Population Ecology of the Invasive American Bullfrog (*Rana catesbeiana*) in Southeastern Arizona

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Cooperator Science Series # 173-2025

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Contractual References:

This document (USGS IPDS #: IP-180289) was developed in conjunction with the University of Arizona and the Arizona Cooperative Fish and Wildlife Research Unit (U.S. Geological Survey, Arizona Game and Fish Department, the University of Arizona, U.S. Fish and Wildlife Service, and Wildlife Management Institute cooperating). The Arizona Game and Fish Department funded this project. In addition, the University of Arizona's Graduate Student Research Grant program, the Audubon Society's Appleton-Whittell Research Ranch Fellowship, and the Cecil Schwalbe Cold-Blooded Research Fund provided additional project funding. This work was conducted under permits from the U.S. Fish and Wildlife Service (TE02234C and ESPER0033846), Arizona Game and Fish Department (SP410389), and the University of Arizona Institutional Animal Care and Use Committee (2022-0871).

Recommended citation:

Sudbeck, E.L. and Bauder, J.M. 2025. Population Ecology of the Invasive American Bullfrog (*Rana catesbeiana*) in Southeastern Arizona. U.S. Department of the Interior, Fish and Wildlife Service, Cooperator Science Series FWS/CSS-173-2025. Washington, D.C. https://doi.org/10.3996/css36183173

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2025

Cooperative Report Funded by Arizona Game and Fish Department Task Order 24-3

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American bullfrogs (*photo: Emma Sudbeck, University of Arizona*)

Suggested Citation: Sudbeck, E.L. and Bauder, J.M. 2025. Population Ecology of the Invasive American Bullfrog (*Rana catesbeiana*) in Southeastern Arizona. U.S. Department of the Interior, Fish and Wildlife Service, Cooperator Science Series FWS/CSS-173-2025. Washington, D.C.
https://doi.org/10.3996/css36183173

Background

Native to eastern North America, the American bullfrog (*Rana catesbeiana*) was introduced to Arizona for sport hunting in the early 1900s and has since come to dominate many aquatic systems and threaten native amphibians through four primary mechanisms (Hayes and Jennings 1986; Bury and Whelan 1985). First, American bullfrogs (hereafter bullfrogs) are carriers of pathogens (e.g., ranavirus, chytrid fungus) and can spread these pathogens to native amphibians that are more susceptible to infection (Hossack et al. 2023; Garner et al. 2006). Second, bullfrogs are voracious predators and are known to prey upon and outcompete many native amphibians (Silva et al. 2009; Werner et al. 1995). Invasive bullfrogs are also known to prey upon other native wildlife including snakes, turtles, birds, small mammals, and fish (Bury and Whelan 1985; Silva et al. 2009; Hensley 1962; Wu et al. 2005; Smith et al. 2024). Third, bullfrog eggs and larvae are unpalatable to many native fish in their invasive range (Kruse and Francis 1977) allowing bullfrog larvae to persist in large numbers. Finally, bullfrogs have high reproductive and dispersal potential allowing them to readily invade previously unoccupied habitats (Peterson et al. 2013; Suhre 2010). For example, a single female bullfrog can lay up to 40,000 eggs in one clutch whereas a native leopard frog (*Rana* spp.) may lay about 300-2,040 eggs per clutch (Wright 1920; Rivera et al. 2023). As a result of these impacts, bullfrog removal is recognized as an important step in native amphibian species recovery plans (U.S. Fish and Wildlife 2007). However, bullfrog removal is challenging at landscape-level scales due to biological (e.g., high reproductive and dispersal potential) and logistical challenges (Bauder and Prewitt 2024; Kamoroff et al. 2020). Understanding region-specific bullfrog ecology is therefore important to help optimize bullfrog eradication efforts (Hall 2021; Drake et al. 2017).

While numerous studies have examined bullfrog reproductive ecology throughout their native (Ryan 1980; Willis et al. 1956; Howard 1981) and nonnative ranges (Urbina et al. 2020 in Oregon), very little published information exists on bullfrog reproductive ecology in southeastern Arizona. Understanding region-specific aspects of bullfrog reproductive ecology within their nonnative range is important for optimizing regional bullfrog eradication efforts. For example, bullfrogs in more temperate regions of their native and nonnative ranges must invariably overwinter multiple times before metamorphosizing (Collins 1979; Govindarajulu et al. 2006) which requires breeding populations of bullfrogs to be associated with permanent water bodies (Bury and Whelan 1985). However, bullfrogs in southeastern Arizona are capable of metamorphosing after a single winter following hatching (Jones 2013). This relatively rapid life history lessens the necessity of consistently permanent water for reproduction and may facilitate more rapid invasions by invasive bullfrogs (Mims et al. 2020) which in turn has implications for the design and implementation of bullfrog eradication efforts in regions with similarly mild climates.

To our knowledge, only one study has previously examined bullfrog dispersal in southeastern Arizona. Suhre (2010) studied bullfrog dispersal using mark-recapture within the Altar Valley, 65 miles southwest of Tucson. This landscape was characterized as a semi-arid grassland and included 58 sites consisting of stock tanks. Using mark-recapture, Suhre reported a mean straight-line dispersal distance of 3.17 km and a maximum distance of 10.2 km within one year. Unmarked bullfrogs were also observed up to 12.8 km from potential source populations (Suhre

2010). However, bullfrog dispersal distances can vary widely within their invasive and native ranges. For example, a study of sexually mature bullfrog dispersal using internally implanted VHF transmitters (n = 9) among ponds in Belgium reported a maximum dispersal distance of 1.8 km (Descamps and De Vocht 2016). Sepulveda and Layhee (2015) radio tracked juveniles (n = 13) in Montana and recorded a maximum distance of 0.37 km. Two studies of bullfrogs in their native range reported straight-line summer dispersal distances between 0.16-1.2 km (n = 264) in Missouri (Willis et al. 1956) and a maximum summer dispersal distance by a juvenile of 0.91 km in New York (Raney 1940).

Weather conditions (e.g., precipitation) and population density can also affect bullfrog dispersal patterns. Suhre (2010) found that dispersal distances were greatest during high rainfall and at high-density sites. Within the arid landscapes of southeastern Arizona, where earthen livestock ponds ("stock tanks") are often widely separated, it is also likely that bullfrog dispersal is facilitated by temporary water bodies or ephemeral water flows within washes that only appear during the rainy summer monsoon (Mims et al. 2015). Many studies have found that juvenile bullfrogs were the most prominent dispersing age class, usually in response to high population densities and the territoriality of larger adult males (Govindarajulu et al. 2005; Cooper 2017; Gahl et al. 2009).

Understanding region-specific size at sexual maturity for bullfrogs in their nonnative range is also important for maximizing the efficacy of eradication efforts. For example, successful bullfrog eradication efforts in southeastern Arizona have prioritized the removal of adults during the first year of eradication efforts to prevent subsequent bullfrog reproduction (D. Hall, University of Arizona, personal communication). The targeting of adults during initial bullfrog eradication efforts can benefit from understanding region-specific patterns of size at sexual maturity. Kaefer et al. (2007) found that bullfrogs in Brazil reproduced year-round and female bullfrogs appeared to be sexually mature when their snout vent length (SVL) was 120.83-174.00 mm and their body mass was 220-550 g. The authors concluded that the mean sizes at maturation for both males and females was similar to those living in their native range in Michigan (Kaefer et al. 2007; Howard 1981). Similarly, Tessa et al. (2016) identified mean SVL at sexual maturity for female bullfrogs at three sites in France and one site in Canada of 140.33, 124.41, 112.80, and 147 mm, respectively. These results collectively illustrate the regional variation in female bullfrog size at maturity which may be due to variation in temperature, site-specific hydroperiods, or other factors (Collins 1979). Urbina et al. (2020) found that male bullfrogs in Oregon that had motile sperm had a SVL of 66 mm. Jones et al. (2023) found histological evidence that male bullfrogs in northwestern Arizona reached maturity at an SVL of 100mm. Although males may become mature at a smaller size than females, female bullfrogs have been shown to exhibit strong selection for lager mating partners (Howard 1978) which may reduce the contribution of smaller males to population reproduction (Jones et al. 2023). However, smaller males may be more susceptible to intra-specific predation and competition from larger males (Govindarajulu et al. 2005; Cooper 2017; Gahl et al. 2009), all of which may increase their likelihood of dispersal. Small adult males could therefore be important facilitators of bullfrog reinvasion through their ability to reproduce with any newly arriving females.

In this report, we present the current results of our project studying the dispersal and reproductive ecology of bullfrogs in southeastern Arizona. The results discussed in this report are from data collected from 14 March 2024 through 15 September 2024. We plan to continue collecting data to address our project objectives during the summer and fall of 2025.

Study Area

Our study area for studying bullfrog dispersal was the San Rafael Valley and the adjacent Canelo Hills in Santa Cruz County, Arizona. This area is topographically variable, including washes, canyons, mesas, and the Huachuca Mountains to the east of the valley and is bisected from north to south by the Santa Cruz River. Vegetation communities include southwestern riparian habitat adjacent to some stock tanks, semidesert grassland, and oak-juniper forest in the higher elevation areas (Brown 1994). The vegetation immediately surrounding the stock tanks can vary widely due to varying hydroperiods but can include bulrush (*Scirpus* spp.), cattail (*Typha* spp.), and sedges (*Carex* spp.). We surveyed 51 stock tanks in the area ranging from 1418-1602 m elevation (Figure 1; Appendix II). Much of our study area was either on private working cattle ranches or on U.S. Forest Service land which was also subject to varying levels of grazing. The valley is home to an abundance of native Arizona species, including an important population of the federally threatened northern Mexican gartersnake (*Thamnophis eques*) within the Santa Cruz River (Bauder et al. 2024).

