike's information criterion
AIC_c	Akaike's information criterion corrected for small sample size
bp	base pairs
BSA	Bovine Serum Albumin
LCR MSCP	Lower Colorado River Multi-Species Conservation Program
CCDI	cost-corrected detection index
COI	cytochrome oxidase I
DAR	digital automated recorder
eDNA	environmental deoxyribonucleic acid
FTA	funnel trap array
GIS	Geographic Information System
GPS	Global Positioning System
m^2	square meter(s)
PCR	polymerase chain reaction
Reclamation	Bureau of Reclamation
SE	standard error
VES	visual encounter survey
VES C/R	visual encounter surveys with an audio broadcast call/response

Symbols

$\approx$	approximately (nearly equal to)
Δ	difference or change
θ	fine-scale occupancy probability
$\leq$	less than or equal to
Ψ	occupancy probability
p	probability of detection

CONTENTS

Page

Abstract i

Introduction 1
Colorado River Toad 1
Lowland Leopard Frog 1
Objective 2
Study Area 2
Colorado River Toad 2
Lowland Leopard Frog 2
Methods 6
Colorado River Toad 6
Visual Encounter Surveys 6
Funnel Trap Arrays 7
Digital Automated Recorders 8
Environmental DNA 8
Trial Monitoring Implementation 11
Lowland Leopard Frog 11
Visual Encounter Surveys 12
Funnel Trap Arrays 12
Digital Automated Recorders 13
Environmental DNA 13
Data Collection and Curation 16
Data Analysis and Processing 16
Results 17
Colorado River Toad 17
Visual Encounter Surveys 17
Funnel Trap Arrays 19
Digital Automated Recorders 19
Environmental DNA 22
Trial Monitoring Implementation 23
Lowland Leopard Frog 24
Visual Encounter Surveys 24
Funnel Trap Arrays 25
Digital Automated Recorders 26
Environmental DNA 27
Multi-Method Occupancy Models 27
Discussion 30
Colorado River Toad 30
Visual Encounter Surveys 30
Funnel Trap Arrays 31
Digital Automated Recorders 32
Environmental DNA 33

Page

Lowland Leopard Frog 34
Visual Encounter Surveys 34
Funnel Trap Arrays 34
Digital Automated Recorders 35
Environmental DNA 35
Lessons Learned 37
Colorado River Toad 37
Lowland Leopard Frog 38
Acknowledgements 39
Literature Cited 41

Tables

Table Page

1 DAR detections per hour and per plot, excluding the incorrectly timed recordings from Plot 6, 2014–15 21
2 Detections from recorder set to Eastern Standard Time on Plot 6 with corrected times 21
3 Potential overlapping detections of Colorado River toads 22
4 DAR detections of Colorado River toads per hour and per plot, 2016 23
5 Candidate models used to estimate occupancy (Ψ) and detection probability (p, constant or varying by week, s) for the trial implementation of acoustic monitoring of Colorado River toads in 2016 24
6 Total number of lowland leopard frog VES detections per plot and per session in the Bill Williams River NWR (BW) and the Big Sandy River (BS) 24
7 Lowland leopard frog VES C/R detections per plot and per session in the Bill Williams River NWR and Big Sandy River 25
8 Lowland leopard frog FTA captures per plot and per session on the Bill Williams and Big Sandy Rivers in 2015 (sessions 1–4) and 2016 (sessions 5–6) 26
9 Lowland leopard frog DAR detections on the Bill Williams and Big Sandy Rivers per hour and per plot in 2015–16 26
10 Candidate models used to estimate large-scale occupancy (Ψ),the probability that an individual is available for detection by a method (small-scale occupancy [θ]), and that detection probability (p) varies by survey session (s) and method (m) for Colorado River toads in 2014 28

Tables (continued)

Table Page

11 Candidate models used to estimate large-scale occupancy (Ψ), the probability that an individual is available for detection by a method (small-scale occupancy [θ]), and that detection probability (p) varies by survey session (s) and method (m) for Colorado River toads in 2015 28

12 Candidate models used to estimate large-scale occupancy (Ψ), the probability that an individual is available for detection by a method (small-scale occupancy [θ]), and that detection probability (p) varies by survey session (s) and method (m) for Colorado River toads in 2015 and 2016 combined 29

13 CCDI calculated by dividing probability of detection from the top-ranked model (P) by the total cost of labor and equipment 29

Figures

Figure Page

1 Colorado River toad survey plots in the Swansea Wilderness on the Bill Williams River. 3

2 Lowland leopard frog survey plots on the Bill Williams River. 4

3 Lowland leopard frog survey plots on the Big Sandy River. 5

4 Locations of DARs for Colorado River toads in the Swansea Wilderness on the Bill Williams River. 9

5 DAR positions in the Bill Williams River National Wildlife Refuge. 14

6 DAR positions in the Big Sandy River. 15

7 Colorado River toad VES detections. 18

8 Colorado River toad FTAs and eDNA sampling locations. 20

9 Overlapping calls of lowland leopard frogs and Arizona Toads. 36

Attachments

Attachment

1 Detections of Non-Target Amphibians and Reptiles for Each Method Across All Study Sites and Years

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 (*Bufo alvarius* [also known as *Incilius alvarius*]) and the lowland leopard frog (*Rana yavapaiensis* [also known as *Lithobates yavapaiensis* and *R. {Lithobates} yavapaiensis*]). Surveys for Colorado River toads occurred along the Bill Williams River in the Swansea Wilderness, 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 seasons, summer monsoons for Colorado River toads and spring for lowland leopard frogs. Surveys for Colorado River toads were completed in the summers of 2014 and 2015. Surveys for lowland leopard frogs were conducted in 2015 and 2016. In 2014, Colorado River toads were detected 11 times with VESs, seven times with VES C/Rs broadcast calls, 0 times with FTAs, and 80 times with the DARs. In 2015, Colorado River toads were detected six times with VESs and were not detected with any other method. Environmental deoxyribonucleic acid was very effective when water was present (toad DNA was detected in 10 of 11 samples), but only three sites in one year had enough water to conduct eDNA sampling.

There were many more detections of lowland leopard frogs in 2015, including 211 VES detections, 152 VES C/R detections, 232 individuals captured in FTAs, and 668 DAR recordings. In 2016, lowland leopard frogs were detected 57 times with VESs, 31 times with VES C/Rs, 12 times with FTAs, and 367 times with DARs. Results from eDNA samples from 2014 and 2015 indicated that eDNA was very effective method of detecting this species, with a higher detection probability than any other method. A cost-corrected detection index was developed to quantitatively compare the amount of effort required to survey and the probability of detecting an animal. This index suggests that the VES method is the most cost-effective method for surveying Colorado River toads and lowland leopard frogs in this implementation, but other concerns such as labor needs and equipment costs affect which method is the best for a given application.

In 2016, a trial monitoring plan for Colorado River toads was tested and confirmed that DARs are an effective and efficient method for detecting Colorado River toads, given that sufficient monsoon rains occur to prompt calling. A monitoring plan for lowland leopard frogs and Colorado River toads will be established following one season of field testing to be conducted for lowland leopard frogs in 2017.

INTRODUCTION

Colorado River Toad

Colorado River toads (*Bufo alvarius* [also known as *Incilius alvarius*]) are believed to be extirpated from the main stem of the Lower Colorado River (Cotten and Leavitt 2014). However, a small population exists on the Bill Williams River (Cotten 2011; Cotten and Leavitt 2014), a tributary to the lower Colorado River. The Colorado River toad 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 action in this portion of its range along the lower Colorado River (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 (mm) (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 (*Rana yavapaiensis* [also known as *Lithobates yavapaiensis* and *R. [Lithobates] yavapaiensis*) are also believed to be extirpated from the main stem of the Lower Colorado River (Cotten and Leavitt 2014). They were known from the Bill Williams River in the early 1990s (B. Raulston 2017, personal communication; Sredl et al. 1997) and were observed on the Bill Williams River National Wildlife Refuge (Bill Williams River NWR) 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 Lower Colorado River or within the Bill Williams River NWR boundaries until two individuals were found in the spring of 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, except during large monsoon flooding. This suggests that a population of lowland leopard frogs could re-establish itself in the Bill Williams River NWR. Reclamation has placed lowland leopard frogs on its list of evaluation species (Reclamation 2004) to determine their status in the LCR MSCP area, to assess the potential effects of covered activities on the species, and to determine appropriate conservation measures. Lowland leopard frogs typically breed 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 lower Colorado River to survey for both species in the future that are cost efficient and effective. Five survey methods were evaluated for their ability to detect each species and then analyzed for cost efficiency and cost effectiveness. 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 was 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 lower Colorado River. It begins at the eastern border of Planet Ranch and proceeds east upstream approximately seven kilometers along the Bill Williams River and into Bureau of Land Management managed public land and the Swansea Wilderness (figure 1).

Lowland Leopard Frog

The study area for lowland leopard frogs was split into two locations within the same drainage. The first location was along the Bill Williams River 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 lower Colorado River. The refuge extends east upstream approximately 16 kilometers to the western boundary of Planet Ranch (figure 2). The Bill Williams River study area extended from approximately Mineral Wash on the west to the eastern boundary of the Bill Williams NWR (figure 2). Plot 4 on the Bill Williams River NWR was replaced with 4A when preliminary surveys revealed extensive beaver impoundments and thick tamarisk (*Tamarix* spp.) across Plot 4 that made sampling it not feasible. The second site was located on the Big Sandy River, a tributary to the Bill Williams River, approximately 11 kilometers south of Wikieup, Arizona (figure 3). Between the two plots lies Alamo Dam, which creates Alamo Lake. Downstream flows on the Bill Williams River are sustained by releases dependent on recent lake levels.

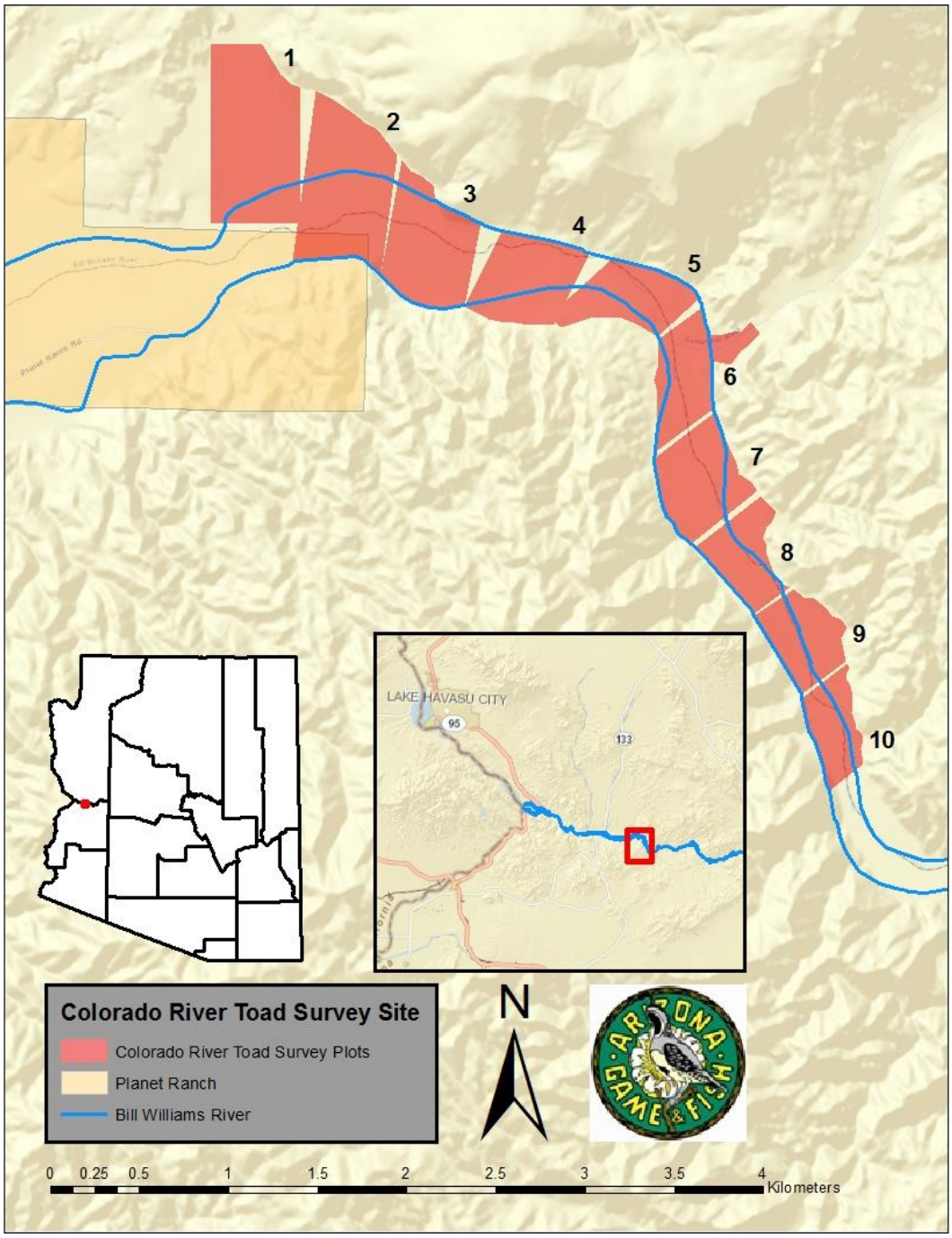

Figure 1.—Colorado River toad survey plots in the Swansea Wilderness on the Bill Williams River.

