

# WESTERN AMPHIBIAN MONITORING INITIATIVE

## STATE WILDLIFE GRANT FINAL COMPLETION REPORT

*Wendy Estes-Zumpf<sup>1</sup>, Zachary Walker<sup>2</sup>, and Douglas Keinath<sup>1</sup>*

<sup>1</sup> Wyoming Natural Diversity Database, University of Wyoming, 1000 E. University Avenue,  
Dept. 3381, Laramie, WY 82071

<sup>2</sup> Wyoming Game and Fish Department, 260 Buena Vista, Lander, WY 82520



October 2014

Prepared for:

Wyoming Game and Fish Department  
Fish Division  
5400 Bishop Blvd  
Cheyenne, WY 82009

## Table of Contents

Abstract.....	3
Introduction .....	5
Purpose & Objectives.....	6
Methods.....	6
Study Area.....	6
Amphibians Species of Interest .....	7
Species' Distributions.....	8
Boreal Toad Breeding Status.....	8
Amphibian Surveys .....	9
Species Occupancy.....	10
Chytrid Fungus .....	11
Boreal Toad Genetics .....	11
Long-term Amphibian Monitoring.....	11
Results.....	13
Amphibian Surveys .....	13
Species' Distributions.....	13
Boreal Toad Breeding Status.....	14
Species Occupancy.....	16
Chytrid Fungus .....	17
Boreal Toad Genetics .....	17
Long-term Amphibian Monitoring.....	18
Discussion.....	19
Future Directions .....	20
Acknowledgements.....	21
Literature Cited .....	22
Tables .....	25
Figures.....	26
Appendix.....	43

## Abstract

Half of amphibians native to Wyoming are considered Species of Greatest Conservation Need by the Wyoming Game and Fish Department (WGFD) largely due to lack of knowledge about the status and distribution of these species in the state. The WGFD and the Wyoming Natural Diversity Database (WYNDD) have worked closely for the past several years to fill information gaps for amphibians across the state to allow for more informed management of amphibians and their habitat in light of recent global amphibian declines. We sought to address the needs of resource managers in western Wyoming by conducting a baseline inventory and developing a long-term monitoring plan for 5 species of amphibians in the Bridger-Teton National Forest (BTNF) and upper Green River drainage.

Biologists from WYNDD, WGFD, and BTNF collaborated to identify gaps in our knowledge of species distributions and then conducted 862 standardized amphibian surveys from 2012-2014 in order to fill those information gaps. The Western (boreal) Toad (*Anaxyrus boreas*) was detected in the northern part of the study area and along the eastern portion of the Wyoming Range. We did not detect the boreal toad during surveys in the western half of the Wyoming Range and only documented the species in 2 isolated drainages in the Wind River Range. Analysis of data on boreal toad breeding sites visited in the past 5 years revealed that the following drainages are important population centers for the species in the study area: South Horse Creek, Pass Creek, North Horse Creek, Buck Creek, Chall Creek, North Fork Middle Beaver Creek, and Gypsum Creek. The Columbia Spotted Frog (*Rana luteiventris*) was found throughout the northern and western portions of the study area but was absent from the Wind River Range and showed evidence of a decline in eastern portions of the Wyoming Range. Boreal Chorus Frogs (*Pseudacris maculata*) were the most widely distributed species in the study area and the only species found throughout the Wind River Range. Tiger Salamanders (*Ambystoma mavortium*) also were widespread in the study area; however, we only detected salamanders at a few low-elevation sites in the Wind River Range. We did not document Northern Leopard Frogs (*Lithobates pipiens*) at any survey sites in the study area.

We modeled occupancy rates for each species across the study area after accounting for species-specific detection probabilities. The Boreal Chorus Frog was the most common species in the study area, occupying approximately 47% of sites. The Tiger Salamander was the second most common species (19%), followed by the boreal toad (13%) and the Columbia Spotted Frog (9%). Boreal Chorus Frogs had the highest detection rates (detection probability = 84%) while boreal toads had the lowest (56%). These occupancy rates can be used as a baseline against which the results of future monitoring efforts can be compared to assess amphibian trends in the BTNF and upper Green River drainage.

Amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) is responsible for amphibian declines worldwide. To determine how prevalent the pathogen is in the study area, we collected a total of 280 chytrid fungus samples from all amphibian species detected. Chytrid fungus was detected in boreal toads, Boreal Chorus Frogs and Columbia Spotted Frogs throughout the study area. Chytrid fungus was documented at many boreal toad breeding sites and was particularly prevalent within the Wind River Range, despite the remoteness of that

range. Currently, we do not know the extent to which chytrid fungus is affecting amphibian species in the study area.

Lastly, we worked with the BTNF and other collaborators to design and implement an effective and sustainable long-term amphibian monitoring program in the region that will allow resource managers to assess the population status of local amphibian species. The occupancy-based study design agreed upon by collaborators is compatible with other monitoring datasets in the region. Standardized monitoring protocols and data collection practices were developed and will be implemented at 36 catchments (5 in each ranger district and 6 in wilderness areas) across the BTNF. Monitoring catchments were selected using stratified sampling across areas most likely to provide amphibian habitat. We will continue to work with all collaborators to evaluate and adjust the monitoring program as needed ensure that it meets the needs of regional resource managers and is both effective and sustainable.

*Recommended Citation:*

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

## Introduction

Historically, amphibians have received little attention from wildlife and resource managers. In Wyoming, half of the native amphibian species are considered Species of Greatest Conservation Need (SGCN) largely due to lack of knowledge about the status and even the distribution of these species in the state. The Wyoming Game and Fish Department (WGFD) and the Wyoming Natural Diversity Database (WYNDD) have been working closely for the past several years to fill information gaps for amphibians across the state. Information on the distribution and status of amphibian populations is essential for the development of effective science-based resource management.

The need to improve our understanding of amphibians in Wyoming is particularly important given recent worldwide amphibian declines. Amphibians are currently considered the most threatened vertebrate class in the world (Stuart et al. 2004, Hof et al. 2011). Thirty-two percent of all amphibian species are threatened with extinction or are already extinct (Stuart et al. 2004). Populations of several amphibian species, including the Western Toad (*Anaxyrus boreas*; hereafter referred to as the boreal toad), Columbia Spotted Frog (*Rana luteiventris*), and Northern Leopard Frog (*Lithobates pipiens*) are known to be declining in the Rocky Mountain region. Known threats to Rocky Mountain amphibians include diseases such as amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) and Ranavirus, pesticides, herbicides, environmental pollutants, invasive species, non-native species, ultraviolet radiation, and habitat loss and fragmentation. Climate change and changes in habitat due to the recent mountain pine beetle outbreak also pose significant potential threats to amphibians in the Rocky Mountains (Jorgensen 1986, Reading 2007, McMenemy et al. 2008, Griffiths et al. 2010). More concerning is that these threats likely work synergistically against amphibian populations. Substantial evidence links climate change with amphibian declines due to chytrid fungus (Pounds 2006, Rohr et al. 2008, Rohr and Raffel 2010, etc.) suggesting that interactions between these threats could lead to significant losses in amphibian biodiversity in the Rocky Mountain region.

Although amphibian populations in the Rocky Mountain west face the combined effects of many threats, effective monitoring largely is lacking. Some monitoring occurs on national park lands and at specific sites for certain species but monitoring at broader spatial extents is rare. Data on Wyoming's amphibians largely are restricted to individual records from one-time projects or incidental sightings, preventing both federal and state resource managers from assessing population trends, identifying problems, and adjusting management plans in a timely manner.

We sought to address the needs of resource managers by conducting a baseline inventory and developing a long-term monitoring plan for amphibians in western Wyoming. We focused on 5 amphibian species in the Bridger-Teton National Forest (BTNF) and upper Green River drainage. These species were the boreal toad, Columbia Spotted Frog, Northern Leopard Frog, Boreal Chorus Frog (*Pseudacris maculata*), and Tiger Salamander (*Ambystoma mavortium*). The first three species are Wyoming SGCN. Currently, population status for all 5 species is unclear and existing data do not allow assessment of population trends. The results

of this study will be used to establish a baseline from which population trends can then be determined following the implementation of the proposed long-term monitoring plan.

## Project Objectives

1. Coordinate with land management agencies and create interest in future amphibian monitoring
2. Compile all previously-collected data on boreal toads, Northern Leopard Frogs, and Columbia Spotted Frogs in their northern range in Wyoming to highlight potential areas for monitoring and future locations for surveys
3. Revisit known boreal toad breeding locations in the upper Green River drainage and survey for all amphibian species
4. Assess changes in population status of revisited boreal toad breeding locations to determine if populations have declined or remained stable over the past decade
5. Conduct additional surveys in previously un-surveyed watersheds in the upper Green River drainage and adjacent mountain ranges for boreal toads, Northern Leopard Frogs, Columbia Spotted Frogs, Boreal Chorus Frogs, and Tiger Salamanders
6. Conduct chytrid testing at all locations found to contain boreal toads
7. Design a monitoring program based on occupancy modeling for amphibians in the Bridger-Teton National Forest and other portions of the upper Green River drainage
8. Collaborate with other agencies to train local biologists including agency personnel, consultants, and other interested parties, to conduct this monitoring in future years
9. Establish a central data repository at the WYNDD where all cooperating agencies can submit and obtain monitoring data.

## Methods

### *Study Area*

We conducted amphibian survey and monitoring efforts in the BTNF and upper Green River drainage in western Wyoming (Figure 1) within historic boreal toad, Northern Leopard Frog, and Columbia Spotted Frog habitat. Lands in the study area are managed for multiple uses, including timber, cattle grazing, recreation, hunting, fishing, and wildlife. Large portions of the study area are designated wilderness areas and are both rugged and remote, requiring access by backpacking or horse-packing.

The study area ranges in elevation from 1712m to 4210m and contains multiple mountain ranges including the Wind River Range on the east, the Wyoming Range and Snake River Range on the west, and the Gros Ventre Range to the north. Mountainous regions of the study area contain mixed conifer and aspen (*Populus tremuloides*) forests, alpine tundra, and mountain meadows. Amphibian habitat in the study area includes riparian areas and oxbow

ponds adjacent large rivers (i.e. the Green River and Snake River) and streams, beaver ponds, pothole ponds, mountain lakes, wet meadows, bogs, and springs. Willows (*Salix* spp.) are common in many riparian areas. Many of the streams and lakes in the study area contain native and non-native trout. Beaver are common in the western and northern portions of the study area, but largely absent from much of the Wind River Range.

### *Amphibian Species of Interest*

Boreal toad - The boreal toad has declined in distribution and abundance in parts of its range in the past 20 years, with dramatic declines and local extinctions throughout the southern Rocky Mountains (BTRT 2001, Keinath and McGee 2005, USFWS 2012). The boreal toad is listed as endangered by Colorado and New Mexico and is ranked as an NSS1 species in Wyoming by the WGFD due to declining populations. The species is genetically divided into several major clades, two of which (Eastern Clade Western Toad and Northwestern Clade Western Toad) occur in Wyoming (Goebel et al. 2009, Switzer et al. 2009). In 2012, the U.S. Fish and Wildlife service issued a positive 90-day finding on a petition to list all or a portion of the Eastern Clade Western Toad as endangered or threatened under the Endangered Species Act (USFWS 2012). Probable reasons for declines include chytrid fungus, habitat modifications, non-native species, environmental pollutants, and changes in micro- and macro-climates. Although previous survey work was conducted in portions of this species' range in Wyoming, monitoring efforts have not been continued for many previously identified populations. Chytrid fungus has been documented in boreal toad populations in the upper Green River watershed and it is imperative to determine if this and/or other threats are effecting local populations. The boreal toad is a USFS Region 4 Sensitive Species.

Columbia Spotted Frog – The Columbia spotted Frog is listed as an NSS3 species in Wyoming by the WGFD and is a USFS Region 4 Sensitive Species. The status of Columbia Spotted Frog populations in Wyoming currently is unknown. The USFWS has listed the Great Basin Distinct Population Segment of this species as a candidate for federal listing (USFWS 2011b). Although Wyoming does not fall within the range of this designated population, additional information on the distribution and status of the species in Wyoming will provide data for any future reassessment of the species' status.

Northern Leopard Frog - Western populations of the Northern Leopard Frog have declined significantly, particularly in higher elevation populations in the Rocky Mountains (Corn and Fogleman 1984, Koch and Peterson 1995, Rorabaugh 2005, Smith and Keinath 2007, USFWS 2011a). Northern Leopard Frogs previously were documented at lower elevations in the BTNF but have not been observed on the forest in several years. This species is listed as an NSSU species in Wyoming by the WGFD. Further information on this species is needed to clarify its status.

Boreal Chorus Frog – The Boreal Chorus Frog is an NSS5 species thought to be widespread throughout Wyoming, however, detailed information on population status and distribution are lacking. The Boreal Chorus Frog is the smallest species of amphibian in Wyoming.

Tiger Salamander - Tiger Salamanders are an NSS4 species in Wyoming. Though believed to be widespread throughout the state, massive die-offs attributed to Ranavirus have recently been documented in the Powder River Basin in both Wyoming and Montana (Estes-Zumpf et al. 2010). Information on current population status, distribution, and disease spread will allow for preemptive conservation planning for this species.

### *Species' Distributions*

Five amphibian species were known to occur within the study area, but our understanding of their distribution and status was severely limited by lack of data because a substantial portion of the study area has not been formally surveyed. We compiled and mapped all available records for amphibians in the region. Data sources included the WYNDD Biotics database, WGFD's WOS and herpetological databases, and the BTNF NRIS database. Over 4800 pre-existing records for amphibians in the study area were compiled at the onset of this study; however many of these records were duplicates found in multiple databases due to regular data exchanges between agencies. Species observation dates for existing records ranged from 1891 to 2011. Existing records ranged in credibility and likely were biased by limited observer identification skills, whether or not observers recorded all species detected, and ease of access to certain parts of the study area. Few records for amphibians existed in the Wind River Range in the eastern portion of the BTNF likely due, in part, to the lack of roads in the area. Furthermore, because most databases store observation records, locations where surveyors searched but did not detect amphibians are rarely reported and archived. Thus, our understanding of amphibian distributions in the study area was based solely on documented occurrences. Inferences about species distributions after inventory surveys conducted during this project were based on both documented occurrences and negative data (i.e., areas that were searched, but where species were not detected).

In order to expand our knowledge of the distribution of species across the study area, we held joint meetings for WYNDD, WGFD, and BTNF wildlife and fisheries biologists in early spring of 2012 and 2013 to identify and prioritize areas to survey for amphibians in each of those years based on gaps in our understanding of species distributions (Figure 2). Priority was given to areas with few or no mapped occurrences but which contained potential amphibian habitat.

### *Boreal Toad Breeding Status*

In addition to filling gaps in our knowledge of amphibian distributions, another study objective was to identify previously reported breeding sites for the boreal toad within the study area and revisit breeding sites to determine if breeding is still occurring at those sites. Herpetologists from WGFD had been revisiting boreal toad breeding sites in the area to evaluate breeding status for several years prior to this study. Thus, we targeted documented breeding sites that had not already been revisited by WGFD in the past five years.