Our study area for studying bullfrog reproductive ecology was the Babocomari River within the Babacomari Ranch. This is the site of an ongoing bullfrog eradication project that began April 2024 on a private working cattle ranch. Bullfrog eradication occurred along an approximately 13 km stretch of the Babocomari River (Figure 2), approximately 6 km of which contained aquatic environments suitable for bullfrog reproduction. Vegetation communities along the Babocomari River are typical southwestern riparian communities and include Fremont's cottonwood (*Populus fremontii*), Gooding willow (*Salix gooddingii*), bulrush (*Scirpus* spp.), and cattail (*Typha* spp.). The elevation of the Babocomari River in this area is approximately 1386 m. The Babocomari River hosts many native aquatic species including invasive northern crayfish (*Faxonius virilis*), largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), yellow bullhead (*Ameiurus natalis*), and mosquitofish (*Gambusia affinis*). The Babocomari River and its tributaries (e.g., O'Donnell Canyon and Post Canyon) may also provide habitat for native fish species such as the Gila chub (*Gila intermedia*) and Sonora sucker (*Catostomus insignis)*.

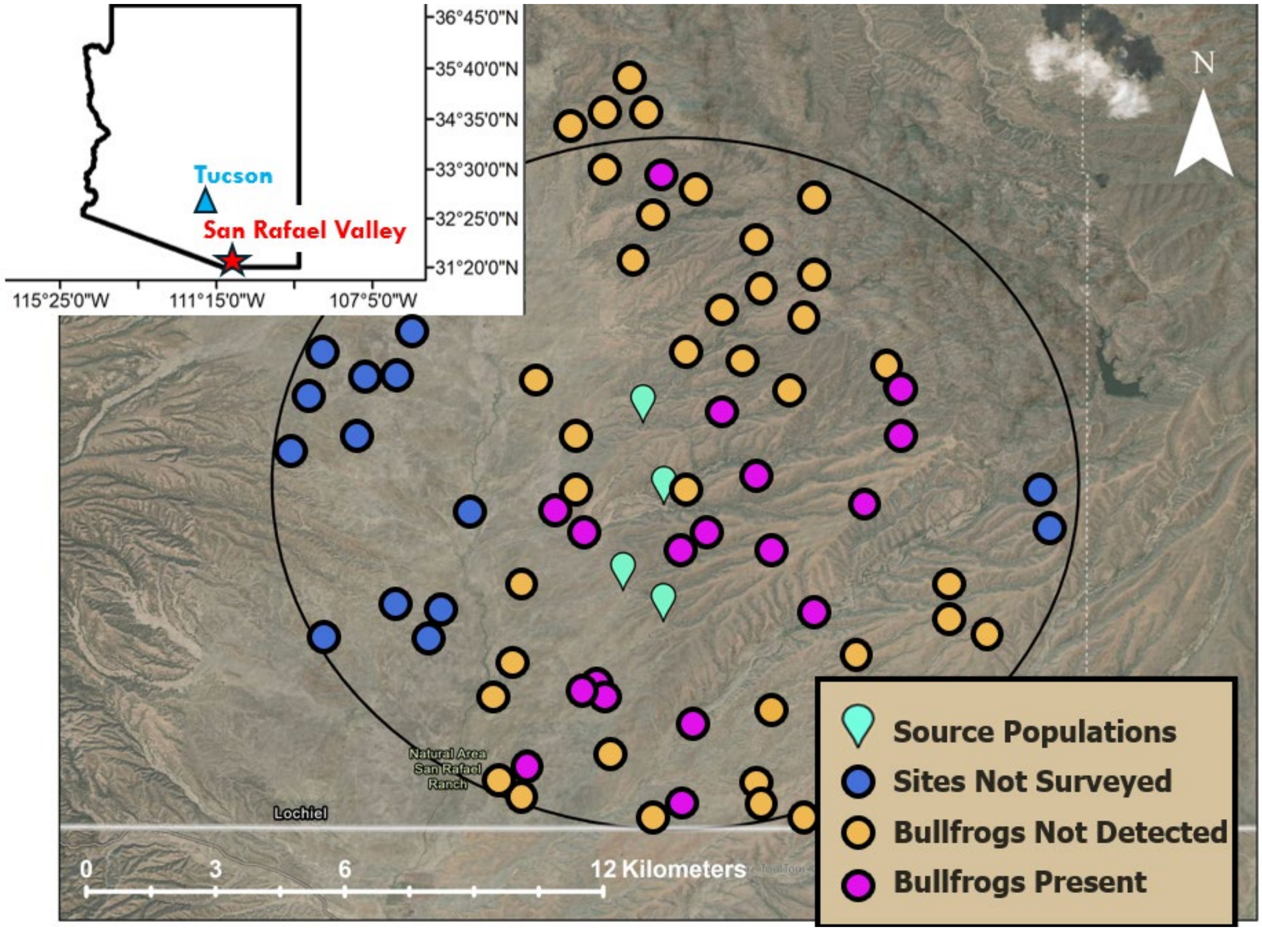

Figure 1: Map of American bullfrog survey and mark-recapture sites in the San Rafael Valley, Santa Cruz County, Arizona, during 2024. Source Populations (turqouise) were sites with large numbers (>20) of bullfrogs and evidence of reproduction (i.e., egg masses and/or tadpoles) where pre-monsoon mark-recapture efforts were focused. Bullfrogs Present (pink) were sites surveyed following the onset of monsoon rains where bullfrogs were detected. Bullfrogs Not Detected (orange) were sites surveyed following the onset of monsoon rains where bullfrogs were not detected. Sites Not Surveyed (blue) were sites within the study area that were not surveyed in 2024 (blue).

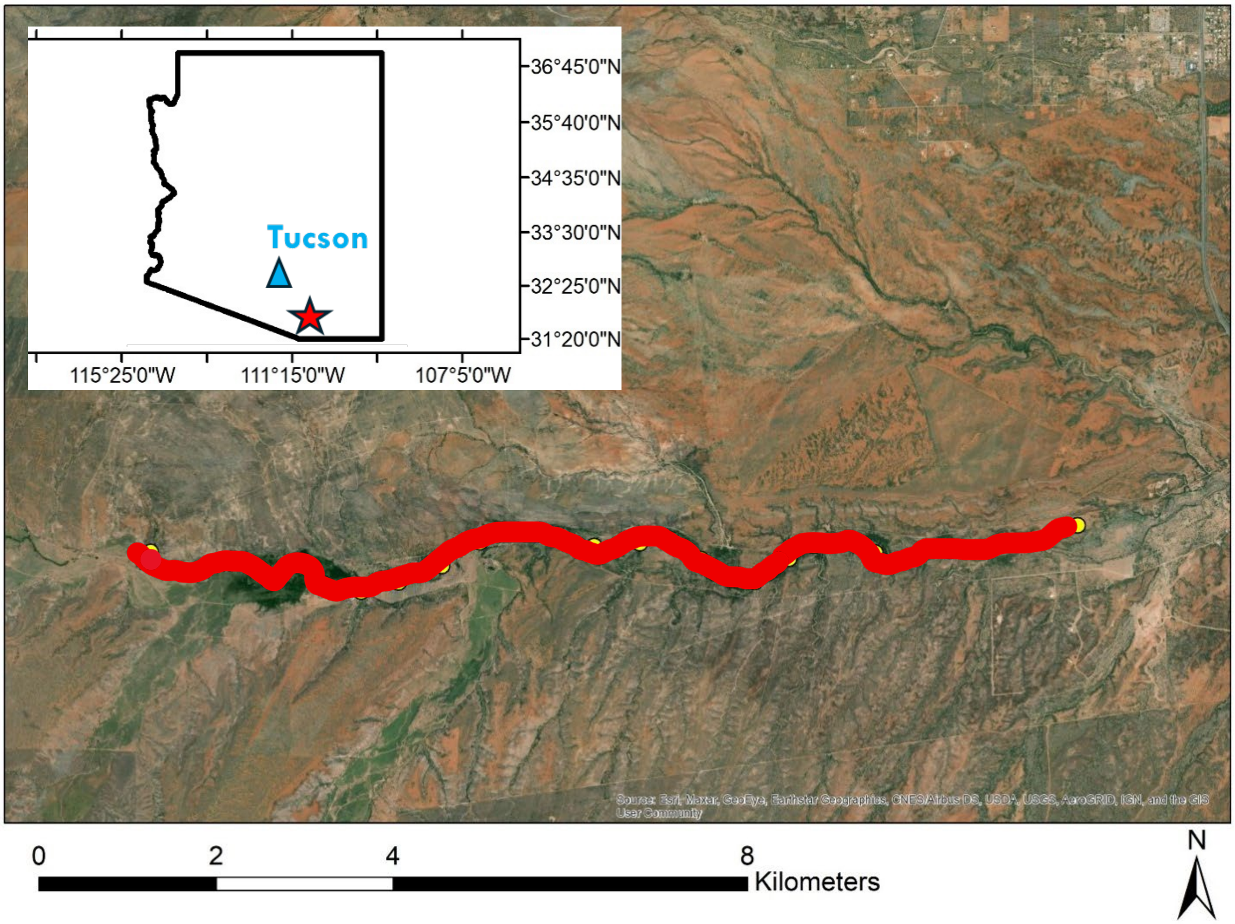

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Figure 2: Map of the 13 km stretch of the Babocomari River in Elgin, Arizona, where American bullfrogs were collected from an ongoing eradication effort for dissection in 2024.

Methods

Bullfrog Mark-Recapture Surveys and Dispersal

We conducted mark-recapture surveys to document bullfrog movements among sites. We first visited stock tanks during May and June to determine if water and bullfrogs were present. We performed nocturnal visual encounter surveys (VES) using two independent observers who began their surveys 20 minutes apart to allow the habitat to settle and any disturbed animals to return to the surface. We recorded all VES data on the Arizona Game and Fish RACH/Riparian Herpetofauna VES Datasheet (see Appendix I). We located four perennial stock tanks with well-water pumps that contained relatively large reproductive populations of bullfrogs and conducted our mark-recapture surveys at these four sites (hereafter source populations) (Figure 1). After performing a single VES at each of the four sites, we captured bullfrogs using a combination of hand-capture and trapping using collapsible minnow traps (Promar Collapsible Live Bait Traps Model TR-501). We conducted multiple capture sessions at each source population to maximize the proportion of individuals marked at each site. We ceased marking efforts when >60% of within-session captures were of marked individuals (i.e., recaptures).