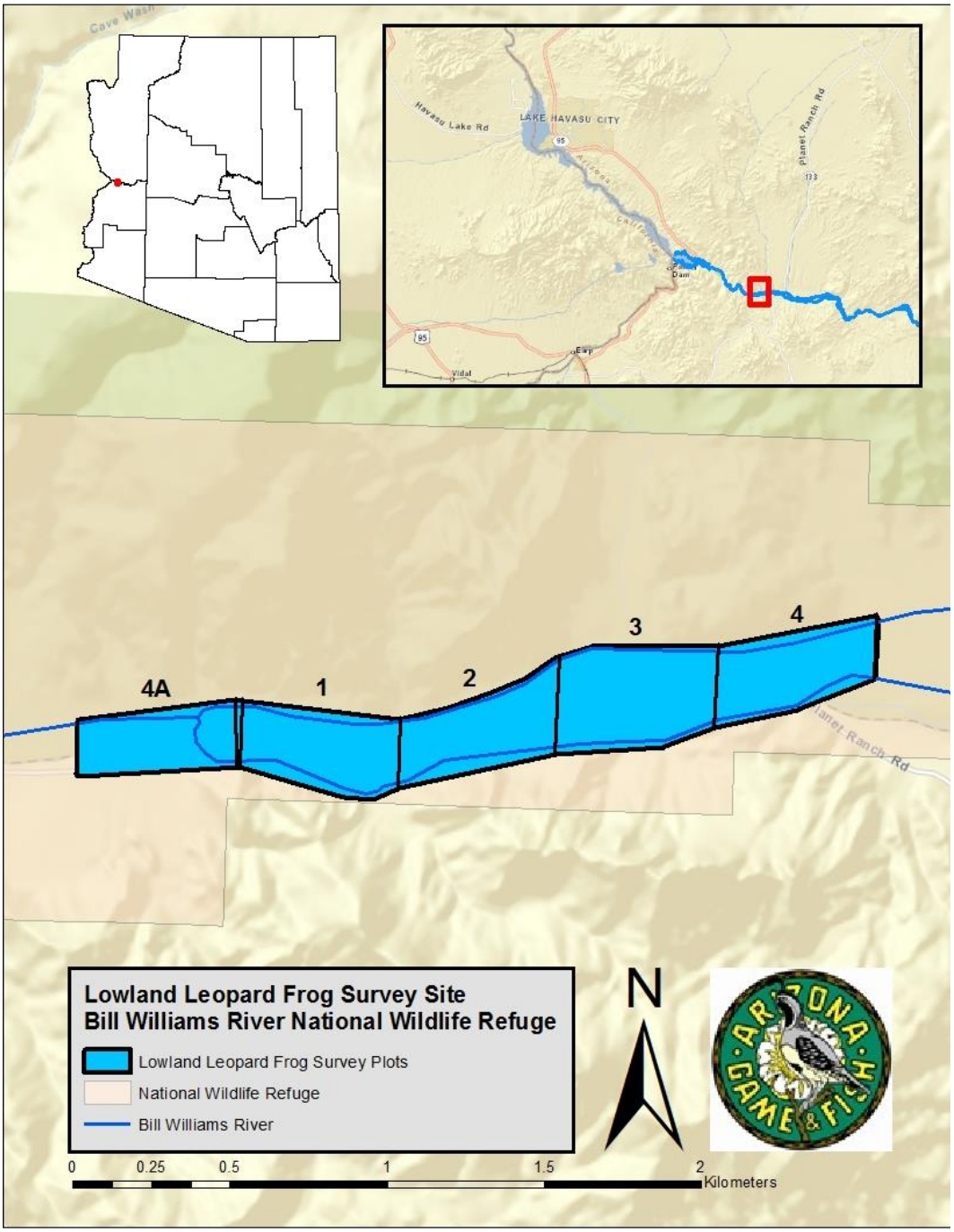

Figure 2.—Lowland leopard frog survey plots on the Bill Williams River.

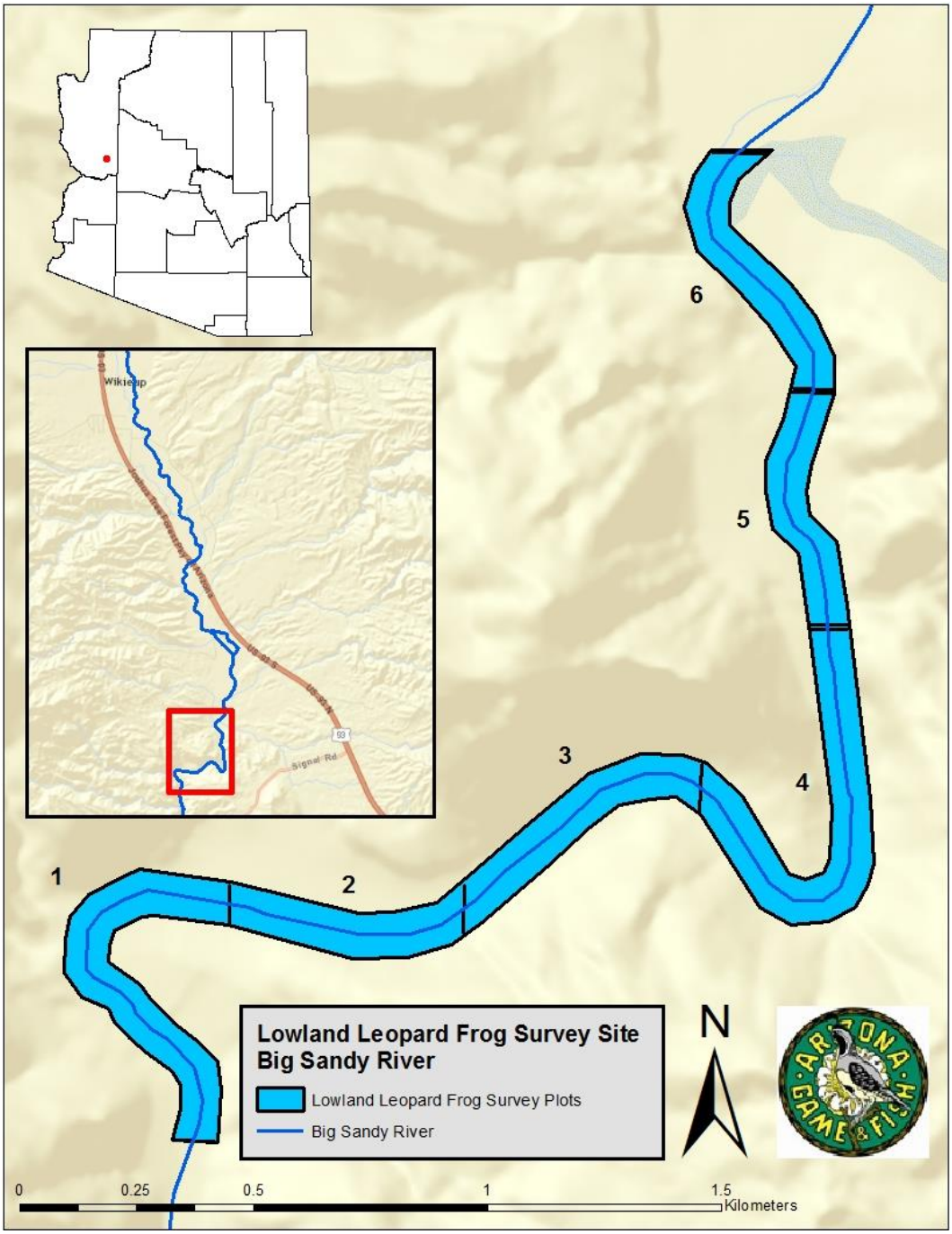

Figure 3.—Lowland leopard frog survey plots on the Big Sandy River.

METHODS

Colorado River Toad

Ten study plots, each 500 × 1,000 meters (m) (5 hectares [ha]), 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 (Swansea Wilderness, Cotten and Grandmaison 2012) (see figure 1). Study plot size was determined by approximate width of the flood plain (1,000 m) and effective range of audio recorders (2,000 m^2) (Saenz et al. 2006). The number of plots surveyed was limited by the budget available for the purchase of DARs. Plots were initially mapped with a Geographic Information System. Preferred habitat of Colorado River toads is Sonoran desert scrub and, more specifically, the lower Colorado River 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*). The only portion of the study plots within this type of biotic community was the river and its historic flood plain represented by the survey area. Only suitable habitat was surveyed within each plot. Within each study site, large portions of the plots did not contain habitat for Colorado River toads. With the exception of Plots 1 and 2, each plot included a significant amount of steep mountainous terrain. Plots 3–10 had 20-60% of 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 numbered upstream (east) 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). Colorado River toads typically 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

Visual encounter surveys consisted of two biologists walking a plot with flashlights for 2 hours to detect adults, egg masses, or tadpoles. Location, life stage, and sex, if known, was recorded for each Colorado River toad. The location of each amphibian or reptile species seen on a plot during a survey was recorded.

Audio broadcast call/responses (VES C/Rs) were included in each VES. At each plot, a VES was conducted for 2 hours, and all amphibians observed by sight or sound were recorded. Every 30 minutes, a VES C/R was added by broadcasting amphibian calls, and amphibians that called in response were recorded as being detected by a VES C/R. A 15-second recording was broadcast two times (30 seconds total), followed by a listening period of 2–3 minutes. This was usually repeated once, but sometimes twice in rare cases in which it was unclear whether a distant frog was calling and further confirmation was needed. Amphibians detected by VES C/Rs might include amphibians already detected by VESs, so there is some overlap between these two methods. Amphibians heard calling before broadcast or more than 3 minutes after broadcast were counted as being detected by VESs whether they were seen or not. Amphibians typically responded immediately to broadcast; amphibians calling during VESs (more than 3 minutes after the end of broadcast) were not considered to be due to delayed responses to VES C/Rs.

Each VES C/R used a FOXPRO, Inc.,® broadcast system 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 4 times by systematically calling every 30 minutes throughout each VES. Broadcast points were spaced out across the study area to ensure optimal coverage of the area: when visual detection was not hindered by vegetation, the perimeter of the plots was accessed; when vegetation blocked the view, complete visual coverage was ensured by crossing through the plot. 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 through 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 surface 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 ten traps were set for 2 nights

and checked once after the first night and again upon removal. Funnel traps were arranged such that all ten traps remained within a 10 × 10 m area in suitable habitat, and thus 0-3 m from a trap to its nearest neighbor. Traps were typically set along banks. No bait was placed in the traps. Traps 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.

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. Recording devices were set along the river channel as close to the center of each plot as possible. Each recorder was set to record one minute of every hour on the hour from 21:00 through 02:00 each night (figure 4). Recordings were downloaded in the field and evaluated later. Any amphibian species heard during the recordings was noted along with time and estimated number of individuals calling.

Environmental DNA

Water samples were filtered at sites where each FTA was set to conduct eDNA sampling using established protocols (Laramie et al. 2015). A maximum of four samples were filtered at each plot during each trapping event. Samples were filtered after the first trap night. One to two liters of water from each site were filtered through a 0.45-micron filter. 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% percent bleach and water solution. Filters were placed in a vial of 95% ethanol solution, labeled, and stored for later analysis. No predetermined number of samples were expected to be taken due to the unreliability of surface water throughout each season.