Because the boreal toad is both a USFS sensitive species and a WGFD species of greatest conservation need, more surveys have been conducted for this species than for any other amphibian species in the study area. Records of boreal toads within and among years were often clustered spatially and each record does not necessarily represent a unique location. Furthermore, the breeding status of clustered records in any one area often varied among years and sometimes within years depending on time of year and survey effort. Because one of our objectives was to revisit and assess breeding status at previously identified boreal toad breeding locations that had not already been revisited within 5 years of the onset of this study, it was necessary to identify unique breeding locations.

We defined a breeding area as a location where eggs, tadpoles/larvae, metamorphs, or adults in amplexus were detected. We considered a location to be a suspected breeding area if older juveniles hatched that summer or large numbers of adults were present during the breeding season, or if calling was heard. If only one or a few adults were observed, we considered the site to be a residence area.

We identified locations where boreal toads were documented breeding while taking into account that spatially clustered records, sometimes only meters apart, likely represent a single breeding location (pond, oxbow, bog, etc.). Because we compiled 2250 boreal toad records from 1900 to 2014 and because breeding ponds and oxbows often are temporary features in a dynamic landscape, we did not assess each cluster of records and digitize boundaries around breeding locations. Instead, we considered records within 100m of each other to be from the same boreal toad location. To do this, we buffered the centroid of each observation record by 100m and combined all points with overlapping buffers into one location polygon (see Figure 3 for example) with an associated attributes table containing records for every unique observation of the species within that site. Thus, all 2250 original records were maintained and linked to the boreal toad location polygon in which they occurred.

To assess status of the boreal toad locations over time, we also included records of residence areas because a breeding site could have one or more years where only adults were detected with no evidence of breeding. Thus, the history of each location is archived over time regardless of what the breeding or occupancy status was in any given year.

### *Amphibian Surveys*

We conducted amphibian surveys in priority survey areas using standardized protocols developed during this and related projects. The goals of amphibian surveys in 2012 and 2013 were to 1) inventory amphibian species across the study and document breeding for each species if it occurs, and 2) collect data on species detectability for use in modeling species occupancy across the BTNF and upper Green River drainage.

Survey methods were similar to guidelines set forth by the USGS Amphibian Research and Monitoring Initiative (ARMI) and permit occupancy-based modeling of amphibian populations (Corn et al. 2005). Surveys were conducted during the breeding season (mid-May to early August, depending on elevation and weather conditions) when species were most detectable and consisted of visual encounter surveys in potential amphibian habitat. 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 (see Appendix A for example datasheet).

Whenever possible, surveys were conducted by 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 survey is complete. The standard dual-observer method has both surveyors 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 allows more area to be inventoried for amphibians but does not contribute to estimates of detectability.

For better detection of tadpoles, we dipnetted every 5-10m or in patches of good habitat (e.g., quiet inlets, backwater areas, or patches of emergent vegetation) for amphibian larvae. Each dipnet event consisted of at least five sweeps with the net. 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 took photographs of any unidentifiable animals or egg masses, and sent photos to WYNDD or WGFD for identification.

We organized and held amphibian survey and monitoring trainings for USFS and WGFD biologists and technicians each spring at the Sherman Guard Station on the BTNF west of Merna, WY. The training involved a background on the project and the need for standardized amphibian monitoring in Wyoming, an explanation of datasheets and protocols, and an overview of amphibian identification. Following training at the guard station, we practiced implementing identification skills and survey protocols in a field setting. In addition to USFS and WGFD biologists and technicians, 4 WYNDD technicians worked in 2 teams of 2 to survey for amphibians in both 2012 and 2013. WYNDD zoologists accompanied by trained volunteers also conducted a number of surveys in remote parts of the study area.

### *Species Occupancy*

Species occupancy refers to presence of a species at a given location, and the proportion of sites occupied (hereafter referred to as occupancy rate) is often used as a variable describing the state of a population in an area of interest (Hecnar and M'Closkey 1997, Bailey et al. 2004). Because many species, especially amphibians, can be difficult to detect, failure to document a species at a site does not necessarily mean the species is truly absent from that site. An estimate of the probability of detecting a species when it is present is needed to correct naïve occupancy rates (MacKenzie et al. 2002). We used occupancy modeling to reduce bias in estimates of occupancy rate due to imperfect detection and to

investigate how survey and habitat conditions influence species detectability and occupancy (MacKenzie et al. 2002, MacKenzie et al. 2006).

We modeled occupancy rate for each species across the study area using data from 727 sites where standardized surveys were conducted over the course of this study. The dual-observer method was used at 222 sites, allowing us to estimate the probability of detecting a species when present. We estimated species occupancy rate within the study area and the probability of detecting a species when present using program PRESENCE (Hines 2006). We present results from a simple constant occupancy, constant detectability model for each species. Results provides conservative estimates that can be used in power analyses to assess amphibian monitoring study design questions in the region.

### *Chytrid Fungus*

We sampled a subset of amphibians detected for amphibian chytrid fungus following procedures outlined by Livo (2004). Using a sterile cotton swab, we sampled the ventral surface, inner thighs and feet of individual amphibians. We placed swabs in screw-cap vials containing 95% ethanol and refrigerated vials until they were sent to the lab for testing. Samples were analyzed at Pisces Molecular, LLC (Boulder, Colorado, USA) using polymerase chain reaction (PCR). Individual samples were tested whenever possible; however, some samples were pooled within drainages due to funding limitations. To prevent the spread of chytrid fungus among sites by surveyors, all survey and sampling gear were decontaminated with either a 10% bleach solution or commercial fungicide between drainages and between isolated sites within drainages.

### *Boreal Toad Genetics*

Boreal toads in portions of the Wyoming Range have been identified as being part of the Eastern Clade of the Western Toad, the distinct population segment recently petitioned for listing under the Endangered Species Act (USFWS 2012). Because the northern boundary of the Eastern Clade is ambiguous, field efforts in the project area also included collection of boreal toad DNA through buccal swabs, tadpole collection, and/or toe clips. Samples were stored in 95% ethanol and placed in a freezer for future analysis if the need arose.

### *Long-term Amphibian Monitoring*

The final phase of this project was to develop a sustainable long-term amphibian monitoring program in the region, encourage participation of local organizations and agencies, and train local biologists and other participants to conduct surveys. The BTNF expressed interest in helping to develop and implement such a monitoring plan. In January 2014, an amphibian monitoring working group consisting of BTNF, WGFD, and WYNDD biologists met in Jackson to discuss monitoring needs, options, and challenges. The amphibian monitoring working group decided to move forward with an occupancy-based amphibian monitoring program at sites selected randomly from good quality amphibian habitat on the forest. We

worked closely with collaborators in 2014 to develop a study design that was compatible with other amphibian monitoring programs in the region. The monitoring program considers ARMI guidelines and resembles the mid-level occupancy-based modeling proposed by the USGS (Corn et al. 2005).

The study design for this amphibian monitoring program followed methods used by Yellowstone and Grand Teton National Parks (Bennets et al. 2013). The primary sampling unit consists of all aquatic sites within a designated survey area (hereafter catchment; Figure 4). A site is defined as a unique aquatic feature (wetland, pond, wet meadow, bog, stream reach, etc.) within a catchment. Surveying multiple sites within a catchment not only increases the likelihood of detecting a species if it is present in the catchment, but also accommodates annual variability in the persistence of wetlands and/or the use of a specific wetland by amphibians. Amphibian surveys are conducted following the dual-observer method outlined above to allow for estimation of detection probabilities. Protocols are optimized for long-term implementation by various land managers in the region.

Selection of monitoring catchments - We selected catchments from areas in the BTNF most likely to provide amphibian habitat. This was done by developing an index of the likelihood that an area contained amphibian habitat based primarily on the amount of wetland edge habitat in a given area. Areas with large amounts of wetland edge habitat were considered more likely to provide habitat for breeding amphibians. Vegetation data were not considered in habitat analyses because the narrow extent of riparian and wetland vegetation surrounding aquatic habitat is not well represented in existing GIS layers. We used ArcGIS (ESRI Inc., Redlands, CA) to conduct all amphibian habitat analyses.

Water dataset consisted of wetland polygons from the National Wetlands Inventory (NWI). Wetland polygons were converted to linear features representing wetland perimeter, the area most likely to contain amphibian breeding habitat. We did not include stream data in habitat analyses because initial trials resulted in too many areas chosen that had fast moving streams but no amphibian breeding habitat (ponds, wet meadows, etc.). Pilot data indicated that a mean catchments size of 35ha typically could be surveyed in 1 working day (Estes-Zumpf et al. 2012). Therefore, wetland edge layers were converted to a raster layer and focal statistics were used to calculate the proportion of cells in a 35ha area (335m circular radius) around each raster cell.

We used stratified sampling to identify survey catchments in potential amphibian habitat across the BTNF. To select catchments more likely to contain good amphibian breeding habitat, we restricted selection to wetlands with a high proportion of adjacent wetland edge cells in the surrounding 35ha area. The selection process was weighted based on ease of access, with areas < 3km from a road given higher probability of selection than areas farther than 3km from road (80:20). Because a large amount of the BTNF is roadless (wilderness), we also randomly sampled catchments with potential amphibian habitat within 1km of a trail in designated wilderness areas on the BTNF. In order to ensure that sampling was balanced across an elevation gradient, we stratified sampling across 3 elevation classes (low, medium, and high). Elevation classes were sampled in proportion to their representation on each of the 6 ranger districts on the BTNF. Lastly, we stratified sampling by ranger district. In each ranger

district, we randomly selected the desired number of catchments from areas identified using the criteria above. We also selected a number of alternative catchments to replace primary catchments if access to primary catchments was restricted or habitat in primary catchments was deemed unsuitable for amphibians. We then digitized at least 4 survey sites encompassing all presumed amphibian habitat within each primary catchment.

## Results

### *Amphibian Surveys*

Working together, WYNDD, WGFD, and USFS survey crews conducted amphibian surveys in all but 2 of the target survey areas identified during spring organizational meetings, surveying 862 sites across the study area (Figure 5). Some data from collaborators were not available at the time this report was drafted, so herein we limit analyses to the 862 sites for which we have information. Collaborators also conducted several targeted surveys in 2014 at previously reported boreal toad and Columbia Spotted Frog locations to determine current status. We included in our analyses all 2014 surveys in which all amphibian species detected were recorded. Results from surveys were used to update our understanding of species distributions across the region.

### *Species' Distributions*

Boreal toad – Prior to this study, boreal toads primarily had been documented along the eastern slope of the Wyoming Range and in the northern half of the BTNF. A few records occurred in isolated parts of the Wind River Range (Figure 6a). Despite extensive surveys, we failed to detect boreal toads outside of their previously mapped distributional extent within the study area. Boreal toads appear to be throughout the northern half of the BTNF but confined to the east slope and southern end of the Wyoming Range (Figure 6b). We confirmed that boreal toads still occurred in the Wind River Mountains; however, the viability of Wind River populations is uncertain. For example, only one adult toad was documented in the Dutch Joe Creek area in the southern Wind River Range in 2003 and only one adult was detected in the same area during this study in 2012. No evidence of breeding has been documented in this area and the nearest recent boreal toad locations in the study area are over 36km northwest. Although surveyors documented boreal toads at 4 additional sites in the Wind River Mountains, all were in the upper Boulder Creek drainage along Macs Creek. Two of these sites were suspected breeding sites, however, the Macs Creek sites are located over 50km from the nearest known active breeding location in the study area. Thus, the Wind River boreal toad locations appear isolated from the rest of the species range.

We identified 51 new boreal toad locations (i.e., occupied locations >100m from previously documented locations), of which 23 were in drainages where the species had not previously been documented (Figure 7). Surveyors detected boreal toads at new sites in the Noble Basin (n=2), near Ann Mountain (n=1), in Nugent Park (n=1), and along Big Twin Creek (n=1), Miner Creek (n=3), Packer Creek (n=1), Slide Creek (n=3), Tosi Creek (n=1), North Piney Creek (n=2), Middle Piney Creek (n=1), South Piney Creek (n=2), Fontenelle Creek (n=1), and

upper Macs Creek (n=4). Twenty-seven of the new boreal toad locations found during this project were new breeding areas or suspected breeding areas for the species.

*Boreal Toad Breeding Status* - Including observations from this study, we compiled 2250 occurrence records representing 449 locations, where all records within 100m of each other were considered to be from a single site. Of the 449 boreal toad locations, roughly half (45%) contained evidence of breeding, but only 24% of locations were confirmed to have had breeding since 1900.

We assessed boreal toad breeding status over the past 15 years in two ways. First, we identified 145 locations that had breeding or were suspected to have breeding at least once between 2000 and 2009. We excluded all locations where only adults were detected over this time period. WGFD, WYNDD, or the BTNF revisited 33 of these locations 1 or more times from 2010-2014. Of these revisited locations, 22 (67%) had breeding or were suspected to have had breeding in the past 5 years. This analysis includes only previously mapped breeding locations and does not include result from new locations found in the past 5 years.

Second, we looked at breeding status over the past 15 years by examining the proportion of all locations (breeding, suspected breeding, resident only) visited each year where breeding was known or suspected to have occurred. For example, 28 locations were visited in 2000, of which 20 (71%) were confirmed or suspected to have had breeding in 2000. This analysis included new boreal toad locations that may have been detected in a given year. Results of this analysis showed extreme variation among years in the proportion of sites visited where breeding occurred (Figure 8). Results ranged from no breeding locations detected in 2004 to 100% of visited locations having breeding in 2007. Much of this variation can be explained by the variation in number of locations visited in any one year, which ranged from 114 locations visited in 2002 to only 1 visited in 2004 and 2005.

The spatial distribution of surveys within and among years also likely contributed to variation in the results. Oftentimes resource managers focus survey effort in different target areas in different years. All records in a given year could have come from surveys along 1 target drainage and results for that year reflect only whether or not toads bred in that drainage. Therefore, results from any one year cannot be used to infer breeding status for toads across the study area in that year. Lastly, many observations of boreal toads over the past 15 years are not from formal amphibian surveys. They are simply observation records. We do not know if an observer looked for tadpoles in an area where they only reported an adult. Because survey methodologies, locations, and spatial distribution of locations were not standardized among years, these data cannot be used to assess population trends for boreal toads in the study area over time. The data simply reflect whether or not breeding was documented at visited locations in a given year.

Although inferences about population trends for boreal toads should not be made with data available at this time, we can use these data to identify important breeding areas for boreal toads in the study area. By mapping the breeding status of all boreal toad locations visited in the past 5 years (n=102) we can identify recent breeding areas that are important for

boreal toads in the study area (Figure 9). Important breeding areas for boreal toads on the BTNF largely occur in drainages along the east slope of the Wyoming Range and in the upper Green River drainage. Key drainages include (from south to north) South Horse Creek, Pass Creek, North Horse Creek, Buck Creek, Chall Creek, North Fork Middle Beaver Creek, and Gypsum Creek. Although other recent breeding areas are apparent, the drainages listed above have a large number of breeding records across the past 5 years and likely represent important population centers for boreal toads in the study area. It should be noted, however, that this information is not comprehensive and does not include breeding areas not surveyed in the past 5 years.