We recorded the snout-vent-length (SVL), weight, and sex of each captured bullfrog (individuals with SVL < 90 mm were recorded as juveniles). An individual was determined male based on presence of secondary characteristics such as nuptial pads on an enlarged thumb, yellow-throat pigmentation, and the tympanum diameter being greater than the diameter of the eye. Females had relatively similar tympanum and eye size and no nuptial pad on their forefeet. We gave all individuals a toe-clip combination unique to the tank at which they were captured (i.e., cohort markings). The cohort markings were a combination of two toe-clips, typically one non-dominant toe on a forefoot and one non-dominant toe on a hindfoot, which will not inhibit movement (McCarthy and Parris 2004). We recorded the combinations using the Martof numbering system (Martof 1953b) (i.e., Hind Right 20 + Fore Left 300) (Figure 3). This is a standard procedure in amphibian mark-recapture studies (Beaupre et al. 2004) and all necessary state, federal, and university permits were obtained prior to field work. All marked frogs were released at their site of capture.

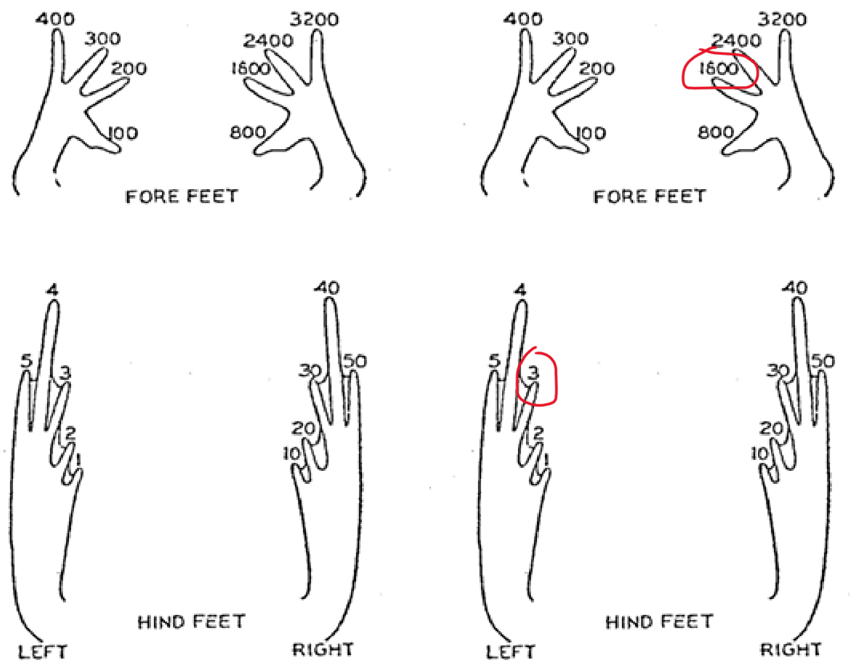

Figure 3: Schematic drawing showing the Martof numbering system of assigning numbers to each digit (left) (Martof 1953b) and which was used to mark American bullfrogs in southeastern Arizona during 2024. The figure on the right shows an example of a cohort marking using two toe clips that would be recorded as Fore Right 1600 + Hind Left 3.

Following the onset of the monsoon (first rainfall 20 June 2024), we began conducting VES at stock tanks within an 8 km radius of our source populations to identify dispersing bullfrogs. Although bullfrogs are known to disperse up to 10.2 km in southeastern Arizona (Suhre 2010), we used an 8 km radius around our source populations due to logistical constraints (Figure 1). We used the same nocturnal VES survey protocol with both observers attempting to capture bullfrogs to look for the presence of a mark. We marked any unmarked bullfrogs captured at these surrounding sites with cohort markings for their respective site of capture and released any within-site recaptures. These frogs were released back into the tank at which they were recaptured. When we recaptured a marked individual, we measured the Euclidean distance between the source and recapture sites. We also adjusted this distance for topography using the topoDistance package (Wang 2020) in R v. 4.4.1 (R Core Team 2024).

Bullfrog Telemetry

We used two types of externally attached transmitters to monitor the movements of individual bullfrogs. The first type was a VHF transmitter (2.4 g PD-2T and 1.8 g BD-2T; Holohil Systems Ltd., Carp, Ontario, Canada) that we attached using an adjustable silicon belt epoxied to the transmitter (Burow et al. 2012) (Figure 4). The second was a 4.8-g dual GPS-VHF transmitter (PinPoint VHF-75, Lotek, New Market, Ontario, Canada; hereafter GPS transmitters). We attached GPS transmitters using waist harnesses made from craft elastic thread and size 14 Japanese glass seed beads in olive matte (Muths 2003) (Figure 4). We passed the elastic thread through the manufacturer-provided holes on the posterior end of the transmitter. We programmed each GPS transmitter to record a GPS location every hour between 1800 and 0600 hr local time and a single GPS location at 1200 hr. We programmed the VHF signal within the GPS transmitter and the remote download option to be active between 1300 and 0400 hr. The estimated minimum and average battery life under these programmed schedules was 14 and 17 days, respectively.

We conducted beacon tests of our GPS transmitters to evaluate their location precision prior to deploying these transmitters on bullfrogs. We attached four GPS transmitters to the backs of plastic dinosaur figures to simulate the possible above-ground height (40-70 mm) of a transmitter attached to a bullfrog. We placed these figures in four microhabitats: on upland habitat in dense vegetation, partially submerged in low density vegetation, completely submerged in medium density vegetation, and one on upland habitat in low density vegetation. We collected the figures after 8 days and downloaded the GPS locations.

We deployed 24 VHF transmitters and 4 GPS transmitters over the course of 3 days: 31 July, 1 August, and 5 August 2024. We monitored the location of these transmitters approximately twice a week until 12 September 2024. We remotely downloaded data from the GPS transmitters using the Lotek PinPoint VHF Commander. We confirmed locations of VHF transmitters using a 3-element Yagi antennae attachment and Telonics Model TR-8 receiver. During each locating event we recorded air temperature, water temperature, relative humidity, presence of precipitation, and relative wind speed. At the conclusion of the telemetry study, we attempted to locate and retrieve all transmitters.

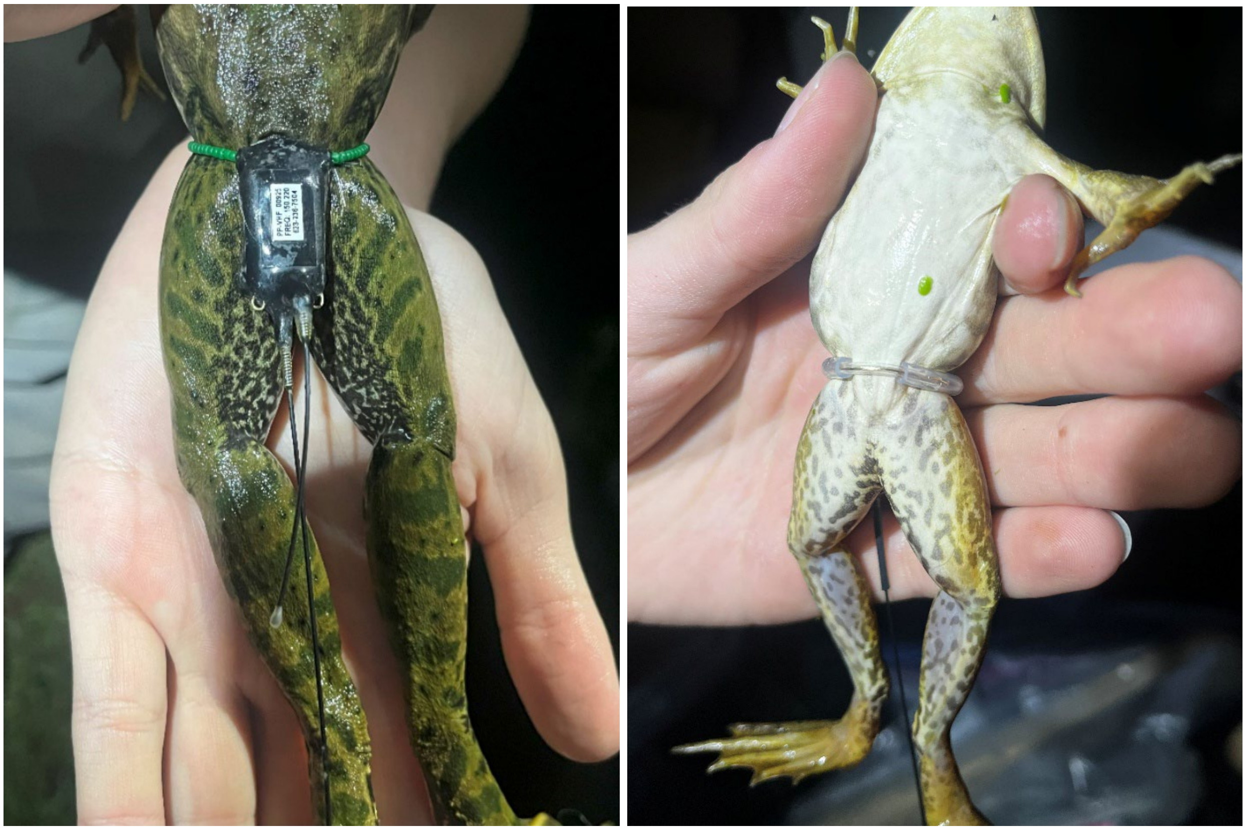

Figure 4: Attachment styles used for externally attaching GPS and VHF transmitters onto American bullfrogs in the San Rafael Valley, Arizona, during August 2024. **Left:** Attachment method for GPS transmitters using waist harnesses made from craft elastic thread and size 14 Japanese glass seed beads in olive matte using design by Muths 2003. **Right:** Adjustable silicon belt epoxied to the transmitter using design by Burow et al. 2012. Photographs by Emma Sudbeck, University of Arizona.

