Designing a Standardized Survey Framework for Inventorying Amphibians and Reptiles on Bureau of Land Management Lands in Wyoming

Final Report, March 2017

Prepared by:

Wendy Estes-Zumpf[1,2], Zach Wallace[1], Lusha Tronstad[1], Mark Andersen[1]

[1] Wyoming Natural Diversity Database, University of Wyoming, 1000 East University Avenue, Department 3381, Laramie, Wyoming 82071

[2] Current address: Wyoming Game and Fish Department, Laramie Regional Office, 528 South Adams Street, Laramie, Wyoming 82070, Email: wendy.estes-zumpf@wyo.gov

Prepared for:

Bureau of Land Management

Wyoming State Office

5353 Yellowstone Rd

Cheyenne, Wyoming 82009

Recommended Citation: Estes-Zumpf, W., Z. Wallace, L. Tronstad, and M. Andersen. 2017. Designing a Standardized Survey Framework for Inventorying Amphibians and Reptiles on Bureau of Land Management Lands in Wyoming. Prepared for the Bureau of Land Management Wyoming State Office by the Wyoming Natural Diversity Database, Laramie, Wyoming.

Cover photos by Sierra Jech, Sarah-Jane Gerstman, and Ben Thesing.

W Y N D D
Wyoming Natural
Diversity Database

Table of Contents

List of Tables

List of Figures

Abstract

Reptiles and amphibians remain chronically understudied taxa in much of North America, despite growing concern over reported declines. In Wyoming, 9 of 12 amphibians (75%) and 24 of 29 reptiles (83%) are identified by the state as Species of Greatest Conservation Need; however, in many cases this designation is mainly due to lack of information. To address this knowledge gap, we collected and field-tested a suite of survey protocols for Wyoming's herpetofauna, including visual encounter surveys of 1) wetland and riparian areas, 2) rock outcrops, and 3) transects in reptile habitat, as well as 4) nocturnal call surveys for amphibians, and 5) incidental and opportunistic observations made while travelling between formal surveys. We tested the performance of this suite of survey methods on lands managed by the Bureau of Land Management (BLM) Newcastle Field Office (NFO) in 2014, Kemmerer Field Office (KFO) in 2014–2015, and Rawlins Field Office (RFO) in 2016. We used existing records of amphibian and reptile locations to focus survey efforts on areas where a number of species were suspected to occur, but had never been documented. During summer field seasons in 2014–2016, we surveyed a total of 303 sites or routes and detected 7 amphibian and 5 reptile species: Tiger Salamander (*Ambystoma mavortium*), Boreal Chorus Frog (*Pseudacris maculata*), Northern Leopard Frog (*Lithobates pipiens*), Great Plains Toad (*Anaxyrus cognatus*), Rocky Mountain Toad (*A. woodhousii woodhousii*), Plains Spadefoot (*Spea bombifrons*), Great Basin Spadefoot (*S. intermontana*), Greater Short-horned Lizard (*Phrynosoma hernandesi*), Northern Sagebrush Lizard (*Sceloporus graciosus graciosus*), Wandering Gartersnake (*Thamnophis elegans vagrans*), Plains Gartnersnake (*T. radix*), and Prairie Rattlesnake (*Crotalus viridis viridis*). This report summarizes results by field office and provides the protocols and datasheets for each sampling method.

Introduction

Reptiles and amphibians remain chronically understudied taxa in much of North America, even in the face of reported declines (Bonnet et al. 2002; Stuart et al. 2004; Pounds et al. 2006; Todd et al. 2010; Hof et al. 2011). In Wyoming, 9 of 12 amphibians (75%) and 24 of 29 reptiles (83%) are listed as Species of Greatest Conservation Need (SGCN; WGFD 2017). In many cases, however, this conservation designation is mainly due to lack of information on distributions, population trends, or both. A recent assessment of the vulnerability of 156 wildlife species to energy development in Wyoming lists 5 amphibians and 8 reptiles as having high or potentially high exposure risk, with the Great Plains Toad (*Anaxyrus cognatus*) and Rocky Mountain Toad (*A. woodhousii woodhousii*) listed as the top 2 most at risk species (Keinath and Kauffman 2014). All of these 'at risk' reptiles and amphibians and many of the SGCN species occur in areas managed by the Bureau of Land Management (BLM). Despite growing concern over the status of Wyoming's reptiles and amphibians, most survey and monitoring efforts have been species- or project-specific, short-term, and/or relatively limited in spatial extent.

Recent herpetological inventories in Wyoming have greatly increased our understanding of species distributions in some areas of the state. These include surveys conducted by the Wyoming Game and Fish Department (WGFD) and Wyoming Natural Diversity Database (WYNDD) in the Powder River Basin (Estes-Zumpf and Keinath 2012), Bighorn National Forest (Estes-Zumpf et al. 2012), and Bridger-Teton National Forest (Estes-Zumpf et al. 2014); monitoring by the National Park Service (NPS) in Yellowstone and Grand Teton National Parks (Murphy et al. 2010, Gould et al. 2012); and the Rocky Mountain Amphibian Project (RMAP)—a standardized monitoring framework for amphibians developed by WYNDD and the University of Wyoming Biodiversity Institute—that is currently being implemented by several National Forests in Wyoming and northern Colorado (RMAP 2017). Additionally, standardized reptile monitoring techniques for use across large landscapes are being developed and tested in Colorado by Danny Martin, a Ph.D. student at Colorado State University (CSU). Despite these efforts, large areas of Wyoming have not been covered by recent herpetological surveys, especially lower-elevation lands that are managed primarily by the BLM. This project was developed in response to the recognition that a number of herpetological monitoring frameworks were in place or being tested in the state, and opportunities existed to integrate these protocols

into a standardized framework to inventory reptiles and amphibians on BLM lands in understudied areas of Wyoming.

Objectives

The primary objectives of this project were to (1) test a suite of amphibian and reptile survey protocols in order to develop a standardized survey methodology for Wyoming's herpetofauna and (2) collect location data to improve our understanding of the distribution of amphibians and reptiles on BLM lands in Wyoming.

Project Summary

In 2014, the BLM expressed interest in developing a standardized survey methodology for reptile and amphibian species considered Sensitive by the BLM or Species of Greatest Conservation Need by WGFD in Wyoming. WYNDD worked with WGFD herpetology staff and the BLM to develop and field-test a survey strategy for reptiles and amphibians on BLM lands in Wyoming. Because the target species group included a wide range of aquatic and terrestrial species, many of which are cryptic and occur at low densities across the landscape, identification of areas most likely to contain several species of interest was important to maximizing survey efficiency. Another important goal was that survey methodology considered future BLM monitoring needs and realistic funding constraints.

To accomplish this, we coordinated with biologists at the state and field offices to discuss information and future monitoring needs as well as available resources. We used GIS to identify areas of highly suitable habitat for groups of taxa (e.g., amphibians, grassland reptiles, desert scrub reptiles) and worked with the BLM to develop standardized survey protocols based on priority species. Unlike montane amphibians, most plains and basin amphibians are more easily detected using nocturnal call surveys. However, this method is not as effective for some sensitive species, like the Northern Leopard Frog (*Lithobates pipiens*), which is better detected using Visual Encounter Surveys (VES) due to its faint calls. Thus, WYNDD implemented a suite of survey protocols to assess the advantages and disadvantages of different techniques that might be included in a comprehensive monitoring framework to satisfy the information needs of the BLM. Once survey methodologies were developed, we used existing pilot data from similar studies in Wyoming and Colorado (e.g., data from WYNDD, WGFD, CSU) and GIS habitat models to inform the final sampling design.

Methods

Study Area

The study area included BLM lands in the Kemmerer Field Office (KFO) in 2015 and the Rawlins Field Office (RFO) in 2016, with a limited number of surveys in priority areas identified by BLM biologists in the KFO and Newcastle Field Office (NFO) in 2014. Our study area focused on low-to-middle elevations (1200 m to 2600 m) in intermountain and prairie basins, and adjacent mountain foothills of Wyoming. Dominant vegetation included sagebrush-steppe, prairie grasslands, salt desert shrublands, and foothill shrublands, with a smaller number of sites in mid-elevation forests. Amphibian and reptile habitat in the study area included stockponds and reservoirs, creeks, oxbows, wetlands, wet meadows, beaver ponds, playa lakes, ephemeral ponds, rock outcrops, scree slopes, small mammal colonies, ant colonies, sandy areas, and foothill canyons.

Site Selection

Our objective was to improve understanding of the ranges and distributions of multiple amphibian and reptile species in Wyoming; thus, we selected survey areas where a number of species were suspected to occur, but had never been documented. Survey areas in 2015 and 2016 were defined by drainages, identified using 10-digit Hydrologic Unit Code (HUC) boundaries. In order to select a target set of HUCs where only a few records from a low number of species had been detected in the past, we identified all HUCs with a low number of reptile and amphibian (hereafter "herp") occurrence records (<10) and low Shannon Diversity Index values. Within each target HUC we selected 5 Public Land Survey System (PLSS) sections with a high probability of having multiple herp species. To do this, we overlaid 36 herp species distribution models (Keinath et al. 2010) and calculated the mean probability of occurrence across all species for each section. We then used a spatially balanced random sampling method (generalized random tessellation stratified sampling) to select 5 sections in each target HUC with a high probability of containing multiple herp species. All survey site selection was done using GIS (ESRI 2011) and Program R (R Core Team 2014).

We identified 2 of the 5 selected sections in each HUC for field surveys. We evaluated sections in the order they were selected in the spatially balanced random sample design. In 2015, we excluded the 1st or 2nd selected sections if they were inaccessible by public roads or contained

very little BLM land. However, due to the extremely low number of detections in 2015 and limited public access to BLM lands in the Rawlins Field Office in 2016, we modified our selection method to also include both accessibility and presence of key habitat components (water bodies and/or rock outcrops). In many cases in 2016, all 5 selected sections were not accessible by public roads and/or did not have key habitat features. When this happened, we selected the nearest accessible sections with similar habitat to the top 2 selected sections.

For each of the 2 high probability sections selected for surveys in each HUC, we also conducted surveys at a nearby random section. Surveys at random sections were conducted to account for incomplete knowledge of reptile and amphibian habitat in Wyoming, especially as reflected in predicted distribution models restricted by available data layers. We typically selected an adjacent section that was accessible via public roads. Due to the low number of species detected in 2015, however, we modified selection of random sections in 2016 to include sections with at least some key habitat features for amphibians or reptiles. Lastly, we conducted opportunistic surveys at individual water bodies, rock outcrops, and small mammal or ant colonies identified by aerial imagery or when driving between formal survey areas.

Survey Methodology

In order to detect the suite of amphibians and reptiles likely to occur on BLM lands in Wyoming, we used several survey techniques to target species with different life history traits and habitat requirements. Methods included Visual Encounter Surveys (VES) of water bodies, rock outcrops, and upland transects, and nocturnal call surveys along roads. We also conducted timed "expert opinion" searches of potential reptile habitat in 2016 only, and recorded incidental detections of all herps found while driving or walking between formal searches. All survey techniques are described below, with datasheets and field protocols include in Appendices 1A–1G.

Wetland and Riparian VES

We used VES along riparian areas, and around wetlands and water bodies to search for amphibians and reptiles that are associated with water during the breeding season. In Wyoming, all amphibian species require water to breed, though species often vary in their preferred type of water body, water quality, permanence of wetland habitat, and the duration of their seasonal association with water. Thus, we conducted wetland and riparian VES surveys at all waterbodies

within selected sections during the breeding season (late-May through July). We recorded evidence of breeding (egg masses, larvae, metamorphs) as well as the presence of any adults and juveniles of each species at each site. We also recorded information on site and survey conditions. Datasheets and protocols are provided in Appendix 1A.

VES were similar to guidelines set forth by the USGS Amphibian Research and Monitoring Initiative (ARMI) to permit occupancy-based modeling of amphibian populations (Corn et al. 2005). Whenever possible, surveys were conducted by a minimum of 2 observers working independently at each site (dual-observer method) to allow for estimation of detection probabilities for each species. Under the dual-observer method, each survey is conducted independently with no discussion of findings or peer correction of datasheets after the survey is complete. The standard dual-observer method has both observers survey around the perimeter of a water body in opposite directions until they meet at the far end, wait for a short period of time (approximately 10 minutes) for animals to settle, and then switch sides and complete the survey. To search more amphibian habitat in remote locations in a short amount of time, however, we sometimes used single-pass surveys where all surveyors spread out to search all potential habitat at a site in a single sweep. This survey method allowed more area to be inventoried for amphibians, but resulted in lower detection rates because each individual of a given species was not available for detection by both observers.

For better detection of tadpoles and salamander larvae, we dip-netted for amphibian larvae every 5–10 m and in patches of high-quality habitat (e.g., quite inlets, backwater areas, or patches of emergent vegetation). If tadpoles could not be identified to the species level in the field, 1–2 individuals were collected, preserved in vials containing a 95% ethanol solution, and sent to WYNDD for identification under a dissecting scope. Surveyors also took photographs of any unidentifiable animals or egg masses and sent photos to WYNDD or WGFD for identification.

Rock Outcrop VES

We surveyed for reptiles on south-facing rock outcrops, where lizards and snakes often concentrate. South-facing rock outcrops provide thermal cover, basking surfaces, cover from predators, potential hibernacula, and invertebrate and small mammal prey. Surveys were conducted primarily during morning hours or evening hours, when reptiles are most likely to be active and visible. Rock outcrop surveys consisted of walking along rocky slopes looking for

basking reptiles in exposed areas as well as individuals resting on shaded ledges, in crevasses, or under rocks. Rocks lifted or flipped over during searches were replaced in their original position to minimize disturbance to habitat (Pike et al. 2010). Habitat, total survey time, and species detected were recorded. Datasheets and protocols are provided in Appendix 1B.