Columbia Spotted Frog - At the onset of this study, Columbia Spotted Frogs had been documented in at least portions of most mountain ranges within the study area, except the Wind River Mountains. Knowledge of the species' distribution in the southern Wyoming Range also was limited to the Greys River corridor. We documented Columbia Spotted Frogs at a number of sites during this study; however, all observations occurred within the previously mapped distributional extent for this species (Figures 10a & b) within the study area. We did not detect Columbia Spotted Frogs at survey sites with the Wind River Range or the southern Wyoming Range outside of the Greys River Corridor (Figure 10b).

Not only were we largely unable to expand the distribution of this species in the study area with our extensive sampling, but we also detected a possible decline in Columbia Spotted Frogs in the northeastern portion of the Wyoming Range and along Gypsum Creek in the Upper Green River drainage. Amphibian surveys conducted by D. Patla in 1999 detected a breeding site for Columbia Spotted Frogs along North Horse Creek. From 2001- 2003, WGFD fisheries and herpetological crews also documented Columbia Spotted Frogs at sites in 6 drainages in the northeastern half of the Wyoming Range (including North Horse Creek) and along Gypsum Creek. Evidence of breeding was documented at most of these sites, and three drainages (North Horse Creek, South Horse Creek, and Gypsum Creek) appear to have had substantial breeding populations of Columbia Spotted Frogs. We found no records for this species between 2003 and 2012 in the area of concern except for a report of a Columbia Spotted Frog along North Horse Creek in 2010 (M. Anderson, USFS, *personal communication*). In 2012 and 2013, WYNDD, WGFD, or USFS crews conducted amphibian surveys at or near all but one of these previously documented sites. Columbia Spotted Frogs (3 adults) were documented at only one site (along Packer Creek) just north of a previously identified site along Spring Creek (Figure 11). A tadpole collected from North Horse Creek in 2012 also may have been a Columbia Spotted Frog tadpole; however, the tadpole was very young and diagnostic features had not yet developed.

In 2014, WYNDD, WGFD, and the BTNF conducted additional targeted surveys at the previously documented locations in the area of concern as well as locations along the neighboring drainages to determine if the lack of detections at the sites were the result of survey timing or conditions in 2012 and 2013 or an actual lack of frogs at these locations. Amphibian crews found a Columbia Spotted Frog and documented at least one individual calling in June along North Horse Creek in the same area as the majority of breeding locations records documented from 1999-2003. A juvenile Columbia Spotted Frog also was detected along Miner Creek, immediately south of the previously documented Spring Creek site and

approximately 2.7km from the Packer Creek site where frogs were detected in 2013. However, no Columbia Spotted Frogs were detected at the other previously documented sites. The cause of the loss of Columbia Spotted Frogs from these locations is currently unknown. Although a few of the previously documented locations, such as those along Nylander Creek, no longer provide habitat for the species possibly due to loss of beaver ponds, other locations appear to provide ample habitat. It is unknown at this time if the decline is temporary (sites will be recolonized) or permanent. Other amphibian species including the boreal toad still occur at several of the locations from which the Columbia Spotted Frog has disappeared.

Boreal Chorus Frog – Boreal Chorus Frogs are the most widespread amphibian species in Wyoming. The species was previously documented in almost all mountain ranges in the study area, including the Wind River Mountains (Figure 12a). Most locations for this species were clustered in easily accessible areas with the remaining records scattered sparsely throughout the rest of the study area.

We detected Boreal Chorus Frogs at many of the sites surveyed during this study and throughout the species' previously identified range within the study area. We also improved our understanding of the distribution of Boreal Chorus Frogs within the study area by documenting the species in a number of areas in which no previous records existed (Figure 12b). In particular, we detected Boreal Chorus Frogs throughout much of the Wind River Mountains across a range of elevations. We also detected the species at additional drainages in the southern and western portions of the Wyoming Range. The central portion of the Wyoming Range, along parts of the Greys River drainage, had the fewest number of survey sites at which Boreal Chorus Frogs were detected. Overall, however, the species was distributed across most of the study area.

Tiger Salamander – Like the Boreal Chorus Frog, the Tiger Salamander was previously known from most of the mountain ranges in the study area (Figure 13a). The species' distribution remained largely unchanged following project surveys (Figure 13b). We detected Tiger Salamanders at most sites at or near where the species was previously known to occur. The species remains largely absent from most of the Wind River Mountains, occurring in only a few lower elevation drainages.

Northern Leopard Frog – We found very few records for the Northern Leopard Frog within the study area. Furthermore, because both Columbia Spotted Frogs and Boreal Chorus Frogs have or can have spots, reports of this species in the BTNF by observers of unknown credibility could be of misidentified frogs. Northern Leopard Frogs are known to have occurred at lower elevation portions of the study area and previously may have occurred at higher elevations; however, few reliable records exist (Figure 14a). We did not detect Northern Leopard Frogs at any sites during this project (Figure 14b). The species might still occur in lower elevations of the study area which were sparsely sampled during this project.

### *Species Occupancy*

The Boreal Chorus Frog was the most common species in the study area, occupying approximately 47% of sites (Table 1). The Tiger Salamander was the second most common



species (19%), followed by the boreal toad (13%) and the Columbia Spotted Frog (9%). Boreal Chorus Frogs had the highest likelihood of being detected when present (detection probability = 84%) while boreal toads had the lowest (56%). Some bias likely exists in our estimate of site occupancy because survey sites were not chosen at random for the inventory portion of this study. Instead, we conducted inventories for amphibians in predefined areas (either where few or no surveys had occurred or where boreal toads had been documented breeding in the past). Also, we modeled occupancy across all sites visited during this study because we initially assumed that each species could be distributed across the entire study area. However, updated species' distributions resulting from this study reveal that some species are absent from large portions of the study area, particularly the Wind River Range. Future occupancy modeling should consider restricting models to current species-specific distributions in the study area to prevent overestimating the proportion of area occupied in areas outside a species' local distribution.

### *Chytrid Fungus*

Together, WYNDD, WGFD, and the BTNF collected a total of 280 chytrid fungus samples from all amphibian species detected in the study area in 2012 and 2013 (Figure 15). We typically pooled 3 to 8 samples to reduce analysis cost. If one or more samples within a pooled batch tested positive for chytrid fungus, we were unable to identify which sample(s) was positive and which might have been negative unless we had samples reanalyzed individually. However, we generally pooled samples only if they were from the same species within the same drainage, so there are likely no cases where individual analysis would have markedly altered the distribution of chytrid fungus in our study area.

Chytrid fungus was detected in boreal toads, Boreal Chorus Frogs, and Columbia Spotted Frogs throughout the study area. The only species in which we did not detect the fungus was the Tiger Salamander; however, we only had samples from 8 salamanders from one location in the southern Wyoming Range. Of the 70 individually-analyzed samples, 34 samples from Boreal Chorus Frogs (7 of 24), boreal toads (15 of 29), and Columbia Spotted Frogs (12 of 17) tested positive for chytrid fungus. We also tested 44 pooled batches of samples. Thirty-four pooled batches tested positive for chytrid fungus and revealed chytrid fungus in drainages tested throughout the study area and in the three frog and toad species noted above. Chytrid fungus was documented at many boreal toad breeding sites, and was particularly prevalent within the Wind River Range, despite the remoteness of this mountain range.

### *Boreal toad genetics*

We collected 68 boreal toad genetic samples from different parts of the BTNF and upper Green River drainage in 2012 and 2013 (Figure 16). Samples were collected by WYNDD, WGFD, and BTNF biologists. All samples were cataloged and stored in a freezer at the University of Wyoming Biodiversity Institute. All samples were shipped to Dr. Anna Goebel's laboratory at Florida Gulf Coast University in July 2014 when funding for genetic analyses became available.

Results from these samples will be used by Dr. Goebel and others to elucidate the boundary between the Eastern and Northwestern Clades of the Western (boreal) Toad.

### *Long-term Amphibian Monitoring*

Standardized long-term amphibian monitoring is critical to understanding and obtaining defensible population trends. We worked with multiple collaborators to design an effective and sustainable long-term amphibian monitoring program in much of the study area. The resulting study design was decided upon by all collaborators after discussing data needs, funding and personnel limitations, and the strengths and weaknesses of different monitoring design options. The occupancy-based study design that was decided upon is compatible with other monitoring datasets in the region including USGS ARMI amphibian monitoring efforts in Grand Teton, Yellowstone, and Glacier National Parks, and recently implemented long-term amphibian monitoring in the Medicine Bow and Routt National Forests in southern Wyoming and northern Colorado.

We used preliminary occupancy modeling results to inform the number of survey catchments to monitor in the study area. Because of low amphibian diversity in the Wind River Mountains, the BTNF chose to focus monitoring on the forest west of the Wind River Range. The BTNF also chose to minimize monitoring efforts in wilderness areas due to difficulty of access. Therefore, we randomly sampled and mapped 36 catchments (5 in each ranger district and 6 in wilderness areas) across the BTNF (Figure 17). The BTNF plans to monitor 28 of the catchments in the core portion of the forest and outside of wilderness areas. The 8 catchments falling in the Wind River Range and wilderness areas will be surveyed by trained volunteers or by the BTNF if time and funds allow.

We provided the BTNF with GIS shapefiles and spreadsheets with the locations of the 36 primary survey catchments and their respective sites. Shapefiles also included a number of alternative catchments within each ranger district to be substituted for primary catchments should problems arise with access or habitat quality upon initial visits to catchments. We also provided each ranger district with survey packets consisting of 1) a catchment overview sheet (with basic information, directions, and maps), 2) site-specific datasheets (1 for each site in the catchment) prepopulated with site and catchment name, all relevant navigation and recommended photo points and a site map on the back, and 3) surveyor 2 site-specific datasheets to be used by the second surveyor when using the dual survey method. See Appendix A for example catchment information and site-specific datasheets.

Because this and similar amphibian monitoring efforts across the state must be sustainable over the long-term despite fluctuations in annual funding to resource managers, we partnered with the University of Wyoming Biodiversity Institute to coordinate and train citizen scientists to assist with data collection. This partnership has resulted in additional financial and logistical support for amphibian monitoring and allowed development of critical training and organizational tools such as a website ([www.toadtrackers.org](http://www.toadtrackers.org)), survey training videos, and a laminated pocket ID guide to the amphibians of Wyoming. WYNDD worked closely with WGFD and UW Biodiversity Institute staff and interns to develop the content for these resources.

In order to make this monitoring program sustainable and effective, we developed partnerships with local agencies and organizations. We especially worked closely with the BTNF to make sure that the resulting monitoring program will meet their data needs and be feasible to implement most years. The BTNF is planning to survey a core set of catchments (~28) each year. By partnering with the UW Biodiversity institute, we not only have a means to coordinate and train surveyors, but we also can use trained outdoor-savvy citizen scientists to survey catchments that the BTNF is unable to visit in any given year. Thus far, citizen scientists have included members of Trout Unlimited, The Nature Conservancy, University of Wyoming faculty and staff, Boy Scouts, and other outdoor enthusiasts. We also have had discussions with USGS ARMI biologists and statisticians about combining datasets for future regional-level analyses to examine trends in amphibian occupancy at larger spatial extents.

Lastly, WYNDD developed a temporary database to store all data from this and related amphibian survey and monitoring efforts. This database stores not only observation records, but also negative survey data, allowing partners to identify where surveyors looked for but failed to detect amphibians. Occurrence data will regularly be added to the WYNDD Biotics database and made available to requestors. The UW Biodiversity Institute, however, is currently funding the UW Wyoming Geographic Information Science Center (WyGISC) to develop an online-accessible amphibian monitoring database where surveyors can enter and view data online and where partners can access and download data. This will be a much-needed tool that will speed up data entry and data dissemination to all collaborators.

## Discussion

Results of this project have added greatly to our understanding of amphibian distributions across the study area. By collaborating with multiple resource managers and coordinating survey efforts annually, we were able to survey over 862 sites across a rugged and difficult-to-access landscape. This allowed us to fill in gaps in our knowledge of the spatial distributions of amphibian species in western Wyoming.

At the onset of this study, we expected to detect amphibians in many previously unsurveyed portions of the study area. In particular, we expected to document boreal toads further west in the Wyoming Range and scattered throughout the Wind River Mountains. However, we did not discover boreal toads outside their previously mapped distribution despite our extensive sampling across the study area. Within the southern half of the study area, most boreal toads occurred along a handful of drainages along the eastern slope of the Wyoming Range and in the upper Green River area, especially along Gypsum Creek.

One of the more surprising results of this study was the lack of amphibian species diversity in the Wind River Range. Abundant wetland habitat exists throughout much of the range and we detected Boreal Chorus Frogs at many of the sites surveyed. However, no Columbia Spotted Frogs or Northern Leopard Frogs were detected in Wind River Range. Boreal toads were documented in only 2 drainages and Tiger Salamanders were documented at only a few sites at lower elevations. Because very little historical data exists for amphibians in the Wind River Range, we are unsure whether this mountain range always has had low amphibian

species diversity or if most amphibian species have been extirpated from the range. A number of known threats to amphibians exist in the Wind River Range; however, we are unable to assess impacts of most of these threats with data collected during this study.

Another unexpected result of this study was the widespread occurrence of amphibian chytrid fungus across in the study area, even in remote wilderness areas. Chytrid fungus is believed to be one of the leading causes of amphibian declines globally (Skerratt et al. 2007). The fungus was first discovered and identified as a threat to amphibians in 1998 (Berger et al. 1998). Scientists continue to expand our understanding of this disease, its origins, and its impacts on amphibians. Recently, scientists have begun to document the persistence (McDonald et al. 2005, Newell et al. 2013) and even range re-expansion (Scheele et al. 2014) of remnant populations of amphibians that initially exhibited severe declines due to chytrid fungus. Such studies are encouraging, suggesting that some amphibian species susceptible to chytrid fungus may be able to adapt to the disease and persist despite the continued presence of chytrid fungus in the landscape. In order for adaptation to occur in species susceptible to the fungus, however, managers must ensure that species abundance and the number and distribution of remnant populations are sufficient to maintain viable populations through any initial population decline caused by the pathogen.

## **Future Directions**

We will continue to work with collaborators to implement, evaluate, and adjust the new amphibian monitoring effort started under this project. In the summer of 2014, biologists and technicians from the BTNF and WYNDD, as well as citizen science volunteers, conducted the first year of surveys at mapped catchments using standardized survey protocols. We participated in a meeting in September 2014 organized by the BTNF at which biologists and technicians responsible for conducting the first year of monitoring surveys could provide feedback on access to and size of catchments, presence of potential amphibian habitat at mapped sites, ease of surveying designated areas, and the usefulness of datasheets and maps. We received excellent feedback and recommendations which will be used to modify and update catchments and datasheets. Later this fall, we also will meet with district biologists from the BTNF to discuss additions and possible modifications to the study design that could improve our ability to make inference about amphibian populations on the BTNF.