Bullfrog Collections and Dissections

We collected bullfrog carcasses from the Babocomari River near Elgin, Arizona, during a bullfrog eradication project initiated during April 2024 on the Babacomari Ranch. Bullfrogs were lethally removed using .22 rifles during nocturnal VES. We collected carcasses by hand or with a dip-net and placed them into individual Ziploc® bags. Because the landowners requested the bullfrog legs for consumption, we recorded the SVL of each carcass in the field, wrote it on the outside of each individual bag, and then removed the legs and placed them in a separate bag for transport to the landowners. We placed carcasses in a cooler with ice and transported them back to the University of Arizona where they were typically dissected within 1-2 days. If dissections were to take place >2 days after collection, the carcasses were frozen.

We recorded data on the size and status of reproductive organs and stomach contents from each carcass. We recorded oviduct diameter, egg diameter, and egg color for each female and used these characteristics to determine the ovarian stage following the definitions of stages used by

Urbina et al. (2020) and Jones et al. (2023) developed by Costa et al. (1998). Stage 1 is a reproductively immature individual, with very thin, orange ovaries and no distinguishable eggs. Stage 2 is an individual beginning to reach sexual maturity, with distinguishable pale-colored eggs and a paler, yellow-colored ovary. Stage 3 is an individual nearing sexual maturity with a mix of post-vitellogenic black eggs and yellow eggs. Stage 4 is a sexually mature individual with a high proportion of black differentiated eggs (Costa et al. 1998). We also included a Stage 0, a reproductively immature individual where no oviduct is detected but fat bodies are present. As noted in Jones et al. (2023), eggs do not become vitellogenic until late-stage 3 and females do not lay until stage 4 so the definition of sexual maturity for this report is any individual in stages 3 or 4. For male frogs, we recorded the average length and width of testes. Although it is unclear whether testes size in bullfrogs is correlated with sexual maturity, it has been found that testes weight increases during the breeding season (Chavadej et al. 2000) and may fluctuate throughout the year (Huang et al. 2004).

We evaluated whether the ratio of males to females in our sample differed from 1:1 using a chi-square goodness-of-fit test. We compared SVL between males and females using a two-sample t-test. We evaluated differences in SVL among ovarian stages using a generalized linear model with a Gaussian error distribution and an identity link. We set Stage 4 as our reference level because we were specifically interested in evaluating differences between Stage 4 and lower stages.

We examined stomach contents of both female and male frogs by emptying the stomach during dissection. We identified contents to higher taxonomic groups, typically a general group, such as beetles, crayfish, plant matter, bees, etc. We evaluated the relationship between an individual's SVL and the presence of three prey groups (crayfish, insect, vertebrate) using three generalized linear models with binomial error distributions and logit links.

Disease Sample Testing

We collected tissue samples and skin swabs from 50 bullfrog carcasses collected from the Babocomari River. We collected 25 mg of liver tissue from each of these 50 carcasses which were placed in a tube and promptly frozen. These tissue samples were paired with a skin swab of the same frog so that researchers at Northern Arizona University (NAU) can compare the pathogen load of skin swabs versus tissue samples for ranavirus. We collected swabs prior to the dissections using cotton swabs by swabbing each appendage, mouth, and ventral side of the frog 15 times each. We then placed each swab into 1ml of ethanol within a 2ml tube and stored them in a freezer. Gloves were worn during this process and changed between each sample collected. We mailed these samples to NAU on 18 September 2024 for testing. We also collected additional skin swab samples from 41 bullfrogs across 5 of our mark-recapture sites in the San Rafael Valley.

Results and Discussion

Bullfrog Movement and Dispersal

We conducted surveys at 34 sites prior to the onset of the 2024 monsoon, which started 20 June. Of these 34 sites, 11 did not have water. We detected bullfrogs at 5 of the remaining 23 sites, including two of our four source population sites (Figure 1). We located our other two source population sites ("Jackpot Tank" and "Pasture 9 Tank") shortly after the start of the monsoon.

We captured and marked a total of 323 individuals at our four source populations ("Jackpot Tank", "Pasture 9 Tank", "Bobcat Tank", "Big Boy Tank") starting on 3 June and continuing into the monsoon (Table 1). The combination of an unexpected early start of the monsoon (first rainfall 20 June 2024) and mark-recapture (MR) efforts taking longer than expected resulted in our continuation of MR efforts into the monsoon. Our MR efforts at Jackpot Tank were particularly time consuming due to the large numbers of bullfrogs at this site. Jackpot Tank contained an estimated 200-400 individuals (primarily juveniles) during our initial VES at the site. We conducted a total of 15 MR sessions (about twice per week) over 8 weeks at Jackpot Tank and still did not achieve our goal of having >60% of captures during a single session consist of previously marked individuals. This was likely due to the high abundance of metamorphosing juveniles within that period, and potentially the immigration of new individuals to the site. We decided to cease MR efforts at Jackpot Tank on 14 August to fully devote our efforts to surveying surrounding sites. We conducted 86 VES at 30 sites around the four source populations between 20 June and 13 September 2024. During these monsoon surveys, we captured and marked an additional 33 individuals at nine sites.

We recaptured a total of five marked individuals that had moved from a source population to another tank (Figure 5). Four out of five of these individuals came from Jackpot Tank. These five individuals dispersed to three different sites. Three juveniles (SVL = 54, 60, and 54 mm) were recaptured at "Pasture 9 Tank" on 1 and 5 July (anywhere between 4 to 11 days after initial marking), a 1.11 km topographic distance from Jackpot Tank. One juvenile (SVL = 69 mm) was recaptured at "Max Tank" on 7 August, a 2.28 km topographic distance from Jackpot Tank. Finally, one adult female (SVL = 160 mm) initially marked at Big Boy Tank on 6 June (inferred from SVL measurement) was recaptured at Max Tank on 6 August, a 1.15 km topographic distance from Big Boy Tank. Max Tank is ephemeral and was dry during our VES on 15 May and 24 June. Our third VES at this site on 6 August was the first time we detected bullfrogs and water at this site. All three movement paths were uphill (25, 47, 61 m). Uphill dispersal was also documented in other ranid species including the Columbia spotted frogs (*Rana luteiventris*) (Funk et al. 2005) and green frogs (*Rana clamitans*) (Martof 1953a). However, Suhre (2010) documented mostly downstream movements of bullfrogs in the Altar Valley.

The individual that moved the furthest distance between capture events (a juvenile, SVL 69 mm) most likely utilized a drainage connecting Jackpot Tank and Max Tank (Figure 5) due to the presence of ephemeral pools along this drainage. The Euclidean distance paths between Big Boy Tank and Max Tank (adult female, SVL = 160 mm) and between Jackpot Tank and Pasture 9 Tank (juveniles; SVL = 54, 60, and 54 mm) are not connected via drainages suggesting that these individuals dispersed across land or a more complex path using interconnected drainages if they used environmental cues that might indicate access to water. However, our data did not allow us to determine the actual movement paths used by these individuals during dispersal.

Our dispersal distance results are similar to dispersal distances for bullfrogs in Belgium where the maximum reported dispersal distance within a 5 month period was 1.8 km (Descamps and De Vocht 2016). Suhre (2010) found a maximum dispersal distance of 10.2 km in a similar habitat in southern Arizona, the Altar Valley using mark-recapture. This far exceeds the maximum distance we recorded during our 5-month study period. The greater dispersal distances reported by Suhre (2010) could be due to their larger study area and greater number of lower elevation stock tanks in the Altar Valley. Suhre (2010) found little to no dispersal up the slopes of the Baboquivari Mountains which might also suggest that bullfrog dispersal may be more limited in areas with greater topographic relief although additional data are needed to evaluate this prediction.

Table 1. Summary statistics for the mark-recapture efforts for American bullfrogs at the four source populations in the San Rafael Valley in southeastern Arizona during the summer of 2024. Summary statistics include the site name, the range of dates during which we conducted mark-recapture (MR) sessions, the total number of frogs marked at each site, the mean, standard deviation (SD), minimum and maximum snout-vent length (SVL) in millimeters (mm), and the mean, SD, minimum and maximum weight in grams (g).

Site Name	Range of MR Dates	Number of MR Sessions	Total Number of Frogs Marked	Mean SVL (mm)	SD SVL (mm)	Minimum SVL (mm)	Maximum SVL (mm)	Mean Weight (g)	SD Weight (g)	Minimum Weight (g)	Maximum Weight (g)
Big Boy Tank	6/3/2024 to 6/20/2024	6	25	105	27	80	170	144	174	36	639
Bobcat Tank	6/11/2024 to 7/3/2024	5	28	98	32	43	180	107	125	10	484
Jackpot Tank	6/24/2024 to 8/14/2024	15	244	59	16	35	125	23	26	5	180
Pasture 9 Tank	7/1/2024 to 7/18/2024	5	26	72	23	52	131	43	54	8	186

Table 2. Summary statistics for the mark-recapture efforts for American bullfrogs at the four source populations in the San Rafael Valley in southeastern Arizona during the summer of 2024. Summary statistics include the site name, the range of dates during which we conducted mark-recapture (MR) sessions, the total number of individual frogs marked at each site, the total number individuals recaptured at that site, the total number of individuals from each source population that were recaptured at a different site, the total number of males, females, and juveniles that were captured and marked at each site, and percentages of males, females, and juveniles captured at each site.