Reptile Transect VES

To detect species of snakes and lizards that occur in habitat other than rock outcrops and riparian zones (e.g., Plains Hog-nosed Snake, *Heterodon nasicus*; Greater Short-horned Lizard, *Phrynosoma hernandesi*), we also searched for reptiles using paired belt transects across alternative habitat (e.g., prairie dog or ground squirrel colonies, sandy substrates, areas with high densities of ant mounds). In 2015, we conducted at least 2 reptile transect surveys per section. Each transect survey consisted of 2 surveyors walking parallel belt transects that were 300 m long, 5 m wide, and spaced 10 m apart. Surveyors recorded all herp species encountered within each transect. Because no species were detected on reptile transects in 2015, we replaced this method with less formal "expert knowledge" searches in 2016. These surveys consisted of each surveyor searching the best potential reptile habitat and recording the amount of time spent surveying, habitat present, and species detected. Although this method was not as standardized as the reptile transects, expert knowledge searches still allowed comparison of detection rates per unit search effort, and resulted in a greater overall number of detections. Datasheets and protocols are provided in Appendix 1C.

Nocturnal Call Surveys

Nocturnal call surveys can be an effective way to survey for anurans (frogs and toads), especially in areas where access to habitat is restricted. Males of all anuran species in Wyoming vocalize to attract females to breeding sites under suitable weather conditions. Species can be identified by their calls and some can be heard up to 2 km away, depending on environmental conditions. Road-based nocturnal call surveys are, thus, an effective method of detecting species presence across relatively large areas without physical access to adjacent land.

Nocturnal call surveys involved a two-person crew starting at a fixed location on a public road and driving a predefined distance (0.2 miles) between a series of listening points. Each surveyor independently recorded all amphibians heard at each point, allowing estimation of detection rates. All surveys were conducted after dark and detailed weather information was

collected at the beginning and end of the survey (e.g., wind speed, barometric pressure, relative humidity, cloud cover, and air temperature). At each listening point, surveyors stood outside and away from the vehicle and listened for 3 minutes, recording the coordinates, species, calling intensity, direction and distance to caller(s), and ambient noise level. Datasheets and protocols are provided in Appendix 1D.

Opportunistic and Incidental Observations

We also reported incidental detections of all herps found while driving or hiking between formal searches. Because reptiles and amphibians basking on or crossing roads are often killed by vehicles, searching for herps along roads can be a productive inventory method (Heyer et al. 1994). Accordingly, surveyors recorded all incidental sightings of reptiles and amphibians found dead or alive while driving between survey sites. Surveyors also recorded all incidental observations of herps seen while hiking between formal surveys. Datasheet is provided in Appendix 1E.

Disease Sampling and Decontamination Protocols

A subset of amphibians detected each year were swabbed for chytrid fungus (*Batrachochytrium dendrobatidis; Bd*) following procedures outlined by Livo (2004). *Bd* samples were sent to Pisces Molecular (Boulder, Colorado) for PCR testing. All survey and sampling gear were decontaminated with either a 10% bleach solution or commercial fungicide between drainages or isolated sites within drainages to prevent the spread of *Bd* among sample locations. The *Bd* sampling protocol is included in Appendix 1F and the fungal decontamination protocol in Appendix 1G.

Summary Statistics and Analyses

We summarized the number of species and individuals detected within each field office by survey method. We calculated effort in person-time per site as the survey duration multiplied by the number of observers. For species detected at ≥10 sites by surveys with dual-observer designs, we estimated probabilities of detection (p) and occupancy (ψ) using single-season, single-species occupancy models (MacKenzie et al. 2005) fitted with package unmarked (Fiske and Chandler 2011) in Program R (R Core Team 2014). For each combination of species and survey method, we used the Akaike Information Criterion adjusted for small sample sizes (AIC_c) to compare

models that pooled data across years (i.e., intercept-only) with models in which p and/or ψ varied by year. We evaluated model fit (MacKenzie and Bailey 2004), and adjusted standard errors of models with moderate evidence of over-dispersion ($2 < \hat{c} < 4$) and discarded models with evidence of severe over-dispersion ($\hat{c} > 4$). Unfortunately, most species were detected in such small numbers that models did not converge, fit poorly (i.e., highly over-dispersed with $\hat{c} > 4$), or had parameter estimates with extremely wide confidence intervals. As an alternative, we calculated simple detection probabilities for each double-observer survey method across all species and sites. At each site, we assigned a detection probability of 1.0 to species detected by both observers and 0.50 to species detected by only one observer. We then averaged these detection probabilities across sites and species within each survey method. We present these simple detection probabilities to emphasize that detection was imperfect during surveys, but caution that this information should be used only as a general guide for design of future surveys.

Results

Newcastle Field Office 2014

In 2014, we conducted surveys on lands in the NFO using the Wetland and Riparian VES, Rock Outcrop VES, and Nocturnal Call survey protocols, as well as incidental and opportunistic observations. Common and scientific names of all species are included in Table 1. We surveyed a total of 12 sites or routes and detected 6 amphibian (Figure 1, Table 2, Table 3) and 4 reptile species (Figure 2, Table 2).

Wetland and Riparian VES

We surveyed 7 wetland or riparian sites. Total survey effort was 5 h 45 min person-time with 1–2 independent observers and averaged 31 person-min per site (range = 10–50 min). We detected 7 species, all of which occurred in reservoir/stockpond habitats: Boreal Chorus Frog, Northern Leopard Frog, Plains Spadefoot, Rocky Mountain Toad, Tiger Salamander, Plains Garter Snake, and Wandering Garter Snake. Average number of species detected per survey was 0.68 (range = 0–4). Observer data required to calculate detection probability was not collected in 2014. Boreal Chorus Frog detections consisted of 13 adults, 3 metamorphs, and ≥1100 tadpoles found at 3 sites. We detected 1 Northern Leopard Frog adult, ≤201 Plains Spadefoot tadpoles found at 3 sites, approximately ≤1100 Rocky Mountain Toad tadpoles found at 1 site, 7 juvenile and ≤ 13

larvae of Tiger Salamander found at 2 sites, and ≤130 unidentified tadpoles found at 2 sites. Additionally, we detected 4 adult Wandering Garter Snake at 1 site, and a total of 5 adult Plains Garter Snake at 2 sites.

Table 1. Common and scientific names of amphibian and reptile species detected during surveys of the Wyoming BLM Newcastle Field Office in 2014, Kemmerer Field Office in 2014–2015, and Rawlins Field Office in 2016.

Common Name	Scientific Name
Tiger Salamander	*Ambystoma mavortium*
Boreal Chorus Frog	*Pseudacris maculata*
Northern Leopard Frog	*Rana pipiens*
Rocky Mountain Toad or Woodhouse's Toad	*Anaxyrus woodhousii woodhousii*
Great Plains Toad	*Bufo cognatus*
Plains Spadefoot	*Spea bombifrons*
Great Basin Spadefoot	*Spea intermontana*
Greater Short-Horned Lizard	*Phrynosoma hernandesi*
Northern Sagebrush Lizard	*Sceloporus graciosus graciosus*
Prairie Rattlesnake	*Crotalus viridis viridis*
Wandering Garter Snake	*Thamnophis elegans vagrans*
Plains Gartner Snake	*Thamnophis radix*

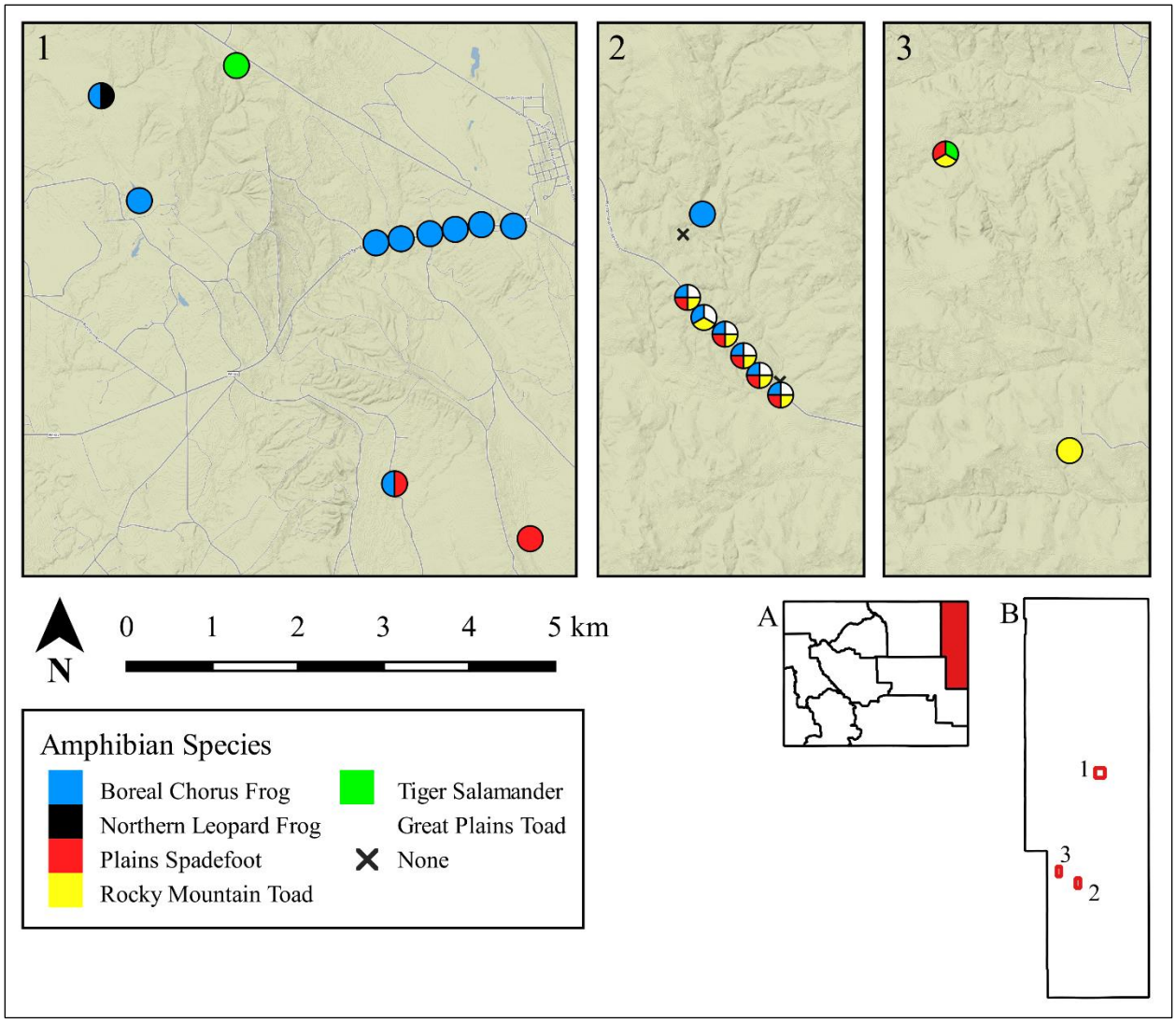


Figure 1. Locations of amphibians detected during surveys of the BLM Newcastle Field Office (NFO) in 2014 using three different protocols. Pie charts show the number of species detected at each site, but do not indicate their relative abundance. Insets show (A) location of the NFO in Wyoming and (B) survey areas 1–3 in the NFO. Scientific names of species are included in Table 1.

Rock Outcrop VES

We surveyed 3 rock outcrop sites. Total survey effort was 5 h 30 min person-time with 2–4 independent observers and averaged 2 h 45 min person-time per site (range = 1 h 30 min – 4 h). We detected 2 species, consisting of 2 Northern Sagebrush Lizard and 1 Prairie Rattlesnake. Average number of species detected per site was 1 (range = 0–1). Observer information necessary to calculate detection probability was not collected.