## Acknowledgements

This project was funded by a Wyoming Game and Fish Department State Wildlife Grant. We sincerely thank biologists from the Bridger-Teton National Forest including G. Hanvey, D. DeLong, M. Anderson, K. Murphy, D. Fogle, and A. Roberts, for their support, ideas and assistance throughout all aspects of this project. We appreciate the help of numerous BTNF biological technicians who helped to conduct surveys and organize data over the past several years. We also thank WGFD fisheries biologists D. Miller and H. Sexauer, as well as WGFD technicians K. Leuenberger, B. Zook, S. Fellows, and Z. Lange for help with logistics and surveys. We sincerely thank WYNDD technicians A. Bowe, C. Zars, S. Weinmann, M. Ochsenfeld, T. Hamilton, D. Jones, O. Wilmot, and V. Ly for hiking and backpacking hundreds of miles of mountainous terrain in search of amphibians. We also thank volunteers I. Abernethy, A. Pocewicz, and S. Zumpf, and interns M. Vujovich, G. Vujovich, and C. Martinez del Rio for help conducting backcountry surveys. We thank I. Abernethy and M. Andersen for help with GIS analyses. The BTNF provided much appreciated logistical support including use of USFS radios and guard stations during field surveys. We sincerely thank the University of Wyoming Biodiversity Institute, its interns, and its staff, especially B. Marsicek, for additional support for the development of training materials, coordination tools, and the long-term amphibian monitoring program. We thank D. Patla for advice and information on regional amphibians. J. Erdmann worked hard to compile and map results from chytrid samples, and M. Hughes cataloged all boreal toad genetic samples.

## Literature Cited

- Bailey, L.L., T.R. Simons, and K.H. Pollock. 2004. Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecological Applications* 14:692-702.
- Bennetts, R., S. Corn, R. Daley, W.R. Gould, C. Jean, D. A. Patla, C. R. Peterson, and A. Ray. 2013. Cooperative amphibian monitoring protocol for the Greater Yellowstone Network: Narrative, version 1.0. Natural Resource Report NPS/GRYN/NRR—2013/654. National Park Service, Fort Collins, Colorado.
- Berger, L., R. Speare, P. Daszak, D.E. Green, A.A. Cunningham, C.L. Goggin, R. Slocombe, M.A. Ragan, A.D. Hyatt, K.R. McDonald, H.B. Hines, K.R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Science* 95:9031–9036.
- Boreal Toad Recovery Team (BTRT). 2001. Conservation plan and agreement for the management and recovery of the southern Rocky Mountain populations of the boreal toad (*Bufo boreas boreas*), Boreal Toad Recovery Team. Colorado Division of Wildlife, Denver, CO.
- 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.
- Corn, P.S. and J.C. Fogleman. 1984. Extinction of montane populations of the northern leopard frog (*Rana pipiens*) in Colorado. *Journal of Herpetology* 18:147-152
- Estes-Zumpf, W.A., H. Griscom, and D. Keinath. 2010. Inventory of amphibians and reptiles in the Powder River Basin area of Wyoming: Year two progress report, 2009. Prepared for the Bureau of Land Management Buffalo Field Office.
- Estes-Zumpf, W.A., D. Keinath, and A. Bowe. 2012. Monitoring amphibian population responses to a changing environment: Power analysis based on 2011 pilot study. Prepared for the Medicine Bow and Routt National Forests and the Thunder Basin National Grassland by the Wyoming Natural Diversity Database, Laramie, WY.
- Goebel, A.M., T.A. Ranker, P.S. Corn, and R.G. Olmstead. 2009. Mitochondrial DNA evolution in the *Anaxyrus boreas* species group. *Molecular Phylogenetics and Evolution* 50: 209-25.
- Griffiths, R.A., D. Sewell, and R.S. McCrea. 2010. Dynamics of a declining amphibian metapopulation: Survival, dispersal and the impact of climate. *Biological Conservation* 143:485-491.
- Hecnar, S. J. and R. T. M'Closkey. 1997. Spatial scale and determination of species status of the green frog. *Conservation Biology* 11:670–682.
- Hines, J. E. 2006. PRESENCE2-Software to estimate patch occupancy and related parameters. USGS-PWRC. <http://www.mbr-pwrc.usgs.gov/software/presence.html>.

- 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.
- Jørgensen, C.B. 1986. External and internal controls of patterns of feeding, growth and gonadal function in a temperate zone anuran, the toad *Bufo bufo*. *Journal of Zoology* 210:211–241.
- Keinath, D. A. and M. McGee. 2005. Boreal toad (*Bufo boreas boreas*). Prepared for the USDA Forest Service, Rocky Mountain Region, Species Conservation Project.
- Koch, E.D. and C.R. Peterson. 1995. Amphibians and Reptiles of Yellowstone and Grand Teton National Parks. University of Utah Press, Salt Lake City, UT. 188 pp.
- 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, G.B. Lachman, S. Droege, J.A. Royle, and C.A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83:2248-2255.
- Mackenzie, D.I., J.D. Nichols, J.A. Royle, K.H. Pollock, L.L. Bailey, and J.E. Hines. 2006. Occupancy estimation and modeling: Inferring patterns and dynamics of species occupancy. Elsevier, Burlington, MA.
- McDonald, K.R., I.D. Mendez, R. Muller, A.B. Freeman, R. Speare. 2005. Decline in the prevalence of chytridiomycosis in frog populations in North Queensland, Australia. *Pacific Conservation Biology* 11:114–120
- McMenamin, S.K., E.A. Hadly, and C.K. Wright. 2008. Climatic change and wetland desiccation cause amphibian decline in Yellowstone National Park. *Proceedings of the National Academy of Sciences* 105:16988-16993.
- Newell, D.A., R.L. Goldingay, and L.O. Brooks. 2013. Population Recovery following Decline in an Endangered Stream-Breeding Frog (*Mixophyes fleayi*) from Subtropical Australia. *PLoS ONE* 8(3): e58559. doi:10.1371/journal.pone.0058559
- 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.
- Reading, C.J. 2007. Linking global warming to amphibian declines, through its effects on female body condition and survivorship. *Oecologia* 151:125–131.
- Rohr, J.R. and T.R. Raffel. 2010. Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proceedings of the National Academy of Sciences* 107:8269-8274.
- Rohr, J.R., T.R. Raffel, J.M. Romansic, H. McCallum, and P.J. Hudson. 2008. Evaluating the links between climate, disease spread, and amphibian declines. *Proceedings of the National Academy of Sciences U.S.A.* 105:17436–17441.

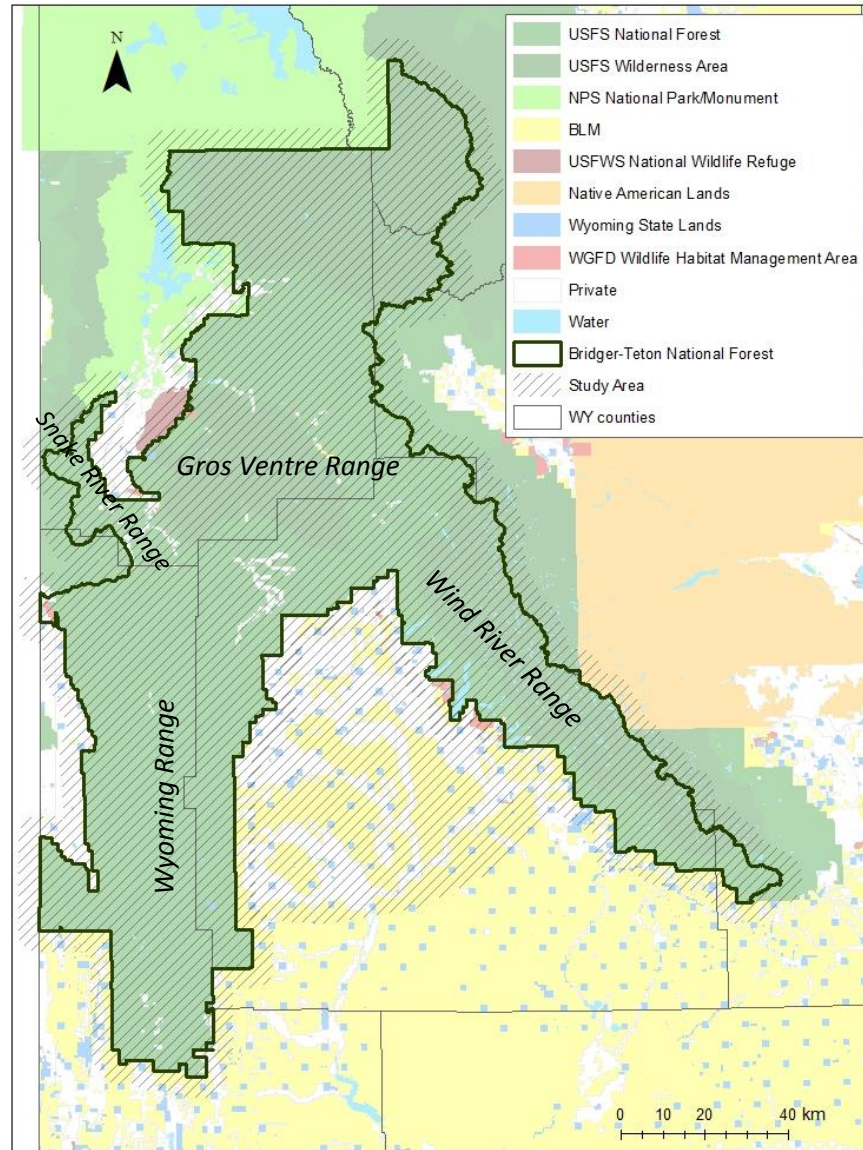
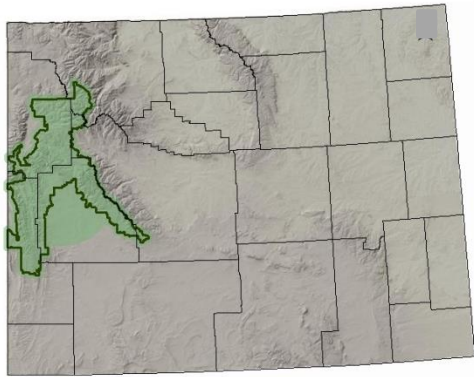
- Rorabaugh, J.C. 2005. *Rana pipiens*. Pages 570-580 in M. Lannoo, editor. Amphibian Declines: The Conservation Status of North American Species. University of California Press, Berkeley, CA.
- Skerratt, L. F., L. Berger, R. Speare, S. Cashins, K. R. McDonald, A. D. Phillott, H. B. Hines, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *Ecohealth* 4:125–134.
- Smith, B.E. and D.A. Keinath. 2007. Northern Leopard Frog (*Rana pipiens*): A technical conservation assessment. Prepared for the USDA Forest Service Rocky, Mountain Region, Species Conservation Project.
- 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.
- Switzer, J. F., R. Johnson, B.A. Lubinski, and T. L. King. 2009. Genetic structure in the *Anaxyrus boreas* species group (Anura, Bufonidae): an evaluation of the Southern Rocky Mountains population. Final Report Submitted to the U.S. Fish and Wildlife Service.
- USFWS. 2011a. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the Northern Leopard Frog in the western United States as threatened. Federal Register 76:61896-61931.
- USFWS. 2011. Endangered and threatened wildlife and plants; Review of native species that are candidates for listing as endangered or threatened; Annual notice of findings on resubmitted petitions; Annual description of progress on listing actions. Federal Register 76:66370-66439.
- USFWS. 2012. Endangered and threatened wildlife and plants; 90-day finding on a petition to list the Eastern or Southern Rocky Mountain population of the boreal toad as an endangered or threatened distinct population segment. Federal Register 77:21920-21936.



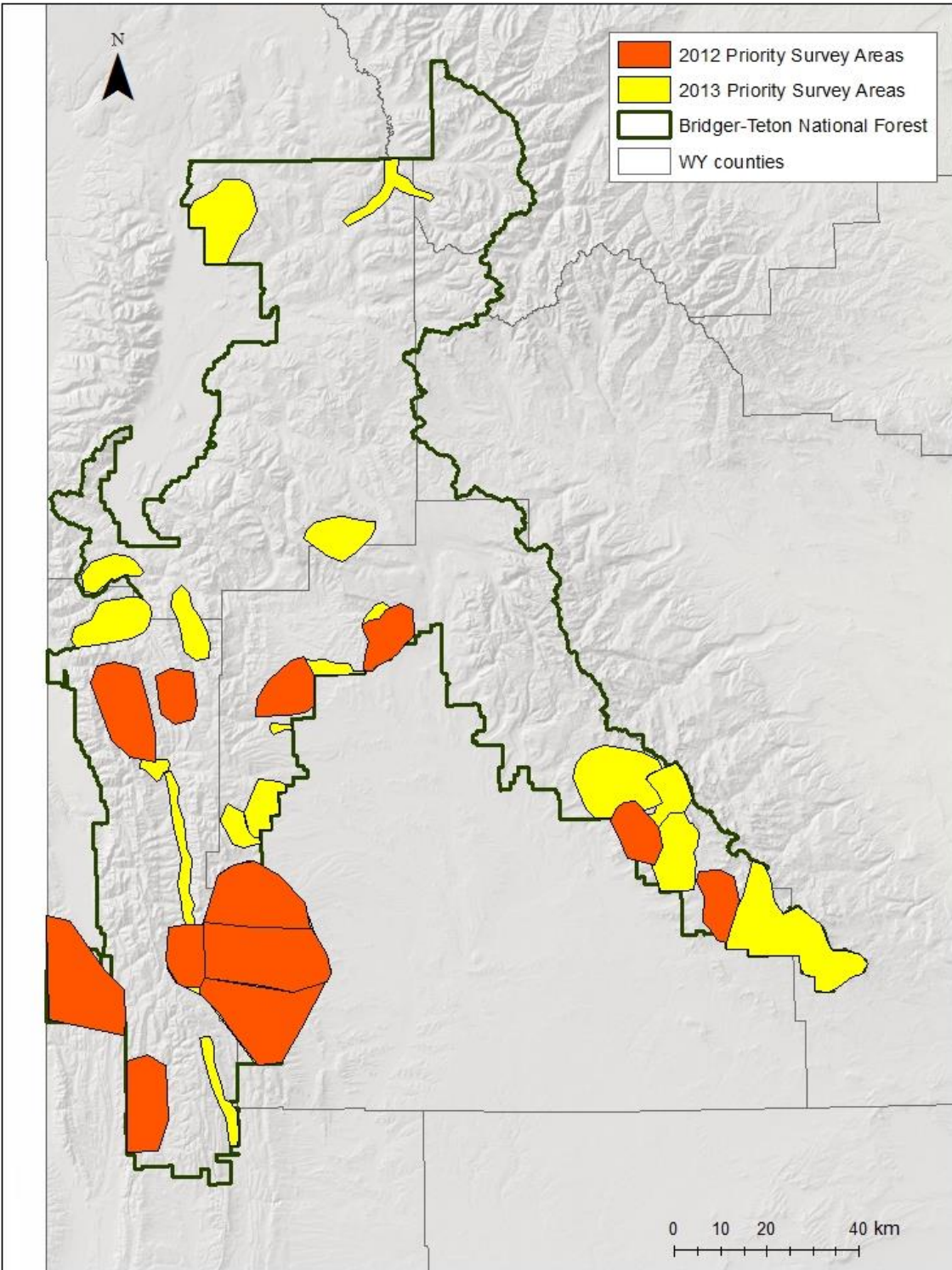
**Table1.** Occupancy ( $\Psi$ ) estimates and detection probabilities ( $p$ ) for amphibians in the Bridger-Teton National Forest and upper Green River drainage based on visual encounter surveys conducted from 2012-2014 and modeled using PRESENCE (Hines 2006).