Site Name	Range of Capture Dates	Number of Capture Sessions	Total Number of Frogs Marked	Number of Recaptures	Number of Individuals Recaptured at Another Site	Number of Males Marked	Number of Females Marked	Number of Juveniles Marked	% Males	% Females	% Juveniles
Big Boy Tank	6/3/2024 to 6/20/2024	6	25	11	1	10	8	1	40	32	4
Bobcat Tank	6/11/2024 to 7/3/2024	5	28	33	0	13	7	8	46	25	29
Jackpot Tank	6/24/2024 to 8/14/2024	15	244	31	4	4	10	229	2	4	94
Pasture 9 Tank	7/1/2024 to 7/18/2024	5	26	5	3*	0	6	20	0	23	77

*Number of individuals initially marked at Jackpot Tank that were recaptured at Pasture 9 Tank

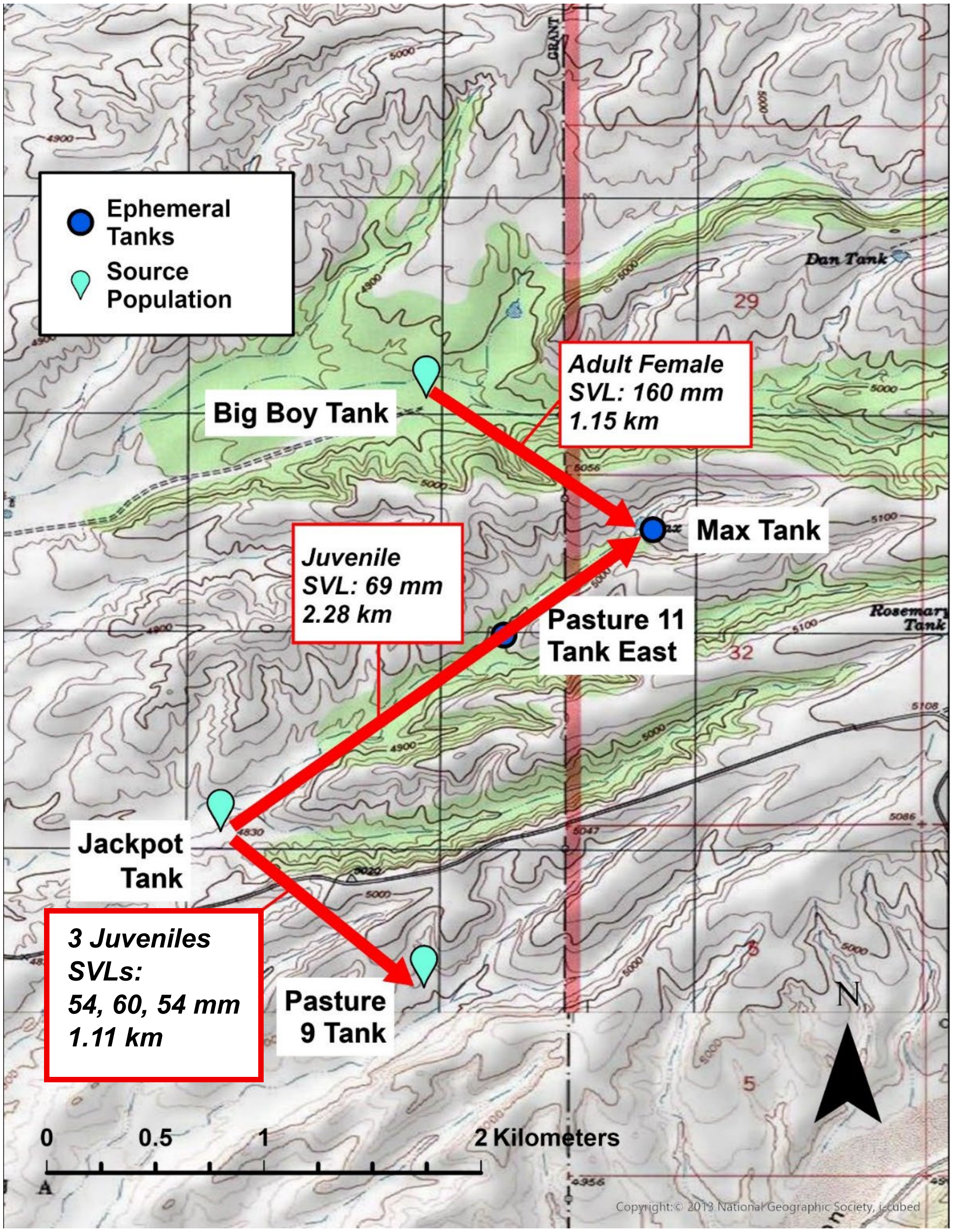

Figure 5: Topographic distances between marking and recapture sites of dispersing American bullfrog during May–September 2024 field season in the San Rafael Valley in southeastern Arizona.

Bullfrog Telemetry

Our GPS transmitters used in beacon testing that were placed under water in cattails did not collect any GPS locations. The GPS transmitter attached to a figure that was partially submerged within grass collected GPS locations at 78 of 187 hourly intervals (42%). Of these 78 GPS locations, two had horizontal dilution of precision (HDOP) >5 (6.4 and 15.7). An additional point with HDOP = 2.4 was approximately 416 m from the centroid of all other locations with HDOP < 5. This outlier point was the only location to receive signals from only three of three available satellites and we suspect this contributed to its outlier position. We therefore excluded these three locations from further summaries. Mean HDOP among the remaining 75 locations with HDOP < 5 was 2.0 (SD = 0.9, range = 1.1–4.9). The vast majority of these locations were within 5 m (80%) and 10 m (95%) of the centroid of those locations. We collected 186 hourly GPS locations from the GPS transmitter placed on bare open ground. Two of these locations had HDOP > 5 (21.9 and 68.2) and we excluded these two locations from further summaries. Mean HDOP for locations with HDOP < 5 was 1.6 (SD = 0.7, range = 0.9–4.8). The vast majority of these locations were within 5 m (88%) and 10 m (99%) of the centroid of those locations. We collected 187 hourly GPS locations from the GPS transmitter placed in thick grass in the upload. Six of these locations had HDOP > 5 (HDOP = 5.4–208.2) and we excluded these six locations from further summaries. Mean HDOP for locations with HDOP < 5 was 1.7 (SD = 0.8, range = 1.0–4.7). Most of these locations were within 5 m (72%) and 10 m (96%) of the centroid of those locations.

Of the four adult bullfrogs with GPS transmitters, we obtained usable GPS locations from three individuals. One individual (tag #925) had five usable GPS locations (HDOP = 1.2–2.1, number of satellites = 3–8) collected over two days (8 and 10 August 2024). These five locations were approximately 4–12 m from their centroid. One individual (tag #921) had GPS coordinates for only a single location from 1 August 2024 but the altitude of this location was very inaccurate (3297 m) and we therefore considered this location unusable despite a HDOP = 4.6. The third individual (tag #914) had only a single usable location with a HDOP = 6.0. We recovered one GPS transmitter that had apparently fallen off its individual. None of these GPS locations indicated that these three bullfrogs left the pond.

We did not detect evidence that any of our VHF-telemetered bullfrogs left the pond. At the end of the study, we found 13 VHF transmitters that had fallen off their respective bullfrog in dense perimeter vegetation and suspected the other 11 to have also fallen off but were too deep in the water and/or mud to retrieve. We were unable to recapture any individuals with transmitters to retrieve those transmitters.

There are few studies that show successful telemetry efforts on the American bullfrog (Chance 2002; Cooper 2017; Descamps and De Vocht 2016; Sepulveda and Layhee 2015). We might have seen better results at a stock tank with very low vegetation and therefore less chance of transmitter entanglement. Attachment methods may have also played a role in the success of this effort. We suspect that the attachment of the VHF transmitter (2.4 g PD-2T and 1.8 g BD-2T; Holohil Systems Ltd., Carp, Ontario, Canada) using an adjustable silicon belt design epoxied to the transmitter (Burow et al. 2012) (Figure 4) was more secure than the elastic bead belt. The attachment for the 4.8-g dual GPS-VHF transmitters (PinPoint VHF-75, Lotek, New Market, Ontario, Canada; hereafter GPS transmitters) using waist harnesses made from craft elastic thread and size 14 Japanese glass seed beads in olive matte (Muths 2003) often broke when we were crafting them. These belts were likely more fragile and may have broken shortly after attachment. However, we were only able to recover one of the GPS transmitters so there is no evidence of this. For future studies telemetry studies of bullfrogs using external attachment methods, we recommend the use of the silicon belt design.

Bullfrog Collections and Dissections

We dissected 363 bullfrogs from the Babocomari River that were removed during March–July 2024. Our sample included more males (n = 203) than females (n = 151; χ^2 = 7.64, P = 0.0057). Males were generally smaller (mean = 106 mm, SD = 36 mm, range = 45-187 mm) than females (mean = 115.8, SD = 40 mm, range = 36-205 mm; t_{352}= 2.46, P= 0.014).

Most females were in either Stage 4 (27.8%), Stage 1 (27.8%), or Stage 0 (23.2%; Figure 6). Relatively few females were at the intermediate stage of maturation (Stages 2 [6.6%] and 3 [14.5%]). Snout-vent-lengths of bullfrogs generally increased with increasing ovarian stages (Figure 7). There was little evidence that individuals in Stage 4 were consistently larger (mean = 156 mm, SD = 17 mm, range = 123–205 mm) than individuals in Stage 3 (mean = 150 mm, SD = 13 mm, range = 129–171 mm; β = -6.67, P = 0.112). Individuals in Stage 4 were generally larger than individuals in Stage 2 (mean = 132 mm, SD = 19 mm, range = 99–157 mm; β = -23.77, P < 0.0001). However, females with SVL of 130–170 mm were frequently found across Stages 2–4. The smallest SVL for a female with mature ova (defined as late-stage 3 to stage 4) was 123 mm (Figure 8). This female was collected 18 April 2024 and had an oviduct diameter of 2 mm. There was no overlap in SVL between individuals in Stage 4 and individuals in Stage 1 (mean = 96 mm, SD = 17 mm, range = 52–122 mm; β = -60.33, P < 0.0001) or Stage 0 (mean = 65 mm, SD = 13 mm, range = 36–94 mm; β = -90.82, P < 0.0001).

