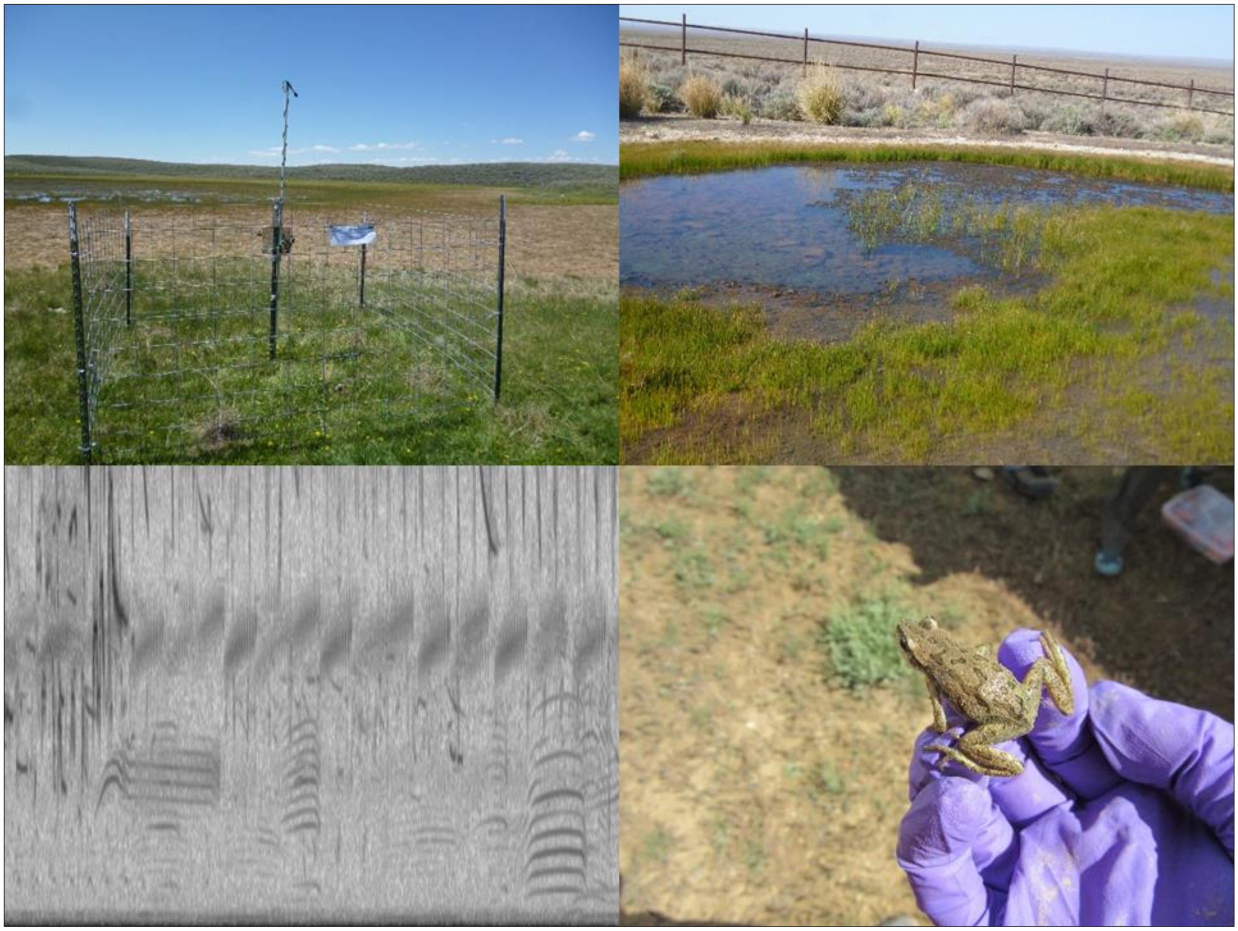
# Wyoming BLM Amphibian Survey Project



Final Report

December 30, 2021

Prepared for:

Bureau of Land Management Wyoming State Office

Assistance Agreement # L16AC0039

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Wyoming Natural Diversity Database

Recommended citation: *Jones, D., Z. Wallace, K. Cook, and I. Abernethy. 2021. Wyoming BLM Amphibian Survey Project: Final Report. Report prepared for Bureau of Land Management Wyoming State Office by University of Wyoming, Wyoming Natural Diversity Database.*

Cover photographs by Katrina Cook, Emily Schmidt, Ken Honeycutt, and Ryan Burns.

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## Abstract

Amphibians are experiencing rapid population declines and unprecedented extinction rates worldwide. In Wyoming, 75% of amphibians are classified as Species of Greatest Conservation Need by the Wyoming Game and Fish Department. Understanding the status of amphibian species in the state is limited by a lack of information on their distribution, especially in the arid desert and prairie basins of central and southwestern Wyoming. To address this gap in knowledge, we surveyed for amphibians at low-to-middle elevation sites in the Bureau of Land Management Rawlins, Rock Springs, Pinedale, and Casper Field Offices during 2019 and 2021. We consulted habitat models and existing data to prioritize areas with high likelihood of having multiple amphibian species, but where few or no surveys had previously been conducted. We then used a suite of methods to survey priority areas for amphibians, including visual encounter surveys, road-based nocturnal call route surveys, and passive acoustic monitoring with automated recording units. We conducted surveys at 73 sites and detected 6 species of amphibians: boreal chorus frog (*Pseudacris maculata*), northern leopard frog (*Lithobates pipiens*), Great Basin spadefoot (*Spea intermontana*), Plains spadefoot (*Spea bombifrons*), Rocky Mountain toad (*Anaxyrus woodhousii*), and tiger salamander (*Ambystoma mavortium*). Each of the survey methods generated detections of at least one unique species. To test the utility of automated recording units for multi-taxa inventories, we deployed ultrasonic microphones to record bat vocalizations concurrently with amphibian calls, resulting in detections of 7 species of bats. Finally, we developed recommendations for future use of passive acoustic monitoring for amphibians in Wyoming. We recommend that automated recording units be deployed for long-term monitoring at wetland sites where amphibians are known or strongly suspected to occur, and that they are best suited for sampling of explosive-breeding species with weather-dependent calling behavior.

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# Introduction

Amphibians are one of the most threatened classes of vertebrates both worldwide and in North America (Stuart et al. 2004, Miller et al. 2016). Despite historically being an understudied group, amphibians have received increasing attention in recent decades, and many of the factors driving their population declines and extinctions have become clearer. Chief among these is the rapid global spread of Chytridiomycosis, an emerging infectious fungal disease that has been well-documented as a cause of declines and extinctions of hundreds of frog species worldwide (Fisher and Garner 2020). Anthropogenic climate change is another major driver of amphibian declines, both as a direct result of higher temperatures (e.g. Cohen et al. 2018) and because increasing frequency and intensity of droughts threaten aquatic habitat in arid environments (Grant et al. 2016). Land use change and habitat degradation have also been linked to amphibian declines for many species (Hof et al. 2011). The threat of climate change, land use change, and aquatic habitat degradation is particularly acute in desert and prairie biomes across western North America, where surface water and amphibian habitat are spatially isolated and sensitive to forces such as regional drought and livestock grazing (National Park Service 2015).

In Wyoming, 9 of the 12 regularly-occurring amphibians are classified as Species of Greatest Conservation Need (SGCN) by the Wyoming Game and Fish Department (WGFD 2019). Although several species are believed to have declined in Wyoming, many of these conservation designations are due partially or wholly to a lack of data on species' distributions and long-term population trends in the state (Estes-Zumpf et al. 2017). Beginning in the early 2010s, the Wyoming Natural Diversity Database (WYNDD) and the Wyoming Game and Fish Department (WGFD) initiated a number of herpetological inventory projects to improve understanding of the status and distribution of amphibians in the grassland and shrubland habitats of the major low-elevation basins of Wyoming. To date, WYNDD has conducted surveys in the Powder River Basin (Estes-Zumpf and Keinath 2012) and Bureau of Land Management (BLM) lands in the Kemmerer and Rawlins Field Offices (Estes-Zumpf et al 2017), and the WGFD has conducted surveys in southwestern (Snoberger and Walker 2012a, 2012b), and central Wyoming (Lange and Estes-Zumpf 2016). Concurrently, BLM biologists in central and western Wyoming have conducted inventory and monitoring efforts to satisfy regional information needs and management objectives in the Rawlins Field Office (Bridger 2017, Bridger 2018, Bridger 2019), Rock Springs Field Office (P. Lionberger, pers. comm.) and Pinedale Field Office (D. Woolwine, pers. comm.). Collectively, these surveys have significantly improved our understanding of amphibian distribution in desert and prairie areas of Wyoming.

Despite this body of research, there remain large areas of public land in the state where few or no dedicated field surveys for amphibians have been conducted, especially in lower-elevation basins of central and southwestern Wyoming. Additionally, although large-scale inventory projects have been instrumental in shedding light on the ranges and habitat requirements for different species of amphibians, there is increasing interest in establishing long-term monitoring projects capable of detecting population changes over time. Because Wyoming is a vast state and wetland habitat in the basins is patchily distributed, amphibian monitoring projects must balance the need to collect as much data as possible with the logistical challenges of finding habitat and successfully detecting species at sites on a single visit. It is therefore critical that researchers and land managers use the most efficient methods available when designing surveys for different species of amphibians in Wyoming.