Filters were sent to the Reclamation's Environmental Applications and Research Group in Denver, Colorado, for analyses. Total DNA was isolated from filters using a protocol modified from Goldberg et al. (2011). Briefly, filters were placed in petri dishes, and one half of each filter was excised and cut to 1 mm. The fragmented filters were placed in 2.0-milliliter (mL) microcentrifuge tubes, and left open to dry for 24 hours in a vented laboratory fume hood. Following drying, samples were digested for 24 hours at 55° Celsius in Buffer ATL + Proteinase K from a DNEasy Blood and Tissue Kit (Qiagen Inc., Valencia, California). 0.36 mL of ATL buffer and 0.04 mL of Proteinase K were used for each sample, twice the manufacturer's specified amount, to ensure immersion and

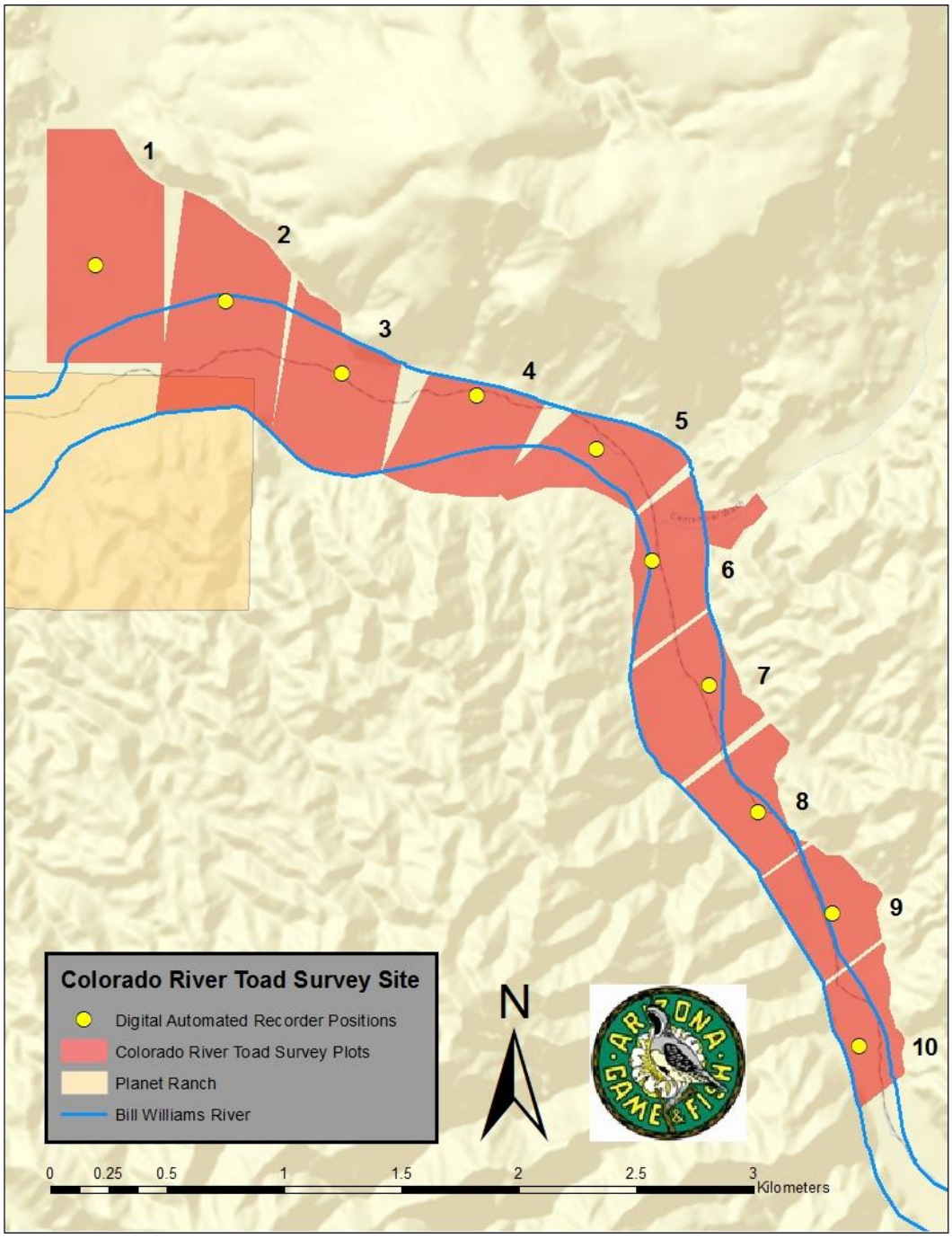

Figure 4.—Locations of DARs for Colorado River toads in the Swansea Wilderness on the Bill Williams River.

complete digestion of the sample. Digested samples were transferred to Qiashredder tubes (Qiagen Inc., Valencia, California), and spun at 20,000 relative centrifugal force for 2 minutes to ensure maceration of collected material and liberation of DNA. Collected supernatant was then processed with the DNEasy Blood and Tissue Kit following the manufacturer's protocol, with the modification that 0.4 mL of Buffer AL and 0.4 mL of ethanol was added to each supernatant, consistent with the increased volume of the digestion solution. The resultant 1.2-mL sample solution was then centrifuged through the DNEasy Blood and Tissue Kit filter tubes in two rounds of 0.6 mL each. Following DNA extraction, all samples were stored at -20° Celsius when not in use. For DNA extraction, and all subsequent protocols, sterile disposable pipet tips and tubes were used for all procedures.

Oligonucleotide primers were designed to specifically amplify fragments of the Colorado River toad mitochondrial cytochrome oxidase I (COI) gene. Primers were designed by alignment of COI gene sequences from Colorado River toads and other toads occurring in Arizona. Reference sequences were obtained from the publically available National Center for Biotechnology Information GenBank database (www.ncbi.nlm.nih.gov/genbank/) and aligned using the MUSCLE software server (www.ebi.ac.uk/Tools/msa/muscle). Primers were designed to regions with the lowest levels of sequence similarity between the COI sequences of Colorado River toads and those of other species. Amplicons were designed to be between 100 base pairs (bp) and 150 bp in length, to minimize the impact of degradation in environmental samples, and thereby improve sensitivity. Four primer pairs were tested. One primer pair, referred to as "INAL COI primer pair 3" was selected for use on environmental DNA (eDNA) samples based upon high sensitivity and specificity (e.g., minimal cross-reactivity when tested with total DNA from the Arizona toad (*Anaxyrus microscaphus*) or the red-spotted toad (*Anaxyrus punctatus*). The two primers, INAL_COI_F3 (5'- GGG CTG ACT GGT ATT GTA CTA -3') and INAL_COI_R3 (5'- AAA CAG CTC CCA TAG ACA GGA -3') amplify a 106 bp fragment of the Colorado River toad COI gene.

A polymerase chain reaction (PCR) was performed using AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific, Inc., Waltham, Massachusetts). PCR reaction mix was optimized to contain the following amounts of reagents per 15 µL reaction: 0.2 µM concentration of each primer, 1× Buffer X (without $MgCl_2$), 1.0 mM $MgCl_2$, 0.2 mM dNTPs, 6 µg Bovine Serum Albumin (BSA), and 0.75 units AmpliTaq Gold DNA polymerase. During protocol optimization, addition of BSA to the amplification reaction was found to reduce PCR inhibition caused by enzyme inhibitors (e.g., humic acid) present in some environmental samples and not fully removed during DNA isolation with the DNEasy Blood and Tissue Kit. All samples were tested at least twice by PCR to ensure

reproducibility of positive results. All PCR runs included positive (total Colorado River toad DNA) and negative (no template DNA) controls to ensure quality control.

All positive PCR reactions were sent for DNA sequencing at a commercial laboratory. Both the forward and reverse stands of PCR products were sequenced and aligned to ensure no errors occurred in sequencing. Primer sequence was removed from the sequencing reads, and the trimmed reads were subjected to blastn search against the NCBI GenBank database (www.ncbi.nlm.nih.gov/BLAST/) to identify sequences of highest similarity. For phylogenetic analysis, COI sequences from the environmental samples were aligned to reference sequences retrieved from the NCBI GenBank database. Alignment was performed with MUSCLE software, and unaligned ends were trimmed. Phylogenetic reconstruction was performed on the aligned sequences using MrBayes v3.2.1 software (Ronquist and Huelsenbeck 2003), with 4 independent runs of 5,000,000 generations each. A consensus tree was calculated and drawn from the results.

Trial Monitoring Implementation

DARs were selected to be deployed in a trial implementation of monitoring suggestions (Miller and Leavitt 2015). DARs were placed in each plot to record calling male Colorado River toads. A Wildlife Acoustics Song Meter SM2+ automated recording device was set in each study unit for a total of 98 nights between July 13 and October 19, 2016. DARs were set along the river channel as close to the center of each plot as possible. Each recorder was set to record one minute of every hour on the hour from 21:00 through 04:00. This extended period relative to the initial trials was intended to assess whether calling might peak later in the night than indicated in initial trials. Recordings were downloaded in the field and evaluated with headphones in a quiet office setting. Each amphibian species heard during the recordings was noted along with time and estimated number of individuals calling.

Lowland Leopard Frog

Ten study plots, each 500 × 1,000 m (5 ha), were split between two study sites. Plot size was determined by approximate width of the flood plain (1,000 m) and effective range of audio recorders (2,000 m^2) (Saenz et al. 2006). The number of plots surveyed was limited by the budget available for the purchase of DARs. Plots were initially mapped with a Geographic Information System. Within those plots only suitable habitat was surveyed. Lowland leopard frogs are greatly

dependent on aquatic habitat (Sredl 2005). The plot size was used to establish the survey area but only the river itself and any pools, channels, or backwaters within the plot were included.

Four plots were placed in the Bill Williams River NWR (see figure 2) and six were placed in the Big Sandy River (see figure 3). The Big Sandy River was chosen because it is a tributary of the Bill Williams River, hosts a known population of lowland leopard frogs, and because of its proximity to the Bill Williams River. Six plots were placed in the Big Sandy River 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 the more significant of the two seasons (Sartorius and Rosen 2000). In 2015, surveys began on February 3 and ended on May 2 to encompass the spring breeding season. In 2016, VESs and minnow trap deployment began on February 2 and ended on February 19, and DARs were deployed between February 8 and April 19. Due to a budget shortfall the full complement of surveys required for comparison to previous years was not completed.

Visual Encounter Surveys

One VES was completed each night for lowland leopard frogs. VESs were conducted in the same manner as for Colorado River toads in 2015, including the audio broadcast call/responses (VES C/Rs). 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 a batch mark to indicate the survey session by clipping one toe. Once each frog was marked and given a brief health evaluation, it was released.

Funnel Trap Arrays

Traps were set throughout each plot in an effort to cover the river with traps over the course of the season. Four clusters of 10 funnel traps were set in each plot within the stream channel or side pools. Two plots were surveyed at a time over a two night period; a total of 80 traps, 40 per plot were surveyed each night. 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 were also recorded and released. Traps were disinfected when moving between study sites to reduce the risk of spreading chytridiomycosis by immersion in a 1:640 dilution 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) and Big Sandy River (figure 6). The use of DAR for lowland leopard frogs was similar to the Colorado River toads, one minute every hour from 21:00 to 02:00 was recorded each night. Data storage cards were collected from each unit and processed throughout the season. Each amphibian heard calling was recorded.

Environmental DNA

One sample was collected at each cluster of traps. A total of four samples were collected and filtered per plot, one from each of the four trapping sessions. Sixteen samples were collected from each plot, resulting in a total of 160 samples for the season. Samples were sent to Reclamation's Environmental Applications and Research Group Denver, Colorado, for analyses.