Species	Naïve $\Psi^1$	$\Psi$	S.E.	$p$	S.E.
<b>boreal toad</b>	0.0812	0.1286	0.0255	0.5577	0.1015
<b>Columbia Spotted Frog</b>	0.0715	0.0894	0.0141	0.7459	0.0779
<b>Boreal Chorus Frog</b>	0.4182	0.4734	0.0239	0.8357	0.0303
<b>Tiger Salamander</b>	0.1362	0.1912	0.0240	0.6479	0.0659

<sup>1</sup>Occupancy estimate not corrected for bias due to imperfect detection of the species. Naïve  $\Psi$  = # sites where the species was detected/total # sites surveyed.

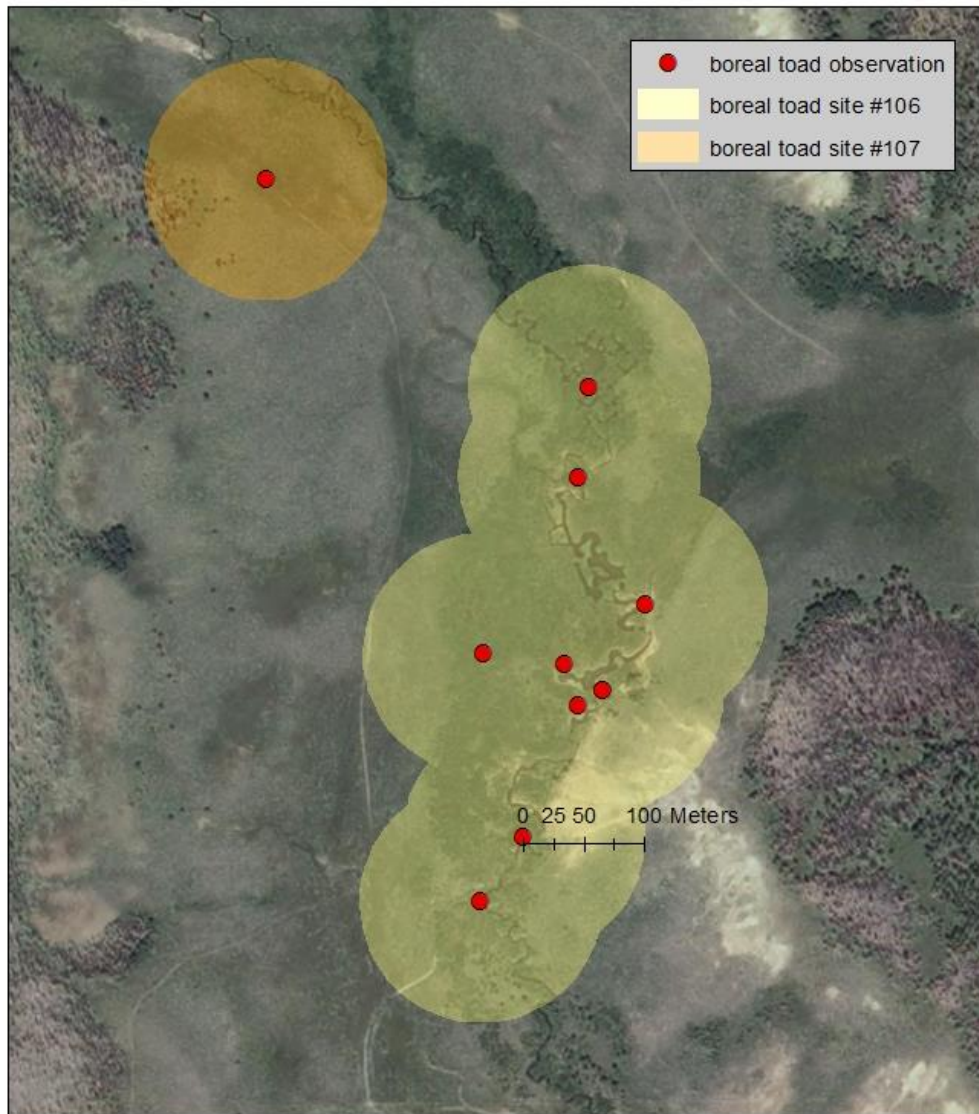


**Figure 1.** Land ownership within and surrounding the study area in the Bridger-Teton National Forest and upper Green River drainage in Western Wyoming, USA.

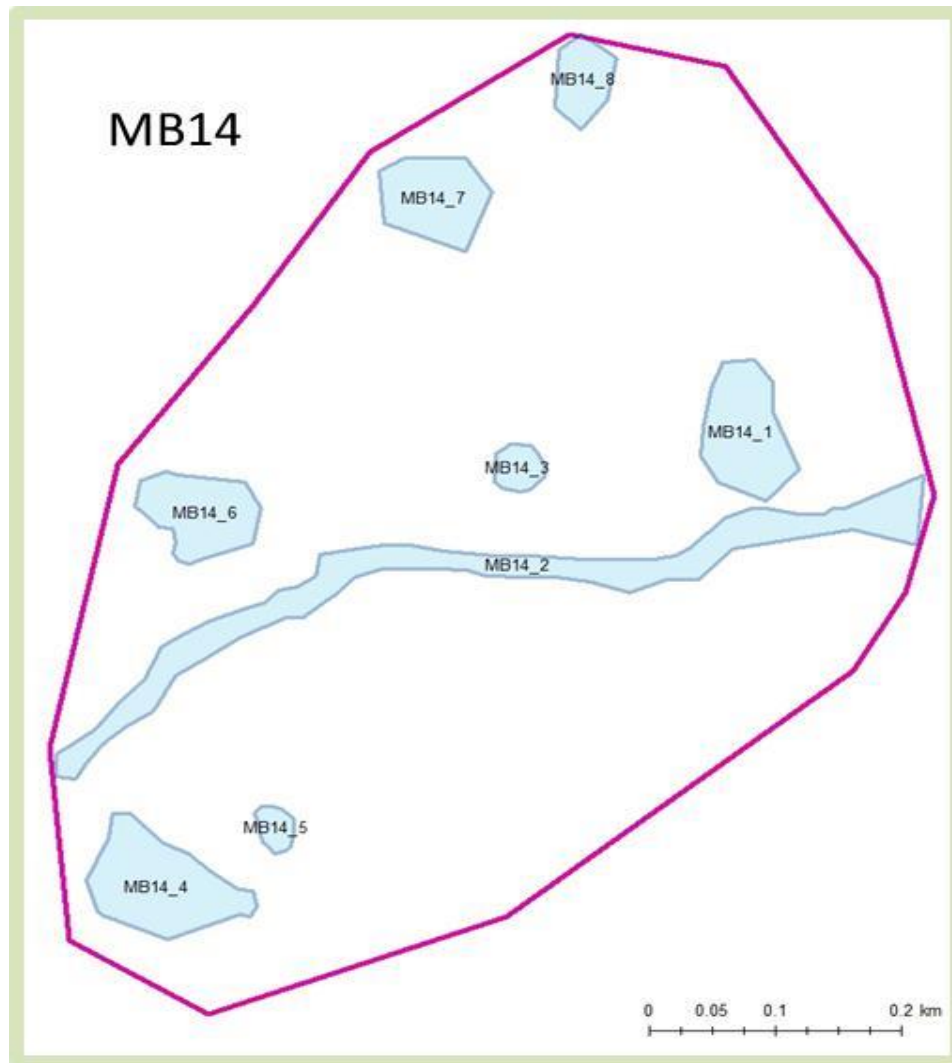


**Figure 2.** Priority areas for amphibian surveys in 2012 and 2013. Priority areas were identified each spring at coordination meetings involving WYNDD, WGFD, and BTNF wildlife and fisheries biologists.

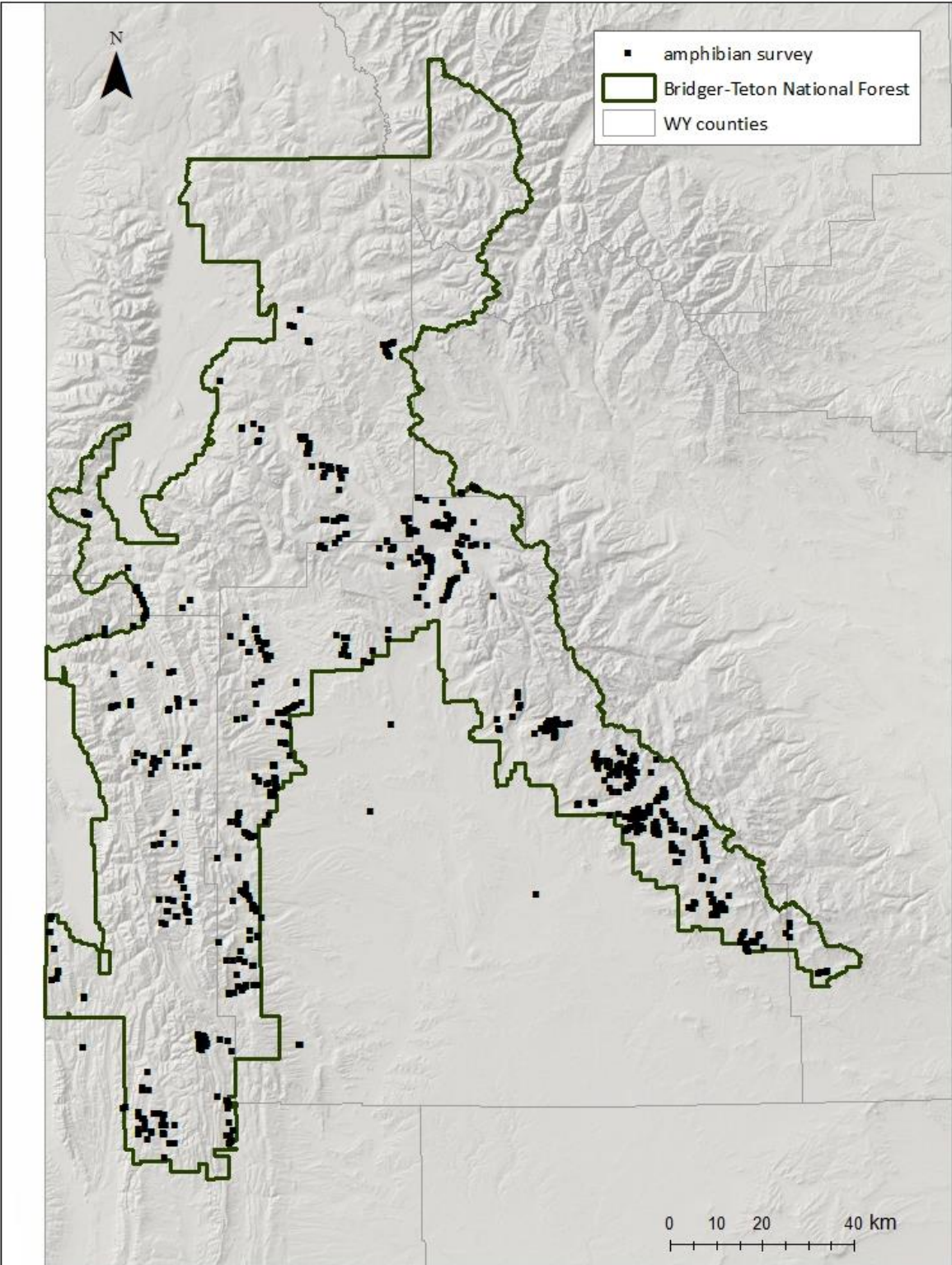




**Figure 3.** Example of how boreal toad locations were created by buffering observation records by 100m and combining all records with overlapping buffers into a single site polygon. Each site polygon retained the original records for every observation falling within that polygon.



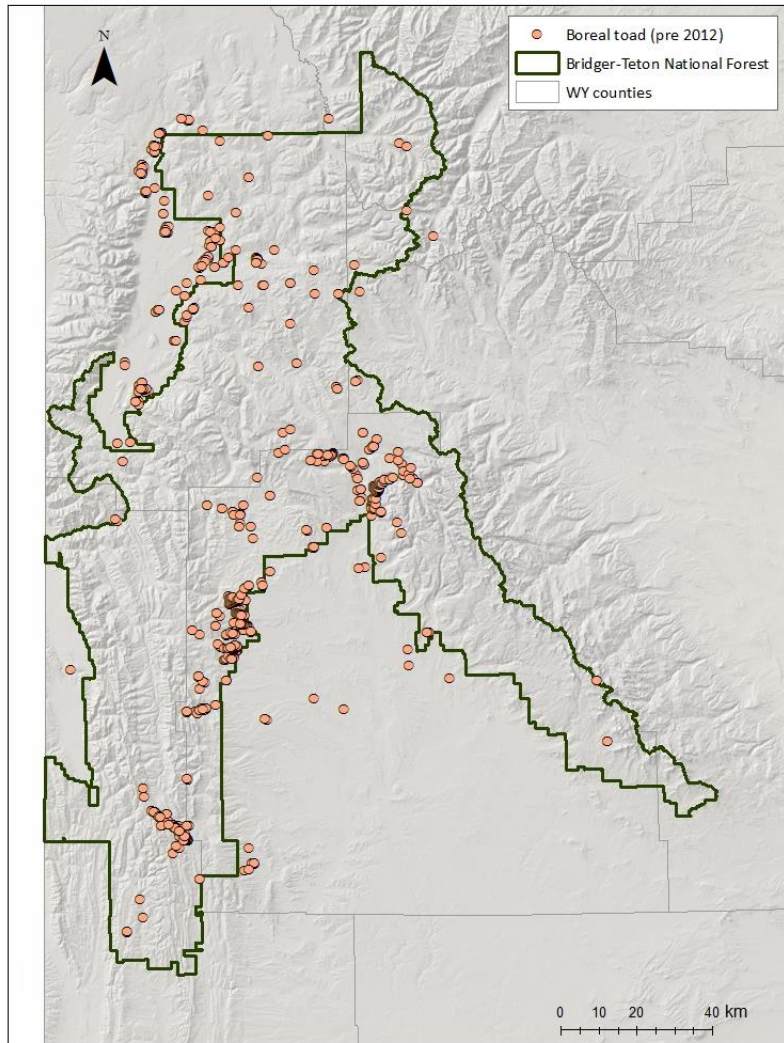
**Figure 4.** Example catchment (MB14) outlined in red with wetland survey sites in blue.