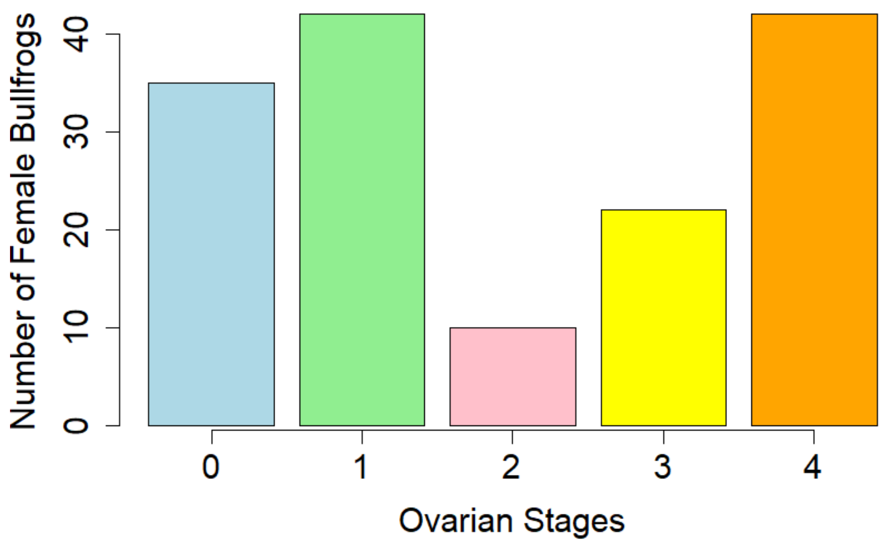

Figure 6: Number of female American bullfrogs collected from the Babocomari River in southeastern Arizona during March–July 2024 by ovarian stage (0-4) as defined by Urbina et al. (2020).

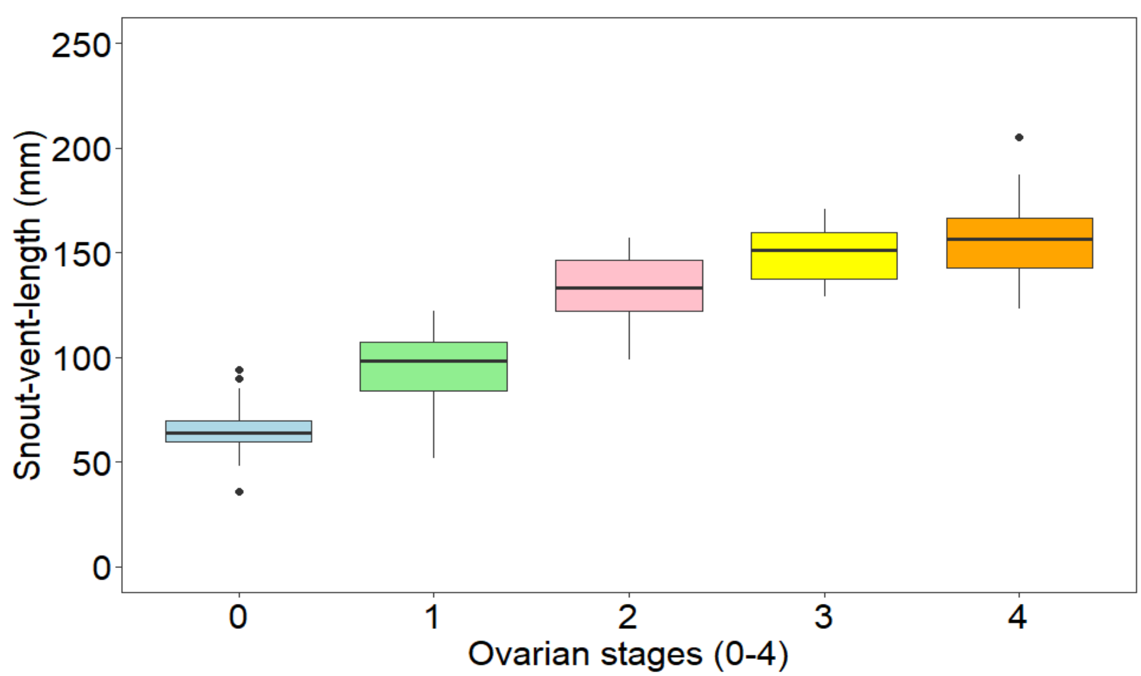

Figure 7: Distributions of snout-vent lengths (mm) of female American bullfrogs collected from the Babocomari River in southeastern Arizona during March–July 2024 by ovarian stage (0-4) as defined by Urbina et al. (2020).

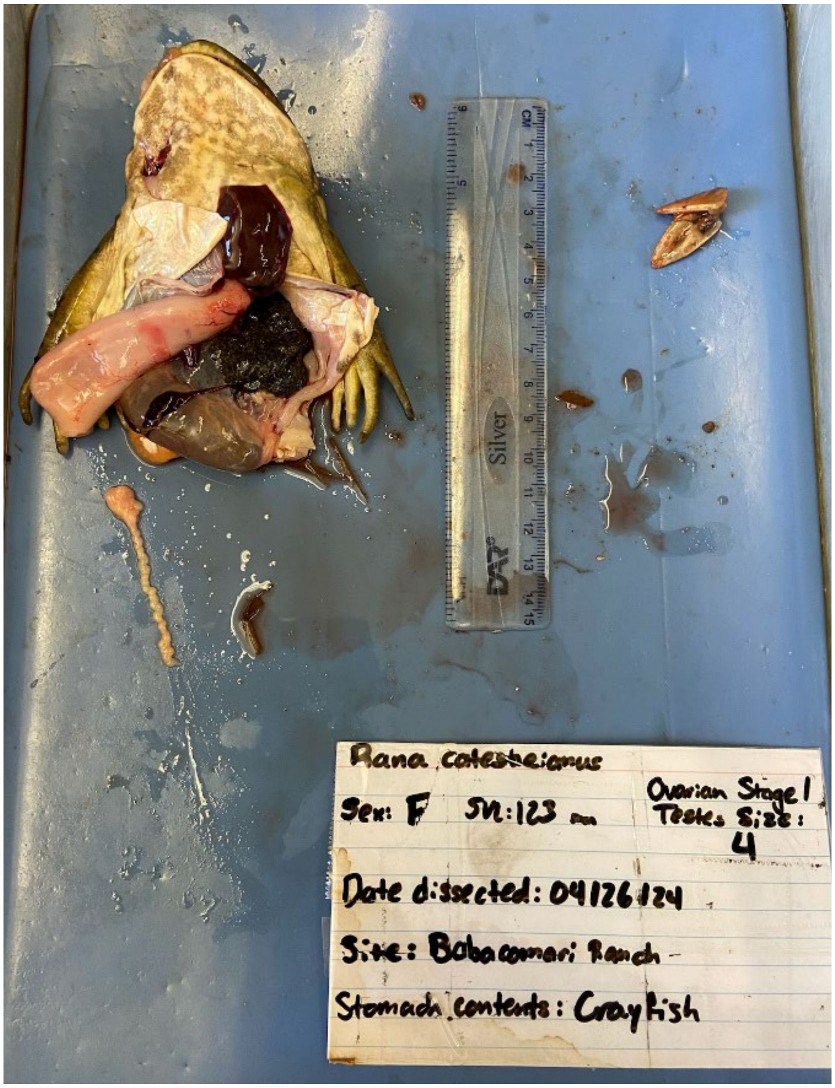

Figure 8: The smallest female American bullfrogs (123 mm snout-vent length) collected from the Babocomari River in southeastern Arizona on 18 April 2024 that showed signs of reproductive maturity with an ovarian Stage 4 and a 2 mm oviduct. Photograph by Morgan McDowell, University of Arizona.

Most dissected bullfrogs contained either crayfish (*Faxonius virilis*) (39%) or insects (e.g., beetles, bees, spiders; 42%) in their stomachs (Figure 9). Only 7% had no prey items within their stomachs (n = 24). The size of the consumed crayfish was highly variable with the largest crayfish being ~110 mm in total length (rostrum to tail) that was found in a 149 mm SVL adult male bullfrog (Figure 10). Evidence of vertebrate prey was found in only 6 bullfrogs (1%). Mean SVL across these six bullfrogs was 110 mm (SD = 29 mm, range = 91–165 mm). Three individuals had evidence of fish species in their stomachs. All three had mosquito fish (*Gambusia affinis*) and one also had two unidentifiable (due to decomposition) centrarchid species that are most likely either bluegill (*Lepomis macrochirus*) or largemouth bass (*Micropterus salmoides*). We identified a small amount of black mammal fur in one individual and bird remnants including black feathers in another individual. We suspect the feathers most likely came from a red-winged blackbird (*Agelaius phoeniceus)* due to their common occurrence around the Babocomari. We identified only one herpetofauna species among the stomach contents of our dissected bullfrogs: a juvenile checkered gartersnake (*T. marcianus)* (total length ≈ 220 mm) within the stomach of a 151 mm SVL adult male bullfrog (Figure 11). We suspect the lack of herpetofauna prey within the bullfrogs we collected from the Babocomari River primarily reflects a relatively low abundance of native aquatic herpetofauna due to the relatively high abundance of nonnative aquatic species in this system (e.g., bullfrogs, crayfish, largemouth bass, mosquito fish). We found evidence that larger frogs were more likely to have crayfish present in their stomachs ($\beta = 0.037$, $P < 0.0001$; Figure 10). Smaller frogs were more likely to have insects present in their stomachs ($\beta = -0.030$, $P < 0.0001$; Figure 12). There was weaker evidence that vertebrate prey items were more likely to be found in larger frogs ($\beta = 0.022$, $P = 0.094$; Figure 12).