Autonomous Recording Units (ARUs) are a relatively new technology with significant applications for amphibian research and monitoring (Brauer et al. 2016). Because many species of frogs and toads emit diagnostic vocalizations during the breeding season, auditory surveys have long been a popular and effective method in amphibian monitoring (Heyer et al. 1994). Passive acoustic monitoring for amphibians is an increasingly feasible alternative method with the advent of ARUs that are small enough to be deployed in remote field settings, rugged enough to withstand the elements, and have long enough battery life and large enough memory space to record nightly for several weeks (Cameron 2019). ARUs also have the potential to increase efficiency of monitoring efforts by sampling multiple taxa simultaneously, including amphibians, bats, and birds (Levandowski et al. 2021). To explore the potential applications of passive acoustic monitoring for amphibians in Wyoming, the BLM provided funding to the WGFD to develop protocols and best-practices for deploying ARUs and analyzing recordings.

In 2017, WYNDD was awarded a grant by the BLM to conduct two years of amphibian inventory and monitoring in wetland and riparian habitats of central and southwestern Wyoming. Our goal was to inventory amphibian species in this area using multiple sampling techniques, including the ARU protocols developed by WGFD. The project's first season of field work was carried out in 2019. The second season of field work took place in 2021, after a one-year hiatus due to the COVID-19 pandemic. The objectives of this project were:

1. Identify and prioritize areas in the Rawlins, Rock Springs, Pinedale, and Casper BLM Field Office regions with potential amphibian habitat that had not previously been surveyed for amphibians.
2. Conduct field surveys for all amphibian species possible in each survey area to generate additional information on their occurrence, range, and distribution.
3. Compare results of visual encounter surveys, nocturnal call surveys, and ARU surveys to inform recommendations on the most efficient methods for future amphibian monitoring.
4. Test the ARU deployment and analysis protocols developed by WGFD and make practical recommendations for future use of passive acoustic monitoring for amphibians in Wyoming.

# Methods

### *Study Area and Site Selection*

The study area for this project was delineated by the boundaries of the Rawlins, Rock Springs, Pinedale, and Casper BLM Field Offices (Figure 1). Surveys were conducted on BLM and State of Wyoming lands at low to middle elevations (5000–9000 ft, 1524–2734 m), including some areas east of the Continental Divide, most of the Red Desert and Great Divide Basin, and the upper Green River Basin. Dominant terrestrial habitat and vegetation types in the study area included grassland, desert shrubland, sagebrush steppe, and foothill shrubland. Amphibian habitat was present throughout the region in the form of permanent lakes and ponds, riparian wetlands, perennial and intermittent streams, springs, stock ponds, playas, and human-made wetlands.

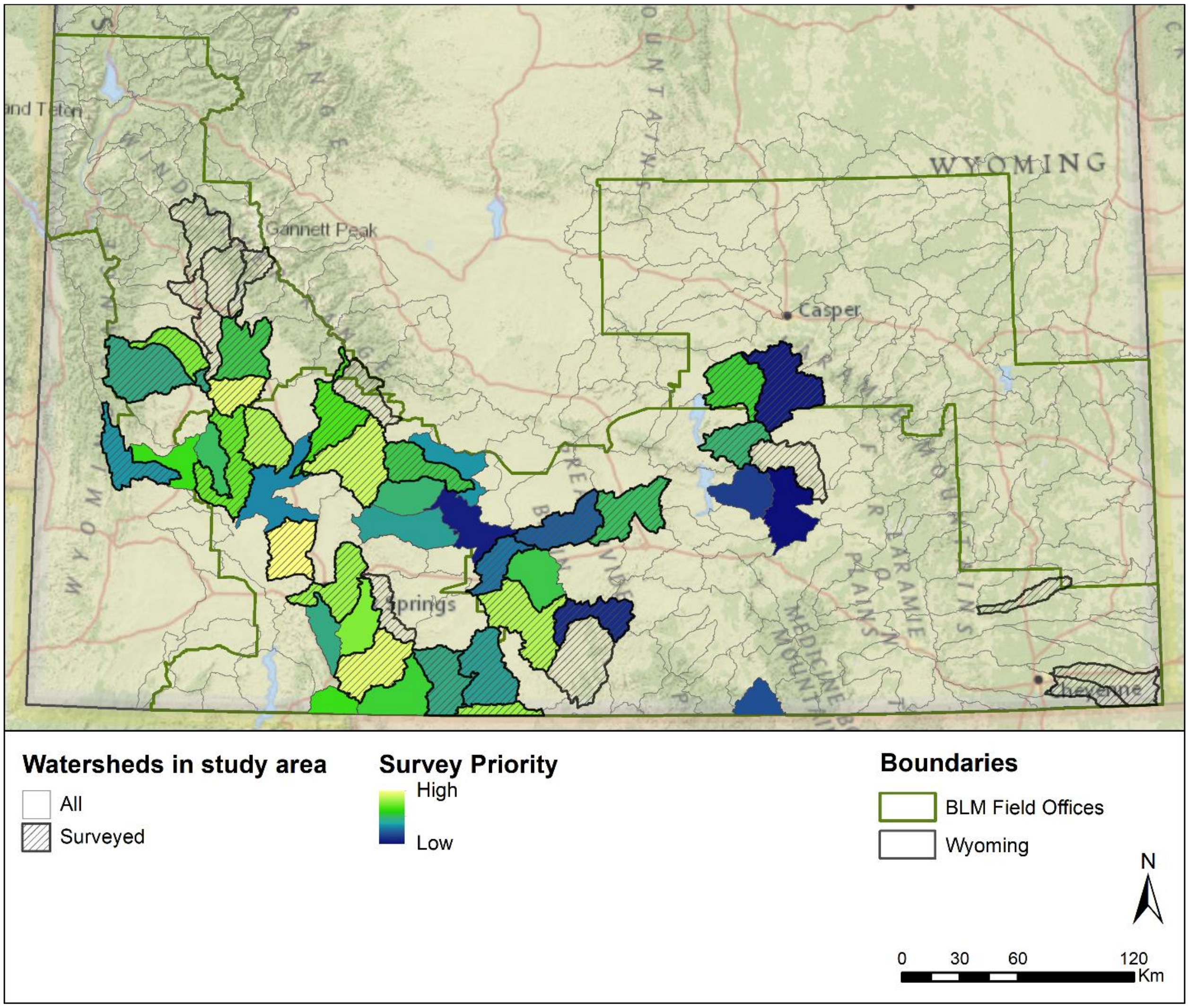


Figure 1. Study area, with target watersheds and watersheds surveyed. Map shows HUCs ranked using predicted species diversity and number of previous surveys (yellow indicates higher priority, blue indicates lower priority), survey status of watersheds (heavy black outlining and stippling indicates surveyed), and boundaries of BLM Field Office in the study area (green lines).

We selected survey sites using a multi-step process to prioritize areas predicted to have high habitat value for amphibians, but where few or no surveys had previously been conducted. The overall goal of the site selection process was not to provide a probabilistic sample of the study area, but to use a combination of data, models, and expert opinion to guide surveyors to areas most likely to fill gaps in existing amphibian data, while also addressing regional information needs of BLM. Our approach was based on a method developed by Estes-Zumpf et al. (2017). First, we identified areas with little or no previous survey effort by ranking the 10-digit Hydrologic Unit Code watersheds (HUCs) in the study area from low to high based on values of the Shannon Species Diversity Index, number of previous negative surveys, and number of species recorded. We then consulted BLM biologists to select from this ranking a set of target HUCs that had little previous survey effort, and also addressed regional information needs. To identify survey sites within HUCs, we selected 10 Public Land Survey System sections (approximately 1-mi$^2$ blocks) with a high probability of having multiple amphibian species on public land. To do this, we overlaid distribution models for amphibians (Keinath et al. 2010) and calculated the mean probability of occurrence across all species in each section. We then selected the 10 sections from each HUC with the highest mean amphibian habitat values on public land. To improve the representativeness of sampling and encourage surveyors to visit diverse areas, we generated a random spatially balanced survey order for the target sections selected in each HUC using the Halton iterative partitioning algorithm. Before conducting field surveys, we evaluated the target sections in their random sample order by reviewing satellite imagery and topographic maps. If the randomly selected sections were inaccessible or did not have potential amphibian habitat, we chose other sites in the HUC based on satellite imagery. We also consulted BLM biologists at this stage to identify any specific sites of interest. In addition to the sites selected using this process, we conducted opportunistic surveys at other sites with suitable habitat identified when traveling between pre-selected survey locations.

*Focal Species*

Of the 12 amphibians that regularly occur in Wyoming, 7 were considered focal species for this project based on their likelihood of occurring in the study area. Boreal chorus frog (*Pseudacris maculata*) is the most widespread and abundant amphibian in Wyoming, occurring in a variety of wetland habitats at almost all elevations (RMAP 2017). During the breeding season, adults often call continuously and can be heard from long distances. Northern leopard frog (*Lithobates pipiens*; SGCN Tier II, NSS4) is also widespread, although it is more common in foothills areas than lower-elevation deserts and prairies (WGFD 2017). Adults vocalize less frequently and more quietly than boreal chorus frogs. Great Basin spadefoots (*Spea intermontana*; SGCN Tier II, NSS4) and Plains spadefoots (*Spea bombifrons*; SGCN Tier II, NSS4) are low-elevation, arid environment specialists that occur west and east, respectively, of the Continental Divide. They are explosive breeders that use temporary playas, puddles, and pools formed after heavy rains. Their calls are loud, but calling activity is highly weather-dependent (Kadi 2012). Rocky Mountain toad (*Anaxyrus woodhousii*) also occurs at lower elevations, but tends to occupy more permanent wetland habitats and calls more frequently and continuously during the breeding season. It is most common in eastern Wyoming (RMAP 2017). Great Plains Toad (*Anaxyrus cognatus*; SGCN Tier II, NSSU) occurs only in eastern Wyoming, where it pulse-breeds in temporary or seasonal wetlands in low elevation grassland habitats after spring and early summer rains (Axley 1999). Tiger salamander (*Ambystoma mavortium*; SGNC Tier III, NSS4) is Wyoming’s only salamander, and is widespread in a variety of habitats at almost all elevations in the state (RMAP 2017). Unlike Wyoming’s anuran amphibians, tiger salamanders do not vocalize.