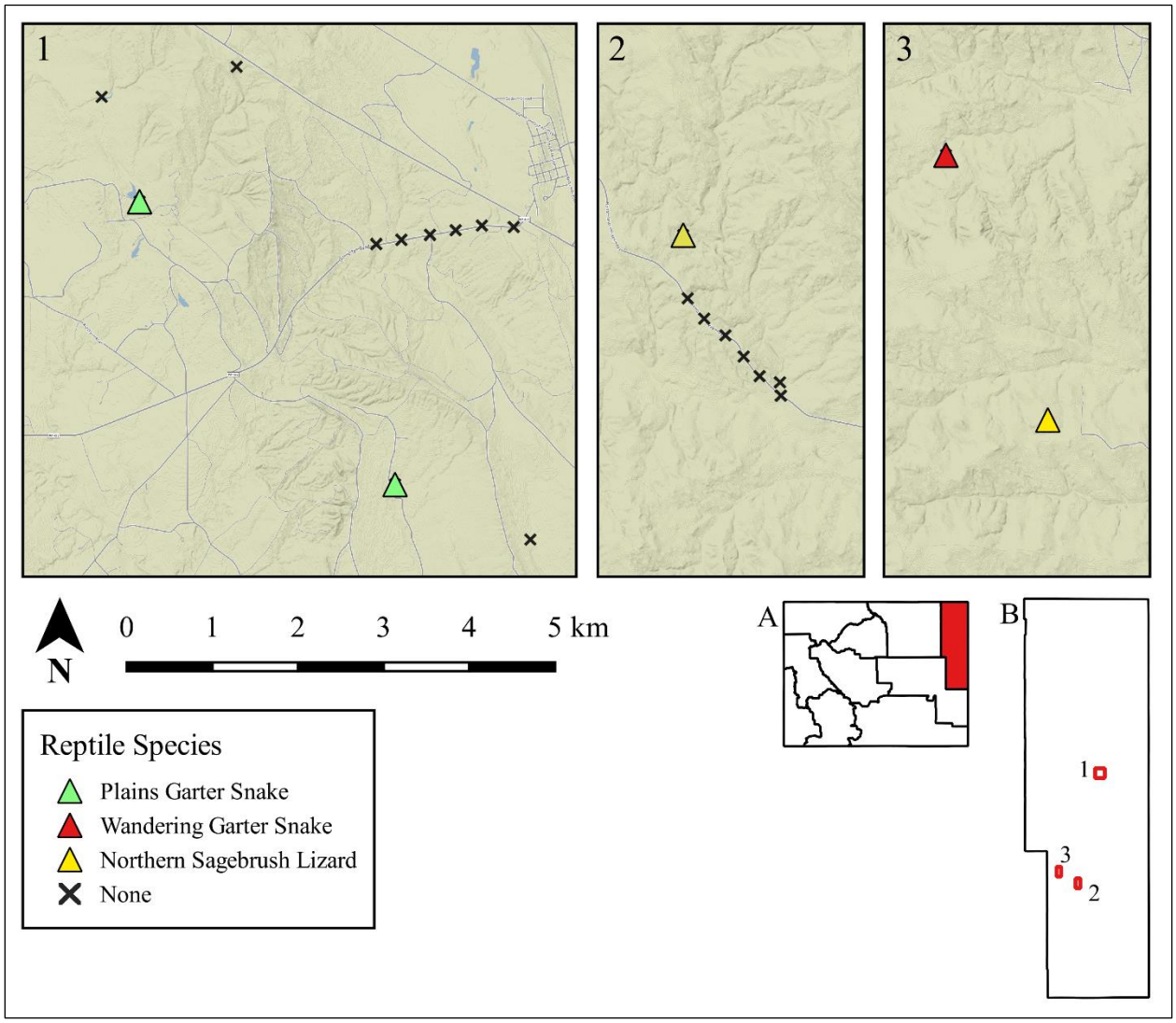


Figure 2. Locations of reptiles detected during surveys of the BLM Newcastle Field Office (NFO) in 2014 using three different protocols. Pie charts show the number of species detected at each site, but do not indicate their relative abundance. Insets show (A) location of the NFO in Wyoming and (B) survey areas 1–3 in the NFO. Scientific names of species are included in Table 1.

Table 2. Number of reptiles and amphibians by age class detected during visual encounter surveys of the BLM Newcastle Field Office area in 2014. Results from nocturnal call surveys are presented in Table 3 and scientific names of species are included in Table 1.

	Age Class			
Species	Adult	Juvenile	Metamorph	Tadpole/Larvae
Boreal Chorus Frog	14	0	3	≥1100
Northern Leopard Frog	1	0	0	0
Plains Spadefoot	0	0	0	≤201
Rocky Mountain Toad	1	0	0	≤1100
Tiger Salamander	0	7	0	≤13
Unidentified Tadpole	0	0	0	≤130
Northern Sagebrush Lizard	3	0		
Wandering Garter Snake	4	0		
Plains Gartner Snake	5	0		
Prairie Rattlesnake	1	0		

Nocturnal Call Surveys

We surveyed 2 routes, comprising 12 points. Total survey effort was 2 h 24 min person-time. We detected 4 species (Table 3). Average number of species detected per route and point was 2.42. Simple detection probability across species averaged 0.85 for 23 detections with dual-observer data.

Table 3. Number of routes and points with detections of amphibian species during nocturnal call surveys of the BLM Newcastle Field Office area in 2014. Scientific names of species are included in Table 1.

Species	Number of Routes	Number of Points
Boreal Chorus Frog	2	12
Plains Spadefoot	1	5
Rocky Mountain Toad	1	6
Great Plains Toad	1	6

Incidental Observations

We recorded incidental observations of 4 species, including 1 Boreal Chorus Frog, 1 Northern Sagebrush Lizard, 1 Rocky Mountain Toad, and 1 unidentified tadpole.

Chytrid Fungus Sampling

We collected *Bd* samples from Boreal Chorus Frogs at 2 sites and Northern Leopard Frog at 1 site in the NFO. None of these samples tested positive for *Bd*.

Kemmerer Field Office 2014–2015

In 2014 and 2015, we conducted surveys on lands in the KFO using the Wetland and Riparian VES, Rock Outcrop VES, Reptile Transect VES, and Nocturnal Call survey protocols. We surveyed a total of 95 sites or routes (Figure 3) and detected 4 amphibian (Table 4, Table 5, Figure 4) and 2 reptile species (Table 4, Figure 5).

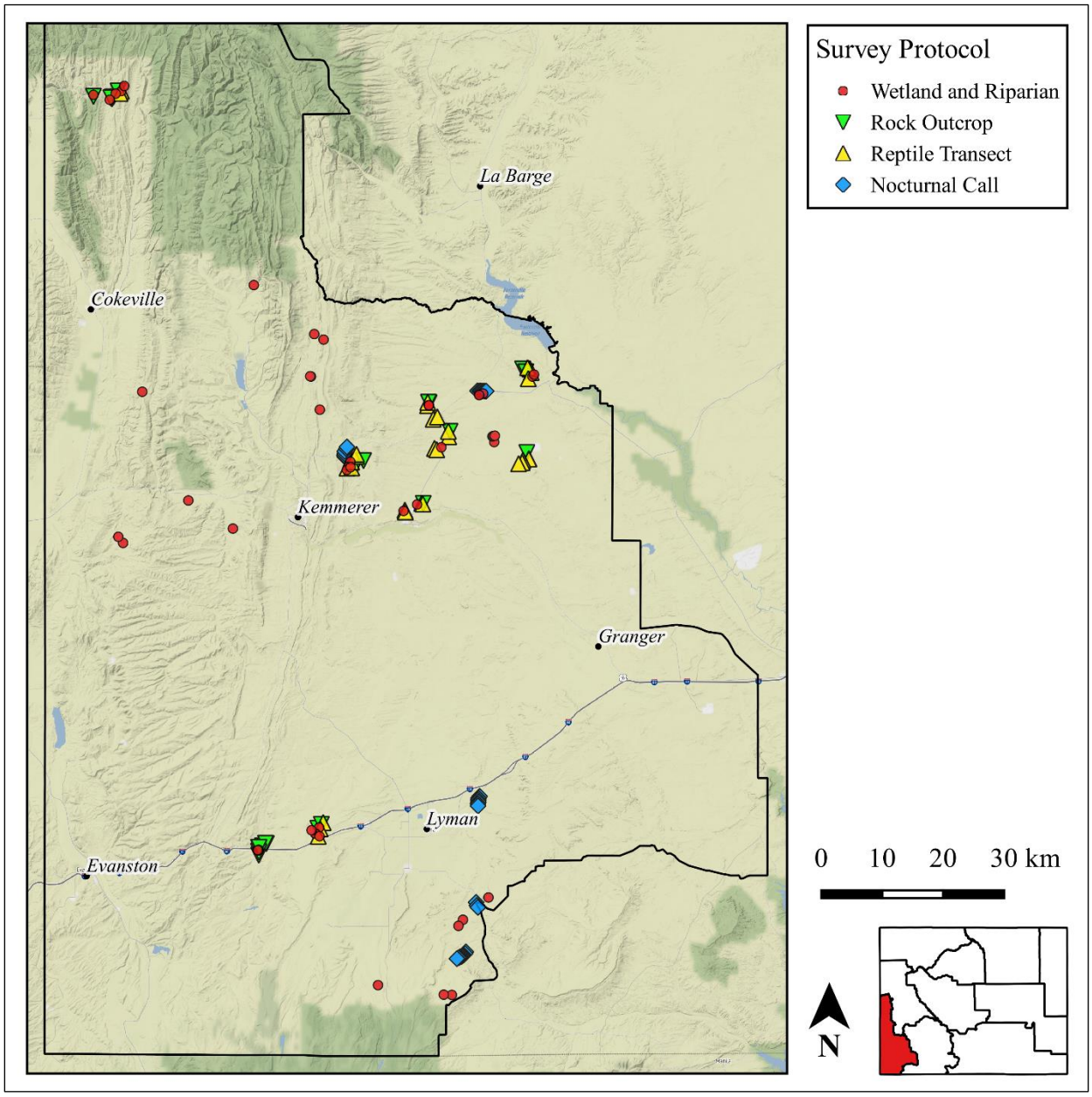


Figure 3. Sites surveyed for reptiles and amphibians in the BLM Kemmerer Field Office (KFO) area (black outline) in 2014–2015 using four different protocols. Inset shows the location of the KFO in Wyoming.

Table 4. Number of reptiles and amphibians by age class detected during visual encounter surveys of the BLM Kemmerer Field Office area in 2014–2015. Results from nocturnal call surveys are presented in Table 5 and scientific names of species are included in Table 1.

	Age Class		
Species	Adult	Juvenile	Tadpole/Larvae
Boreal Chorus Frog	15	0	≤1123
Northern Leopard Frog	2	0	0
Great Basin Spadefoot	1	0	≤1000
Tiger Salamander	3	1	≤121
Northern Sagebrush Lizard	23	0	0
Unknown Lizard	1	0	
Wandering Garter Snake	6	7	
Unknown Snake (Skin)	1	0	

Wetland and Riparian VES

We visited 47 wetland or riparian sites and conducted surveys at 45 sites with sufficient water, including 15 surveys in 2014 and 30 surveys in 2015. Total survey effort was 43 h 6 min person-time with 1–3 independent observers and averaged 56 person-min per site (range = 5 min – 4 h 30 min). We detected 5 species: Boreal Chorus Frog, Northern Leopard Frog, Tiger Salamander, Great Basin Spadefoot, and Wandering Garter Snake. Average number of species detected per survey was 1.13 (range = 0–3). We detected Boreal Chorus Frog at 10 of 45 sites, including 15 adults and ≤1123 tadpoles found in marsh/bog, spring/seep, reservoir/stockpond, and permanent lake/pond habitats. The top model for Boreal Chorus Frog suggested probabilities of detection (p = 0.61; 95% CI: 0.16, 0.93) and occupancy (ψ = 0.13; 95% CI: 0.04, 0.35) did not vary among the KFO in 2015 and the RFO in 2016. We detected Tiger Salamander at 7 sites (5 in 2014 and 2 in 2015), including 3 adults, 1 juvenile, ≤121 larvae found in reservoir/stockpond and permanent lake/pond habitats. We detected 2 adult Northern Leopard Frog at 1 permanent lake/pond site, a total of 1 adult and ≤1000 tadpoles of Great Basin Spadefoot at 1 wetland/marsh and 1 reservoir/stockpond site, and 3 adult and 7 juvenile Wandering Garter Snake at 2 reservoir/stockpond sites. Simple detection probability averaged 0.75 across 6 detections with dual-observer data.

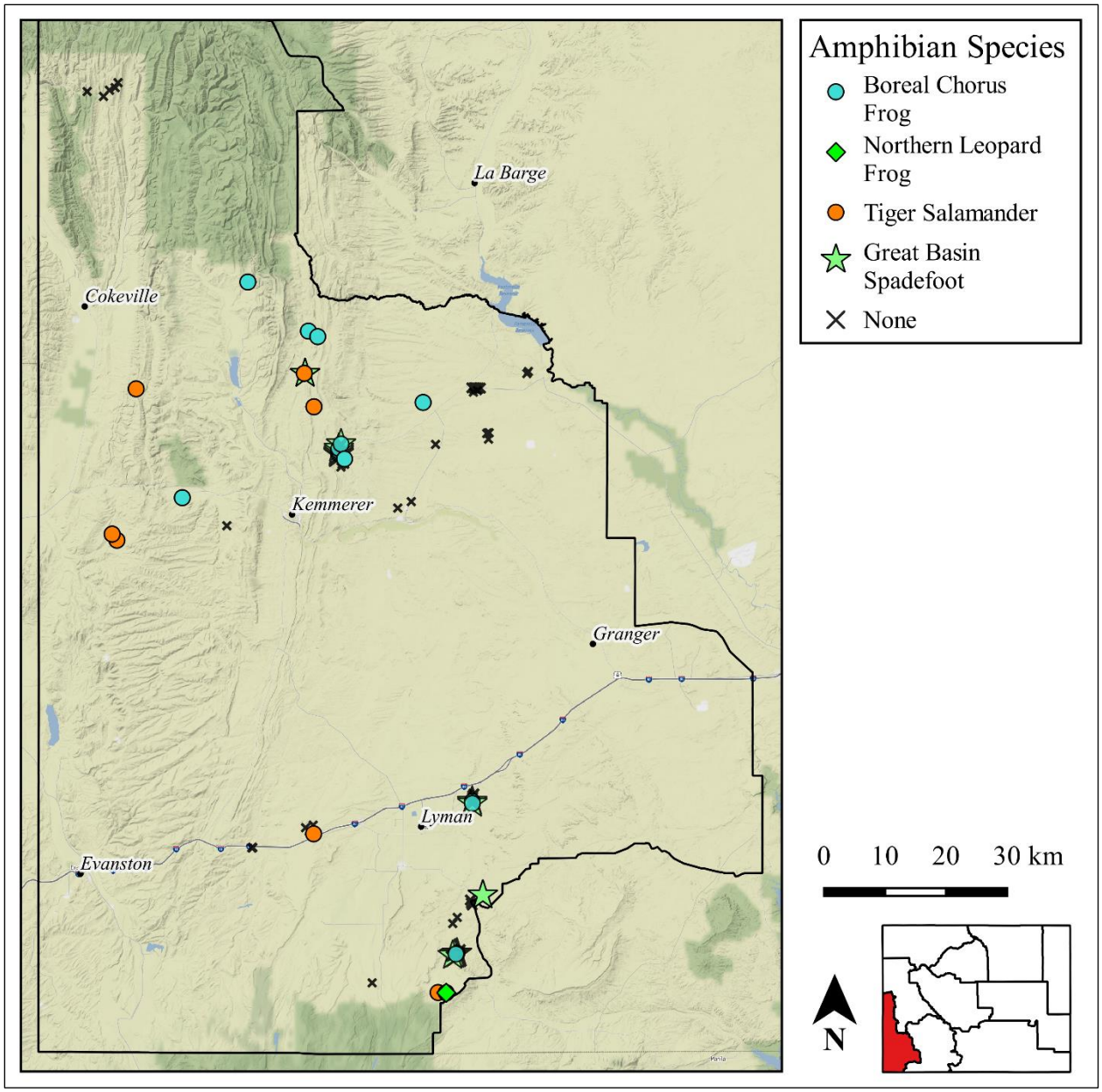


Figure 4. Locations of amphibians detected during visual encounter and nocturnal call surveys of the BLM Kemmerer Field Office (KFO) area (black outline) in 2014–2015. Inset shows the location of the KFO in Wyoming. Scientific names of species are included in Table 1.