Analysis of eDNA from lowland leopard frogs was analogous to that from Colorado River toads with the following exceptions in primer design. Oligonucleotide primers were designed to specifically amplify fragments of the lowland leopard frog COI gene. Because COI gene sequences from lowland leopard frogs were not available in publicly available databases, a 678 bp fragment of the COI gene was amplified from toe clip DNA extractions using the primers COI-Chmf4 (5'- TYT CWA AYC AYA AAG AYA TCG G -3') and COI-Chmr4 (5'- ACY TCR GGR TGR CCR AAR AAT CA -3') (Che et al. 2012). The resultant PCR products were sequenced and aligned to COI reference sequences from American bullfrog (*L. catesbeiana*), plains leopard frog (*L. blairi*), and the northern leopard frog (*L. pipiens*). The American bullfrog was chosen because its geographic range overlaps that of the lowland leopard frog. Plains and northern leopard frogs are closely related to lowland leopard frogs and have proximate geographic ranges. Primers were designed to regions with the lowest levels of sequence similarity between the lowland leopard frog COI sequence and those from other species. Amplicons were designed to be between 100 and 150 bp in length, to minimize the impact of degradation in environmental samples and thereby improve sensitivity. Five primer pairs were tested. One primer pair, referred to as "LIYA COI primer pair 3" was selected for use on eDNA samples based upon high sensitivity and specificity. The two primers, LIYA_COI_F3 (5'- CCG GAA CTG GTT GAA CGG TA -3') and LIYA_COI_R3 (5'- AAT AGA CGA CAC TCC TGC CAG -3') amplify a 110 bp fragment of the lowland leopard frog COI gene.

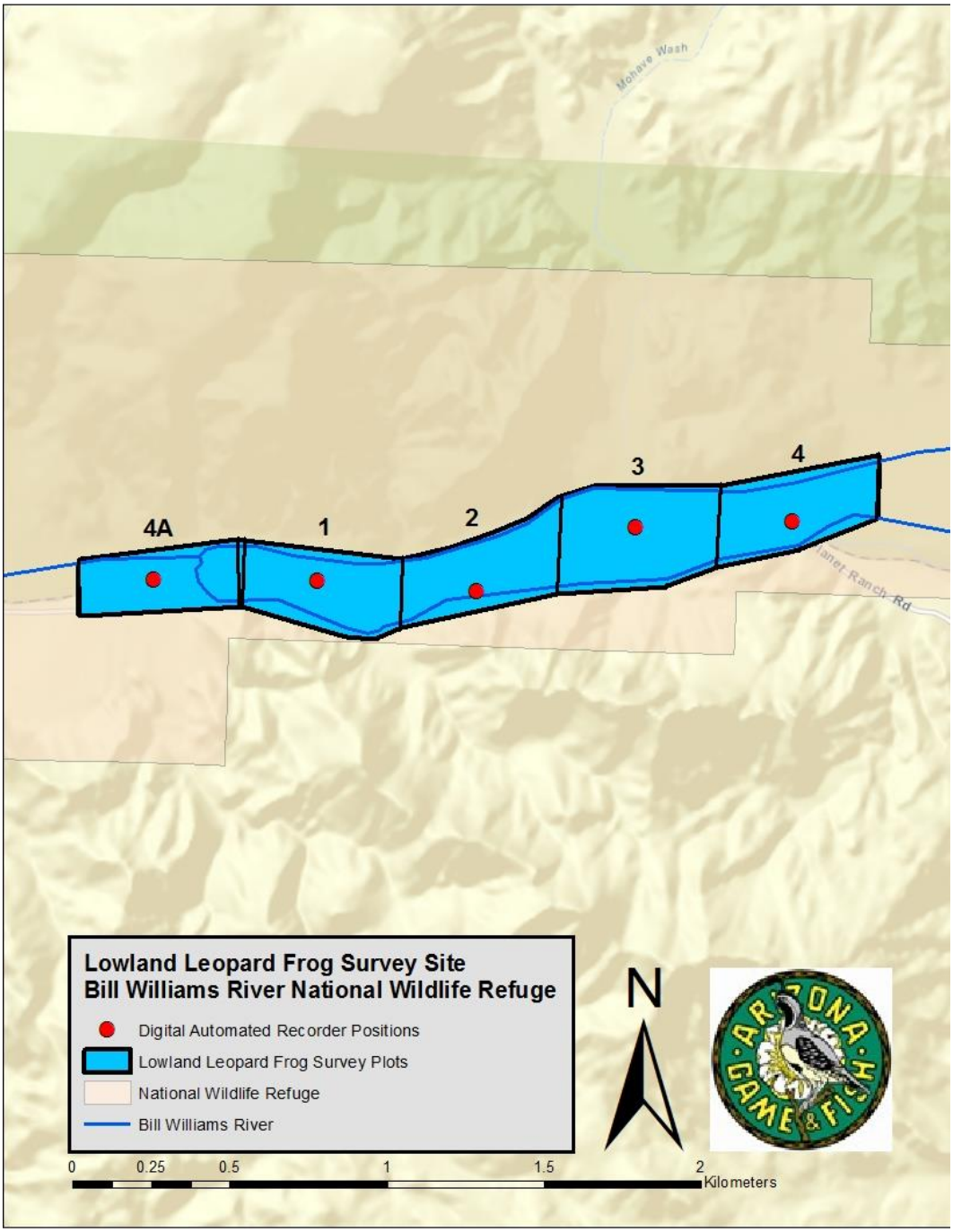

Figure 5.—DAR positions in the Bill Williams River National Wildlife Refuge.

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

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 datasheets 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 exported into a Microsoft Access® database through the program GPS Pathfinder®.

Data Analysis and Processing

To examine differences among the methods of detection, three separate occupancy models were developed. Multi-method occupancy models were developed in Program PRESENCE version 11.7 (Hines 2006) for Colorado River toads separately in 2014 and 2015 and lowland leopard frogs in a combined analysis of 2015 and 2016 (Nichols et al. 2008). Data were combined over years for lowland leopard frogs because VESs and FTAs were only completed for one complete session in 2016. These models left large-scale (sampling unit) occupancy probability (Ψ) as a constant parameter; the probability that an individual was available for sampling by a detection method (fine-scale occupancy, θ, at the scale of the detection method) was allowed to vary by survey session; and probability of detection (p) was evaluated by survey session, method, and for an interaction between survey session and method. (Our previous implementations of this model had also varied θ by method, but this term has been removed here per Nichols et al. 2008). A survey session was defined as 1 week of field work for each method (two surveys per plot for VESs and VES C/Rs, 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 5 to 6 survey sessions for lowland leopard frogs and 4–10 for Colorado River toads. An additional model was also developed for the trial implementation of Colorado River toad monitoring in 2016. All models were ranked according to their Akaike's information criterion (AIC) (Akaike 1973) and were corrected for small sample size (AIC_c) (Burnham and Anderson 2002). Models with $\Delta AIC_c \leq 2$ were considered to be well-supported by the data (Burnham and Anderson 2002). The small number of sites available (10) precluded using a goodness of fit test, as the results would have had low statistical power. Because plots were conterminous, it is likely that detection probabilities may be spatially autocorrelated. This may have resulted in estimated standard errors (SE) for detections that were lower than they would have been if plots were truly independent.

A cost-corrected detection index (CCDI) 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 multiplying by a scaling factor of 10,000. Total cost was calculated as the sum of equipment, analysis, and personnel costs dedicated specifically to that method. Because VES C/Rs were added to VESs, in terms of both cost and detections, VES C/R values represent the additional cost of VES C/Rs over VESs. VES C/R detection probabilities represent only the added detections gained by using call/responses with VESs. Although this coarse CCDI 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

Colorado River Toad

Visual Encounter Surveys

A total of 54 VESs were conducted in 2014. A total of 11 Colorado River toads were detected, including 1 male recaptured in 2014. Each Colorado River Toad that was detected was also captured. Captures occurred on Plots 1, 2, 5, and 6 (figure 7). Plot 6 had seven captures, four of which were on the night of August 7 at a recently flooded ephemeral pool. Single captures on Plot 6 occurred on July 12, September 2, and September 3, the last of which was the recaptured individual. Plot 2 had two captures, one on July 9, and one on September 17. Finally Plots 1 and 5 each had a single capture on August 20 and August 6, respectively. Two amplectic pairs were incidentally observed on Plot 5 on August 5, 2014, as well as two egg masses on August 7, 2014. Calling males were found on Plot 6, but no females or egg masses were observed. Responses were heard during seven VESs in the 2014 season. Call responses were heard from Plots 4, 5, 6, 7, and 8. All VES C/R detections came from outside the surveyed plot except for a single detection on Plot 5.

A total of 91 VESs were conducted in 2015, resulting in 6 detections of Colorado River toads. Three Colorado River toads were found on Plot 6: two on September 22 and one on September 23. Two toads were captured on Plot 9: one on August 25 and the other on September 9. One toad was captured on Plot 5 on July 30. No calling or breeding activity was seen or heard in the 2015 season.

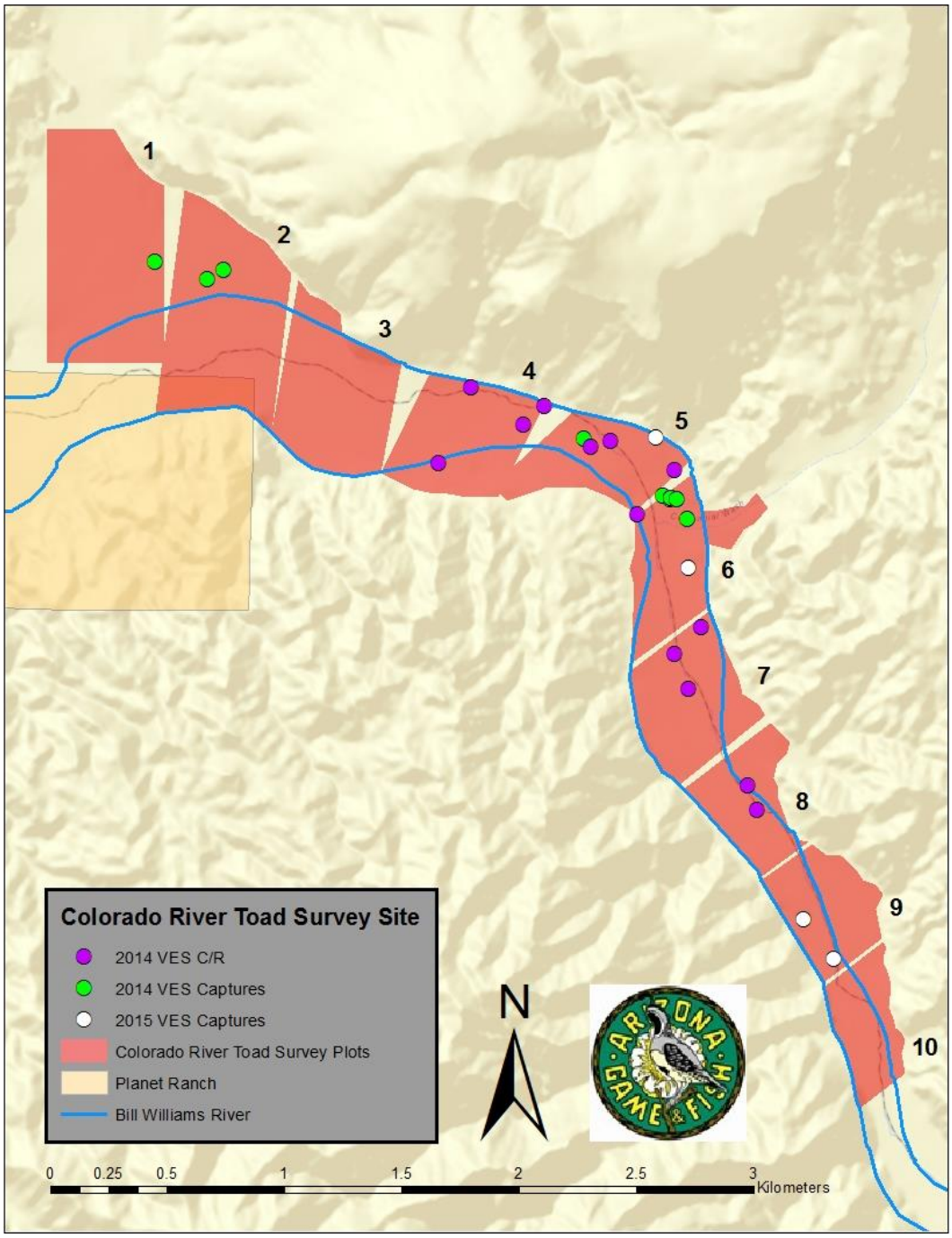

Figure 7.—Colorado River toad VES detections.
Purple represents 2014 VES C/R detections, green represents 2014 VES captures, and white represents 2015 VES captures.