*Nocturnal Call Surveys*

We conducted nocturnal call surveys (NCS) along roads traversing areas of likely amphibian habitat following the protocol developed by the WGFD (Snoberger and Walker 2012). We prioritized sites for NCS where public roads ran parallel to streams or other suitable habitat on private land that would not have been possible to survey using an ARU or visual encounter survey (VES). Surveys were conducted on warm, humid nights, ideally within 2 days of wetting rainfall events. Technicians drove prospective routes in the daylight to scout road conditions and potential amphibian habitat.

Surveys started at least 30 minutes after sunset. Depending on the length of the route, stops were spaced 0.3 or 0.5 miles apart. At the beginning and end of the survey, we collected environmental data including elevation, barometric pressure, temperature, wind speed, and time since last rain. Each stop consisted of a three minute count divided into 3, 1-minute intervals. Two observers independently recorded all amphibian species detected during each of the intervals, estimated the distance and direction to each species group, and recorded the calling intensity and ambient noise level. We calculated simple detection probabilities for each species as the proportion of observers that detected a species, averaged across all sites where the species was detected by at least one observer.

*Visual Encounter Surveys*

We conducted VES in areas of potential amphibian habitat following the dual-observer protocol developed by the Rocky Mountain Amphibian Project (Estes-Zumpf et al. 2014). Visual encounter surveys were conducted at smaller wetland sites with limited potential habitat and areas where longer distance from the nearest road precluded ARU deployment.

Two observers conducted separate surveys at each site. Starting at the same point, observers divided the site in half and surveyed independently for amphibians on each side, focusing on water edges and emergent vegetation for adult amphibians. Observers used dipnets to sample for amphibian larvae at 5–10-m intervals. After surveying their half of the site, observers paused to record a variety of environmental data before surveying the other half. When adult amphibians were captured, they were swabbed for chytrid fungus following a protocol adapted from Livo (2003).

*Incidental Observations*

We recorded incidental observations for all amphibians and reptiles encountered while in the field that were not recorded during formal surveys.

*Automated Recording Units*

Automated recording units (ARUs) were deployed following the protocol developed by the Wyoming Game and Fish Department (Bergman and Honeycutt 2021). We prioritized sites for ARU deployments at large and complex wetlands where VES were unlikely to detect all amphibians, and at locations lacking surface water at the first visit, but where we believed future precipitation might create suitable amphibian breeding conditions.

We used several different ARU models for this project (Wildlife Acoustics Song Meter SM4, SM2+, and SM1). ARUs were programmed to record for 5 minutes every hour starting at approximately 2000 hours and continuing throughout the night. Gain and duty settings were selected based on the WGFD manual (Bergman and Honeycutt 2021) and varied between ARU models (Appendices 1 and 2). ARUs were deployed on relatively flat, solid ground above the high-water line and as close as possible to the best habitat at each site. We mounted ARUs on steel t-posts and constructed temporary, fenced exclosures using t-posts and hog panels to exclude wildlife, livestock, and feral horses that might damage recorders (Figure 2).

At locations where SM2+ units were deployed, we took advantage of this model's separate gain and duty settings for each channel to record bat calls using an ultrasonic microphone (Wildlife Acoustics SMX-U1) on one channel. To reduce ultrasonic echoes, we mounted these microphones on an aluminum pole at least six feet above the ARU, pointing downward in the direction of the surface water.

Figure 2. ARU deployment with fenced exclosure, SongMeter2 recording unit, and ultrasonic microphone.

*Analysis of Acoustic and Ultrasonic Recordings*

Acoustic recordings were stored and analyzed in RFCx Arbimon, a free, cloud-based platform for bioacoustic data (Rainforest Connection 2021). We uploaded our recordings to the Wyoming Amphibians project developed by the WGFD and used the recognizer models developed by WGFD to classify the presence or absence of vocalizations of different amphibian species in each 1-minute recording segment. To reduce analysis time, we ran the recognizer model for each species on recordings only from sites where we judged it was plausible for the species to occur based on ranges and habitat associations (Appendix 3). For species with multiple recognizer models in the Wyoming Amphibians project, we used the top performing model for each species in each region as identified by WGFD (Honeycutt and Bergman 2021). For species with a small number of recordings, we used multiple models to increase the chances of finding a positive detection.

To determine occupancy of amphibian species at each site with the minimum amount of manual review, we began by raising the threshold for each model to achieve as close to 0.95 precision as possible, using the combined Random Forest and threshold model option in Arbimon. This generated predicted presences only for those recordings with the strongest model support, which we then manually validated to determine occupancy. If a site had no validated positive detections at the highest threshold, we lowered the threshold to ~0.75 precision and again reviewed the recordings classified as predicted present. We continued to incrementally lower the model threshold for each site until a positive detection was found or we arrived at the original optimal threshold identified by WGFD (Honeycutt and Bergman 2021). We did not review predicted presences for thresholds below the baseline for each model because this produced a prohibitively large number of recordings to review, given the constraints of the project, most of which were false positives. We repeated this process for all species with existing recognizer models available in the Wyoming Amphibians Arbimon Project (models and thresholds used are presented in Appendix 4). As an index of the availability of species for detection, we calculated the number of days that elapsed between the deployment of the recorder and the first confirmed positive recording classified as present by the model.

For ultrasonic recordings, we first converted files from WAC to WAV format using the program Kaleidoscope (Version 5.1.9i). During conversion, recordings were split into files with a maximum duration of three seconds and noise files were filtered out (i.e., those with ultrasonic noises that triggered a recording but were not made by a bat). Call files containing bat vocalizations were analyzed using SonoBat North America (Version 4.2.2) and Region Pack WY[c20171124] Western Wyoming. We used the SonoBatch automated call analysis algorithm and set the acceptable call quality to 0.70 and the discriminate probability threshold to 0.90. Call characteristics for some bat species have a high degree of overlap and a non-zero probability of misidentification exists; to reduce the risk of misidentification, we used the appropriate species identification algorithms for species assemblages expected to occur within the study area. We also manually verified species presence at each site by visual inspection of call files assigned to each species to ensure at least one call file with diagnostic characteristics was recorded at that site. Recordings deemed unreliable for species identification were removed from species tallies at that site.

# Results

*Nocturnal Call Surveys*

We conducted 17 nocturnal call route surveys, including 13 surveys between 2 May and 10 July 2019 and 4 surveys between 6 May and 17 June 2021. Survey routes had an average of 11.7 stops (range: 4–20 stops) and lasted 99 minutes (range: 52–175 min).

We detected an average of 0.9 species per route, including 5 routes (29.4%) with 2 species, 6 routes (35.3%) with 1 species, and 6 routes (35.3%) with no detections. Boreal chorus frog was the most common species, detected at 64 points (32.1%) on 11 routes (64.7%), followed by Great Basin spadefoot at 29 points (14.6%) on 4 routes (23.5%), and Rocky Mountain toad at 5 points (2.5%) on 1 route (5.9%; Figure 3). Across all routes, 105 points (52.8%) had no detections of any amphibian species.

The average estimated detection distance was greatest for Rocky Mountain toad, shortest for boreal chorus frog, and all three species were detected at the maximum distance cut-off of 1 mile (1609 m; Table 1). The simple detection probability was high (≥ 0.95) for all species, indicating there were very few points where one observer failed to detect a species detected by the other observer.