Rock Outcrop VES

In 2015, we visited 25 potential rock outcrop sites and conducted surveys at 24 sites with suitable habitat. Total survey effort was 16 h 38 min person-time with 2 independent observers and averaged 42 person-min per site (range = 14 min – 2 h 15 min). We detected 2 species, consisting of 23 Northern Sagebrush Lizard and 3 Wandering Garter Snake. Average number of

species detected per site was 0.40 (range = 0–2). Simple detection probability averaged 0.78 for 9 detections with dual-observer data. The top model for Northern Sagebrush Lizard suggested detection probability for the KFO in 2015 (p = 0.72; 95% CI: 0.13, 0.98) was higher than the RFO in 2016 (p = 0.11; 95% CI: 0.01, 0.58), and occupancy probability (ψ = 0.32; 95% CI: 0.08, 0.73) did not vary among years/study areas.

Reptile Transect VES

We conducted 32 Reptile Transect surveys in 2015. Total survey effort was 15 h 53 min person-time with 2 independent observers and averaged 31 person-min per site (range = 16 min – 2 h 39 min). We did detect any reptiles during these surveys.

Nocturnal Call Surveys

We surveyed 6 routes, comprising a total of 33 points, with 2 routes in 2014 and 4 routes in 2015. Total survey effort was 6 h 36 min person-time. We detected 2 species (Table 5). Average number of species detected was 0.42 per point and 0.39 per route. Simple detection probability averaged 0.88 for 12 detections with dual-observer data.

Table 5. Number of routes and points with detections of amphibian species during nocturnal call surveys of the BLM Kemmerer Field Office area in 2015. Scientific names of species are included in Table 1.

Species	Number of Routes	Number of Points
Boreal Chorus Frog	3	9
Great Basin Spadefoot	2	5

Chytrid Fungus Sampling

We collected *Bd* samples from 3 individual Boreal Chorus Frogs at 1 site in the KFO. Of 3 samples, 2 tested positive for *Bd*.

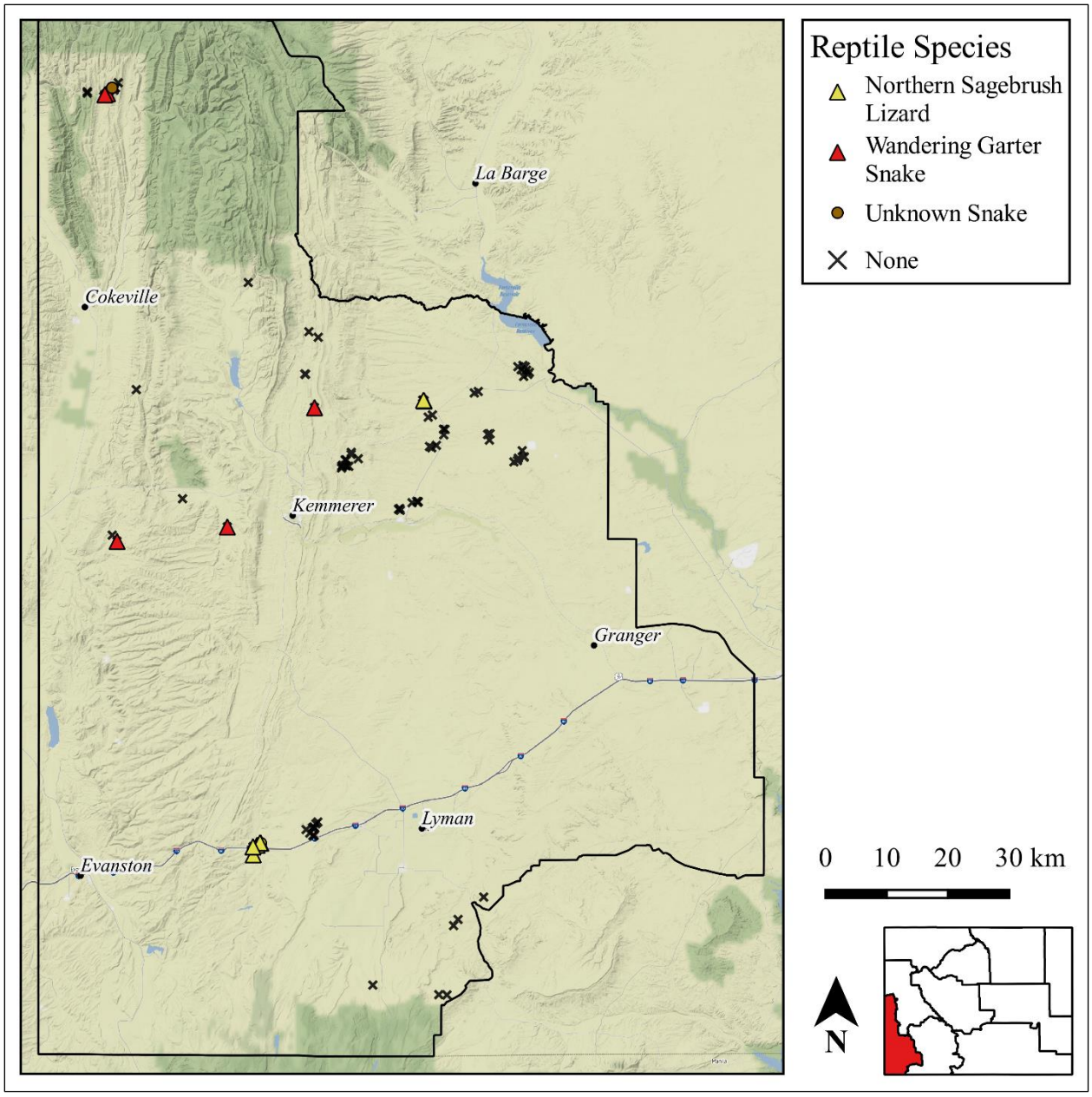


Figure 5. Locations of reptiles detected during visual encounter surveys of the BLM Kemmerer Field Office (KFO) area (black outline) in 2014–2015. Inset shows the location the KFO in Wyoming. Scientific names of species are included in Table 1.

Rawlins Field Office 2016

In 2016, we conducted surveys on lands in the RFO using the Wetland and Riparian VES, Rock Outcrop VES, and Reptile Transect VES protocols, as well as incidental and opportunistic observations. We surveyed a total of 196 sites (Figure 6) and detected 3 amphibian (Table 6, Figure 7) and 4 reptile species (Table 6, Figure 8).

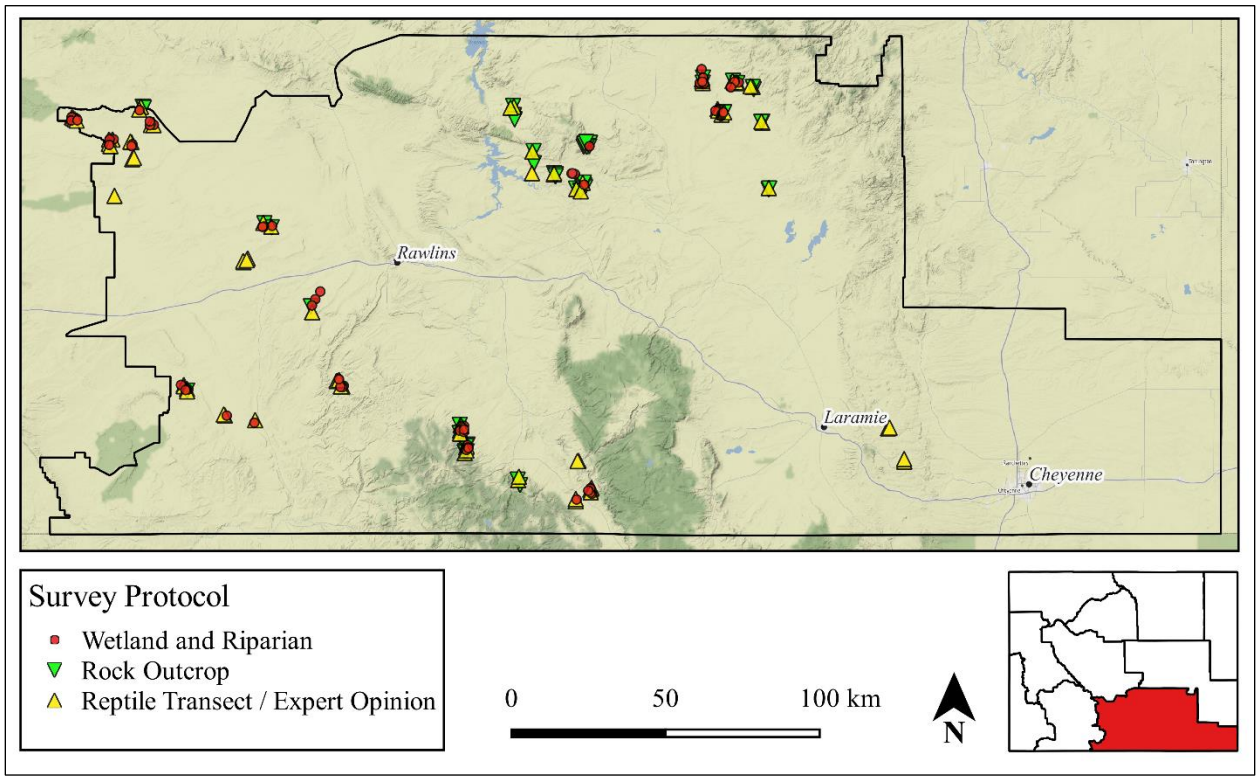


Figure 6. Sites surveyed for reptiles and amphibians in the BLM Rawlins Field Office (RFO) area (black outline) in 2016 using three different visual encounter survey protocols. Inset shows the location the RFO in Wyoming.

Table 6. Number of reptiles and amphibians detected by age class during surveys of the BLM Rawlins Field Office area in 2016. Scientific names of species are included in Table 1.

	Age Class				
Species	Adult	Juvenile	Metamorph	Tadpole/Larvae	Unknown
Boreal Chorus Frog	0	0	26	50–100	0
Great Basin Spadefoot	0	0	0	25–50	0
Tiger Salamander	8	0	0	102–250	0
Possible Tiger Salamander	0	0	0	2–50	0
Northern Sagebrush Lizard	16	0			0
Greater Short-Horned Lizard	6	0			0
Wandering Garter Snake	5	13			0
Unidentified Garter Snake	1	1			0
Prairie Rattlesnake	19	5			2

Wetland and Riparian VES

We visited 85 wetland or riparian sites and conducted surveys at 49 sites with sufficient water. Total survey effort was 25 h 32 min person-time with 2–3 independent observers and averaged 31 person-min per site (range = 6 min – 4 h 4 min). We detected 4 species: Boreal Chorus Frog, Tiger Salamander, Great Basin Spadefoot, and Wandering Garter Snake. Average number of species detected per survey was 0.28 (range = 0–3). Boreal Chorus Frog detections comprised a total of 26 metamorphs and 50–100 tadpoles found at 6 sites in wet meadow, stockpond, permanent lake/pond, temporary lake/pond, and spring/seep habitats. The top model for Boreal Chorus Frog suggested probabilities of detection (p = 0.61; 95% CI: 0.16, 0.93) and occupancy (ψ = 0.13; 95% CI: 0.04, 0.35) did not vary among the RFO in 2016 and the KFO in 2015. Correction of standard errors for over-dispersion ($\hat{c}$ = 2.52) contributed to the poor precision (i.e., wide confidence intervals) of estimates from this model. Tiger Salamander detections comprised a total of 8 adults and 102–250 larvae, as well as 2–50 possible Tiger Salamander larvae, found at 7 sites in temporary lake/pond, permanent lake/pond, stream, stockpond, and wet meadow habitats. We detected 25–50 tadpoles that were likely Great Basin Spadefoot in 1 stockpond. We detected Wandering Garter Snake at 3 sites, including 2 adults and 13 juveniles

in stockponds, wet meadow, and temporary lake/pond habitats. Simple detection probability averaged 0.75 across 23 detections with dual-observer data.