Non-target species detected included Arizona toads (*Anaxyrus microscaphus*), red-spotted toads (*A. punctatus*), and northern Mexican gartersnakes (*Thamnophis eques megalops*), among others (attachment 1). Each of these three species was detected in 2014 and 2015.

Funnel Trap Arrays

In 2014, FTAs were deployed for 6,100 trap-hours on only Plots 5, 6, and 10 due to limited water availability elsewhere (figure 8). Three sessions of trapping were conducted on Plots 5 and 6, totaling 5 nights for each plot. Trapping occurred on both plots on the nights of August 7 and 8, September 3, and September 23 and 24. Plot 5 had seven trap clusters set throughout the season and Plot 6 had eight. Plot 10 only had one night of trapping as water levels receded between the first and second day of the only available trapping event on October 2. A lack of water availability on Plot 10 also allowed for only two clusters to be set. No Colorado River toads were captured in traps. In 2015, traps were not deployed because there was no surface water observed on any of the plots at any point in the season.

Non-target species captured during trapping sessions included Couch's spadefoots (*Scaphiopus couchii*), red-spotted toads, and northern Mexican gartersnakes (attachment 1).

Digital Automated Recorders

DARs operated for 4,301 minutes (almost 72 hours) in 2014. DARs recorded Colorado River toads calling on a total of 21 nights during the 2014 field season. All calling and breeding activity occurred between August 3, 2014, and September 17, 2014. DARs produced 70 detections of target species (table 1) and an additional ten came from a recorder set to the incorrect time on Plot 6 (table 2). Detections occurred on Plots 3–7. The greatest number of detections came from Plots 5 and 6, with 40 and 24, respectively. All other plots had six or fewer total detections. The hour with the most detections was 23:00, which produced 18, followed by 01:00, which produced 15 detections. The fewest detections occurred during the 21:00 hour, with 7.

Part of the detections came from a recorder on Plot 6 that was not set correctly. There was a 3- hour time difference on this set of detections. The internal clock was mistakenly set for Eastern Standard Time, so recordings began at 18:00 and ended at 22:01 local time. Detections from this recording period, which was approximately 36 out of 87 nights, showed the peak of detections (four) was at 21:00.

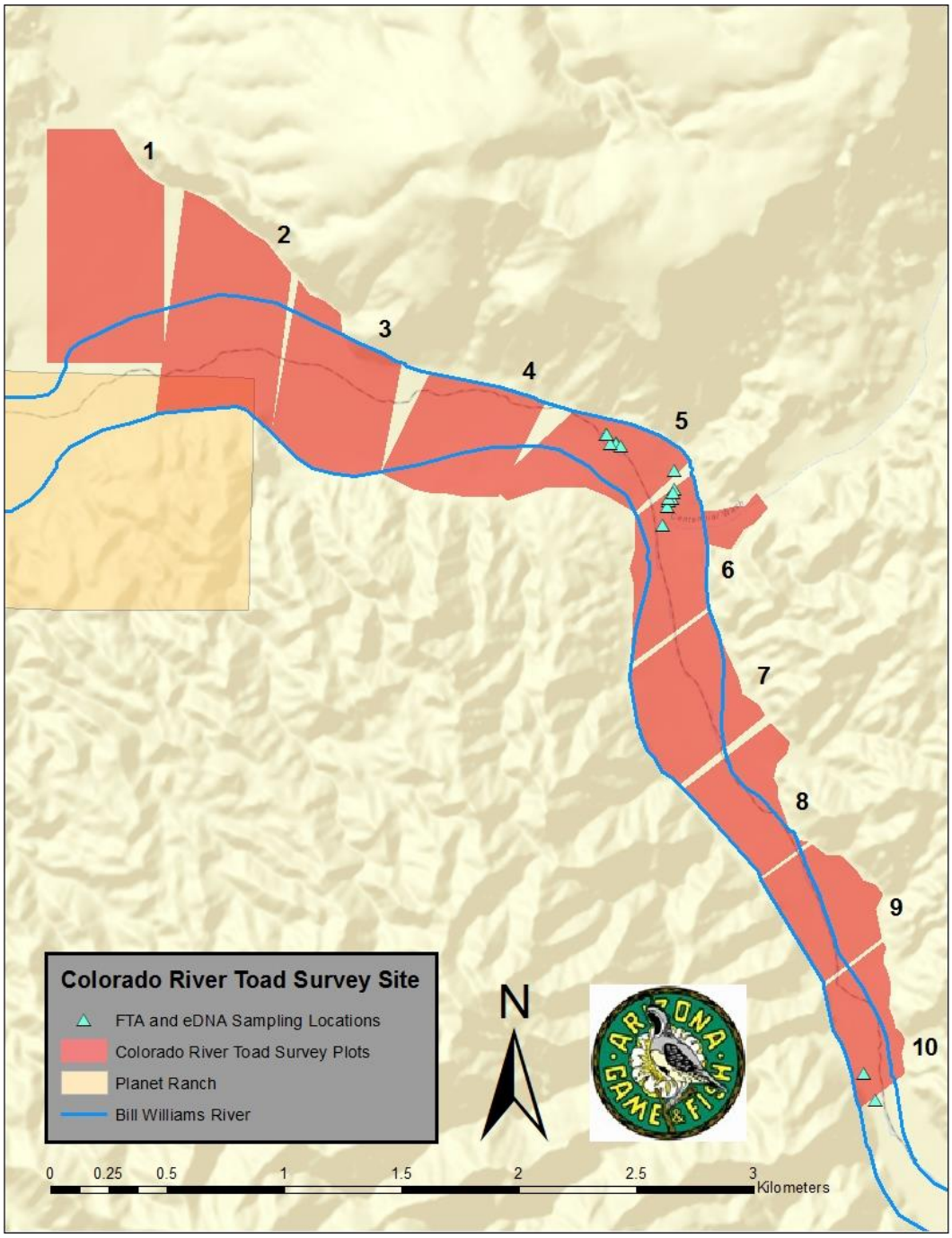

Figure 8.—Colorado River toad FTAs and eDNA sampling locations.

Table 1.—DAR detections per hour and per plot, excluding the incorrectly timed recordings from Plot 6, 2014–15
(All detections occurred in 2014.)

Plot number	Time						Detections per plot
	21:00	22:00	23:00	00:00	01:00	02:00	
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	1	1	1	1	0	4
4	0	0	1	1	3	1	6
5	1	7	10	5	9	8	40
6	2	5	2	1	2	2	14
7	0	0	2	3	0	1	6
8	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
Total detections per hour	**3**	**13**	**16**	**11**	**15**	**12**	**70**

Table 2.—Detections from recorder set to Eastern Standard Time on Plot 6 with corrected times

Estimated time						Total detections
18:00	19:00	20:00	21:00	22:00	23:00	
0	0	2	4	2	2	10

Many DAR detections overlapped in time and were adjacent to one another (table 3). All potential overlapping detections came from Plots 4, 5, 6, and 7. Because of this, the 28 duplicate detections have been reduced down to 13 detections. Recorders can pick up adults calling from over 500 m away, meaning they were recorded on multiple devices. This reduces the total detections from 80 to 65.

DARs were operated for almost 81 hours (4,804 minutes) in 2015. No Colorado River toad calling was recorded during the entire 2015 season. Amphibian species heard during the 2014 season included Arizona toads, red-spotted toads, and Couch's spadefoots. The Couch's spadefoot was the only species heard in 2015 and calling occurred on only one night (attachment 1).

Table 3.—Potential overlapping detections of Colorado River toads
(The first number is the total number of individuals observed for the time period, and the number in parenthesis is the plot where the call was recorded.)

Time	Aug. 8	Aug. 10	Aug. 13	Aug. 14	Aug. 15	Aug. 28	Aug. 29	Aug. 30
21:00	0	0	0	0	0	0	0	0
22:00	0	1 (5) 1 (6)	1 (5) 1 (6)	0	0	0	0	0
23:00	1 (5) 1 (6)	0	0	0	0	0	1 (4) 1 (5)	0
00:00	0	1 (5) 1 (6)	0	0	0	0	0	1 (4) 1 (5)
01:00	0	0	0	1 (5) 1 (6)	0	1 (4) 1 (5)	1 (4) 2 (5)	1 (4) 1 (5)
02:00	0	1 (5) 1 (6) 1 (7)	0	0	1 (5) 1 (6)	1 (4) 1 (5)	0	0
Total	2	7	2	2	2	4	5	4

Environmental DNA

Eleven eDNA samples were collected in 2014 where sufficient water was available. One sample was collected from each trap array that was set at each plot. Four samples were collected from Plot 5, five from Plot 6, and two from Plot 10. Samples were collected from Plot 5 on August 8 and from Plot 6 on September 3. Samples for Plot 10 were collected on October 3. Samples were not collected at FTAs set on Plots 5 and 6 during the September 23–25 deployment. No samples were collected during the 2015 season because there was no surface water to sample.

Ten of the eDNA samples tested for Colorado River toad DNA reproducibly generated PCR bands of the expected size when amplified with *INAL COI* primer pair 3. Sequence alignment and BLAST searches following DNA sequencing of these PCR products showed the Colorado River toad COI gene to be the most likely match for all samples, with 100% identity match for 8 of the 10 sequences, and a single nucleotide difference for the remaining two sequences.

To further confirm the origin of the sequenced Colorado River toad eDNA PCR products, phylogenetic reconstruction was performed. Bayesian maximum likelihood reconstruction of the evolutionary relationships between sequences recovered a consensus tree in which all of the sequenced eDNA PCR products clustered most closely with the reference Colorado River toad COI sequence from NCBI GenBank. There is strong support for the conclusion that the eDNA PCR

products are fragments of the Colorado River toad COI gene. Therefore, Colorado River toad DNA was present in the 10 samples that tested positive by PCR.

Trial Monitoring Implementation

Between July 13 and October 19, 2016, seven thousand six hundred seventy-five 1-minute recordings (almost 128 hours) were recorded and analyzed from 10 sites on 98 nights. Colorado River toads were detected 202 times.

In 2016, the period of each night's audio recordings were extended: in previous years, recording ended at 02:01, and in 2016, recording continued until 04:01. More vocalizing Colorado River toads were recorded in the later hours than earlier hours (table 4). Calling activity peaked at 02:00 and was still higher at 04:00 than it was at 00:00. Indeed, at two sites, toads would not have been detected at all in 2016 without adding the 04:00 sampling period.

Table 4.—DAR detections of Colorado River toads per hour and per plot, 2016

Plot #	Time								Total
	21:00	22:00	23:00	00:00	01:00	02:00	03:00	04:00	
1	0	0	0	0	0	0	0	1	1
2	0	0	0	0	0	0	0	1	1
3	0	0	0	0	0	0	0	0	0
4	0	1	2	1	1	3	3	4	15
5	2	3	4	6	8	8	8	7	46
6	1	4	3	5	6	6	5	5	35
7	0	1	1	3	5	4	5	3	22
8	0	1	2	4	5	5	2	2	21
9	2	3	4	4	3	4	5	4	29
10	1	3	4	5	5	5	5	4	32
Total	**6**	**16**	**20**	**28**	**33**	**35**	**33**	**31**	**202**

The most likely occupancy model indicated that detection probability varied by week (table 5), with detection probability estimates of 0 for most weeks except ranging from 0.70 ± 0.14 SE in week 4, 0.30 ± 0.14 in week 5, and 0.20 ± 0.13 in week 6 of the study (August 3–23, 2016).

Table 5.—Candidate models used to estimate occupancy (Ψ) and detection probability (p, constant or varying by week, s) for the trial implementation of acoustic monitoring of Colorado River toads in 2016

Model	AIC	ΔAIC	AIC weight	Model likelihood	Number of parameters
ψ,p(s)	64.44	0.00	1.00	1.00	15
ψ,p(.)	85.17	20.73	0.00	0.00	2

Lowland Leopard Frog

Visual Encounter Surveys

In 2015, four VESs were conducted on each plot. One VES was completed each night. A total of 211 lowland leopard frogs were caught among both study sites; 32 were recaptures. The Bill Williams River NWR had 70 captures, and the Big Sandy River had 141 (table 6). A total of 152 lowland leopard frogs responded to the VES C/Rs across both sites, 70 from the Bill Williams River NWR and 124 from the Big Sandy River (table 7). Non-target captures included Arizona toads, Sonora mud turtles (*Kinosternon sonoriense*), and northern Mexican gartersnakes (attachment 1).