Table 1. Number of survey points with observations, average estimated distance (with range), and simple detection probability (with standard deviation) for amphibian species detected during nocturnal call surveys in Wyoming, 2019 and 2021.

| **Species** | **Number of points with observations** | **Estimated distance (m)** | **Simple detection probability** |
|---|---|---|---|
| Boreal chorus frog | 64 | 419 (20–1609) | 0.977 (0.107) |
| Great Basin spadefoot | 29 | 663 (75-1609) | 0.948 (0.155) |
| Rocky Mountain toad | 5 | 800 (50-1609) | 1.000 (0.000) |

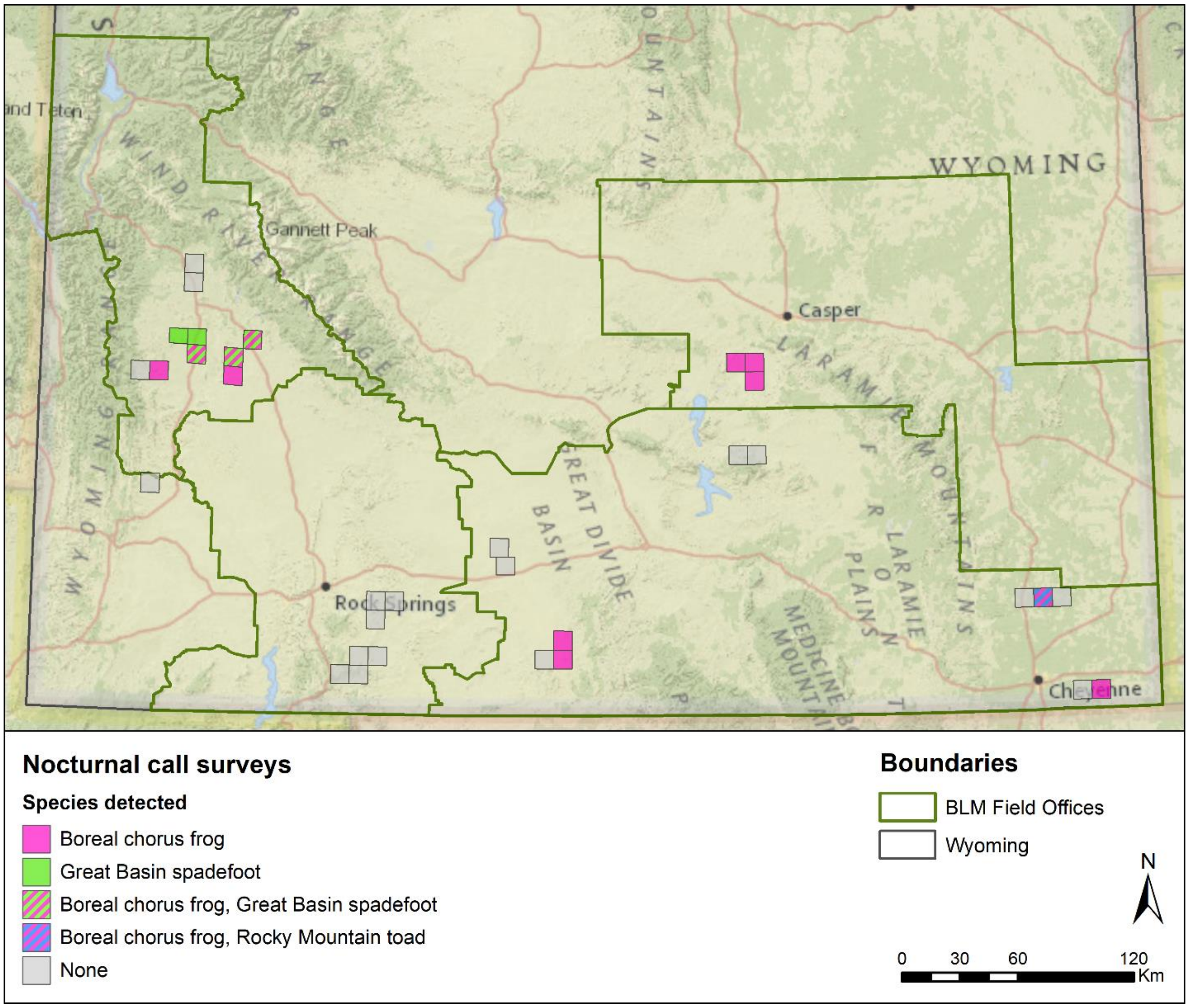


Figure 3. Nocturnal call routes surveyed for amphibians in Wyoming, 2019 and 2021. Map shows locations of surveys (17 routes) and detections of amphibian species within Public Land Survey System townships.

*Visual Encounter Surveys*

We conducted 22 visual encounter surveys, including 10 surveys between 3 June and 11 July 2019 and 12 surveys between 6 May and 18 June 2021. Surveys lasted an average of 76 minutes (range: 15–161 min). We detected boreal chorus frogs at 6 survey locations (27%), tiger salamanders at 4 locations (19%), Great Basin spadefoots at 2 locations (9%), and northern leopard frogs at 2 locations (9%; Table 2 and Figure 4).

Table 2. Numbers of survey locations and detections of amphibian and reptile species by age class from visual encounter surveys in Wyoming, 2019 and 2021. Tadpole numbers are estimates based on the average group size.

| Species | Survey Location | Age Class | | |
|---|---|---|---|---|
| | | Adult | Metamorph | Tadpole |
| Boreal Chorus Frog | 6 | 38 | 0 | 75 |
| Great Basin Spadefoot | 2 | 1 | 0 | 88 |
| Northern Leopard Frog | 2 | 3 | 0 | 0 |
| Tiger Salamander | 4 | 53 | 22 | 0 |
| Unknown amphibian | 1 | 0 | 0 | 75 |
| Wandering Garter Snake | 1 | 3 | NA | NA |
| Short-horned Lizard | 1 | 1 | NA | NA |

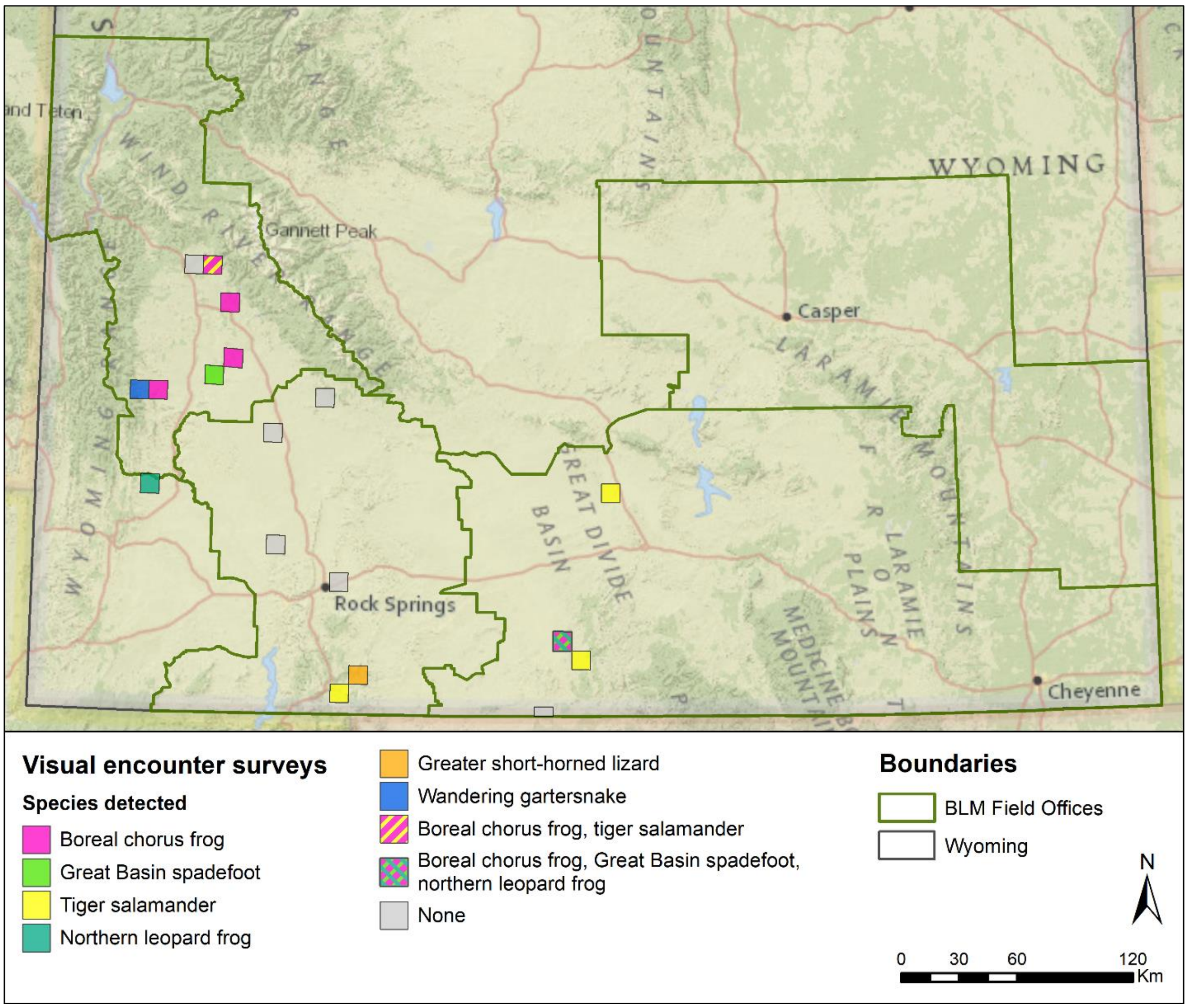


Figure 4. Visual encounter surveys for amphibians and reptiles in Wyoming, 2019 and 2021. Map shows locations of surveys (22 sites) and detections of species within Public Land Survey System townships.

*Incidental Observations*

We collected 17 incidental observations of 6 amphibian and reptile species in 2019 and 2021 (Figure 5). There were 5 observations of greater short-horned lizard (*Phrynosoma hernandesi*), 3 observations each of boreal chorus frog, Great Basin spadefoot, and bull snake (*Pituophis catenifer sayi*), 2 observations of prairie rattlesnake (*Crotalus viridis*), and 1 observation of northern sagebrush lizard (*Sceloporus graciosus graciosus*).