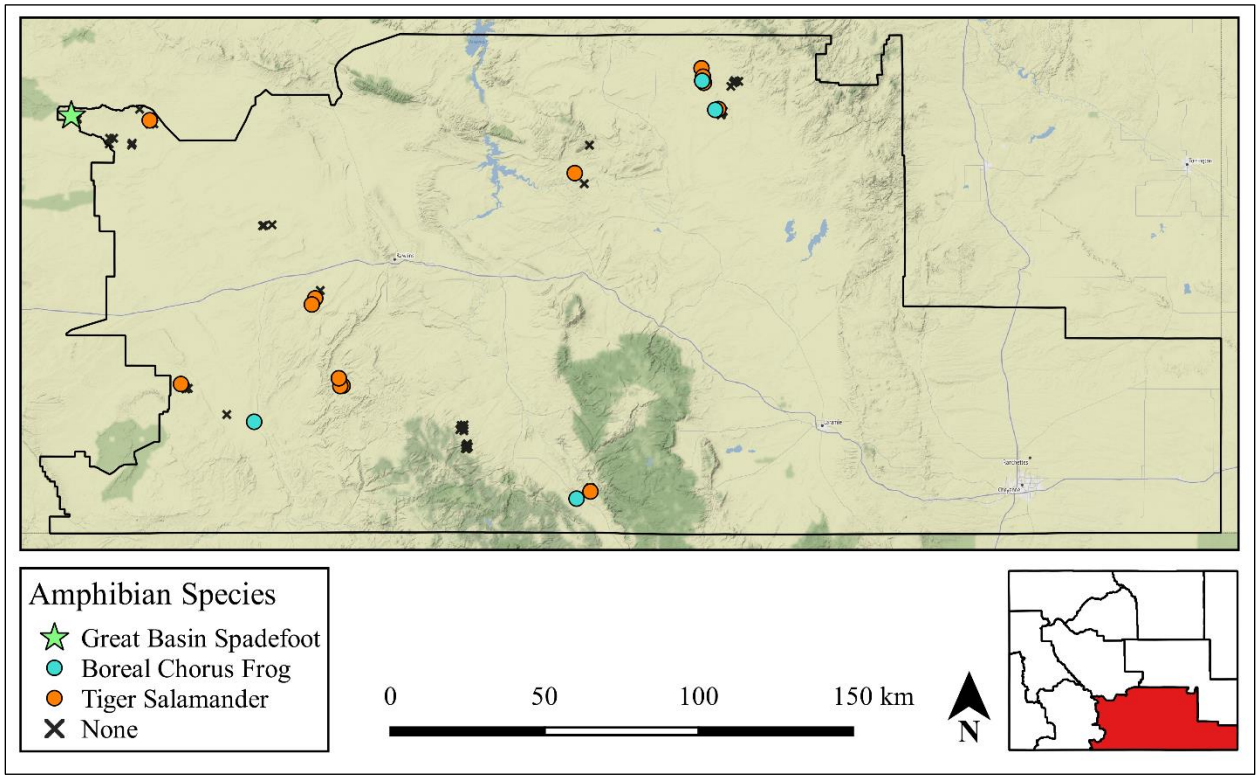


Figure 7. Locations of amphibians detected during visual encounter surveys of the BLM Rawlins Field Office (RFO) area (black outline) in 2016. Inset shows the location of the RFO in Wyoming. Scientific names of species are included in Table 1.

Rock Outcrop VES

We visited 59 potential rock outcrop sites and conducted surveys at 57 sites with suitable habitat. Total survey effort was 14 h 58 min person-time with 2 independent observers and averaged 17 person-min per site (range = 6 min – 1 h). We detected 2 species, consisting of 5 Northern Sagebrush Lizard found at 4 sites and 2 Prairie Rattlesnake found at 2 sites. Average number of species detected per survey was 0.11 (range = 0–1). Simple detection probability averaged 0.50 for 6 detections with dual-observer data. The top model for Northern Sagebrush Lizard suggested detection probability for the RFO in 2016 (p = 0.11; 95% CI: 0.01, 0.58) was lower than the KFO in 2015 (p = 0.72; 95% CI: 0.13, 0.98), and occupancy probability (ψ = 0.32; 95% CI: 0.08, 0.73) did not vary among years/study areas. Correction of standard errors for over-dispersion ($\hat{c}$

= 3.47) contributed to the poor precision (i.e. wide confidence intervals) of estimates from this model.

Expert Opinion VES

We conducted 85 Expert Opinion surveys. Total survey effort was 32 h 18 min person-time with 2 independent observers and averaged 23 person-min per site (range = 12 min – 1 h 28 min). We detected 3 species, comprising 10 Northern Sagebrush Lizard found at 3 sites, 2 Wandering Garter Snake found at 1 site, and 1 Greater Short-Horned Lizard. Average number of species detected per site was 0.06 (range = 0–1). Simple detection probability averaged 0.50 for 3 detections with dual-observer data.

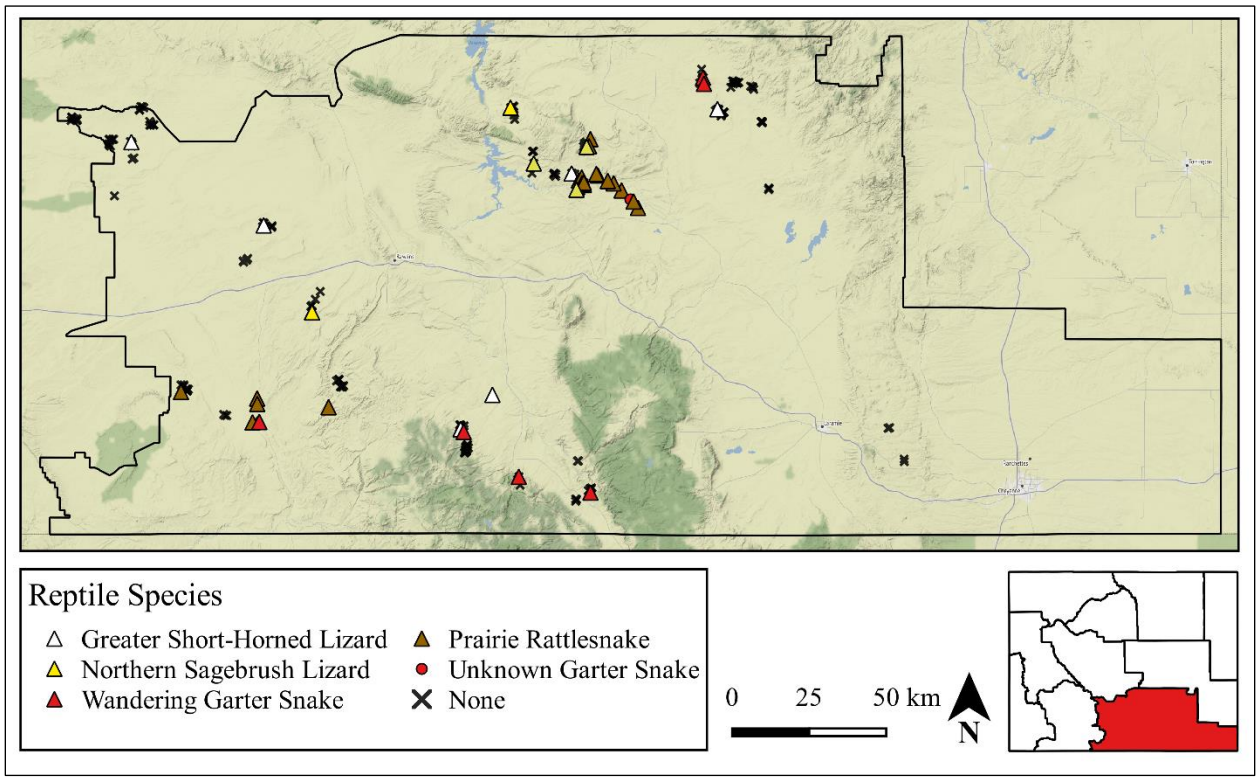


Figure 8. Locations of reptiles detected during visual encounter surveys of the BLM Rawlins Field Office (RFO) area (black outline) in 2016. Inset shows the location of the RFO in Wyoming. Scientific names of species are included in Table 1.

Opportunistic and Incidental Observations

We recorded incidental observations of 4 species, including 5 Greater Short-Horned Lizard, 1 Northern Sagebrush Lizard, 17 Prairie Rattlesnake, 1 Wandering Garter Snake, and 2 unidentified garter snake. Of 20 total snakes, 9 were found alive and 11 dead. Additionally,

observers conducted opportunistic driving surveys of 4 road routes, with 1 route surveyed twice. Total effort for road surveys was 5 h 25 min person-time, with an average of 54 person-min per route (range = 25 min – 1 h 35 min) with 2 observers. These surveys resulted in detections of 6 Prairie Rattlesnake, comprising 2 juvenile, 3 adult, and 1 of unknown age class, with 2 found alive, 2 dead, and 2 unknown. One Prairie Rattlesnake detected during a road survey likely represented the first record of melanism documented in a wild *Crotalus viridis*. While other members of the same genus display this type of morphological variation more frequently (i.e., *C. horridus*), it is seldom seen in *C. viridis*.

Discussion

We field-tested a suite of protocols to survey a diverse group of reptile and amphibian species on BLM lands in Wyoming. Our overall approach was successful in obtaining new location records for 7 amphibian and 5 reptile species. These data will be available to guide planning by BLM field offices and contribute to future efforts by WYNDD to refine distribution models for these species. While our overall approach was successful, the varied life histories and habitat associations of our target species presented challenges to developing an efficient landscape-scale sampling design and comprehensive survey protocol. Reptiles and amphibians in Wyoming are commonly associated with isolated habitat features that are widely dispersed in the landscape, like rock outcrops and wetlands, and the difficulty of identifying these features with remotely sensed data limited the efficiency of our sampling effort. Despite these limitations, we chose to use design-based methods for site selection because of their importance to making rigorous inference on distribution and habitat associations of wildlife.

The Wetland and Riparian VES protocol was effective for detecting amphibians and wetland-associated reptiles, and provided the most observations of any method. Boreal Chorus Frog and Tiger Salamander were the most common species in our study and both were frequently detected with this protocol. Sample sizes of Boreal Chorus Frog detections from this dual-observer survey method were sufficient to estimate probabilities of occupancy and detection using single-season occupancy models, although estimates had relatively poor precision. The Wetland and Riparian VES protocol was also successful in detecting both Plains and Great Basin Spadefoot, and was the source of our only detections of Northern Leopard Frog (2 adults in the KFO and 1 adult in the NFO). Additionally, this protocol was effective for detecting reptiles

associated with wetlands, including two species of garter snake. Site selection and survey methods for Wetland and Riparian VES benefitted from existing spatial data on hydrology and amphibian occurrence, and established methods for visual surveys of wetland habitats. Beginning in 2015, these methods were standardized as the RMAP protocol, and are currently being implemented by biologists and citizen scientists on US Forest Service lands in Wyoming (RMAP 2017).

Nocturnal Call surveys were also effective for detecting Boreal Chorus Frog and both species of spadefoot, but were particularly valuable for detecting species of concern in prairie habitats of the Newcastle Field Office (i.e., Rocky Mountain Toad and Great Plains Toad). Implementing this protocol more widely in eastern Wyoming could improve our understanding of sensitive amphibian species of the Great Plains region, especially on private lands with limited access for visual encounter surveys.

Rock outcrop surveys were effective for detecting Northern Sagebrush Lizard and several species of snakes within the narrowly defined habitats of rocky terrain and outcrops. Although sample sizes of Northern Sagebrush Lizard detections from this dual-observer survey method were sufficient to estimate probabilities of occupancy and detection using single-season occupancy models, the low precision of estimates (i.e., very wide confidence intervals) will limit their value for trend monitoring.

The least effective method was the Reptile Transect VES, which had the broadest habitat criteria among our protocols. After we failed to detect any reptiles using Reptile Transect VES in 2015, we replaced it with Expert Opinion surveys in 2016. Although our objective was to use design-based site selection for all surveys, we acknowledge that ad-hoc approaches, like the Expert Opinion protocol, are sometimes appropriate for areas where information to identify target habitats are lacking or species densities are extremely low. In this case, relying on the expert opinion of biologists to identify suitable habitats in the field can be a more efficient way to obtain location records for rare species, which are essential to improving distribution models to guide systematic surveys in the future. Expert Opinion surveys detected the same reptile species as Rock Outcrop surveys, with the addition of Greater Short-Horned Lizard. While both the Expert Opinion and Reptile Transect protocols included the option of surveying the ant colony habitats used by Greater Short-Horned Lizard, they resulted in only a single detection of this species, with the majority of records from incidental observations in the RFO. Efficiency of

future surveys for Greater Short-Horned Lizard could be improved by focusing effort in ant colony habitats identified using aerial imagery. Lastly, opportunistic observations made along roads comprised a considerable proportion of rattlesnake detections, suggesting systematic road-based sampling could be an effective method to survey snakes in Wyoming.