Table 6.—Total number of lowland leopard frog VES detections per plot and per session in the Bill Williams River NWR (BW) and the Big Sandy River (BS)

(Number in parenthesis is from Plot 4. Sessions 1–4 were in 2015, and sessions 5–6 were in 2016. Session 6 was incomplete – plots not surveyed are indicated by an asterisk [*].)

Session	BW 1	BW 2	BW 3	BW 4A	Total for BW	BS 1	BS 2	BS 3	BS 4	BS 5	BS 6	Total for BS
1	2	0	0	(0)	2	8	3	5	7	1	3	27
2	10	0	1	0	11	11	1	4	7	9	7	39
3	11	6	3	4	24	11	6	3	12	2	6	40
4	26	2	2	3	33	12	1	8	9	1	4	35
5	6	2	0	1	9	1	0	3	4	2	5	15
6	28	*	*	5	33	*	*	*	*	*	*	*
Total per plot	**49**	**8**	**6**	**7**	**70**	**42**	**11**	**20**	**35**	**13**	**20**	**141**

Table 7.—Lowland leopard frog VES C/R detections per plot and per session in the Bill Williams River NWR and Big Sandy River

(Number in parenthesis is from Plot 4. Session 6 was incomplete – plots not surveyed are indicated by an asterisk [*])

Session	BW 1	BW 2	BW 3	BW 4A	Total for BW	BS 1	BS 2	BS 3	BS 4	BS 5	BS 6	Total for BS
1	0	0	1	(1)	2	7	12	0	0	6	4	29
2	3	0	0	3	6	7	7	7	5	4	10	40
3	4	0	5	1	10	5	9	1	4	3	7	29
4	5	0	2	3	10	3	2	12	0	2	7	26
5	0	1	0	5	6	1	0	0	3	3	3	10
6	7	*	*	8	15	*	*	*	*	*	*	*
Total per plot	**19**	**1**	**8**	**21**	**49**	**23**	**30**	**20**	**12**	**18**	**31**	**134**

In 2016, one VES was conducted on eight plots, and two VESs were conducted on two plots. A total of 56 lowland leopard frogs were caught between both study sites, with 42 at the Bill Williams River National Wildlife Refuge and 15 at the Big Sandy River. There were no new incidental species captured in 2016 that had not been captured in 2015.

Funnel Trap Arrays

Four FTAs were deployed per survey session on each plot. A total of 68,519 trap-hours were recorded over the course of the 2015 season for both study sites. The Bill Williams River NWR had 26,774 of those hours and the Big Sandy River had 41,745. Fifty-six captures were made on the Bill Williams River NWR, of which seven were recaptures (table 8). Plot 1 had the most captures with 36 and Plots 4 and 4A had the fewest with only one capture. The Big Sandy River had 176 captures, 26 were recaptures, with a maximum of 54 captures on Plot 6 and a minimum of 19 on Plot 3. Non-target vertebrates caught in FTAs on the Bill Williams River NWR included Arizona toads, western mosquitofish (*Gambusia affins*), and green sunfish (*Lepomis cyanellus*). Non-target species caught in the Big Sandy River included northern Mexican gartersnakes, Arizona toads, red-spotted toads, western mosquitofish, and Sonora mud turtles (attachment 1).

A total of approximately 20,500 trap-hours were recorded over the course of the 2016 season for both study sites, and trap-hours were evenly split between the two sites. Six captures were recorded on the Bill Williams River NWR, of which two were recaptures (table 8). Plot 1 had the most captures with four, and Plots 2 and 3 had no captures. The Big Sandy River had nine captures, four were recaptures, with a maximum of four captures each on Plot 6 and a minimum of zero on Plots 1 and 5. No new non-target vertebrates were caught in FTAs that had not been caught in 2015.

Table 8.—Lowland leopard frog FTA captures per plot and per session on the Bill Williams and Big Sandy Rivers in 2015 (sessions 1–4) and 2016 (sessions 5–6)

(Numbers in parentheses are from Plot 4. Session 6 was incomplete – plots not surveyed are indicated by an asterisk [*].)

Session	BW 1	BW 2	BW 3	BW 4A	Total for BW	BS 1	BS 2	BS 3	BS 4	BS 5	BS 6	Total for BS
1	0	0	1	(0)	1	2	10	0	4	8	0	24
2	11	1	0	0	12	7	5	4	0	3	7	26
3	11	4	4	1	20	3	8	5	11	0	2	29
4	14	7	2	0	23	10	1	10	6	25	45	97
5	0	0	0	0	0	0	1	2	2	0	4	9
6	5	*	*	1	6	*	*	*	*	*	*	*
Total per plot	**41**	**12**	**7**	**2**	**62**	**22**	**25**	**21**	**23**	**36**	**58**	**185**

Digital Automated Recorders

Nearly 81 hours (4,832 minutes) of audio were recorded and processed for lowland leopard frogs in 2015, and about 50 hours (2,986 minutes) were recorded and processed in 2016. There were 521 lowland leopard frog detections on the Bill Williams River NWR and 394 on the Big Sandy River, totaling 915 detections for both sites (table 9). Plot 4A on the Bill Williams River NWR had the most detections with 220 and Plot 3 on the Big Sandy had 122. Detections peaked during the 23:00 and 00:00 hours on the Bill Williams River NWR and during the 21:00 hour on the Big Sandy River. Three plots were missing a significant number of recordings. The device on Plot 3 on the Bill Williams River NWR was stolen between March 22, 2015 and May 2, 2015. At the Big Sandy River, two devices were lost from Plots 1 and 4 due to a large flood event in early March 2015. These devices were replaced on March 6, 2015. Recordings from Plot 1 were lost on February 22, 2015 and February 18, 2015, for Plot 4.

Table 9.—Lowland leopard frog DAR detections on the Bill Williams and Big Sandy Rivers per hour and per plot in 2015–16

Time	BW 1	BW 2	BW 3	BW 4A	Total for BW	BS 1	BS 2	BS 3	BS 4	BS 5	BS 6	Total for BS
21:00	40	12	0	38	90	24	14	33	12	2	20	105
22:00	35	15	0	34	84	15	11	26	11	3	12	78
23:00	38	19	0	41	98	20	7	20	12	1	11	71
00:00	36	19	0	43	98	13	8	16	12	0	10	59
1:00	29	22	0	33	84	12	3	17	6	0	6	44
2:00	30	6	0	31	67	12	3	10	6	0	6	37
Total per plot	**208**	**93**	**0**	**220**	**521**	**96**	**46**	**122**	**59**	**6**	**65**	**394**

Environmental DNA

One eDNA sample was collected at each FTA during each survey. Four FTAs were set per plot and each plot had four sessions of trapping. This resulted in 16 eDNA samples collected per plot for a total of 160 across both sites. All samples were sent to Reclamation's Environmental Applications and Research Group in Denver, Colorado, for analyses.

Of the 160 environmental samples tested for lowland leopard frog DNA, 109 reproducibly generated PCR bands of the expected size when amplified with LIYA COI primer pair 3. DNA sequencing was successfully performed for 105 of these 109 PCR products. Alignment of DNA sequences showed a 100% match to the lowland leopard frog COI reference sequence for 104 of the sequences, with one sample showing a single nucleotide difference from the reference. In comparison, COI sequence from the American Bullfrog showed nine nucleotide differences (81.2% identity) compared to the reference sequence from a lowland leopard frog. Four samples (JDMEBEBEU02_020415 Cluster 4, ERGEBSBSU02_040115 Cluster 2, JDMEBSBSU06_022015 Cluster 3, ERGEBSBSU06_031815 Cluster 2) tested positive, but were not successfully sequenced. In summary, lowland leopard frog DNA was present in 105 samples that tested positive by PCR and confirmed by DNA sequencing. An additional four samples tested positive by PCR and were expected to contain lowland leopard frog DNA, but were not able to be confirmed by DNA sequencing.

Multi-Method Occupancy Models

The top model in the occupancy analysis, as ranked by AIC, indicated that detection probability varied by method for each species and year (tables 10–12). For Colorado River toads, the top model in both 2014 and 2015 indicated that detection probability varied by method. In 2014, θ (small-scale occupancy) varied by session, which is consistent with the short duration of surface activity in this species. In 2015, when only one method detected Colorado River toads and there was no significant monsoon activity, the top model indicated that detection probability varied by method but θ did not vary by season.

A CCDI was developed as a baseline quantity to compare the cost and probability of detection for both species (table 13). The method with the highest CCDI for Colorado River toads in 2014 was the eDNA method at 5.1, followed by VES C/Rs at 0.72. Colorado River toad eDNA was detected at each site where it was sampled, but only 3 of the 10 sites could be sampled due to an absence of water at the other 7. In 2015, only one method detected Colorado River toads, the VES method. The method with the highest CCDI for lowland leopard frogs was the VES C/R method, with a CCDI of 6.4, followed by VESs at 2.7 and FTAs at 1.4. eDNA and DARs had relatively low CCDIs for this species, at 0.33 and 0.30, respectively.

Table 10.—Candidate models used to estimate large-scale occupancy (Ψ), the probability that an individual is available for detection by a method (small-scale occupancy [θ]), and that detection probability (p) varies by survey session (s) and method (m) for Colorado River toads in 2014

Model	AIC	ΔAIC	AIC weight	Model likelihood	Number of parameters
ψ,θ(s),p(m)	115.72	0.00	0.83	1.00	10
ψ,θ(.),p(m)	120.82	5.10	0.06	0.08	7
ψ,θ(.),p(s)	121.12	5.40	0.06	0.07	6
ψ,θ(.),p(ms)	122.28	6.56	0.03	0.04	22
ψ,θ(s),p(.)	124.56	8.84	0.01	0.01	6
ψ,θ(s),p(s)	126.38	10.66	0.00	0.00	9
ψ,θ(s),p(ms)	127.70	11.98	0.00	0.00	25
ψ,θ(.),p(.)	132.19	16.47	0.00	0.00	3

Table 11.—Candidate models used to estimate large-scale occupancy (Ψ), the probability that an individual is available for detection by a method (small-scale occupancy [θ]), and that detection probability (p) varies by survey session (s) and method (m) for Colorado River toads in 2015

Model	AIC	ΔAIC	AIC weight	Model likelihood	Number of parameters
ψ,θ(.),p(m)	47.09	0.00	0.99	1.00	5
ψ,θ(s),p(m)	57.44	10.35	0.01	0.01	14
ψ,θ(.),p(s)	65.36	18.27	0.00	0.00	12
ψ,θ(s),p(s)	83.36	36.27	0.00	0.00	21
ψ,θ(.),p(ms)	93.44	46.35	0.00	0.00	32
ψ,θ(s),p(ms)	111.44	64.35	0.00	0.00	41

Table 12.—Candidate models used to estimate large-scale occupancy (Ψ), the probability that an individual is available for detection by a method (small-scale occupancy [θ]), and that detection probability (p) varies by survey session (s) and method (m) for Colorado River toads in 2015 and 2016 combined

Model	AIC	ΔAIC	AIC weight	Model likelihood	Number of parameters
ψ,θ(.),p(m)	243.49	0.00	0.96	1.00	7
ψ,θ(s),p(m)	249.86	6.37	0.04	0.04	12
ψ,θ(.),p(ms)	273.30	29.81	0.00	0.00	32
ψ,θ(s),p(ms)	275.50	32.01	0.00	0.00	37
ψ,θ(.),p(s)	300.76	57.27	0.00	0.00	8
ψ,θ(s),p(s)	303.19	59.70	0.00	0.00	13
ψ,θ(.),p(.)	306.28	62.79	0.00	0.00	3
ψ,θ(s),p(.)	306.35	62.86	0.00	0.00	8

Table 13.—CCDI calculated by dividing probability of detection from the top-ranked model (P) by the total cost of labor and equipment

Colorado River toad, 2014				
Method	**P ± SE**	**Labor cost ($/session)**	**Equipment/ analysis cost ($)**	**CCDI: probability of detection/cost (×10,000)**
VES	0.28 ± 0.09	4,064	470	0.62
VES C/R	0.20 ± 0.08	2,711	250	0.72
FTA	0	1,597	1,280	0
DAR	0.32 ± 0.10	1,414	6,990	0.38
eDNA	1.00 ± 0	41	1,928	5.1
Colorado River toad, 2015				
VES	1.00 ± 0	7,258	470	1.3
VES C/R	0	2,421	250	0
DAR	0	1,538	6,990	0
Lowland leopard frog, 2015				
VES	0.92 ± 0.04	2,903	470	2.7
VES C/R	0.78 ± 0.06	967	250	6.4
FTA	0.72 ± 0.06	3,865	1,280	1.4
DAR	0.26 ± 0.06	1,553	6,990	0.30
eDNA	0.95 ± 0.04	600	28,050	0.33

DISCUSSION

Colorado River Toad

Visual Encounter Surveys

In 2014, the probability of detection for VESs was 0.28 ± 0.09 and for VES C/Rs it was 0.20 ± 0.08. In 2015, probability of detection with VESs was 1.00 ± 0, and no other method recorded detections. VESs were the only method to detect Colorado River toads over both seasons. VES C/Rs appeared most efficient when cost of labor and equipment was considered. VESs alone at 0.62 CCDI were not as cost effective due to the greater labor required; however, in the study they were the most consistently effective means of detecting Colorado River toads.