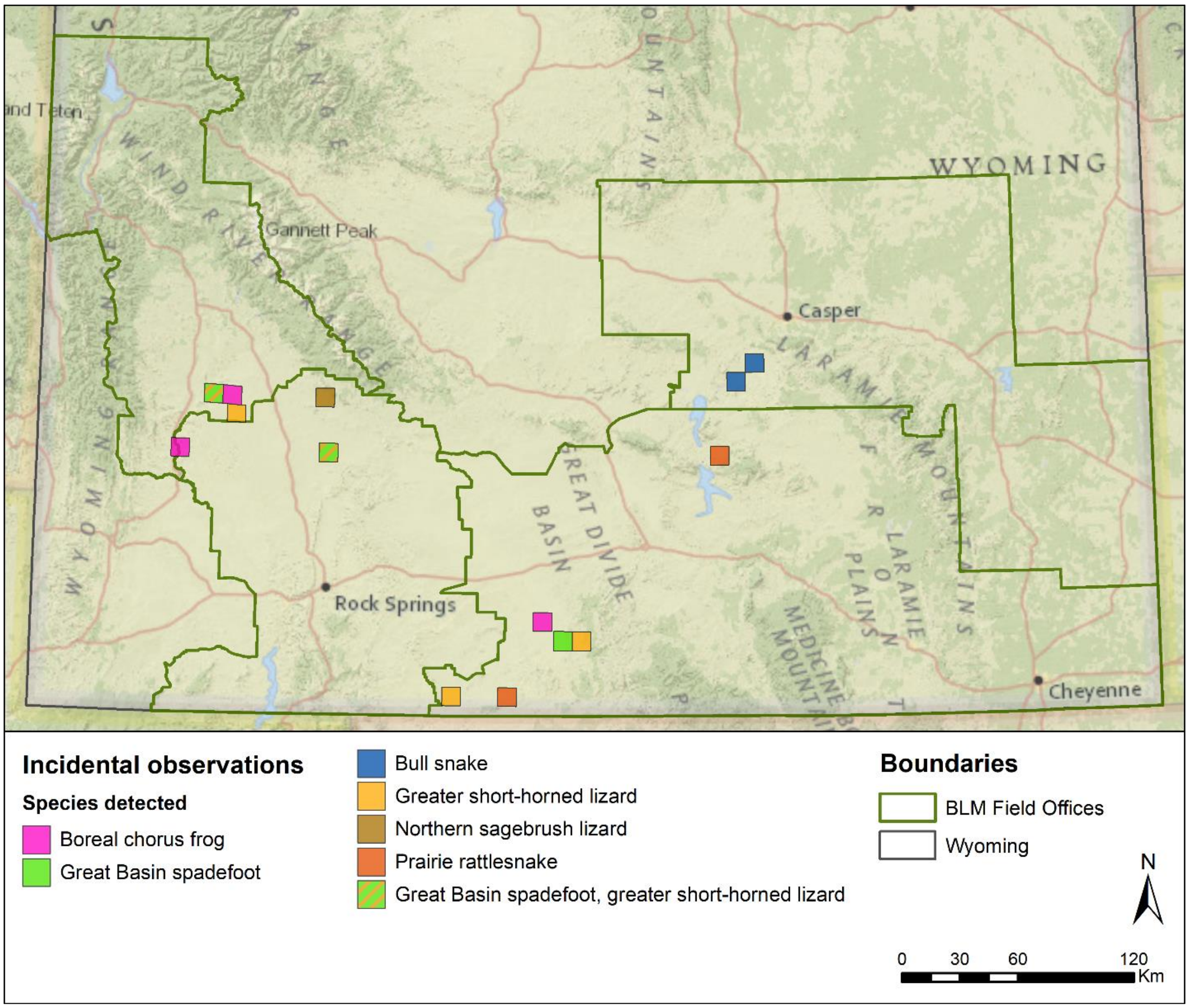


Figure 5. Incidental observations of amphibians and reptiles in Wyoming, 2019 and 2021. Map shows locations of observations by species within Public Land Survey System townships.

*Automated Recording Units*

We deployed 34 ARUs, including 16 in 2019 and 18 in 2021. ARUs were deployed between 7 May and 18 July 2019 and between 5 May and 25 June 2021. Deployments lasted an average of 28 nights (range: 2–43 nights).

Recognizer models were available for 6 species of amphibians occurring in our study area. For each species and site, we analyzed an average of 1103 (SD: 82.5) 1-minute recordings and manually reviewed an average of 122 (SD: 93) recordings (Appendix 3). This resulted in detections of boreal chorus frogs at 16 sites (47% of all sites), Great Basin spadefoots at 9 sites (26%), and plains spadefoots at 1 site (3%; Table 3 and Figure 6). We did not detect Great Plains toads, northern leopard frogs, or Rocky Mountain toads on any recordings classified as predicted positive, given the baseline thresholds of the models.

Table 3. Number of sites surveyed and species detected with passive acoustic monitoring in Wyoming, 2019 and 2021.

| **Species** | **Number of sites** | | |
|---|---|---|---|
| | **Detection** | **No detection** | **Total** |
| Boreal chorus frog | 15 | 22 | 37 |
| Great Basin spadefoot | 9 | 20 | 29 |
| Plains spadefoot | 1 | 3 | 4 |
| Great Plains toad | 0 | 5 | 5 |
| Rocky Mountain toad | 0 | 8 | 8 |
| Northern leopard frog | 0 | 35 | 35 |

At sites with detections, confirmation of species' presence required manual review of only a small number of recordings and the majority of review time was dedicated to sites where we did not confirm presence (Table 4). At sites where they were detected, the first predicted positive recording confirmed presence for boreal chorus frog (15 sites) and plains spadefoot (1 site), and we reviewed an average of only 8.9 recordings per site to confirm presence of Great Basin spadefoot (9 sites). By contrast, at sites where we did not successfully confirm species' presence, we manually reviewed hundreds of recordings that were classified as predicted positive by the model.

Table 4. Number of recordings manually reviewed per amphibian species at sites with detections and no detections from passive acoustic sampling in Wyoming, 2019 and 2021. Table shows for each species the mean, minimum, and maximum number of recordings manually reviewed per site, and the total across sites.

| **Species** | **Detection** | | | | **No detection** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **Min** | **Max** | **Total** | **Mean** | **Min** | **Max** | **Total** |
| Boreal chorus frog | 1 | 1 | 1 | 15 | 23.18 | 0 | 163 | 510 |
| Great Basin spadefoot | 8.89 | 1 | 36 | 80 | 214.90 | 29 | 1011 | 4298 |
| Plains spadefoot | 1.00 | 1 | 1 | 1 | 346.00 | 160 | 558 | 1038 |
| Great Plains toad | 0 | 0 | 0 | 0 | 73.60 | 0 | 307 | 368 |
| Rocky Mountain toad | 0 | 0 | 0 | 0 | 22.13 | 1 | 113 | 177 |
| Northern leopard frog | 0 | 0 | 0 | 0 | 212.37 | 0 | 881 | 7433 |

The number of days that elapsed between the deployment of recorders and the first confirmed positive detection from the model suggested differences among species in their availability for detection. Average time until detection was 0.33 days for boreal chorus frog (range: 0–1, 15 sites), 4.33 days for Great Basin spadefoot (range: 0–11, 9 sites), and 12 days for Plains spadefoot (1 site).