Our results are like Smith et al. (2024) who found that adult bullfrogs in southern California were more likely to consume larger prey such as crayfish and vertebrates and that juveniles were more likely to consume smaller invertebrate prey. Crayfish and various orders of insects were the most prominent prey species found within the bullfrogs from one of their sites in San Diego County. Vertebrates only represented 4.9% of their total prey records (Smith et al. 2024), a small amount similar to our 1%.

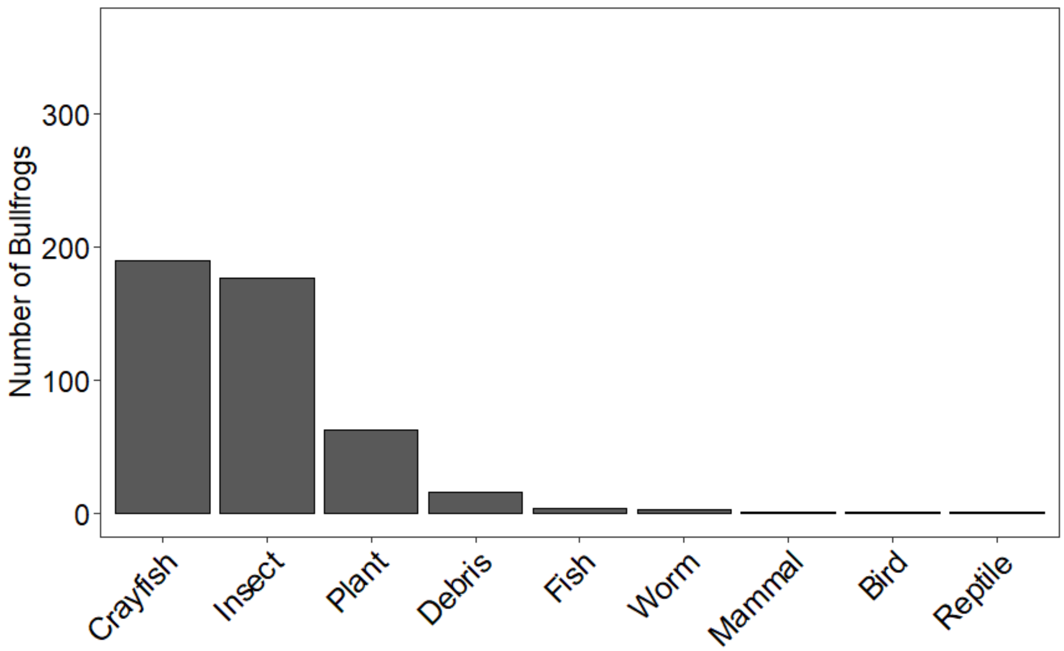

Figure 9: Prevalence of major prey groups found within the stomachs of 363 American bullfrogs collected from the Babocomari River in southeast Arizona during March–July 2024.

Figure 10: An adult (149 mm snout-vent length) male American bullfrog collected from the Babocomari River in southeastern Arizona on 11 April 2024 with a large crayfish (~110 mm) in its stomach. Photograph by Morgan McDowell, University of Arizona.

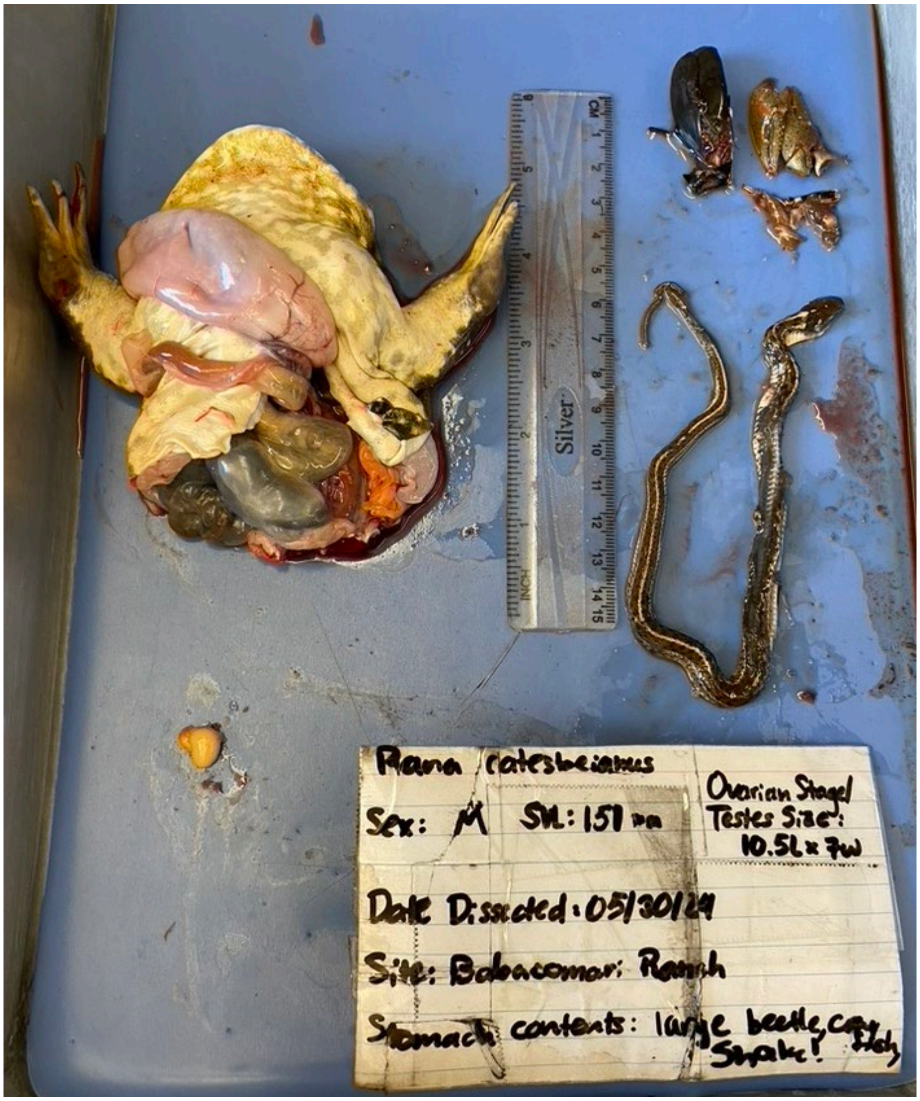

Figure 11: An adult (151 mm snout-vent length) male American bullfrog collected from the Babocomari River in southeastern Arizona on 28 May 2024 with a juvenile checkered garter snake (total length ~220 mm) in its stomach. Photograph by Morgan McDowell, University of Arizona.

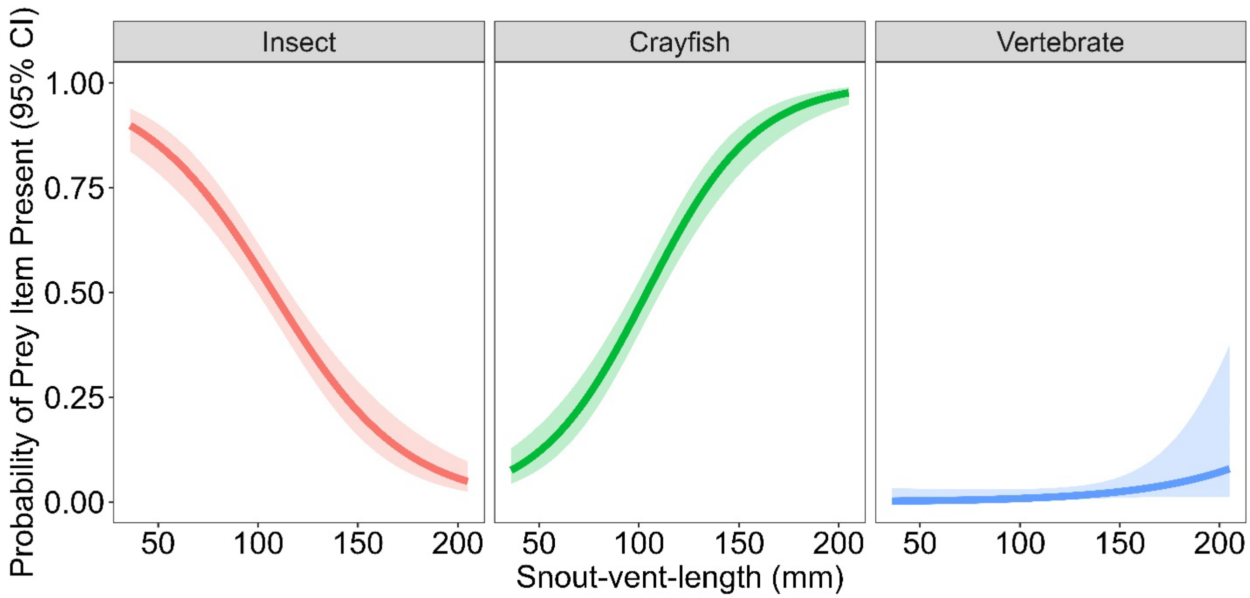

Figure 12: Predicted probabilities and 95% confidence intervals (CI) of the presence of three groups of prey items (insects, crayfish, and vertebrates) in the stomachs of dissected American bullfrog collected from the Babocomari River in southeastern Arizona during 2024 as a function of snout-vent-length.

Data Organization

We compiled and organized data collected during 2018-2023 from previous bullfrog field dissections by David Hall and Chris Prewitt (University of Arizona) from various sites throughout southern Arizona into a single spreadsheet. We recorded the results of all dissections of carcasses from the Babocomari River in a similar spreadsheet for ease of combination in the future. We photographed each carcass following dissection and stored these photos within a cloud folder with a unique picture ID that was also recorded in the spreadsheet for easy reference.