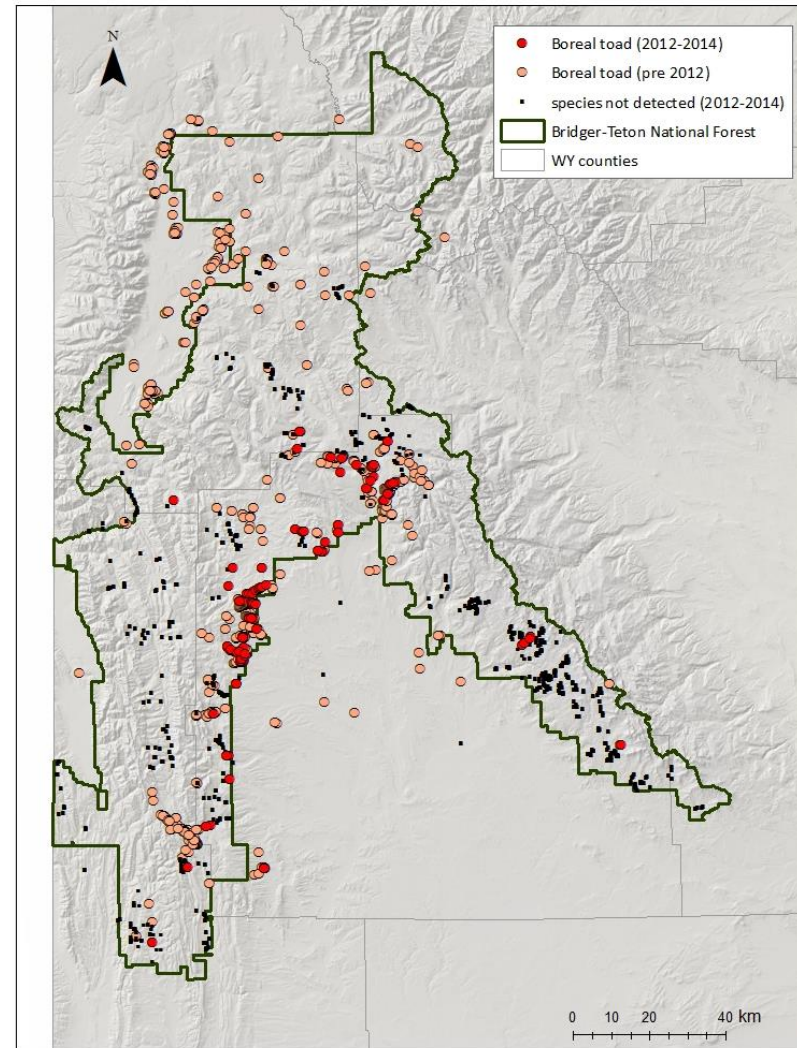
**Figure 5.** Location of 862 sites surveyed for amphibians in the Bridger-Teton National Forest and upper Green River drainage from 2012-2014.



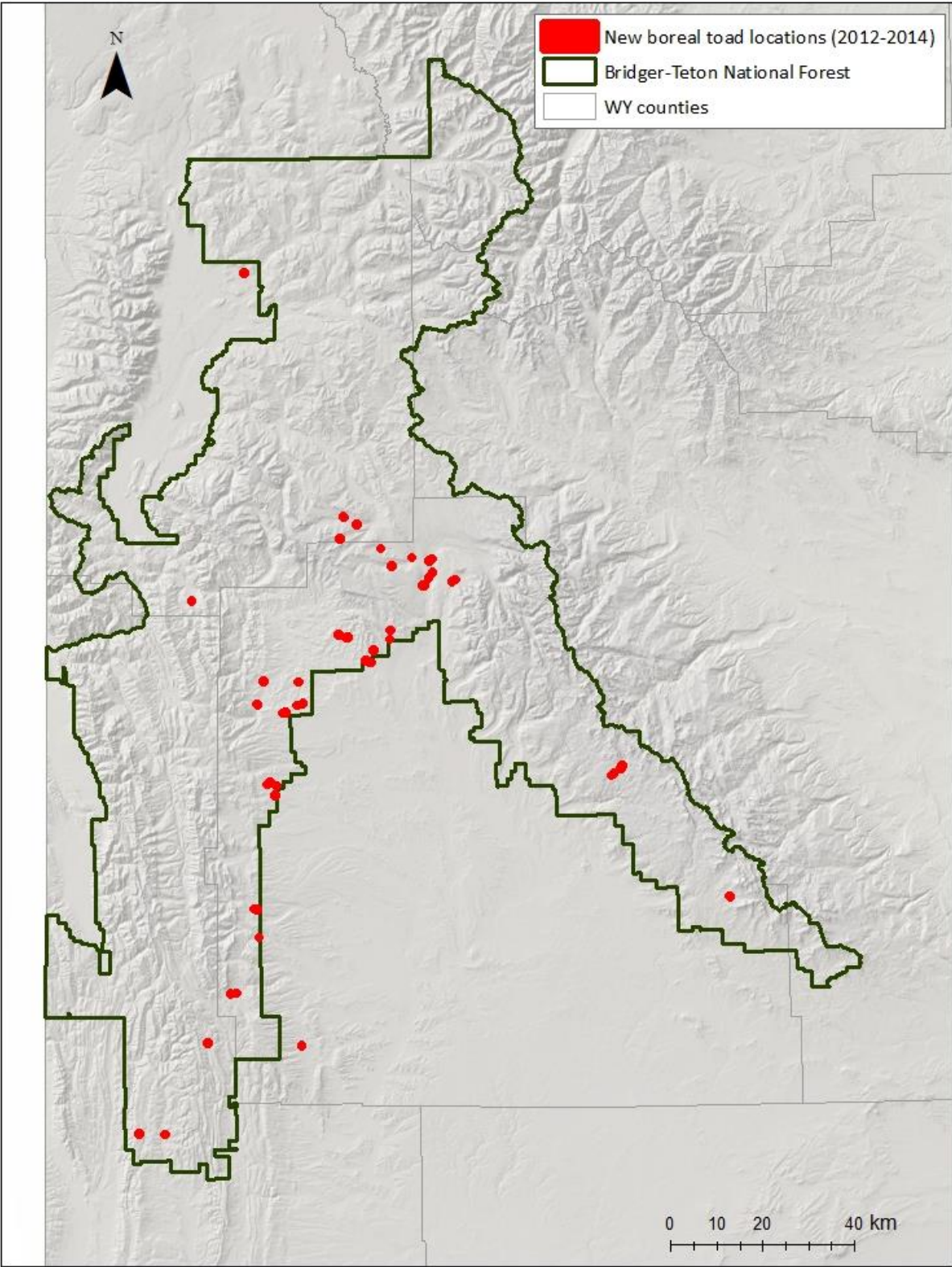
a)



b)

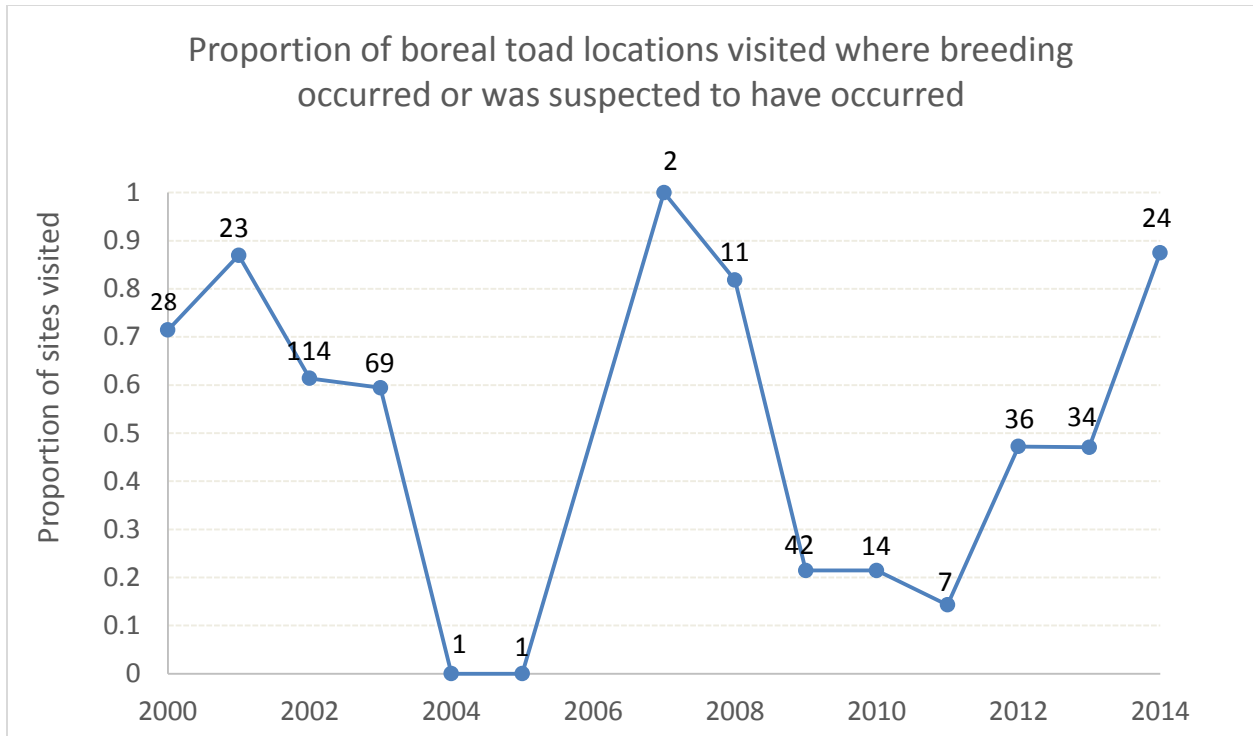


**Figure 6.** Distribution of boreal toads in the Bridger-Teton National Forest and upper Green River drainage based on data compiled a) prior to this study (<2012), and b) as of 2014.

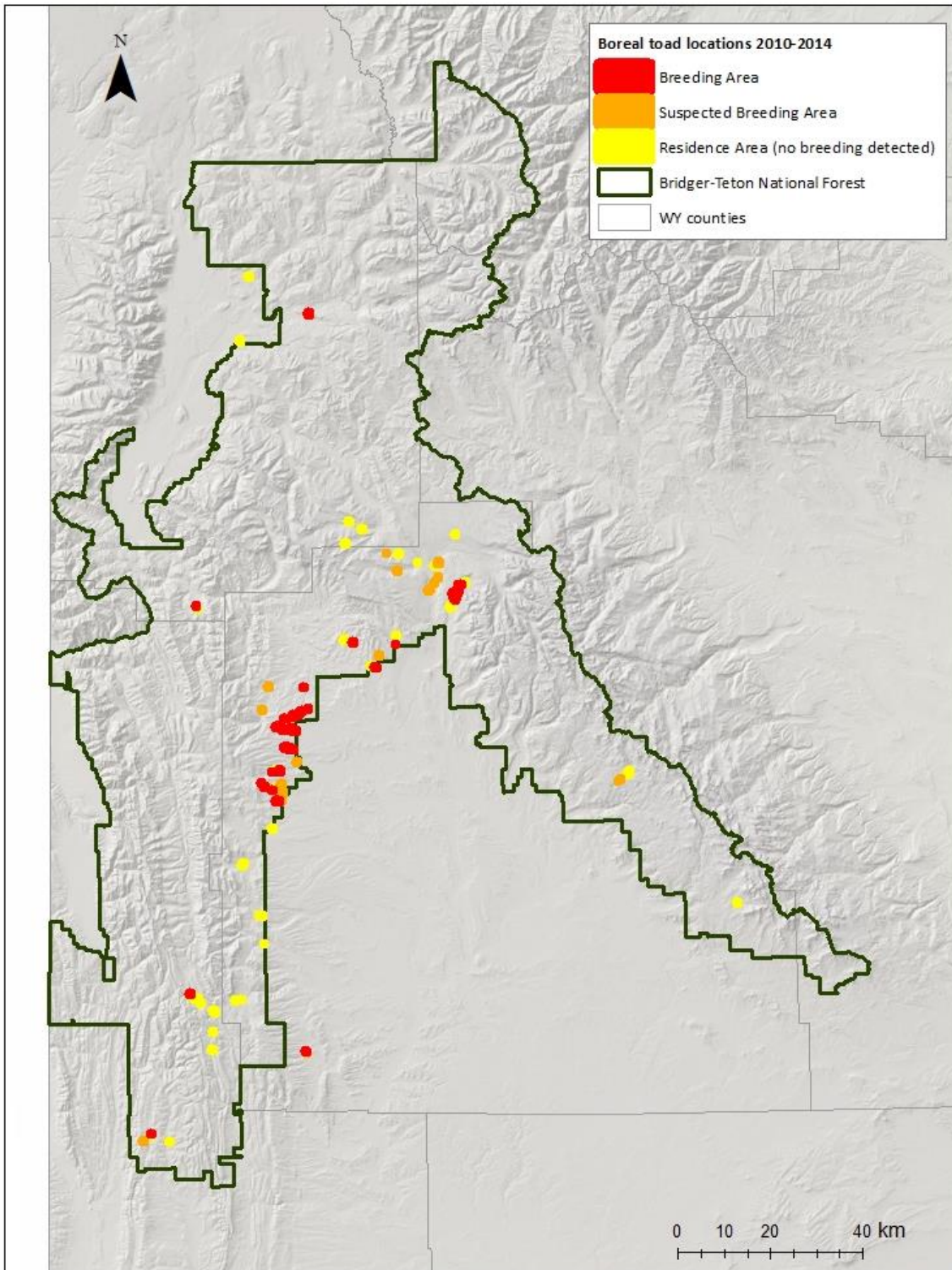


**Figure 7.** New boreal toad locations discovered during surveys from 2012-2014. New locations did not fall within 100m of previously documented boreal toad occurrence records.