Our objective was to field-test a suite of survey protocols and we were, thus, able to dedicate only a moderate level of effort to each sampling approach. Combined with the sparse distributions of our target species and the difficulty of sampling their habitats, our level of survey effort resulted in relatively few detections for each species and method. Only the most effective methods and common species had sufficient numbers of detections to support occupancy modeling of dual-observer data. Allocation of effort is a key consideration in the development of monitoring programs, and the limited sample sizes reported here point to the trade-offs inherent in using multiple protocols to sample multiple species. Future efforts to design an integrated monitoring program for reptiles and amphibians will need to balance the need to sample multiple species with the importance of concentrating effort on fewer species and/or survey protocols to obtain sample sizes sufficient to estimate occupancy and trend. The information presented here provides a toolbox of survey protocols and baseline data on occurrence and detectability that will be useful to inform the design of future survey programs for reptiles and amphibians in Wyoming.

Acknowledgments

We would like to thank our partners and funders at BLM. Z. Walker, C. Snoberger, and V. Zero of WGFD collaborated on design of survey protocols. Special thanks to all field technicians: S. Jech, S. Gerstman, B. Thesing, J. Erdmann, C. Faidley, and T. Tronstad.

Literature Cited

Bonnet, X., R. Shine, and O. Lourdais. 2002. Taxonomic chauvinism. Trends in Ecology and Evolution 17:1–3.

Corn, P. S., M. J. Adams, W. A. Battaglin, A. L. Gallant, D. L. James, M. Knutson, C. A. Langtimm, and J. R. Sauer. 2005. Amphibian research and monitoring initiative—concepts and implementation. U.S. Geological Survey Scientific Investigations Report 2005–5015.

ESRI 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.

Estes-Zumpf, W. A., Z. J. Walker, and D. A. Keinath. 2012. Status and distribution of amphibians in the Bighorn Mountains of Wyoming. Report prepared for the Wyoming Game and Fish Department by the Wyoming Natural Diversity Database and Wyoming Game and Fish Department Aquatic Assessment Crew, Wyoming.

Estes-Zumpf, W. A., and D. Keinath. 2012. Monitoring of amphibians and reptiles in the Powder River Basin of Wyoming. Prepared for the Buffalo Field Office of the Bureau of Land Management by the Wyoming Natural Diversity Database, University of Wyoming, Laramie, Wyoming.

Estes-Zumpf, W., Z. Walker, and D. Keinath. 2014. Western amphibian monitoring initiative State Wildlife Grant final completion report. Report prepared for the Wyoming Game and Fish Department Fish Division by the Wyoming Natural Diversity Database, Laramie, Wyoming.

Fiske, I., and R. Chandler. 2011. unmarked: an R package for fitting hierarchical models of wildlife occurrence and abundance. Journal of Statistical Software 43:1–23. Available: http://www.jstatsoft.org/v43/i10/.

Gould, W. R., D. A. Patla, R. Daley, P. S. Corn, B. R. Hossack, R. Bennetts, and C. R. Peterson. 2012. Estimating occupancy in large landscapes: evaluation of amphibian monitoring in the Greater Yellowstone Ecosystem. Wetlands 32:379–389.

Hof, C., M. B. Araújo, W. Jetz, and C. Rahbek. 2011. Additive threats from pathogens, climate and land-use change for global amphibian diversity. Nature 480:516–519.

Keinath, D. A., M. D. Andersen, and G. P. Beauvais. 2010. Range and modeled distribution of Wyoming's species of greatest conservation need. Report prepared by the Wyoming

Natural Diversity Database, Laramie, Wyoming for the Wyoming Game and Fish Department, Cheyenne, Wyoming and the U.S. Geological Survey, Fort Collins, Colorado.

Keinath, D. and M. Kauffman. 2014. Quantifying exposure of Wyoming's wildlife to energy development in the face of expanding production. Report prepared by the Wyoming Natural Diversity Database, Laramie Wyoming and Wyoming Cooperative Fish and Wildlife Research Unit for the Wyoming Game and Fish Department, Cheyenne, Wyoming and the U.S. Geological Survey, Fort Collins, Colorado. December 30, 2014.

Livo, L. J. 2004. Methods for obtaining *Batrachochytrium dendrobatidis* (*Bd*) samples for PCR testing. Department of Integrative Physiology, University of Colorado, Boulder, CO.

MacKenzie, D. I., J. D. Nichols, J. A. Royle, K. H. Pollock, L. Bailey, and J. E. Hines. 2005. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press, Burlington, Massachusetts.

Murphy, M. A., J. S. Evans, and A. Storfer. 2010. Quantifying *Bufo boreas* connectivity in Yellowstone National Park with landscape genetics. Ecology 91:252–261.

Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P .L. Fogden, P. N. Foster, E. La Marca, K .L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. Arturo Sanchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439:161–167.

Rocky Mountain Amphibian Project [RMAP]. 2017. Rocky mountain amphibian project website. Available: http://www.toadtrackers.org/.

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster. 1994. Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institution Press, Washington D.C.

MacKenzie, D. I., and L. L. Bailey. 2004. Assessing the fit of site-occupancy models. Journal of Agricultural, Biological, and Environmental Statistics 9:300–318.

Pike, D. A., B. M. Croak, J. K. Webb, and R. Shine. 2010. Subtle–but easily reversible–anthropogenic disturbance seriously degrades habitat quality for rock-dwelling reptiles. Animal Conservation 13:411–418.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Todd, B. D, J. D. Wilson, and J. W. Gibbons. 2010. The global status of reptiles and causes of their declines. Pp. 47–67 *In* Ecotoxicology of amphibians and reptiles (2nd edition). Sparling, D.W., G. Linder, C.A. Bishop, and S. Krest (eds.). CRC Press, Boca Raton, Florida.

Wyoming Game and Fish Department [WGFD]. 2017. State Wildlife Action Plan. Available: https://wgfd.wyo.gov/Habitat/Habitat-Plans/Wyoming-State-Wildlife-Action-Plan/.

Appendices

Appendix 1A: Wetland and Riparian VES

Rocky Mountain Amphibian Project

Site

Catchment:

Survey Date:		Method	Dual Team
Total # of people helping with surveys:			
Sufficient water present for survey?		Yes No	

Datum: WGS 84

Start Waypt:	**End Waypt:**	*Note if coordinates differed and why
Latitude:	Latitude:	
Longitude:	Longitude	

Approx. survey time (min.):

Start Time	
End Time	

Photo Waypt.: **Photo Easting**

Photo Direction: **Photo Northing**

Photo #	
Who's camera was used?	

Survey Conditions (circle appropriate conditions or fill in blank)

Air Temp	F or C?	Wind	Calm Light Moderate Strong	Cloud Cover
Water Temp @ best habitat (shallows)	F or C?	Precipitation	None Rain Snow Hail	0-25% 25-75% 75-100%
Survey Comments				
Waterbody Type (circle all that apply):	Permanent Lake/Pond Temporary Lake/Pond Active Beaver Pond Inactive Beaver Pond Wet Meadow Marsh/Bog Spring/Seep Backwater/Oxbow Stockpond Stream			

Water pH		pH Method	Indicator strip pH meter	Maximum Water Depth	< 3 ft > 3 f
Water color	Clear Stained	Water Turbidity	Clear Cloudy		
Primary Substrate:	silt/mud sand gravel cobble boulder/rock			Fish Present?	Yes No
Evidence of Cattle Grazing	None Light Heavy veg Heavy shore Heavy veg and shore			Fish Species:	
% of shoreline with emergent vegetation (top of plant sticks out of water):	0% 1-25% 25-50% 50-75% 75-100%				
Site Notes (please provide description!)				Conductivity (if taken):	

Species Detected

Survey 1 *Please mark on map (back of sheet) where you found these species!

Surveyor Name(s)

				*Fill below if sample taken (e.g. chytrid) or if comments			
Species:		Detection Method:	Call Visual	Sample #	Latitude	Longitude	Comments (sex, SVL,...)
# Adults		# Metamorphs					
# Juveniles		# Egg Masses:					
# Tadpoles	1-25 25-50 50-100 100-500 >500						

				*Fill below if sample taken (e.g. chytrid) or if comments			
Species:		Detection Method:	Call Visual	Sample #	Latitude	Longitude	Comments (sex, SVL,...)
# Adults		# Metamorphs					
# Juveniles		# Egg Masses:					
# Tadpoles	1-25 25-50 50-100 100-500 >500						

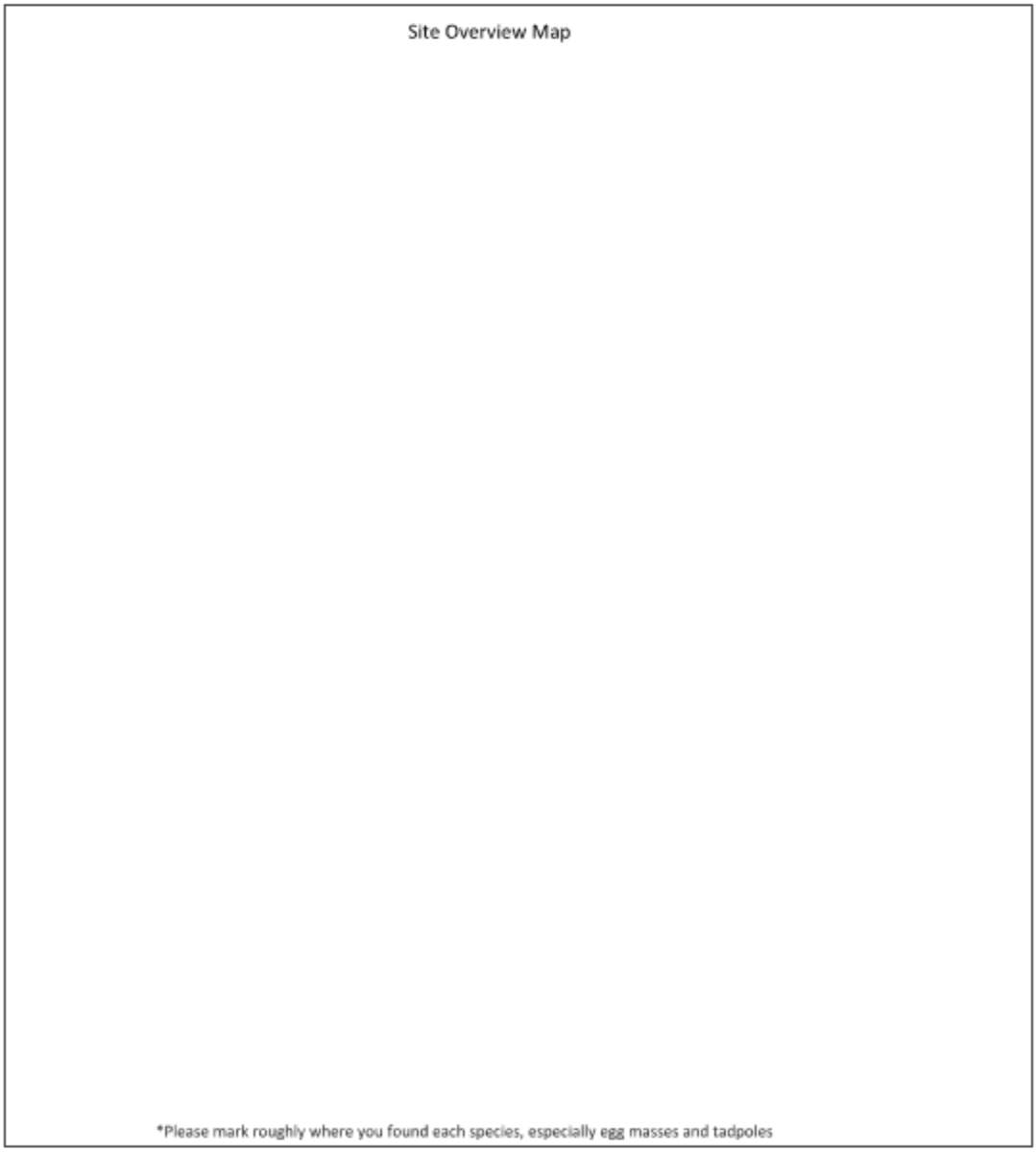
				*Fill below if sample taken (e.g. chytrid) or if comments			
Species:		Detection Method:	Call Visual	Sample #	Latitude	Longitude	Comments (sex, SVL,...)
# Adults		# Metamorphs					
# Juveniles		# Egg Masses:					
# Tadpoles	1-25 25-50 50-100 100-500 >500						

				*Fill below if sample taken (e.g. chytrid) or if comments			
Species:		Detection Method:	Call Visual	Sample #	Latitude	Longitude	Comments (sex, SVL,...)
# Adults		# Metamorphs					
# Juveniles		# Egg Masses:					
# Tadpoles	1-25 25-50 50-100 100-500 >500						

Enter data at www.toadtrackers.org

Send completed datasheets to: Wendy Estes, Dept. 3381 University of Wyoming 1000 E. University Ave., Laramie, WY 82071

Site Overview Map



*Please mark roughly where you found each species, especially egg masses and tadpoles

Note additional species seen and identified (i.e. birds, invertebrates, mammals, plants):

Species Codes :
BCF = Boreal Chorus Frog NLF = Northern Leopard Frog WF = Wood Frog BT = Western (Boreal) Toad
TS = Tiger Salamander CSF = Columbia Spotted Frog

Rocky Mountain Amphibian Project

Site:

Catchment:

Survey Date:	
Start Time	
End Time	

Species Detected*

Survey 1

*Please mark on map (back of site sheet) where you found these species!