Colorado River toads do not actively call throughout the season; they respond only to significant rain and typically call for a few nights each year. This may limit the ability to use VES C/Rs because of the unpredictable nature of monsoon storms in Arizona. This method was not able to produce detections in 2015 because there were no significant monsoon events during the sampling period.

Both VESs and VES C/Rs were effective in finding toads if surveys were conducted shortly after a rain event. During the 2014 season toads were found foraging on nights during which no rains occurred. However, on nights shortly after (≈ 2–5 days) a heavy monsoon event, pools were found with breeding Colorado River toads. Of the 11 detections, 5 occurred during a 2-night period (August 6 and 7, 2014) following a monsoon event where breeding occurred. The monsoon event occurred on August 3 and 4, 2014. Additionally two amplectic pairs were observed on August 5, 2014, while hiking to the target plot. All known breeding activity, including calling, amplexus, or egg mass deposition, occurred on Plot 5 and 6. These two plots were the only ones to collect and maintain water in the 2014 season.

There were no significant rains during the 2015 season. However, it did rain lightly several times, which encouraged activity from all toad species even though water did not pool. On September 21, 2015 a dampening rain hit the study site and Colorado River toads were found the following 2 nights in an ephemeral pool on Plot 6, the same pool in which toads were observed calling the previous year.

There are a few logistical constraints to this method relating to timing and labor. A single VES required 2 hours to conduct. As shown by the DAR recordings, calling activity tended to begin about midnight. Many surveys started 1 hour prior to sunset, between 1815 and 1930. This suggests that peak nightly activity of Colorado River toads was missed due to VES being conducted too early. Also, the need to be onsite shortly after a significant rain event is problematic

depending on conditions and safety concerns. VES C/Rs, while conducted throughout the season regardless of monsoon activity, are unlikely to be productive without monsoon rain.

One unexpected benefit of VESs was the ability to capture northern Mexican gartersnakes, which are a threatened species. The continued ability to record this species is a benefit of this method in the study area.

Funnel Trap Arrays

FTAs were the least effective survey method tested. FTAs had a probability of detection and CCDI of 0 because there were no detections in either year. FTAs, along with eDNA, are only effective when biologists can be onsite within a few days after a storm. When the water table is low in this system, as in 2014 and 2015, surface water does not persist long. Active monsoon storms prohibited access to the study site twice in 2014. Damage to roadways made navigating the terrain challenging and sometimes dangerous. Water evaporating or percolating in 1 or 2 nights prevented trapping in potential breeding pools on additional plots other than the two main pools found on Plots 5 and 6. Water was likely available temporarily within the study area other than Plots 5 and 6 corresponding with rain events, but the inability to get to the study site, coupled with percolation and evaporation, prevented confirmation of this. Successful breeding could not have occurred in these areas because the water did not persist for more than one day and larvae can take up to one month to metamorphose (Fouquette et al. 2005). During the 2015 season there was no monsoon event large enough to create surface water or encourage a breeding event, so traps were never set.

FTAs were not effective for detecting adult Colorado River toads because the funnel openings were too small for them to enter. The main goal with this method was to catch tadpoles and immature toads, but these were never caught. Several clusters of traps in areas where Colorado River toad tadpoles and eggs were seen, and the lack of captures highlights the weakness of this particular method at catching the target species. Because the breeding season for Colorado River toads is relatively short and significant local rain events are limited and unpredictable, the effectiveness of the FTA method has been difficult to test. More significant rain events in the area, which would increase trapping effort, would likely increase detection rates.

Like VESs, FTAs also captured a few northern Mexican gartersnakes throughout the 2014 season.

Digital Automated Recorders

The probability of detection with DARs was 0.32 ± 0.10 in 2014 and 0 in 2015. DARs had the second lowest CCDI of the methods tested in 2014. Detections from DARs were very high in that season, with 80 detections overall, especially on Plots 5 and 6. These results could be misleading for several reasons. The detection range of the recorders overlapped with one another, which means an individual could have been detected on two or more recorders. They could have called on successive nights, which would reduce the number even further, but this is hard to determine. Future applications should ensure DARs are established at least 500 m apart to reduce the risk of overlap in detection. This approach would reduce the amount of recorders needed to cover a given area as well as reduce the cost of processing recordings.

One challenge faced was the detection of VES C/R broadcasts by the DARs. Detections that did not fit into the overall breeding window of the toads on this site were eliminated from the data set, that is, detections that occurred before the first major monsoon event when water was not present. During the period of time that Colorado River toads were known to be breeding, some individual calls from broadcasts may have been detected. Most if not all of the false detections were eliminated by comparing the pattern of call in the broadcast to those in the recordings. Nonetheless, in the future, incorporating a distinctive audio signature in the broadcast recording, such as a beep or other simple tone, when VES C/Rs are used concurrently with DARs is recommended.

Colorado River toads generally began calling late at night, starting at 21:00, increasing at 22:00, and continuing through 02:00 with a maintained higher frequency. Indeed, in the trial implementation in 2016, calling activity had a broad peak between 00:00 and 04:00, and was greatest at 02:00. Future DAR surveys should begin recording at 00:00 and continue until 05:00 to minimize effort when calling is less frequent in the early hours of the night and to ensure coverage of the period of peak calling activity.

Out of all four methods, DARs produced the highest number of detections. This fact is obscured in the CCDI analysis because only data from recordings on nights when VES C/Rs were also conducted were analyzed. This enabled a direct comparison of the probability of detection for each method since all the other methods are limited in the number of days they are deployed. The sheer number of detections over the course of the season greatly outweighs other methods when all data is included. Although DARs have a high equipment cost, their labor cost is very low, and their equipment cost could be reduced by using fewer recorders (at least 500 m apart). Bridges and Dorcas (2000) suggest that DARs have an added benefit of reduced disturbance to the target species during calling and the ability to retain a saved recording for further analysis. The results show that DARs can be very effective at determining presence. This method does not require a biologist presence onsite to record data. The recorders can be deployed

at the beginning of the survey season and left until the end of the season and can be setup to store large amounts of audio files which reduce time needed to replace storage cards. They will automatically record data during any monsoon event, thus reducing safety concerns related to field biologists traveling to or through washes during monsoon rains. Data analysis costs can be further reduced by analyzing only recordings from dates during and after rain. DARs collect data every day for very little additional cost over the first day. They also inherently save permanent digital data that can be analyzed at a later date, although archiving this data may have its own costs associated with maintaining digital records. DARs were particularly effective with Colorado River toads because of the long range at which a Colorado River toad can be heard.

Environmental DNA

Environmental DNA can reliably determine presence of a species by identifying trace amounts of genetic material of the target species suspended in the water column and is especially useful at detecting species that are either hard to find, difficult to survey, or occur at low densities (Ficetola et al. 2008). Samples of eDNA were collected in 2014 but not in 2015 because there was no water onsite to collect. Due to technical issues, samples were not collected during the September 23 and 24, 2014 trap event, which reduced the total sample size by six.

Environmental DNA sampling, like FTAs, needs to be conducted shortly after rain as water does not persist long at many of these sites. As long as there is some water in a plot, samples can be collected and used. When water is present, this method is highly effective: Colorado River toads were detected at each of the three sites sampled, and in ten of eleven samples. One limitation of this method is that it can only be used to test for species presence; it does not provide an estimate of abundance. Because monsoon pools are temporary, it is likely that any genetic material detected was recent; the ability to detect the presence of toads from previous seasons has not been tested.

eDNA sampling had the highest detection probability and the highest CCDI for Colorado River toads in 2014. However, this could be misleading because the method could only be implemented on three sites in one year (out of ten sites in two years). The cost could be much higher (and CCDI lower) when surface water is more prevalent and more sites can be sampled. Because this method relies on the presence of surface water, it requires both significant rain events and that biologists are onsite to conduct sampling shortly after these events. Together these logistical challenges reduce the practical appeal of this method in this system, although it may still be a preferred method in cases where it is easier for biologists to respond quickly to rain events and when surface water is reliable.

Lowland Leopard Frog

Visual Encounter Surveys

VESs were very effective at detecting lowland leopard frogs, with a detection probability of 0.92. Broadcasting calls added additional responses, with an additional detection probability of 0.78. Together, these two methods gave a total detection probability of approximately 0.98. (Calculated as 1-the probability of not detecting a frog by either method, i.e. 1-((1-0.92)×(1-0.78)).). Broadcasting calls took little time and was very effective in eliciting responses. If funds are not available for extensive surveys, just the C/R component could be conducted during the breeding season and could be a very effective and efficient method to determine presence.

The habitat at the Bill Williams River NWR was complex compared to the Big Sandy River, which made visually locating lowland leopard frogs challenging. The Bill Williams River NWR contains dense emergent vegetation on the banks of the river and includes a dense canopy and understory that provide plenty of cover for lowland leopard frogs. In contrast, the Big Sandy River is relatively open with little emergent vegetation. This may not be normal for the Big Sandy River as vegetation was removed by a large rain event in the early spring, however the Big Sandy River was still relatively sparse compared to the Bill Williams River NWR under normal conditions. Visual detection of lowland leopard frogs is easier in this system compared to the Bill Williams River NWR. This may also affect the results of the VES C/Rs and DARs as sound may not carry as well in the Bill Williams River NWR.

Northern Mexican gartersnakes were passively captured during lowland leopard frog VESs on the Big Sandy River. This data may be beneficial for future monitoring of this species if it is added to the LCR MSCP.

Funnel Trap Arrays

FTAs were also effective at capturing lowland leopard frogs. Probability of detection for FTAs was 0.72 ± 0.06. The probability of detection ranked second to last among the tested methods. However, the CCDI for FTAs was the third-highest, because FTAs are relatively cheap compared to eDNA and DARs.

Several benefits of FTAs include the ability to passively capture individuals, especially in habitats where visual detections are not reliable due to the complexity of the vegetative structure. They are also a great way to capture target species while conducting other survey methods. Finally, traps have the ability to capture individuals at all hours of the day. This enables them to be present during high activity times that might be missed by other methods. Generally, VESs are conducted during the night because lowland leopard frogs are easier to detect then, but that may not be when they are most active.

One side benefit of using traps is the ability to conduct surveys for different species at the same time. Funnel trap surveys for northern Mexican gartersnakes generally are thought to be the most effective means of capturing individuals, and funnel traps are also effective at capturing lowland leopard frogs, so setting traps to catch both species at the same time could reduce costs. Northern Mexican gartersnakes could theoretically reduce the probability of detecting lowland leopard frogs by feeding on lowland leopard frogs in funnel traps, but none of the northern Mexican gartersnakes detected had any recent prey in their stomachs (noted by visual inspection).

Digital Automated Recorders

DARs had the lowest probability of detection, 0.26 ± 0.06, and because of their high equipment cost they ranked last with a CCDI of 0.30. Like DAR analysis with Colorado River toads, only calls heard on days that the other methods were tested were analyzed. There were 915 total detections over the course of the two seasons; though, when comparing this method to all others only 197 were included in this analysis. If only presence is needed, DARs still may be a very effective method for surveying lowland leopard frogs.

In analyzing audio recordings, it was often difficult to detect lowland leopard frogs because of Arizona Toads calling at the same time (figure 9). Lowland leopard frog calls are comparatively quiet and can be overpowered by Arizona Toad calls. Their relatively weak call also requires individuals to be close to the recorder, unlike Colorado River toads which can be heard over 500 m away. The estimate is that lowland leopard frogs need to be 150 m or less from a DAR to be audible in a recording. This means more recorders may be needed to cover the same area compared to Colorado River toads.