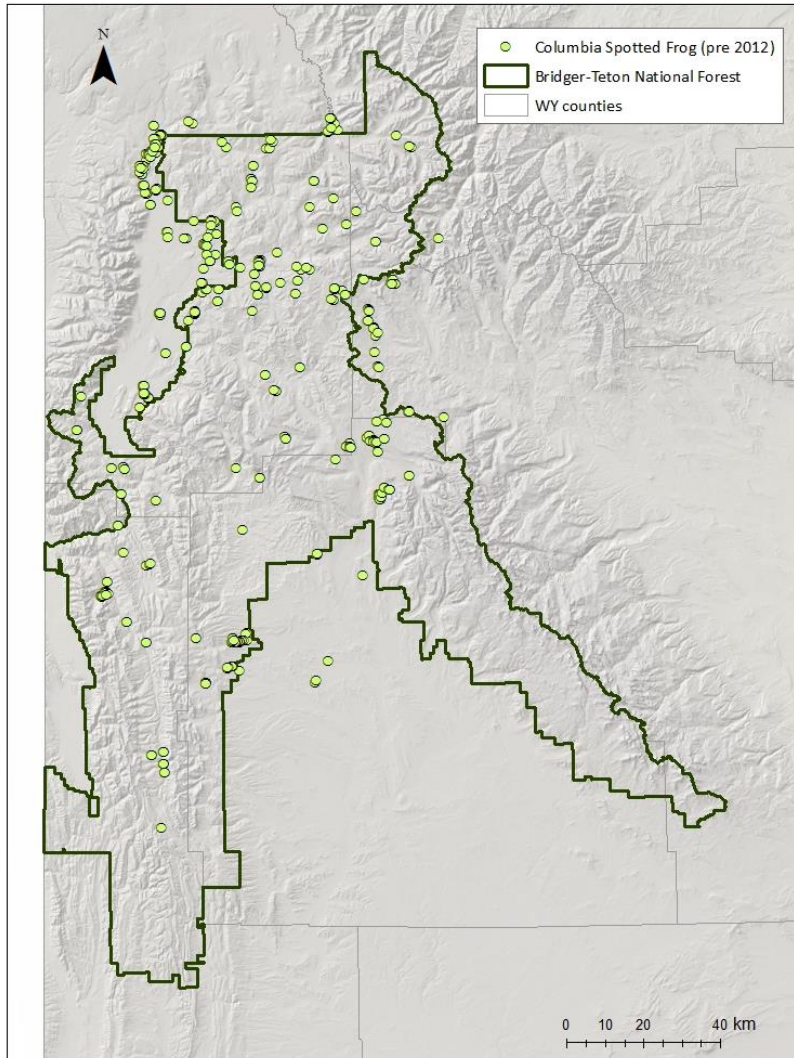


**Figure 8.** Proportion of boreal toad locations visited in a year where breeding was known to occur (eggs, tadpoles, metamorphs, or adults in amplexus detected) or suspected to occur (juveniles, breeding calls, or aggregations of adults detected) that year. Numbers above points indicate how many sites were visited in a given year. Boreal toad locations were in the Bridger-Teton National Forest and Upper Green River drainage in Wyoming.

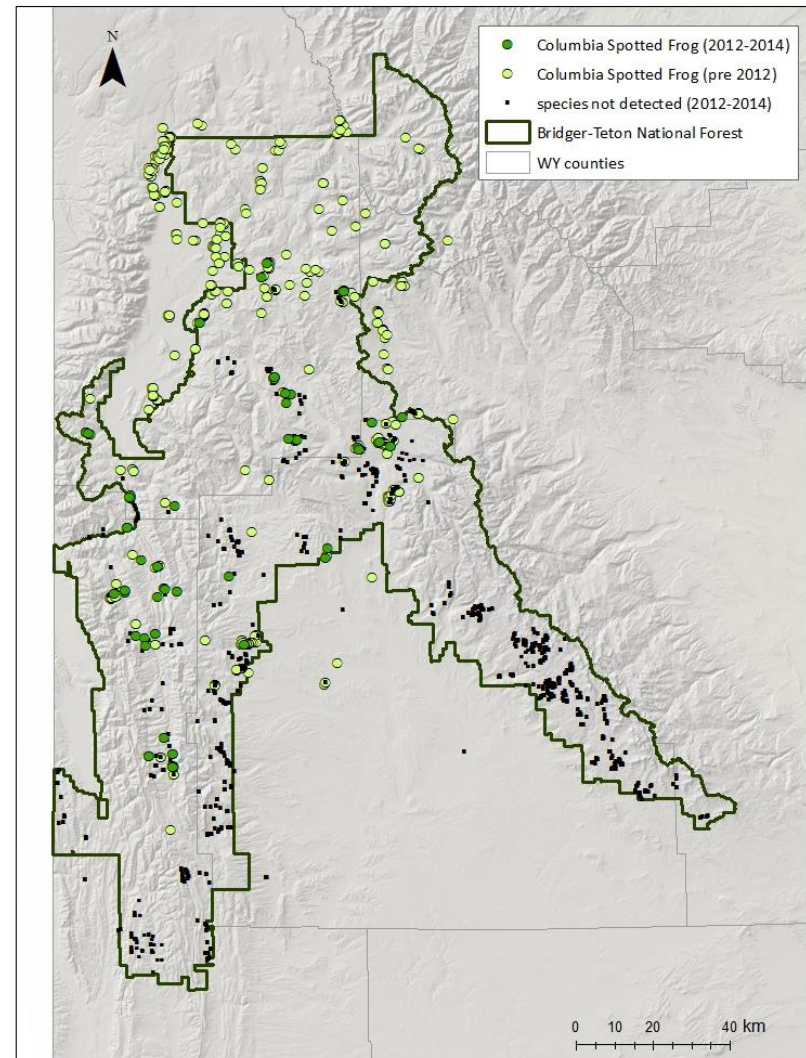


**Figure 9.** Locations of important breeding areas for boreal toads in the Bridger-Teton National Forest and upper Green River drainage based on breeding status at boreal toad locations visited from 2010-2014. Breeding status is displayed giving priority first to breeding areas, then to suspected breeding areas.

a)

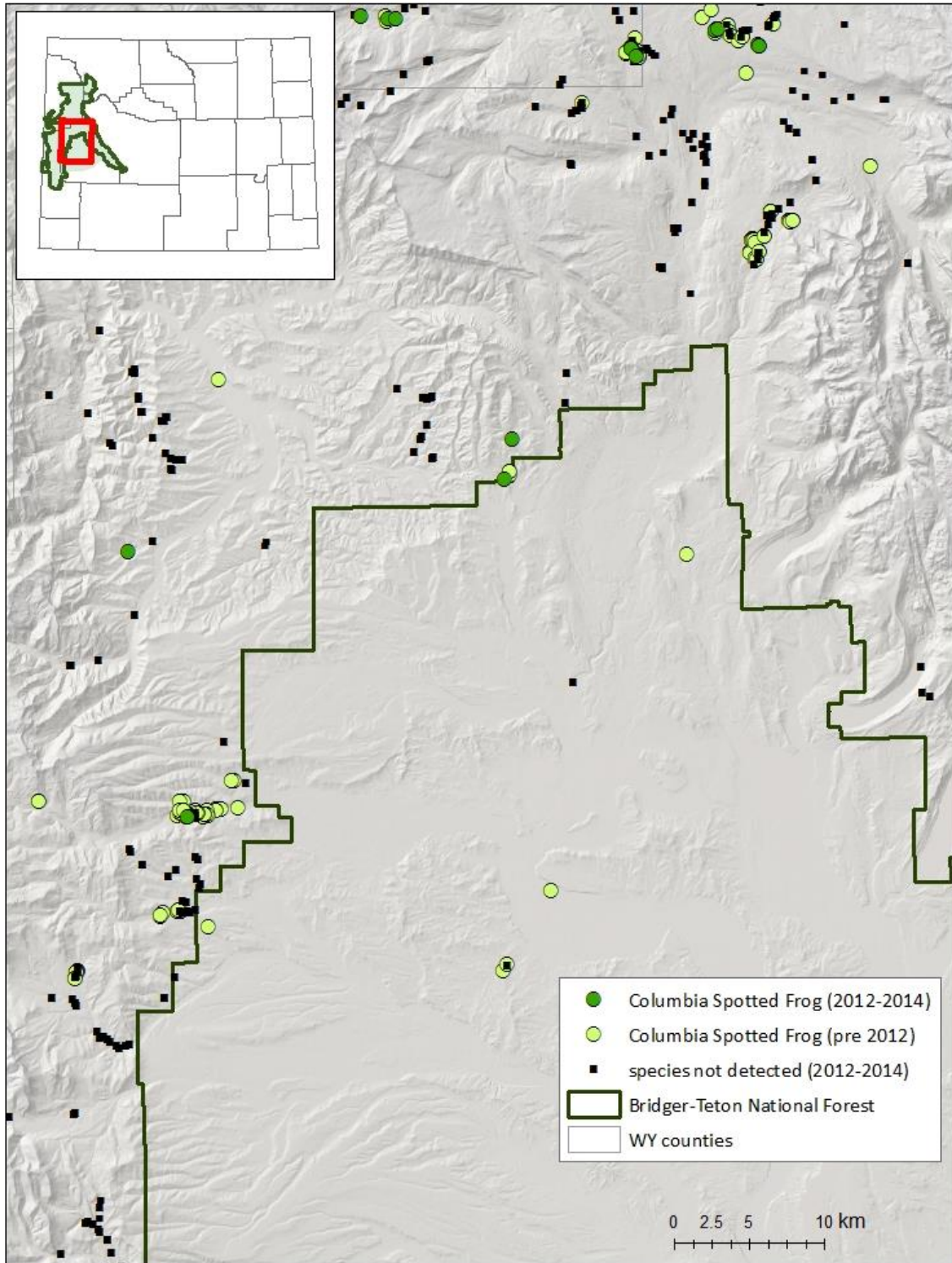


b)



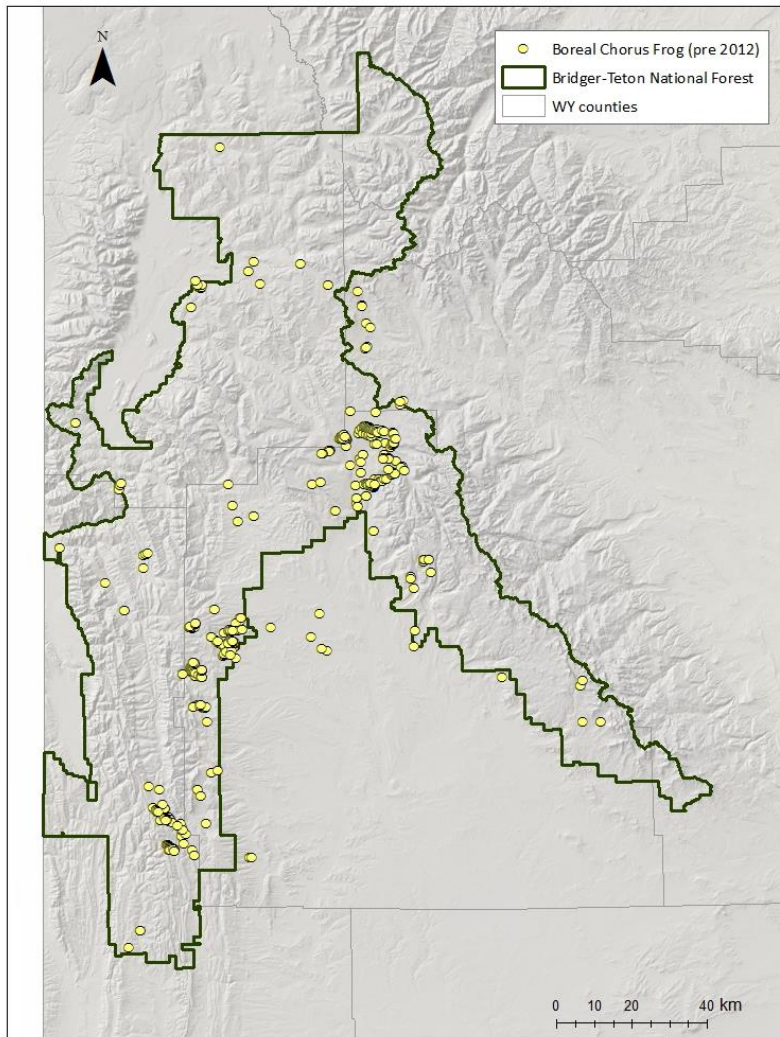
**Figure 10.** Distribution of Columbia Spotted Frogs in the Bridger-Teton National Forest and upper Green River drainage based on data compiled a) prior to this study (<2012), and b) as of 2014.



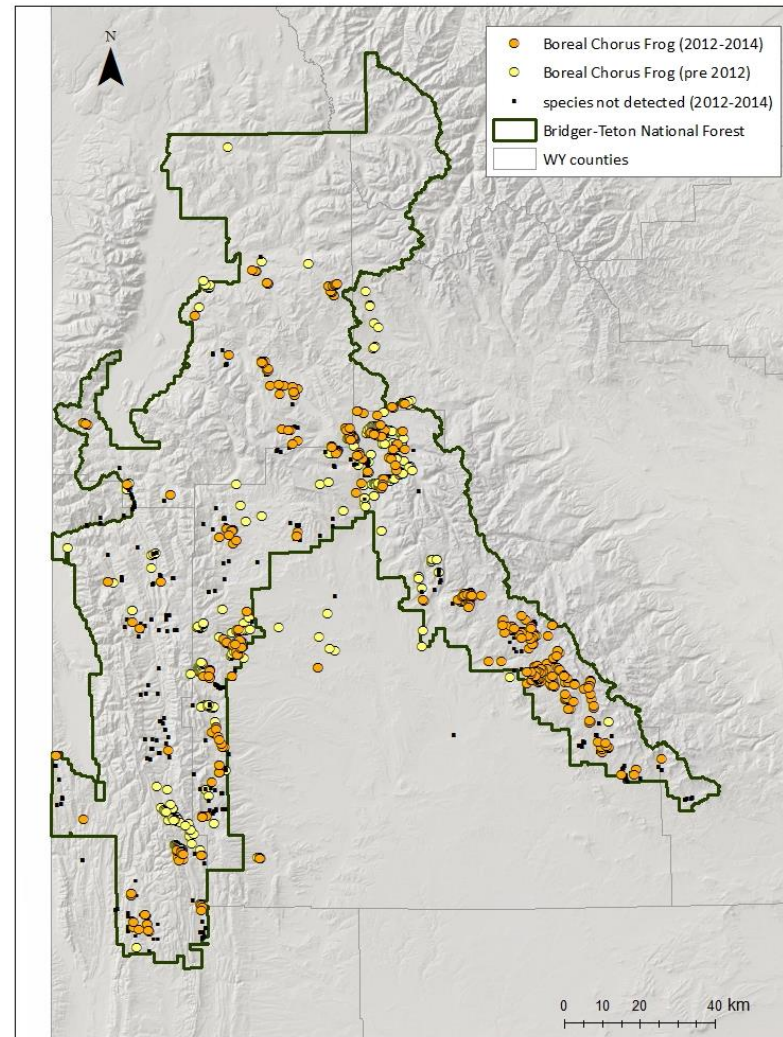


**Figure 11.** Distribution of Columbia Spotted Frog locations before and after this study in the northeastern Wyoming Range and upper Green River drainage. Most pre-2012 records in this region were from amphibian surveys conducted between 2001 and 2003.

a)