Surveyor Name(s)

*Fill below if sample taken (e.g. chytrid) or if comments

Species:		Detection Method:	Call Visual	Sample #	Latitude	Longitude	Comments (sex, SVL,...)
# Adults		# Metamorphs					
# Juveniles		# Egg Masses:					
# Tadpoles	1-25 25-50 50-100 100-500 >500						

*Fill below if sample taken (e.g. chytrid) or if comments

Species:		Detection Method:	Call Visual	Sample #	Latitude	Longitude	Comments (sex, SVL,...)
# Adults		# Metamorphs					
# Juveniles		# Egg Masses:					
# Tadpoles	1-25 25-50 50-100 100-500 >500						

*Fill below if sample taken (e.g. chytrid) or if comments

Species:		Detection Method:	Call Visual	Sample #	Latitude	Longitude	Comments (sex, SVL,...)
# Adults		# Metamorphs					
# Juveniles		# Egg Masses:					
# Tadpoles	1-25 25-50 50-100 100-500 >500						

*Fill below if sample taken (e.g. chytrid) or if comments

Species:		Detection Method:	Call Visual	Sample #	Latitude	Longitude	Comments (sex, SVL,...)
# Adults		# Metamorphs					
# Juveniles		# Egg Masses:					
# Tadpoles	1-25 25-50 50-100 100-500 >500						

Note additional species seen and identified (i.e. birds, invertebrates, mammals, plants):

Species Codes :
BCF = Boreal Chorus Frog NLF = Northern Leopard Frog WF = Wood Frog BT = Western (Boreal) Toad
TS = Tiger Salamander CSF = Columbia Spotted Frog

Enter data at www.toadtrackers.org

Send completed datasheets to: Wendy Estes, Dept. 3381 University of Wyoming
1000 E. University Ave., Laramie, WY 82071

ROCKY MOUNTAIN AMPHIBIAN PROJECT

DUAL OBSERVER METHOD

These surveys were designed to accommodate occupancy modeling of amphibians which will be used to assess long-term trends in Wyoming's amphibian populations. Estimating the probability of detecting a species during a survey is critical to occupancy modeling. Thus, protocols are designed maximize the ability to estimate detection probability. Because egg and larval stages of amphibians are most sensitive to environmental conditions, recording evidence of breeding is critical to any amphibian monitoring program. Survey protocols and datasheets allow surveyors to detect and record evidence of breeding.

General Instructions:

1. Check your catchment information sheet for dates between which you should conduct your surveys. In general, surveys are conducted from early June through late July depending on elevation.

2. Sites should be visited at least twice during the spring/early summer if possible.

3. Do not conduct surveys when it is very windy, snowing, or raining hard.

4. Beware of dead trees! There are a large number of dead trees in Wyoming's forests due to the mountain pine beetle infestation. These trees **WILL** fall in strong winds. Try to park away from trees and avoid hiking or traveling in the forest on windy days or during thunderstorms!

5. Surveys should be conducted between approx. 8AM and 3PM as amphibian activity tends to slow down during the afternoon on hot days. If your catchment requires a long drive or hike to reach it, consider camping the night before so you can begin your surveys in the morning.

6. Each survey area (catchment) has multiple wetland sites to be surveyed. **Consult your catchment map for the location of all survey sites in you catchment.** Coordinates for navigation points on your map are provided and should be used to guide surveys.

7. It is extremely important that **all wetland sites within a catchment be surveyed**. Approximate survey times for each site are listed on your datasheets. Pay attention to your pace and adjust as necessary to finish all sites by mid-afternoon.

8. Survey all amphibian habitat in your catchment. See *Important Tips for Surveyors* for tips on where to survey (i.e. what is habitat). Note if mapped sites are dry. Use a blank datasheet and fill in appropriate information for any new unmapped wetland sites that may be present (e.g. new beaver pond).

9. Record all species detected during surveys, and the number adults, metamorphs, tadpoles (OK to estimate!), and egg masses found. **It is important to record any evidence of breeding (metamorphs, tadpoles, egg mass, or adults in amplexus (mating behavior)).**

10. **Please take at least 1 photo of each species found to confirm species ID.**

11. Photographs also should be taken of animals or egg masses that cannot be identified. Photos can be sent to WGFD or WYNDD for possible ID. If possible photos should include a head, belly, and dorsal (back) photo.

12. Tadpoles should be identified to species if possible. Specimens of unidentified tadpoles can be stored in vials with ethanol and sent to WGFD or WYNDD for possible ID. Only a few tadpoles from each aggregation should be collected for this purpose.

13. Within your catchment, sample **up to** 5 individuals of each species for chytrid fungus (see chytrid sampling procedure). Try to collect chytrid samples from several sites in your catchment rather than all taking all 5 samples from 1 site. ***NOTE: Catchments on the Bridger-Teton National Forest do not need to have chytrid samples collected in 2015.**

Dual Observer Protocols:

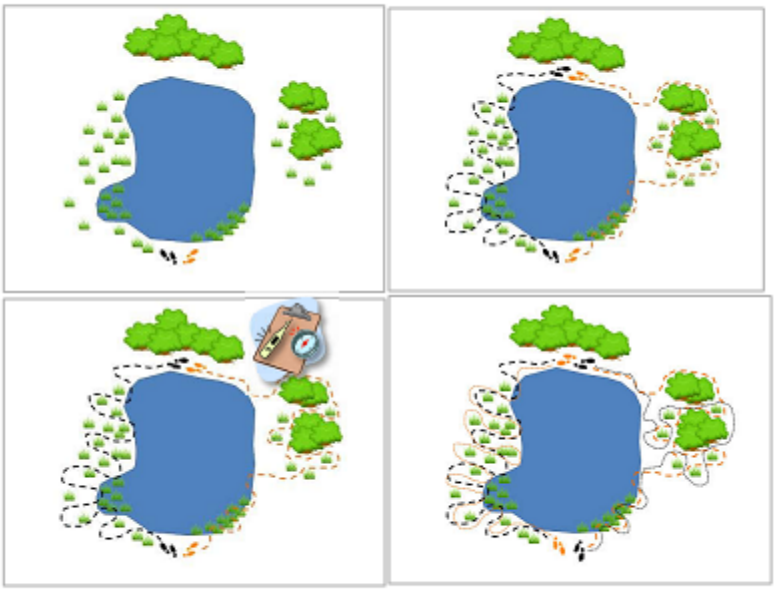
1. Under the Dual Observer Method, two people conduct **separate** surveys at a site. This provides 2 independent surveys of each site for each visit to the catchment and allows us to estimate detection probability. **Each surveyor fills out a separate datasheet!**

2. You will be given 2 datasheets for each site - one for each of the 2 surveyors. Use the appropriate datasheet for each site within a catchment.

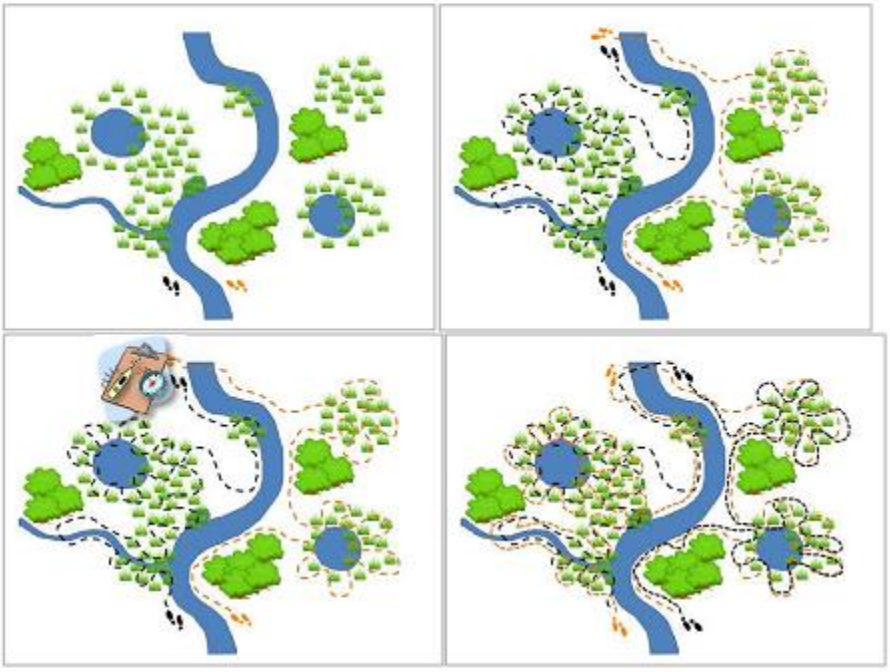
3. Each site should be surveyed **independently** by each surveyor with **no discussion of amphibians found.** Do not alter your datasheet based on what your partner found. Knowing how often amphibians present are actually detected is CRITICAL to estimating trends in amphibian populations.

4. Walk slowly in a zig-zag pattern, surveying evenly across **accessible moist habitat** to cover as much potential habitat as possible. Consult catchment maps for any "hidden" water bodies.

5. At pond/lake sites, surveyors should walk in opposite directions around the pond searching for amphibians. When surveyors meet at the far end, stop for 10 minutes and quietly **fill out the site and survey conditions** on the main site datasheet before continuing with searches. This procedure allows amphibians disturbed by the first surveyor to resume normal behavior before the second surveyor searches that shore.



6. Along streams and in complex wetlands, surveyors should walk on either shore without discussing what species they find or adding amphibians brought to their attention by their partner. Each surveyor must survey both sides of the stream, so a surveyor should survey down a stream on the opposite side he/she walked up it.



7. **Dipnet** every 5-10m or in patches of good habitat for amphibian larvae (quiet inlets/backwater areas or patches of emergent vegetation). Each dipnet event should consist of at least five sweeps with the net.

8. **Surveyors should make sure that datasheets are completely filled out and that all site and survey conditions have been recorded before leaving the catchment.**

9. Surveyors **MUST** follow chytrid fungus decontamination procedures after leaving the sites and before surveying another site **in a different watershed**. Decontamination should also occur between isolated sites (i.e. upland ponds and potholes) within a catchment whenever possible.

Equipment List:

- Tall boots/waders
- Dip net
- Watch
- GPS unit
- Compass
- Camera
- Datasheets & survey protocols
- Pencil or pen
- Thermometer for air and water temperature
- Sterile swabs and vials for chytrid sample collection
- pH indicator strips
- Powder-free latex or nitrile gloves
- Decontamination equipment (kept in vehicle unless surveying in different watersheds). Includes 10% bleach solution, water, scrub brush, sprayer/bucket. Commercial fungicides such as Virkon can be used as well.

Important Tips for Surveyors!

****** You will likely get wet! ☺ Bring a change of clothes (at least extra socks)!! *******

- ***Where do I look for amphibians?***
  - Different amphibians and life stages prefer different habitat. But during the spring and early summer, most are found in moist areas near water bodies.
  - Survey from the shoreline out to the edge of the riparian area or to where the soil is no longer moist.
  - Make sure to sample different habitat types within the survey site (i.e. emergent vegetation along the shoreline, mud flats, sedges, meadow grasses, willows).
    - Tadpoles and egg masses will be found in the water along the shoreline.
    - Tadpoles love warm water and can also be found in several inches of water in wet meadows.
    - Metamorphs (toadlets and froglets) like warm moist areas where they can bask in the sun but that also offer some cover (grasses, sedges, willows) to hide them from predators.
    - Adults only need water for breeding. After that, they can be found across a range of moist habitats within the riparian area depending on the species, time of day, and weather.
- ***How will I see amphibians?***
  - Most amphibians are not seen until they move, and many are quite small. Look for movement in the grass (even tall grass) and water to help you find amphibians.
  - Most amphibians won't move until you get close to them. You can increase your chances of seeing an amphibian by walking in zig-zags rather than straight lines and by using your dip net to part the grass or poke under willows, stream banks, etc.
  - Fortunately, the most difficult amphibian to see (because it's so small) is the easiest to hear. Boreal Chorus Frogs have a very distinctive call and often call all day long during the breeding season. So keep your ears open while at each site and make sure you learn the call of Wyoming tiniest frog!
- ***How do I hold an amphibian?***
  - Carefully and only when you need to (for identification or chytrid sampling)!
  - If necessary to catch an amphibian to ID it, catch and hold it in your dip net while you look at it.
  - NEVER HANDLE AN AMPHIBIAN WITH YOUR BARE HANDS IF YOU HAVE BUG-SPRAY ON YOUR HANDS!!!
    - Amphibians are VERY sensitive to chemicals in bug spray, lotions, and sunscreens.
    - Use powder-free latex or nitrile gloves…or keep hands free of chemicals and rinse well in pond or creek water before and after handling.
    - Use a different set of gloves for each amphibian handled to reduce the risk of transferring chytrid among individuals.

Appendix 1B: Rock Outcrop VES

Rock Outcrop Survey Form

Survey Name:		Date:		Time:	Observer:
Town/Range/Sect.	Picture of Habitat:		Aspect of slope:	Habitat Description:	
	Air Temp:				
Datum:	Start GPS waypt:		Start Latitude:		Start Longitude:
	End GPS waypt:		End Latitude:		End Longitude:

Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Other species present? (describe sign or observations of other taxa detected during survey):				Total Search Time:

Survey Name:		Date:		Time:	Observer:
Town/Range/Sect.	Picture of Habitat:		Aspect of slope:	Habitat Description:	
	Air Temp:				
Datum:	Start GPS waypt:		Start Latitude:		Start Longitude:
	End GPS waypt:		End Latitude:		End Longitude:

Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Other species present? (describe sign or observations of other taxa detected during survey):				Total Search Time:

ROCK OUTCROP VES

Overview: Because reptiles have a dispersed distribution on the landscape, it is difficult to find survey methods that efficiently locate them. One potentially high impact of oil and gas development on reptiles is mortality from being hit by vehicles while basking or crossing roads. Reptiles are particularly vulnerable when migrating from their winter hibernaculum (often shared between species) to feeding grounds in early spring. These surveys attempt to locate important hibernating locations to measure the potential impact of nearby roads and inform future mitigation measures.