Environmental DNA

In 2013, an eDNA study was conducted on lowland leopard frogs in several locations on the Bill Williams River and Lower Colorado River, one being the Bill Williams River NWR and a portion upstream of the refuge that had a known population of lowland leopard frogs (Cotten and Leavitt 2014). Fifty samples were collected from the Bill Williams River NWR and two came back with positive results for eDNA of lowland leopard frogs. Twenty samples were collected in the known population upstream of the refuge and resulted in 13 positive detections. At that time lowland leopard frogs had just been rediscovered on the Bill Williams River NWR and population size was estimated to be extremely small. This method clearly can determine presence at low population levels but is one of the more costly methods tested.

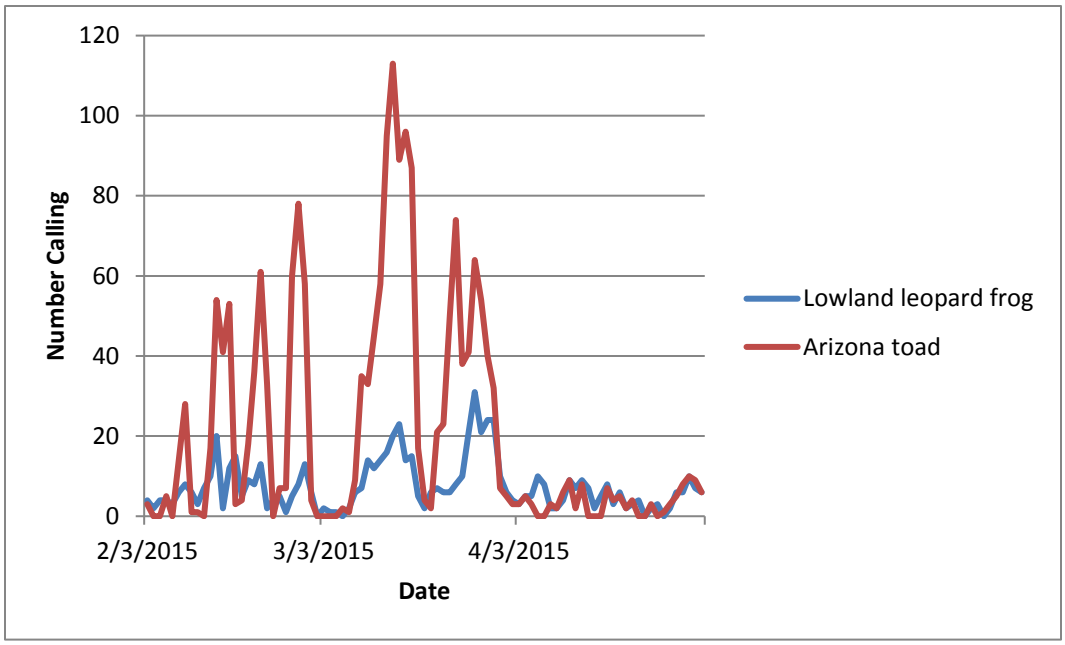

Figure 9.—Overlapping calls of lowland leopard frogs and Arizona toads.

Lowland leopard frog eDNA was detected in 105 of 160 samples, making this the most effective single method tested, with a detection probability of 0.95 ± 0.04. Because lowland leopard frogs inhabit areas with permanent or nearly permanent surface water, and do not rely on fast-drying ephemeral pools for breeding, these frogs may be more effectively sampled by eDNA than Colorado River toads would be. One caveat is that it is unknown how long eDNA remains detectable in these systems, or how far it can flow downstream, so it is possible that this method might be slow to detect losses of local populations, or might detect upstream populations in downstream locations where frogs are not present. The high sensitivity of this method gives it great promise for monitoring lowland leopard frogs in areas where presence or absence must be rapidly assessed and abundance data are not required.

Because minnow traps were installed in pools for 1 day before collecting water samples from the same pools, and minnow traps were not cleaned when moved from one plot to another within a drainage, it is possible that some eDNA detections were false positives due to detecting lowland leopard frog DNA transported on minnow traps. If this is the case, the detection probability might be overestimated. It is important that if this method is implemented in the future, extra care should be taken to eliminate the possibility of spreading frog DNA among plots.

LESSONS LEARNED

Colorado River Toad

For Colorado River toads, VES C/Rs and DARs were the most cost efficient methods of detection. VESs are still the preferred method when rain is not predicted and surveys must be conducted regardless of weather, because VES C/Rs and DARs are only suitable at detecting Colorado River toads after a monsoon event. When surveys can be conducted during or shortly after a rain event, VES C/Rs are likely to be effective, although it has high labor costs and requires larger crews spending longer time conducting surveys. DARs have the added benefit of requiring very little labor, and thus are very cost effective once the recording devices have been purchased. They do require at least some significant rain during a season to be effective, and in some years they may fail to detect a species even when it is present if there is insufficient rain to prompt a breeding event.

Surveys (VESs) of Colorado River toads in the Bill Williams River from 2011 through 2013 found no breeding behavior, with the exception of one male that had fresh egg mass strands on its back (Cotten and Leavitt 2014). Use of only DARs and VES C/Rs could have missed this event simply because there was no calling detected by biologists for this breeding event. VESs can provide the identification of potential or current breeding sites and associated activity like amplexus, egg masses, or tadpole success as well as general activity not associated with breeding behavior such as foraging. The drawback of this method is that it is costly in terms of time and labor. DARs and VES C/Rs lack the ability to detect foraging adults or potential breeding locations. If setting DARs in a new study site, one must place the devices near areas where water will later pool.

DARs have a high upfront cost which contributes to their lower overall CCDI. However, over a multi-year span their effective cost will decrease while other methods will remain relatively constant. Therefore, they become more cost efficient and have a higher CCDI over time compared to the other methods tested, making them a very valuable tool for long term monitoring.

Since DARs have the capability to record a large amount of audio recordings, they can be left onsite for extended periods. The recorders were checked, and the data storage cards were replaced approximately every 3 weeks. However, to further reduce overall cost of DARs, one could limit the removal of data storage cards to once or twice a season and therefore lessen the travel time to the study site. (Doing so increases the risk of losing more data from equipment loss or failure, however.) All other methods require personnel to be onsite to record data and this includes a great amount of travel that will increase costs.

The one-season trial implementation of DARs in the Swansea area of the Bill Williams River (see figure 1) demonstrated that this is an effective and efficient method for monitoring the presence of Colorado River toads. The best model indicated that detection probability varied by week, with detection probability estimates of 0 for most weeks but ranging from 0.2 to 0.7 in weeks 4–6 of this study (August 3–23, 2016). This again emphasizes the importance of correct timing and a reliance on unpredictable monsoon rains in detecting this species. The efficiency of this method could be further increased by analyzing only recordings from periods where rain occurred.

Lowland Leopard Frog

For lowland leopard frogs, the most cost-effective method for detecting presence was the VES method. Its low overall cost and high probability of detection make it an effective method for determining presence of lowland leopard frogs. The efficiency of this method could probably be increased by conducting VES for shorter periods but combining it with VES C/Rs. For example, surveys could be stopped once a frog was detected, saving the cost of additional searching after presence had been established. Unlike Colorado River toads, the breeding window and calling window of lowland leopard frogs is much larger and not dependent on isolated weather events. This allows more flexibility when surveying new study sites. These methods will be tested during one more field season in spring 2017.

ACKNOWLEDGEMENTS

Allen Calvert, Michael Ingraldi, Pam Kennedy, Sonja Kokos, Barbara Raulston, Michelle Reilly, Jeneal Smith, Tim Wade, and Renée Wilcox provided logistical support. Sky Arnett-Romero, Colin Beach, Allen Calvert, Taylor Cotten, Emily Grabowsky, Rachel Harrow, Jeffrey Hill, Hillary Hoffman, Erin Lees, Keith Sullivan, Ashley Grimsley, Jeneal Smith, Jennifer White, and Sam Williams contributed to field work and analysis of audio recordings. Jon Miller contributed to field work, data analysis, and report preparation. Yale Passamaneck conducted analysis of eDNA samples and wrote drafts of those sections.

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. Monthly Weather Review 117:1423–1438.

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle *in* B.N. Petrov and F. Csaki (editors). Second International Symposium on Information Theory (pp. 267–281). Budapest: Academiai Kiado.

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

Bridges, A.S. and M.E. Dorcas. 2000. Temporal variation in anuran calling behavior: implications for surveys and monitoring programs. Copeia 2000:587–592.

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

Burnham, K.P. and D.R. Anderson. 1998. Model Selection and Inference: A Practical Information-Theoretic Approach. Springer-Verlag, New York.

Che, J., H.-M. Chen, J.-X. Yang, J.-Q. Jin, K. Jiang, Z.-Y. Yuan, R.W. Murphy, and Y.-P. Zhang. 2011. Universal COI primers for DNA barcoding amphibians. Molecular Ecology Resources 12:247–258.

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. Arizona Game and Fish Department, Phoenix, Arizona. 25 p.

Cotten, T. and D. Grandmaison. 2012. Lowland Leopard Frog and Colorado River Toad Distribution and Habitat Use in the Greater Lower Colorado River Ecosystem: 2012 Annual Report. Arizona Game and Fish Department, Phoenix, Arizona. 40 p.

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, Flagstaff, Arizona. 45 p.

Ficetola, G.F., C. Miaud, F. Pompanon, and P. Taberlet. 2008. Species detection using environmental DNA from water samples. Biology Letters 4:423–425.

Fouquette, M.J., Jr., C.W. Painter, and P. Nanjappa. 2005. *Bufo alvarius* Girard, 1859. Colorado River Toad. Pages 384–386 *in* M. Lannoo (editor). Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley, California.

Goldberg, C.S., D.S. Pilliod, R.S. Arkle, and L.P. Waits. 2011. Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. PLoS One 6:e22746.

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.

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

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

Laramie, M.B., D.S. Pilliod, C.S. Goldberg, and K.M. Strickler. 2015. Environmental DNA Sampling—Filtering Water to Capture DNA from Aquatic Organisms. U.S. Geological Survey Techniques and Methods, book 2, chapter A13. 15 p.

Miller, J.D. and D.J. Leavitt. 2015. Development and Implementation of a Repeatable Monitoring Plan for Lowland Leopard Frogs and Colorado River Toads on the Lower Colorado River: 2015 Annual Report. Arizona Game and Fish Department, Phoenix, Arizona. 44 p.

Nichols, J.D., L.L. Bailey, A.F. O'Connell Jr., N.W. Talancy, E.H. Campbell Grant, A.T. Gilbert, E.M. Annand, T.P. Husband, and J.E. Hines. 2008. Multi-scale occupancy estimation and modelling using multiple detection methods. Journal of Applied Ecology 45:1321–1329.

Raulston, B. 2017. Bureau of Reclamation, Boulder City, Nevada, personal communication.

Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

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. Southwestern Naturalist 45:267–273.

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.

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

Sullivan, B.K. and K.B. Malmos. 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-–43.

ATTACHMENT 1

Detections of Non-Target Amphibians and Reptiles for Each Method Across All Study Sites and Years

Species (common name)[1]	Big Sandy River, 2015			Big Sandy River, 2016			Bill Williams River National Wildlife Refuge, 2015			Bill Williams River National Wildlife Refuge, 2016			Swansea Wilderness, 2014			Swansea Wilderness, 2015			Swansea Wilderness, 2016
	VES[2]	FTA[3]	DAR[4]	VES	FTA	DAR	VES	FTA	DAR	VES	FTA	DAR	VES	FTA	DAR	VES	FTA	DAR	DAR
Arizona toad	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X			X
Red-spotted toad	X		X					X	X				X	X	X	X		X	X
Canyon treefrog	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			
California kingsnake													X			X			
Western threadsnake													X			X			
Spotted leaf-nosed snake																X			
Northern Mexican gartersnake	X	X											X	X		X			

[1] Scientific names can be found on the next page.
[2] VES = visual encounter survey.
[3] FTA = funnel trap array.
[4] 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*
California kingsnake	*Lampropeltis californiae*
Western threadsnake	*Leptotyphlops humilis*
Spotted leaf-nosed snake	*Phyllorhynchus decurtatus* (Cope, 1868)
Northern Mexican gartersnake	*Thamnophis eques megalops*