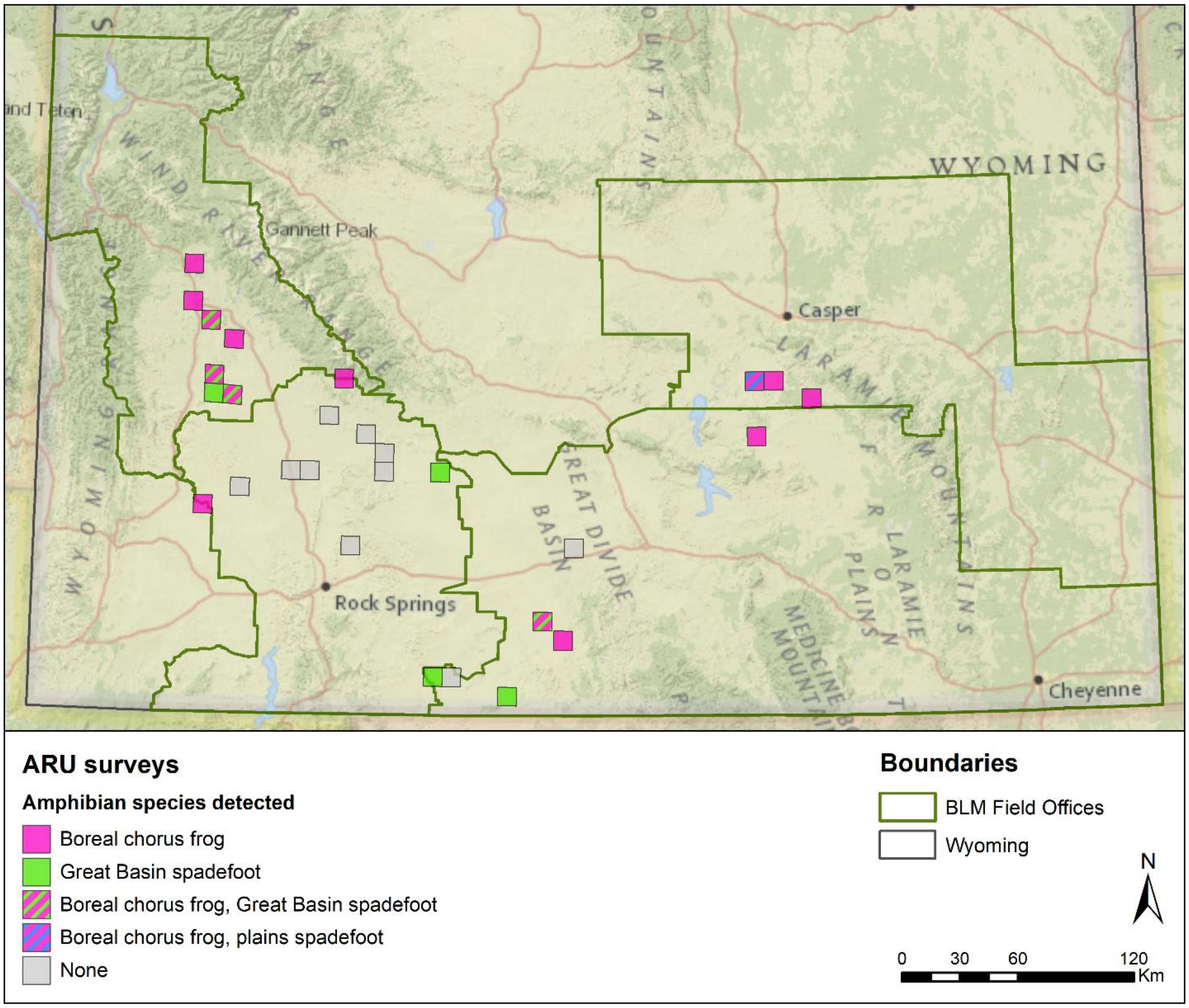


Figure 6. Locations of amphibian surveys using Automated Recording Units (ARUs) in Wyoming, 2019 and 2021. Map shows locations of surveys (34 sites) and detections by species within Public Land Survey System townships.

Recordings from ultrasonic microphones yielded detections of 7 species of bats (Table 5). Big brown bat (*Eptesicus fuscus)* was detected at 5 sites (15%), hoary bat (*Lasiurus cinereus*) at 9 sites (26%), silver-haired bat (*Lasionycteris noctivagans*) at 19 sites (56%), Western small-footed Myotis (*Myotis ciliolabrum*) at 15 sites (44%), long-eared Myotis (*M. septentrionalis*) at 8 sites (24%), little brown bat (*M. lucifugus*) at 14 sites (41%), and long-legged Myotis (*M. volans*) at 6 sites (18%). Through visual assessment, we determined that recordings of three additional bat species did not contain diagnostic features required to confirm presence. The Sonobat algorithms classified a total of 12 calls across three sites as California Myotis (*Myotis californicus*), five calls at two sites as spotted bat (*Euderma maculatum*), and one call at one site as fringed Myotis (*Myotis thysanodes*). These species are known to occur in southwest Wyoming, but visual assessment of all call sequences classified to these species did not provide sufficient evidence that they actually occurred at our study sites.

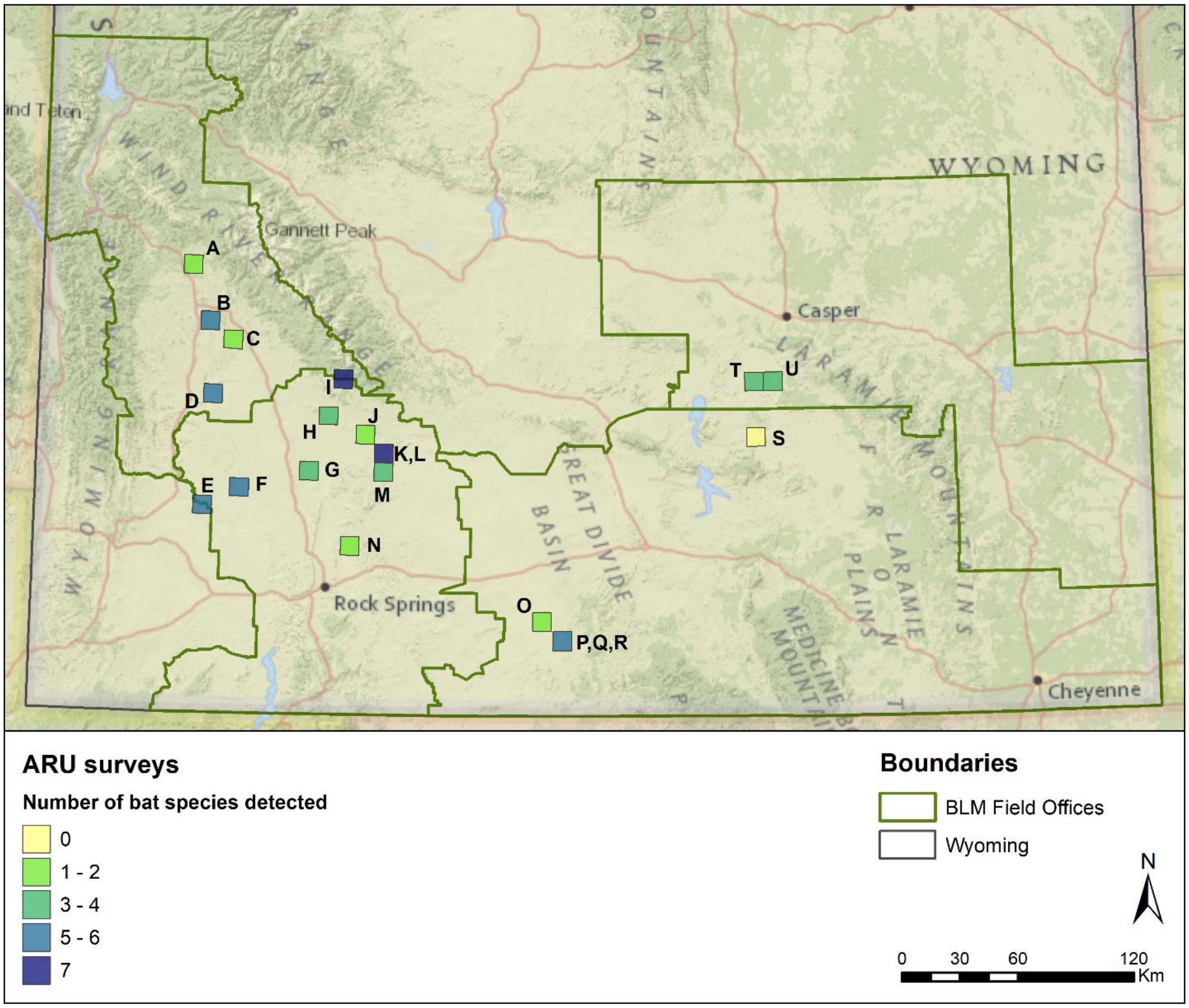


Figure 7. Locations of bats surveys using Automated Recording Units (ARU) in Wyoming, 2019 and 2021. Map shows locations of surveys (21 sites) and number of species detected within Public Land Survey System townships. Site labels correspond to Table 1, which includes counts by species.

Table 5. Number of bat vocalizations detected using Automated Recording Units with ultrasonic microphones in Wyoming, 2019 and 2021. Site labels correspond to map in Figure 7.

| Site | Species | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Big brown bat** | **Hoary bat** | **Silver-haired bat** | **Western small-footed Myotis** | **Long-eared Myotis** | **Little brown bat** | **Long-legged Myotis** |
| A | 0 | 0 | 13 | 0 | 0 | 2 | 0 |
| B | 0 | 1 | 13 | 1 | 6 | 27 | 0 |
| C | 0 | 0 | 6 | 0 | 0 | 0 | 0 |
| D | 1 | 0 | 5 | 31 | 86 | 159 | 1 |
| E | 5 | 3 | 231 | 1 | 0 | 94 | 0 |
| F | 0 | 2 | 9 | 24 | 26 | 0 | 1 |
| G | 0 | 2 | 7 | 0 | 0 | 1 | 0 |
| H | 0 | 12 | 137 | 2 | 0 | 0 | 0 |
| I | 1 | 11 | 27 | 1 | 1 | 219 | 6 |
| J | 0 | 0 | 5 | 0 | 0 | 2 | 0 |
| K | 1 | 0 | 3 | 23 | 7 | 39 | 14 |
| L | 0 | 2 | 37 | 1 | 2 | 3 | 0 |
| M | 0 | 1 | 9 | 1 | 2 | 0 | 0 |
| N | 0 | 0 | 15 | 18 | 0 | 0 | 0 |
| O | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| P | 0 | 0 | 2 | 8 | 0 | 6 | 2 |
| Q | 0 | 0 | 2 | 511 | 14 | 56 | 3 |
| R | 0 | 0 | 2 | 9 | 0 | 2 | 0 |
| S | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | 5 | 0 | 57 | 0 | 43 | 0 |
| U | 4 | 0 | 1 | 13 | 0 | 2 | 0 |
| All sites | 12 | 39 | 527 | 701 | 134 | 655 | 27 |

# Discussion

Field surveys conducted as part of this project during 2019 and 2021 were successful in detecting the majority of amphibian species present in central and southwestern Wyoming. Using multiple survey techniques, we generated new occurence records for 6 amphibian and 2 reptile species in areas with few or no previous records. These data will be stored by WYNDD and will be available to inform planning and management decisions by BLM, future distribution modeling and range mapping by WYNDD, and shared with other interested parties. We also gained valuable information about the utility and efficiency of different amphibian survey methods, and developed practical recommendations for future field projects using ARUs for passive acoustic monitoring of amphibian populations.