Protocol: Identified south-facing, rocky slopes, will be canvassed for basking reptiles by cautiously turning over rocks, logs, etc. Keep your ears out for prairie rattlesnakes when doing this and avoid confrontations. GPS the locations where surveying starts and stops. Take pictures when possible.

Rock outcrops can be critical habitat for other species as well. Please note all evidence or visual observations of other species (bats, rabbits, birds, etc.) detected. **Note coordinates of all bat locations.

Some Species Codes:

NSL – Northern Sagebrush Lizard
NRB – Northern Rubber Boa
EYR – Eastern Yellow-bellied Racer
PR – Prairie Rattlesnake
PHS – Plains Hog-nosed Snake
PM – Pale Milksnake
BS – Bullsnake
WGS – Wandering Gartersnake
PGS – Plains Gartersnake
VGS – Valley Gartersnake
RSGS – Red-sided Gartersnake

Appendix 1C: Reptile Transect VES

Reptile Transect Survey Form

Transect Name:		Date:	Time:	Observer:
Town/Range/Sect.	Picture of Habitat:	Habitat Description:		
	Air Temp:			
Datum:	Start GPS waypt:	Start Latitude:		Start Longitude:
	End GPS waypt:	End Latitude:		End Longitude:

Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Other species present? (describe sign or observations of other taxa detected during survey):				Total Search Time:

Transect Name:		Date:	Time:	Observer:
Town/Range/Sect.	Picture of Habitat:	Habitat Description:		
	Air Temp:			
Datum:	Start GPS waypt:	Start Latitude:		Start Longitude:
	End GPS waypt:	End Latitude:		End Longitude:

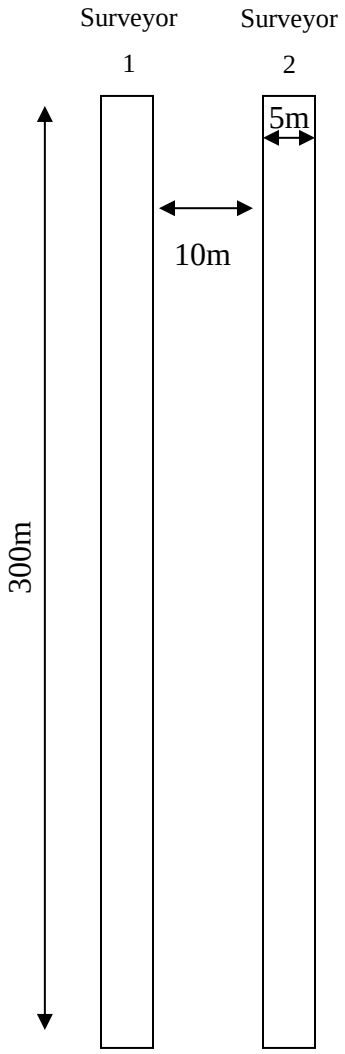
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Other species present? (describe sign or observations of other taxa detected during survey):				Total Search Time:

Reptile Transect Survey Protocols

Protocol: Conduct 1 reptile transect for each wetland/riparian amphibian survey, or **at least 2 reptile transects per section**.

1. After each wetland survey, walk ~200m in the direction of best reptile habitat (sandy openings with ant mounds, prairie dog colonies, base of rock outcrops, etc.).

   ***NOTE**: If no wetlands in section, walk to best habitat (above) and follow direction below.
2. Take a starting waypoint. Choose next available random compass direction from back of notebook and project your endpoint 300m in the chosen direction.
3. Surveyors should start about 10m apart and survey parallel transects in the direction of the endpoint.
4. Each transect should be ~5m wide and 300m long.
5. Record all species detected during surveys.
6. Make sure to look under any debris (including trash), shrubs, and around ant mounts for lizards and snakes.



Appendix 1D: Nocturnal Calling Route

Nocturnal Calling Route Data Sheet

Date_____ Observer _____
Route Name_____ Catchment Name _____

Start Time_____ End Time _____ Distance btw stops _____ mi.
Starting GPS Coordinates: Latitude (X)_____ Longitude (Y)_____
Ending GPS Coordinates: Latitude (X)_____ Longitude (Y) _____
Time since rain: _____days
Barometric Pressure: Start_____ End_____
Relative Humidity: Start_____ End_____
Weather Code[1]: Start_____ End_____
Wind Speed: Start_____ End_____ GPS Unit #:_____
Temperature: Start_____ End_____

Stop Number	Species Code	Intensity[2]	X	Y	Bearing	Estimated Distance	Ambient Noise[3]	Waypoint Number

Nocturnal Call Surveying Protocol and Code Keys

Warm and humid evenings (especially after rain) should be targeted for nocturnal call surveys. Target roads will be driven **at least 30 minutes after sunset**. The route name, starting time of the survey, starting location, time since last rain, barometric pressure, relative humidity, weather code, and wind speed will all be measured at the beginning of each route. GPS location, species, intensity, etc will be measured at each stop.

Survey Protocol

Begin survey at the predefined location. Get out of vehicle and record all starting location and weather information on the datasheet.

Surveyors may record location and weather data together but must collect species calling data independently. Surveyors should only write down what they hear (or see) and not change their datasheet based on what their partner heard or saw.

Listen and record for 3 min. Slowly and quietly turn in a circle to pick up calling in all directions. Record the stop number (1-6), species code, calling intensity, bearing and estimated distance to calling individuals, and other relevant data for each individual or group heard calling.

Stop every 0.2 mi along the route for 1 mile (6 stops) listening for 3 minutes at each stop for a total of eighteen survey minutes. Record all ending information on datasheet.

Once the survey of the route is complete, the time, GPS location, barometric pressure, relative humidity, weather code, and wind speed will once again be measured. If the species heard is identified and not on the species code list, the technician will abbreviate the new species and make a note at the bottom of the data sheet.

Equipment List

GPS + extra batteries
Headlamp
Countdown timer
Maps
Recording of species' calls
Datasheets
Weather collecting device (i.e., Kestrel)
Compass

CODES

1. Weather Code: **0** = 0-15% cloud cover; **1** = 16-50% cloud cover; **2** = 51-75% cloud cover; **3** = 76-100% cloud cover; **4** = fog; **5** = light rain; **6** = downpour heavy rain; **7** = snow
2. Calling Intensity: **0** = no calling; **1** = number of calling individuals countable, calls not overlapping; **2** = number of calling individuals countable, calls overlapping; **3** = chorus constant, individuals not countable; **4** = animals visually observed
3. Ambient Noise: **0** = no noise at all; **1** = very little noise, whisper audible; **2** = medium noise, normal speaking voice audible; **3** = high noise, yelling audible

Species Code Key: **BCF**: Boreal Chorus Frog **BF**: Bullfrog **NLF**: Northern Leopard Frog
GPT: Great Plains Toad **RMT**: Rocky Mountain Toad **PSFT**: Plains Spadefoot Toad
GBSFT: Gr. Basin Spadefoot

Appendix 1E: Opportunistic and Incidental Observations

Incidental Observation Data Sheet

Observation #:	Observer(s):	Date:	Time:	Habitat Description:
Datum, Zone:	X-coordinate (Easting or Longitude):	Y-coordinate (Northing or Latitude):	GPS waypoint #:	
Species:	Number of individuals:	Dead or Alive	Picture #:	Specimen Collected? Y N
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
Datum, Zone:	Y-coordinate (Northing or Latitude):	X-coordinate (Easting or Longitude):	GPS waypoint #:	
Species:	Number of individuals:	Dead or Alive	Picture #:	Specimen Collected? Y N
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
Datum, Zone:	Y-coordinate (Northing or Latitude):	X-coordinate (Easting or Longitude):	GPS waypoint #:	
Species:	Number of individuals:	Dead or Alive	Picture #:	Specimen Collected? Y N
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
Datum, Zone:	Y-coordinate (Northing or Latitude):	X-coordinate (Easting or Longitude):	GPS waypoint #:	
Species:	Number of individuals:	Dead or Alive	Picture #:	Specimen Collected? Y N
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
Datum, Zone:	Y-coordinate (Northing or Latitude):	X-coordinate (Easting or Longitude):	GPS waypoint #:	
Species:	Number of individuals:	Dead or Alive	Picture #:	Specimen Collected? Y N
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
Datum, Zone:	Y-coordinate (Northing or Latitude):	X-coordinate (Easting or Longitude):	GPS waypoint #:	
Species:	Number of individuals:	Dead or Alive	Picture #:	Specimen Collected? Y N
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
Datum, Zone:	Y-coordinate (Northing or Latitude):	X-coordinate (Easting or Longitude):	GPS waypoint #:	
Species:	Number of individuals:	Dead or Alive	Picture #:	Specimen Collected? Y N
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
Datum, Zone:	Y-coordinate (Northing or Latitude):	X-coordinate (Easting or Longitude):	GPS waypoint #:	
Species:	Number of individuals:	Dead or Alive	Picture #:	Specimen Collected? Y N
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
Datum, Zone:	Y-coordinate (Northing or Latitude):	X-coordinate (Easting or Longitude):	GPS waypoint #:	
Species:	Number of individuals:	Dead or Alive	Picture #:	Specimen Collected? Y N

HOW TO SWAB AMPHIBIANS FOR CHYTRID FUNGUS[1]

Animals should be collected with clean decontaminated equipment and individually handled with **fresh** disposable gloves. Chytrid fungus can be transferred between individual amphibians, so please **do not handle multiple animals with the same gloves** or place multiple animals in the same container. Otherwise, you risk infecting a previously uninfected animal.

Chytrid fungus infects the skin of amphibians. Sample for chytrid fungus by gently but firmly swabbing skin on the ventral (belly) surface of an amphibian with a sterile cotton swab. The swab is then placed in a vial with 95% ethanol and sent to a lab for testing. It is important to follow instructions to: 1) not transfer chytrid fungus between animals, and 2) not contaminate samples taken from different animals.

To sample an amphibian for chytrid fungus, you will need:

- Clean disposable powder-free latex or nitrile gloves
- A sterile cotton swab
- A vial with 95% ethanol for storage of sample

Here is how to collect a chytrid sample from an amphibian:

1) Hold the animal gently but firmly so that you can swab the lower half of the underside of the animal. Use a rolling/scraping motion to sample skin cells on the belly, groin, inner thighs, and hind foot webbing (Figure 1).

FIGURE 1. Swabbing ventral surface of amphibian.

2) Place the swab in the vial. Cut or break the stick short enough so that the cotton swab sits in ethanol and the lid can be securely tightened (Figure 2).

FIGURE 2. Insert swab or stick into tube with sample at bottom of tube.

3) Record the sample number (written on the vial) on your datasheet, along with the species name and other relevant information when available (age, sex, GPS location, abnormalities, etc.).

[1]Adapted from: Livo, L.J. 2003. Methods for obtaining *Batrachochytrium dendrobatidis* (Bd) samples for PCR testing. Department of Integrative Physiology, University of Colorado, Boulder, Colorado.

Appendix 1G: Fungal Decontamination Procedures

Fungal and Viral Pathogen Decontamination Procedures and Useful References on Fungal Pathogens

Bryce A. Maxell, Grant Hokit, Jeff Miller, Kirwin Werner

When to Decontaminate

1. After any site where dead, dying, or ill animals are encountered
2. Between sites located in different watersheds
3. Between individual sites that are surveyed when traveling distances greater than 5 kilometers or between definitive clusters of sites.
4. Between all breeding sites of sensitive species that are surveyed and separated by more than 1 kilometer.

What to Decontaminate

1. Boots and waders
2. Dipnets
3. Fingernails
4. Any other body parts, clothing, or other equipment that was exposed to waters or mud.

Washing and Decontamination Procedures (separate issues)

1. Washing - Once surveys are completed at a site or watershed scrub and rinse all equipment to remove any lingering mud. In general it is a good idea to do this between all sites if possible.
2. Decontamination - Prepare a mixture of 10% bleach by putting 4 ounces of bleach (half cup) in one gallon of clean water in a waterproof tub or bucket that can be carried in your vehicle between watersheds or sites. Use a fresh bottle of bleach each field season for this. Also in order to ensure that concentrations remain around 10%, a new bleach mixture should be made on a regular basis. If the solution of disinfectant becomes cloudy or brown with mud, silt, and vegetation, it should be discarded and a fresh solution made. Diluted bleach solutions should also be discarded after decontaminating equipment from any site where dead, dying, or ill animals are encountered. When discarding used bleach pour it out at least 30-40 meters away from water.
3. After rinsing equipment dip and thoroughly scrub individual items in the container of 10% bleach. An alternative approach for remote sites and where carrying a tub of bleach is impractical is to spray rinsed equipment with a concentrated (25-30%) bleach solution out of a large spray bottle and then let equipment dry between sites.
4. Do not rinse bleached equipment between sites. Instead allow the bleach to remain on the equipment to ensure that all fungal pathogens are killed. Most bleach will evaporate between sites so the amount of bleach introduced at the next site should be quickly diluted.

Handling Ill or Dying Animals

1. When handling ill or dying animals at a site use fresh rubber gloves for each animal to ensure that you are not transferring pathogens between individual animals.
2. Place individual animals in individual zip lock bags and keep them on ice continuously prior to shipping them to a pathologist for analysis.