Future Plans

We plan to commence a second field season beginning 27 May 2025 to collect additional data on bullfrog dispersal and movement excluding a telemetry effort. We plan to use the same study area and survey procedure as we used in 2024. In addition to cohort marking all captured bullfrogs with toe-clipping, we plan to mark all captured individuals with a passive integrated transponder (PIT) tag to identify unique individuals. This will improve our ability to track dispersal among multiple sites. Because we occasionally detected bullfrogs at our surrounding sites but were unable to capture them to document the presence of a mark, we plan to begin lethally removing bullfrogs within our study area at the end of our 2025 field season. This will allow us to positively determine if a given individual was indeed marked.

We plan to continue to collect and dissect bullfrog carcasses from the Babocomari River beginning in March 2025. However, we expect very low numbers of adults due to the success of removing adult bullfrogs during 2024. The last adult bullfrog seen at the Babocomari River was during August 2024 (C. Prewitt, University of Arizona, personal communication). We plan to combine all dissection data from this study with those collected by David Hall and Chris Prewitt to examine regionwide patterns and variation in bullfrog reproductive biology and diet. We plan to present the results of this project during an anticipated thesis defense in May 2026.

Acknowledgements

We want to thank the many individuals who have made this project possible. Arizona Game and Fish Department has provided funding for this project through the Heritage Program, and we thank Tom Jones, Audrey Owens, Mason Ryan, Ian Latella, and Ryn Davison for their advice and support. Mason Ryan allowed us to use the GPS transmitters and Matt Goode provided some of the VHF transmitters. We particularly thank David Hall for his advice and for sharing his data. Chris Prewitt, Jace Lankow, Raul Vega and the 2024 crew of the University of Arizona Frog Project (Stephanie Austin, Harley Davis, Connor McNally, Samantha Crone, Soren Steinfeldt, Kaylee Wilt, and Hannah Youngwirth) have been tremendously supportive in helping to collect bullfrog carcasses from the Babocomari River and for providing advice on bullfrog survey and capture methods. We thank Ellen Williams for allowing us access to the Babacomari Ranch and permission to conduct bullfrog eradication surveys. We thank our 2024 technicians Rylee Reginald, Jeremiah Powers, and Morgan McDowell for all of their help collecting the data for this project and braving the chaos of field work, without them we would not have collected these data. Morgan, in particular, has been an incredibly dependable leader for collecting bullfrog carcasses and performing dissections. We also thank all the volunteers that came out to help with field work. We thank Scott Bonar for providing the lab space for dissections and lending field vehicles when in a pinch. We thank Ross Humphreys for supporting this research and granting us access to his land. We thank the members of the Alston and Bauder labs for their feedback and support during lab meetings. Lastly, I'd like to thank my advisor Javan Bauder for his endless support and guidance through all the obstacles of research. The University of Arizona's Graduate Student Research Grant program, the Audubon Society's Appleton-Whittell Research Ranch Fellowship and the Cecil Schwalbe Cold-Blooded Research Fund have all provided additional project funding. This work was conducted under permits from the U.S. Fish and Wildlife Service (TE02234C and ESPER0033846), Arizona Game and Fish Department (SP410389), and the University of Arizona Institutional Animal Care and Use Committee (2022-0871). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Appendix I

Visual Encounter Survey (VES) data sheet created by Arizona Game and Fish for Chiricahua leopard frog (RACH) and riparian herpetofauna surveys that are used for recording VES data for this project.

Date: ___ / ___ / ___
M M D D Y Y Y Y

RACH/Riparian Herpetofauna VES Datasheet

NUM: ___ - ___

Arizona Game and Fish Department

Last updated February 2020 version 2.5

SITE:		SITE AT: (Additional location info)	
Observer(s):	Affiliation:	Email:	Phone:
Time (24Hr) Start:	Stop:	Dry Site: ◯ Y ◯ N	

ST-CNTY: ___ - ___	UTM ZONE (Circle one)	NAD (Circle one)	*Lotic sites use both start and stop*	UTM EASTING	UTM NORTHING
	◯ 11	◯ 83	UTM Start:		
System Type:	◯ 12				
	◯ 13	◯ 27	UTM Stop:		

System Type: Lentic ◯ Lotic ◯

Water Source: ◯ Spring ◯ Runoff ◯ Well ◯ Unknown

Water Type: ◯ Riverine ◯ Wetland ◯ Earthen tank ◯ Reservoir ◯ Artificial

Surface Area (must be measured)
Lotic sites use width measurement
Long(m): Short(m):

VOUCHERS Habitat Photos: Y ◯ N ◯ Specimen Photos: Y ◯ N ◯
Specimen(s) collected: ◯ Y ◯ N
# of Disease/Genetic Samples: Water ___ Swab/Tissue ___

T_{Air}: ◯ °C ◯ °F T_{Water}: ◯ °C ◯ °F RH: %

Precipitation: (select one) ◯ None ◯ Intermittent ◯ Steady

Wind: ◯ Still ◯ Breezy ◯ Windy

Search Methods: ☐ Visual ☐ Seine ☐ Trap ☐ Dipnet ☐ Snorkel ☐ Boat ☐ Call Playback

Vegetation	%	Prominent Species (if known)
Floating		
Submerged		
Emergent		
Perimeter		

PREDATORS (Non-Herp)*
☐ Native fish ☐ Nonnative fish
☐ Crayfish ☐ Birds
☐ Aquatic invertebrates ☐ Mammals

Grazing Exclosure: ◯ Y ◯ N ◯ Partial

GRAZING ACTIVITY Livestock: ◯ Present ◯ Sign ◯ None Native Ungulate: ◯ Present ◯ Sign ◯ None

SITE/SURVEY NOTES: (*include any predator species from above)

RIPARIAN HERPETOFAUNA (select one life stage per line)

Species	Certainty		Life Stage				Total #	Comments (calling, breeding, behavior, etc)
	Uncertain ◯	Certain ◯	Egg ◯	Larvae ◯	Juvenile ◯	Adult ◯		
	Uncertain ◯	Certain ◯	Egg ◯	Larvae ◯	Juvenile ◯	Adult ◯		
	Uncertain ◯	Certain ◯	Egg ◯	Larvae ◯	Juvenile ◯	Adult ◯		
	Uncertain ◯	Certain ◯	Egg ◯	Larvae ◯	Juvenile ◯	Adult ◯		
	Uncertain ◯	Certain ◯	Egg ◯	Larvae ◯	Juvenile ◯	Adult ◯		
	Uncertain ◯	Certain ◯	Egg ◯	Larvae ◯	Juvenile ◯	Adult ◯		

Reset Bullets

CLEAR FORM

ENTERED BY: ____________ DATE: __/__/__

VERIFIED BY: ____________ DATE: __/__/__

Release (select one) ◯ Y ◯ N

Appendix II

Table of site names and locations of sites surveyed in the San Rafael Valley, Santa Cruz County cross referencing alternative names used by Arizona Game and Fish Department (AZGFD), Coronado National Forest (USFS), and U.S. Geological Survey (USGS).

Site Name (AZGFD)	Coronado NF Name	USGS Name	UTMN	UTME
A-Bar Draw Tank	A Bar Draw Tank	A Bar Draw Tank	3480156	544698
Arlene Tank			3469251	544974
Astragalus or Killder	Jeep Well	59	3477277	547278
Bear Scat		Dam	3481095	545771
Belly Ache Tank			3470833	547095
Big Boy Tank			3474072	542943
Bill Woods Tank	Bill Woods Tank	Bill Woods Tank	3475691	547655
Bobcat Tank			3475955	542522
Bodie Canyon Tank			3471104	549148
Boundary Tank			3469097	543431
Concrete Tank	Corenela Dam	Bad Dam	3479079	544897
Cow Bone	Ki-He-Kah Tank	Unnamed 20	3479746	542242
Dan Tank	Dan Tank	Dan Tank	3474779	544780
Double Reed Tank	Double Tanks	Double Tanks	3478543	544020
Dove Tank	Dove Tank	Dove	3476199	543962
Eddie Tank 2 or Eddie	Gertrudis Tank	Gertrudis Tank	3477480	544531
Faint Track Tank			3467625	544682
Gypsy Tank / Kelly Tank	Payne Tank	Payne Tank	3469691	549565
Heron Spring 2			3468051	540268
High Plains North	Border Tank		3483025	541604
Huachuca Tank	Huachuca Tank	Huachuca Tank	3468836	547112
Inez Tank	Inez Tank	Inez Tank	3471490	548469
Jack Tank	Jack Tank	Jack	3474080	546817
Jackpot Tank			3472079	542142
Jackrabbit Tank	Bar Tank		3481351	543541
Jeep Trail Tank	East Mesa Tank	East Mesa Tank	3467197	544860

Just North Tank	Canelo Tank		3483901	542185
Leslie Tank	Leslie Tank	Leslie	3476729	545439
FS799	Little Outfit Tank	Little Outfit Tank	3482866	540897
Lonely Mesquite			3468228	541826
Max Tank	Max Tank	Max Tank	3473471	543838
Missing Tank		60	3476811	547441
Mystery Tank	Upper Tank		3483085	542423
No Name Tank/ Hilltop Tank	Apache Tanks		3479173	543885
P48			3474019	540824
Parker Canyon #1 (North) / Parker 1N	Antelope Tank	Antelope Tank	3467143	543232
Parker Canyon #2 (South) / Parker 2N	Lower Antelope Tank	Lower Antelope	3466837	542790
Parker Canyon Stream			3471228	545558
Pasture 11 Tank East			3472997	543209
Pasture 15 Windmill Tank			3475774	541114
Pasture 16 Tank			3476976	540318
Pasture 22 Tank			3474405	541131
Pasture 9 Tank			3471366	542945
Pasture 9 Windmill Tank			3469976	541564
Rosemary Tank	Rosemary Tank	Rosemary	3472973	545002
Santa Cruz River South			3469158	538993
Section 17-18 / 17_18	Picnic Tank	Picnic Tank	3477686	543318
Skunk Tank			3477069	539086
Unnamed			3471230	541444
Upper 21 Windmill			3473535	541285
Dense Tank	Woodchopper Tank		3481703	542839