All amphibians were found in expected habitat types, generally within the boundaries of their known ranges. The sites where we detected Great Basin spadefoots in the Pinedale Field Office expanded the northern edge of the species' known range in the Upper Green River Basin. We also detected Great Basin spadefoots at several isolated spring-fed wetlands in the vicinity of the Powder Rim and Adobe Town in the southern Rock Springs Field Office. In 2021, these spring-fed wetlands were some of the only sites with surface water in the Red Desert region, and they likely represent important habitat for spadefoots during drought conditions when playas and other rain-fed wetlands are unsuitable for breeding. The single ARU deployment site where we detected Plains spadefoots, in the Bates Hole area southwest of Casper, was near the westernmost limit of that species' range in Wyoming. Muddy Creek Wetlands in the Rawlins Field Office hosted the most diverse amphibian community of any site surveyed, with detections of boreal chorus frogs, northern leopard frogs, and Great Basin spadefoots, in addition to five species of bats. Due to its large size, relatively stable hydrologic conditions, and extensive emergent vegetation, Muddy Creek is likely one of the most species-rich wetlands in the Rawlins Field Office. Our detections of Rocky Mountain toads occurred east of the Laramie Range in an area with previous reports of that species. Unsurprisingly, we detected tiger salamanders and boreal chorus frogs at sites throughout the study area in a variety of wetland and habitat types, reflecting those species' broad ranges and generalist habitat preferences.

Amphibian habitat in the lower-elevation, arid environments that comprise most of the Rawlins, Rock Spring, Pinedale, and Casper BLM Field Office regions is patchily distributed and highly variable from year-to-year based on precipitation and water availability. During the 2019 field season, precipitation was generally average to above-average throughout the study area, but during 2021 precipitation was far below average and all of central and southwestern Wyoming was experiencing severe drought. The lack of precipitation during the spring and summer of 2021 led to a scarcity of surface water and available amphibian habitat throughout our study area, especially in the lower-elevation sections of the Great Divide, Bitter Creek, and Green River Basins. Many sections selected for surveys based on having a high probability of containing habitat for multiple amphibian species had no surface water even in early May, significantly limiting the number of surveys we were able to conduct. Likewise, the lack of rainfall during the 2021 field season meant that there were very few occasions on which the conditions were appropriate for nocturnal call route surveys. Consequently, because technicians spent less time on nocturnal call route surveys in 2021, we were able to deploy more ARUs during the project's second season. Due to the drought, we shifted some field effort away from target watersheds in the Rawlins and Rock Springs Field Offices and spent more time surveying in comparatively wetter areas in the Pinedale Field Office. Future surveys focused on inventorying amphibian distributions could maximize their return on effort by prioritizing surveys in years and areas where favorable precipitation patterns result in improved habitat conditions for amphibians. By contrast, studies focused on long-term monitoring should include sites and years representing a range of conditions to better understand fluctuations in amphibian populations over time.

*Comparison of Survey Methods*

One of the goals of this project was to compare the results of amphibian surveys conducted using multiple methods and comment on the appropriateness of those methods for different species and field situations. Although this study was not designed to test for differences between methods, we conducted surveys using more than one protocol at some sites, and observed patterns in species detections between survey methods. While no single survey methodology is appropriate for all species of amphibians, some are preferable for certain species, survey conditions, and objectives (Table 6).

Visual encounter surveys yielded detections of more species than any other method, despite being performed opportunistically and often at sites which were generally smaller and more isolated than those where ARUs were deployed. This was the only method that generated observations of northern leopard frogs, which have very quiet vocalizations, and tiger salamanders, which do not vocalize and therefore cannot be detected using nocturnal call surveys or passive acoustic monitoring. Visual encounter surveys also yielded the only reptile detections from this project, aside from opportunistic observations. In addition to detecting quiet and non-vocal amphibians, visual encounter surveys can provide information about population size (i.e., counts) and is the only method capable of detecting egg masses and tadpoles, which allows observers to document amphibian presence and reproduction even when adults are not actively calling.

Nocturnal call route surveys yielded detections of fewer species, but provided the only detections of Rocky Mountain toads. As mentioned above, conditions in 2021 limited the opportunities for nocturnal call route surveys, especially in southwestern portions of the study area. Nocturnal call routes allowed observers to survey wetlands on private property from nearby public roads, which was particularly beneficial where riparian areas, wetlands, and water sources were privately-owned but could be approached along routes with public easements. The maximum estimated distance to calling amphibians was ~1600 meters for Rocky Mountain toads, boreal chorus frogs, and Great Basin spadefoots, indicating that under good conditions, nocturnal call route surveys can be effective wherever public access exists within roughly one mile of potential amphibian habitat. The simple detection probability of 95% or higher for all three species suggests that the double-observer protocol used for this project was unnecessary; if there are amphibians calling at a stop, both observers are very likely to detect them. For amphibian species that reliably produce repeated and conspicuous vocalizations when calling, like boreal chorus frogs and Rocky Mountain toads, we recommend that future nocturnal call route surveys use a single-observer protocol to allow more routes to be covered. Nocturnal call routes can also be used for pulse-breeding species, like spadefoots and Great Plains toad; however, it is critical to conduct surveys after rain fall events, when these species are calling and therefore available to be detected.

Table 6. Conceptual comparison of amphibian survey techniques.

| Attribute | Survey technique | | |
|---|---|---|---|
| | Passive Acoustic (ARU) | Visual Encounter | Nocturnal Call Route |
| Relative effort per site | High | Medium | Low |
| Sampling duration | Long<br>(weeks to months) | Medium<br>(minutes to hours) | Short<br>(minutes) |
| Sampling extent | Small<br>(1 site per recording unit) | Medium<br>(several sites per day) | Large<br>(many sites per night) |
| Detects non-vocalizing species | No | Yes | No |
| Weather dependence | Low | Medium | High |
| Data types | Detection/non-detection; relative abundance (calling intensity) | Detection/non-detection; abundance; age-class; reproductive status; disease status | Detection/non-detection; relative abundance (calling intensity) |
| Characteristics of target species | Pulse-breeding species with loud calls | Non-vocal or quiet species active during daytime | Vocal species with loud, regular calls |
| Example target species | Spadefoots,<br>Great Plains toad | Northern leopard frog, tiger salamander | Boreal chorus frog, Rocky Mountain toad |

Passive acoustic monitoring using ARUs comprised the bulk of the field work and analysis effort of this project. This method yielded the only detection of plains spadefoots, along with multiple detections of boreal chorus frogs and Great Basin spadefoots. Our success detecting spadefoots highlights the key advantage of the greater sampling duration with ARUs to increase the likelihood of capturing suitable weather conditions for activity of explosive-breeding species. This was confirmed by the greater number of days that elapsed between the deployment of recorders and the first confirmed positive detections for both spadefoot species, compared to boreal chorus frog. Additionally, by deploying ultrasonic microphones with units that had the capability to record under separate settings on each channel, we were able to survey for bats at a subset of locations and generated new occurrence records for 7 species of bats at little additional cost. Despite these advantages, organizing, storing, analyzing, and manually reviewing recordings from passive acoustic sampling is a time consuming task that should not be underestimated when planning future amphibian monitoring projects or weighing the advantages of ARUs relative to other methods.

*Considerations for Passive Acoustic Monitoring*

Based on the results of this project, we developed several recommendations to guide future passive acoustic monitoring for amphibians in Wyoming. Foremost among the advantages of ARUs is their ability to autonomously collect recordings over a period of several weeks or months. Calling of many explosive-breeding amphibian species in arid environments is highly dependent on episodic rainfall events, which in some years occur on a very limited number of occasions each spring and summer. ARUs deployed during periods of dry weather when amphibians are not calling can thus continue to record and potentially generate detections if conditions improve and breeding activity increases at any point during the sampling period. With improved storage and battery life of newer model ARUs, it is increasingly feasible for an individual unit to record nightly for 2–3 months, thereby allowing a single deployment to effectively sample for amphibians over the entire duration of the breeding season in Wyoming. We recommend that future amphibian monitoring projects in Wyoming use passive acoustic monitoring for species and in situations where calling is likely to be highly weather-dependent, resulting in low availability for detection on a single visit.

ARUs are also well-suited for monitoring, in which a sample of sites are re-visited over multiple years to track their status. Sites for monitoring could consist of a random sample, priority wetlands for management in a given jurisdiction, revisits to historically occupied sites to determine their current status, or before-after studies on impacts of changes in land use. In this context, ARUs can efficiently sample large and complex wetland sites where visual encounter surveys may be unlikely to detect all amphibians present. ARUs are capable of detecting calling amphibians at distances of several hundred meters, depending on the amplitude of different species' calls and environmental noise, which gives them a range large enough to cover most wetlands in Wyoming. They can also detect amphibians calling from inaccessible parts of wetland sites that may be difficult or impossible to sample with visual encounter surveys, like islands, thick vegetation, and deep water.

We used ARU recordings to document site occupancy, rather than estimate species abundance, because the primary goal of our project was to generate new occurrence records. However, there is an increasing body of research supporting the use of amphibian calling surveys to model species abundance, a technique which has been used with traditional nocturnal call survey data in eastern North America (Royle 2004, Dorcas et al. 2009). This type of modeling is equally well-suited to passive acoustic data, meaning that future amphibian monitoring projects in Wyoming could use repeated ARU deployments at the same site over multiple years to estimate trends in abundance to inform conservation and management decisions. Likewise, future passive acoustic monitoring for amphibians in Wyoming should consider the calling frequency, sound amplitude, and detectability of target species when determining study design and length of deployment (Yip et al. 2017).

Inventorying amphibians with ARUs may be most efficient for relatively small study areas, especially in situations where the capacity to conduct other field protocols is limited. For our study, we found the advantage of passive acoustic monitoring in species detection was generally outweighed by the logistical challenges of deploying and collecting the units across an extensive study area. If the goal of a study is to visit as many sites as possible, the two visits required to deploy and collect an ARU might be better spent surveying multiple sites with other methods. ARUs may be the only option to survey wetland sites for amphibians if personnel are not able to conduct lengthy field surveys, including working at night, or if areas of interest are inaccessible during suitable survey conditions (e.g., roads may be impassible or unsafe following rain).

The ability to sample multiple, cryptic taxa with one method is a key advantage of passive acoustic monitoring. We successfully recorded and analyzed data for amphibians and bats, but also note that the same recordings could be used to document secretive marsh birds and other species. Sampling multiple taxa simultaneously has the potential to increase the efficiency of ARUs as a survey technique and we recommend future studies consider adding other species. Relatedly, we suggest that all recordings should be archived in a long-term, secure repository for future analysis as models for our focal species and other taxa improve over time.

In addition to the field deployment of ARUs, passive acoustic amphibian monitoring requires a substantial office-based effort to process and analyze recordings. The Wyoming Amphibians project maintained by the WGFD in RFCx Arbimon has greatly simplified these tasks by providing a platform to store and manipulate recordings, and creating recognizer models for most amphibian species that occur in the state. We found that the need for manual review was minimal to satisfy the objective of generating presence-only records for species with loud, conspicuous calls. This reflects the success of the calibration of WGFD's models to remove 95% of false positives, while retaining 50% true presences (Bergman and Honeycutt 2021). However, documenting the absence of a species using ARUs (or other survey methods) requires substantially greater effort. For example, we found that it took one technician about 4 weeks (120 hours) to scan and manually validate recordings for species presence/absence across the 636 total hours of predicted positive recordings from our 34 ARU deployments. Based on the calibration of the model, the predicted positive recordings we reviewed would have included only 50% of true positives, with the remaining half mixed with the substantially larger number of true negatives that the model classified as predicted absences. Thus, definitively confirming the absence of a species from a set of recordings would require listening to the recordings in their entirety.

To improve the efficiency and repeatability of the review process for recordings, we developed the standardized approach described above in the methods section. Briefly, we recommend reviewing the first few predicted positive recordings from the default model settings to check for detections. If no detections are found immediately, we recommend raising the threshold using the combined Random Forest-Threshold model approach to return predicted positives for only those recordings with the best model support (i.e., 95% precision threshold). If no detections are found at the highest threshold, we suggest sequentially lowering the threshold and reviewing recordings until the original model threshold is reached. The goal of this approach is to minimize time spent reviewing recordings, while improving the comparability of data among sites by standardizing the amount of effort.

## Acknowledgements

Grant funding for this project was secured by Gary Beauvais, George Jones, Ian Abernethy, and Zach Wallace, working with Chris Keefe at Wyoming BLM. Asila Bergman and Ken Honeycutt with the WGFD provided technical assistance with ARU programming, deployment, and analysis techniques. Wendy Estes-Zumpf reviewed and provided feedback on this report. Tony Bridger, Patrick Lionberger, and Dale Woolwine provided input on survey locations and access conditions in the Rawlins, Rock Springs, and Pinedale BLM Field Offices, respectively. Thanks to our hard working technicians who collected field data: Rebecca Blankenship, Emily Schmidt, and Ryan Burns.

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# Appendices

*Appendix 1: Microphone, recorder, and duty settings for Song Meter recording units.*

| **Setting** | **ARU Model** | | | |
|---|---|---|---|---|
| | **SM1 stereo (acoustic)** | **SM2+ right channel (acoustic)** | **SM2+ left channel (ultrasonic)** | **SM4 stereo (acoustic)** |
| Sampling Rate | 8 kHz | 8 kHz | 192 kHz | 8 kHz |
| Microphone Gain | +0.0 dB | +0.0 dB | +0.0 dB | +16.0 dB |
| Preamp Gain | +48 dB | +48 dB | +48 dB | On (+26 dB) |
| Bias | On | On | On | NA |
| High Pass Filter | NA | Off | fs/12 | Off |
| Low Pass Filter | NA | Off | Off | NA |
| Compression | Off (WAV) | Off (WAV) | Off (WAV) | Off (WAV) |
| Duty Cycle | Start recording at 2000, record 5 minutes every hour, end recording at 0400 | See Appendix 2 | See Appendix 2 | Start recording at 2000, record 5 minutes every hour, end recording at 0400 |

*Appendix 2: Advanced schedule commands for Song Meter SM2+ recording units.*

| **2019** | |
|---|---|
| **Command** | **Translation** |
| AT SSET-00:15:00 | 15 minutes prior to sunset |
| SET 192000xMONO-L | Set L channel to 192 kHz |
| RECORD 00:15:00 | Record for 15 minutes |
| SET 8000xMONO-R | Set R channel to 8 kHz |
| RECORD 00:05:00 | Record for 5 minutes |
| SET 192000xMONO-L | Set L channel to 192 kHz |
| RECORD 00:45:00 | Record for 45 minutes |
| SET 8000XMONO-R | Set R channel to 8 kHz |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 22:00:00 | At 2200 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 23:00:00 | At 2300 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 00:00:00 | At 0000 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 01:00:00 | At 0100 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 02:00:00 | At 0200 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 03:00:00 | At 0300 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 04:15:00 | At 0415 |
| SET 192000xMONO-L | Set R channel to 192 kHz |
| RECORD 00:45:00 | Record for 45 minutes |
| GOTO LINE 01 00X | Repeat the next night |

| **2021** | |
|---|---|
| **Command** | **Translation** |
| AT SSET-00:15:00 | 15 minutes prior to sunset |
| SET 192000xMONO-L | Set L channel to 192 kHz |
| RECORD 00:15:00 | Record for 15 minutes |
| SET 8000xMONO-R | Set T channel to 8 kHz |
| RECORD 00:05:00 | Record for 5 minutes |
| SET 192000xMONO-L | Set L channel to 192 kHz |
| RECORD 00:40:00 | Record for 40 minutes |
| SET 8000xMONO-R | Set R channel to 8kHz |
| RECORD 00:05:00 | Record for 5 minutes |
| SET 192000xMONO-L | Set L channel to 192 kHz |
| RECORD 00:40:00 | Record for 40 minutes |
| SET 8000xMONO-R | Set R channel to 8kHz |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 23:00:00 | At 2300 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 00:00:00 | At 0000 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 01:00:00 | At 0100 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 02:00:00 | At 0200 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 03:00:00 | At 0300 |
| RECORD 00:05:00 | Record for 5 minutes |
| AT TIME 04:00:00 | At 0400 |
| RECORD 00:05:00 | Record for 5 minutes |
| SET 192000xMONO-L | Set L channel to 192 kHz |
| RECORD 00:45:00 | Record for 45 minutes |
| GOTO LINE 01 00X | Repeat the next night |

*Appendix 3: Models, thresholds, and recordings analyzed per species.*

| Species | Total number of recordings analyzed | Model | Threshold | Number of sites tested | Number of sites with predicted positives | Number of sites with validated detections | Total number of recordings with predicted positives |
|---|---|---|---|---|---|---|---|
| Great Basin spadefoot | 33,972 | Sin8/6/19_3ROIs_ ExAwoPmaSboSin488RefinedVals | 0.628 (0.95 precision) | 29 | 13 | 5 | 493 |
| | | | 0.552 (0.75 precision) | 24 | 12 | 1 | 138 |
| | | | 0.333 (0.62 precision) | 23 | 23 | 3 | 1,196 |
| | | | 0.233[1] (0.57 precision) | 20 | 20 | 0 | 3,220 |
| Boreal chorus frog | 38,162 | PMA2.1.9 | 0.344 (0.95 precision) | 34 | 24 | 15 | 5,603 |
| | | | 0.303[1] (0.82 precision) | 21 | 16 | 0 | 406 |
| Plains spadefoot | 4,868 | SboBoth2.2.3 | 0.466[2] (0.80 precision) | 4 | 2 | 1 | 10 |
| | | | 0.311 (0.75 precision) | 3 | 3 | 0 | 272 |
| | | | 0.251[1] (0.67 precision) | 3 | 3 | 0 | 746 |
| Rocky Mountain toad | 9,092 | AWO2.9.12 | 0.243[1,3] (0.72 precision) | 8 | 8 | 0 | 177 |
| Great Plains toad | 4,868 | ACO2.1.2 | 0.160[1,3] (0.91 precision) | 4 | 3 | 0 | 177 |
| | | ACO2.1.1 | 0.095[1,3,4] (0.89 precision) | 4 | 4 | 0 | 332 |
| Northern leopard frog | 38,162 | LPI3.2.2 | 0.314 (0.95 precision) | 34 | 23 | 0 | 164 |
| | | | 0.271 (0.81 precision) | 34 | 32 | 0 | 1,643 |
| | | | 0.240[1] (0.80 precision) | 34 | 33 | 0 | 5,626 |

[1]Original optimal model threshold determined by Honeycutt and Bergman (2021)
[2]Highest level of precision possible with this model
[3]Started with the original threshold due to the smaller overall number of recordings for this species
[4]Ran a second model due to few positive detections using the top performing model