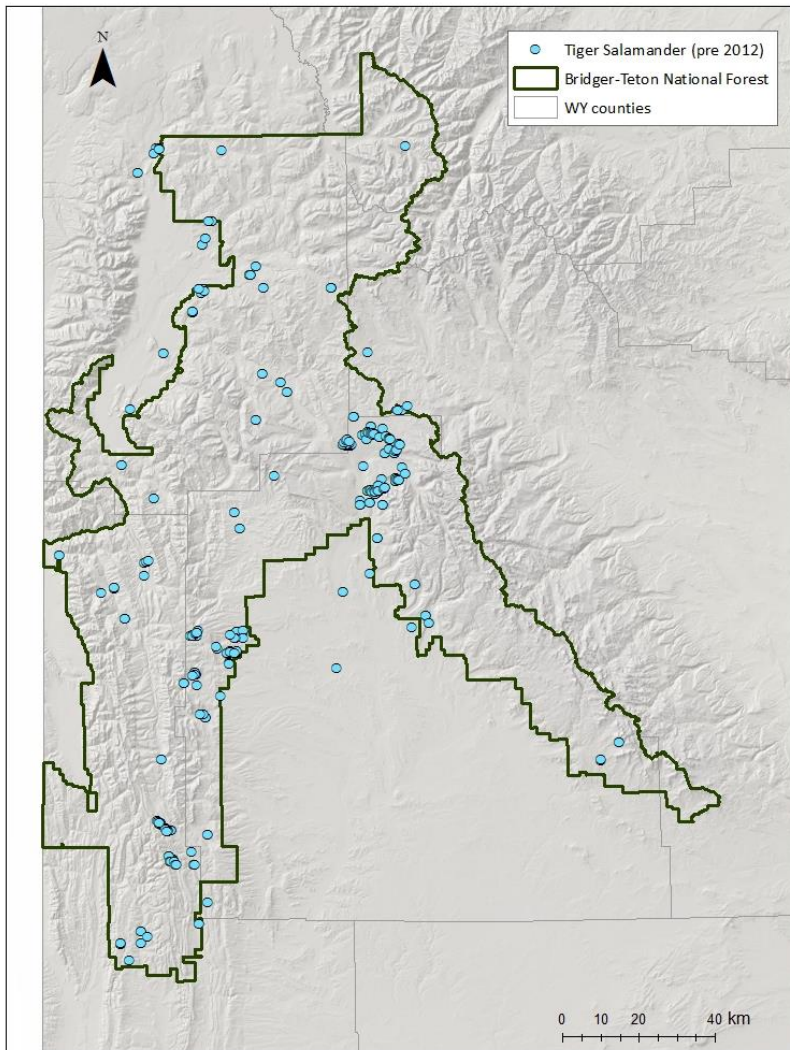
b)



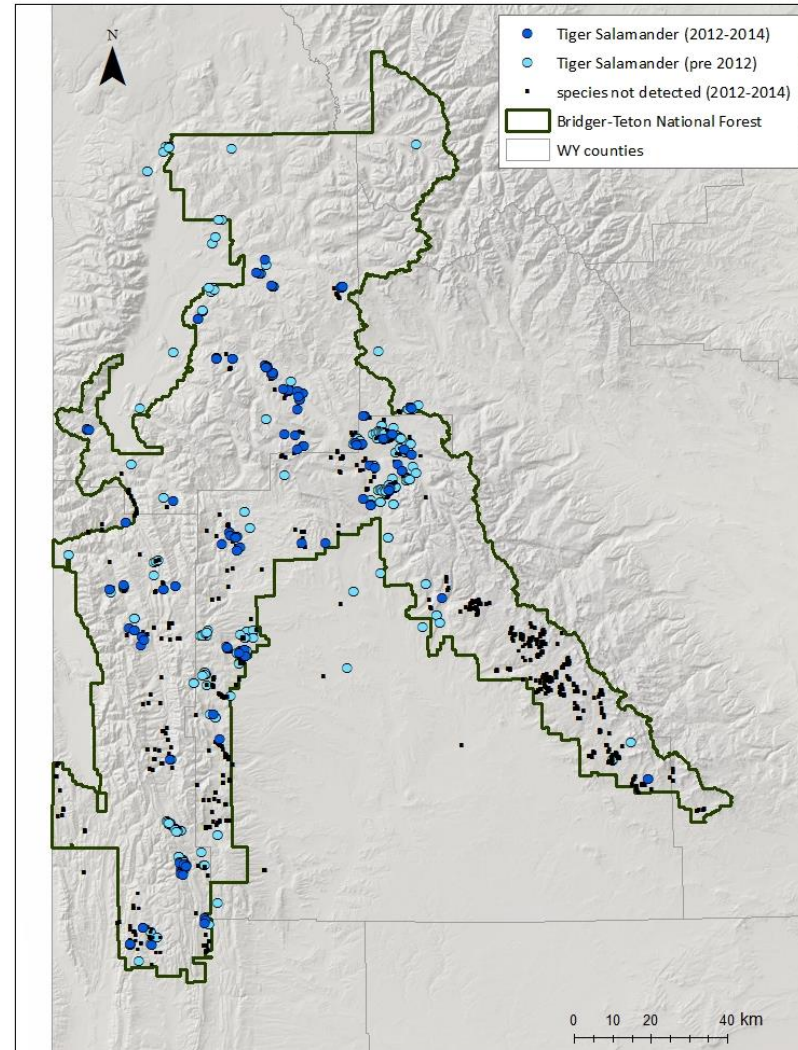
**Figure 12.** Distribution of Boreal Chorus Frogs in the Bridger-Teton National Forest and upper Green River drainage based on data compiled a) prior to this study (<2012), and b) as of 2014.



a)

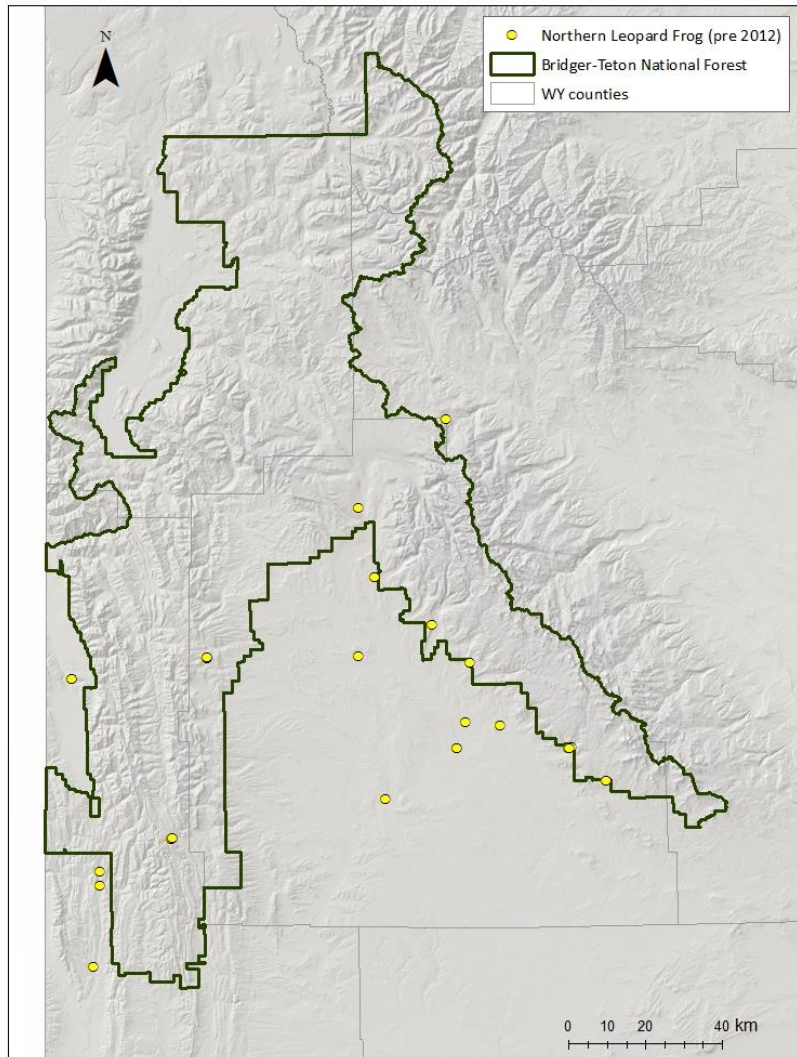


b)

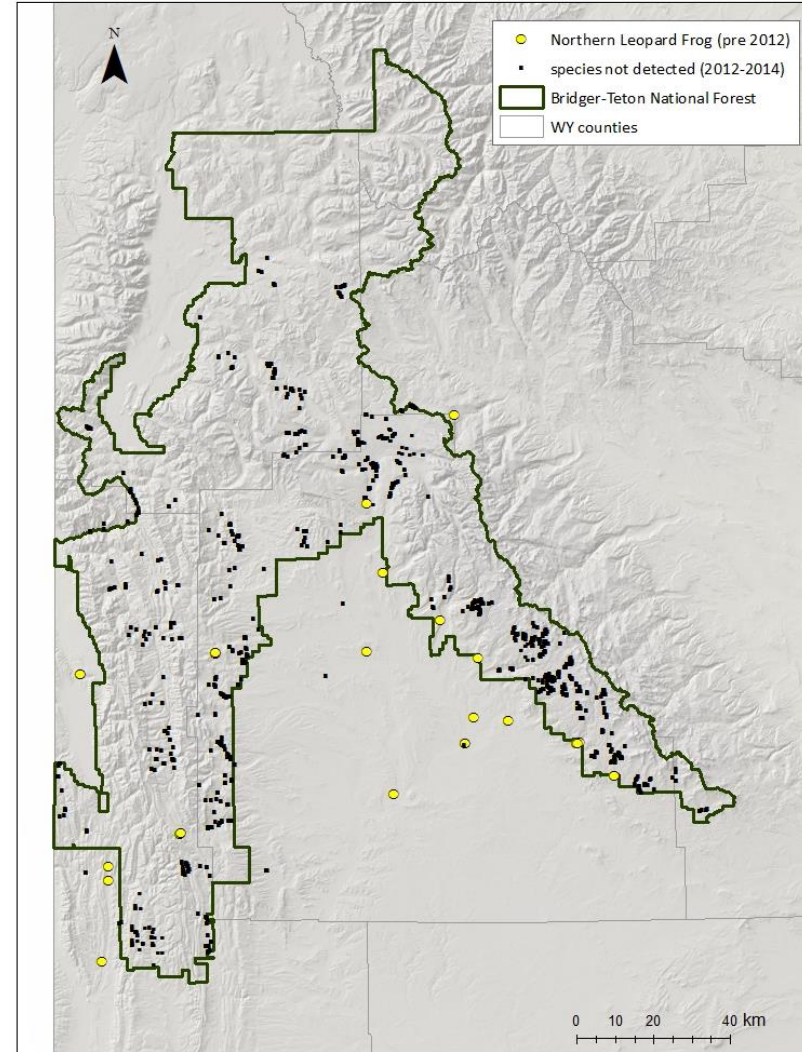


**Figure 13.** Distribution of Tiger Salamanders in the Bridger-Teton National Forest and upper Green River drainage based on data compiled a) prior to this study (<2012), and b) as of 2014.

a)

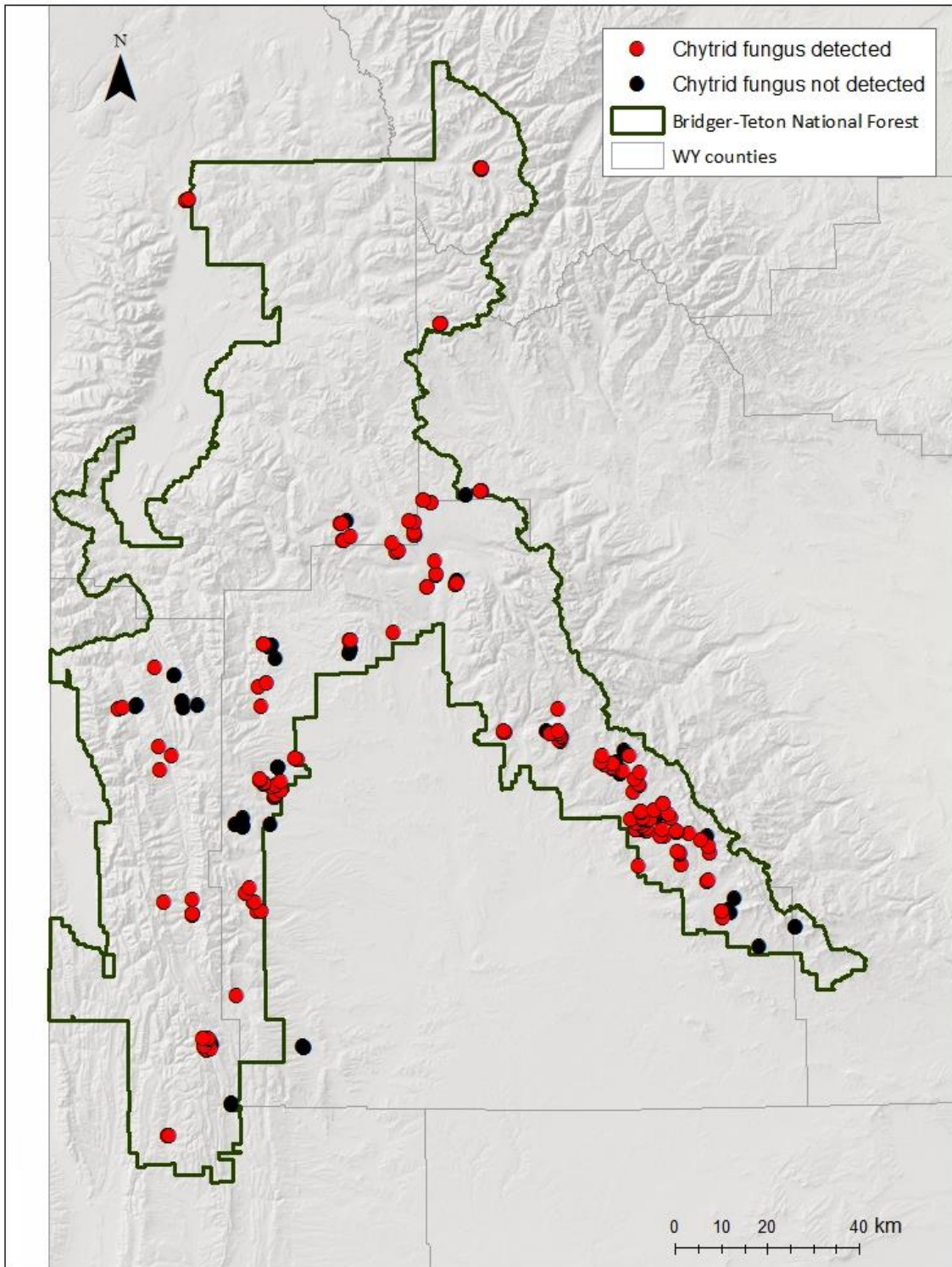


b)



**Figure 14.** Distribution of Northern Leopard Frogs in the Bridger-Teton National Forest and upper Green River drainage based on data compiled a) prior to this study (<2012), and b) as of 2014.

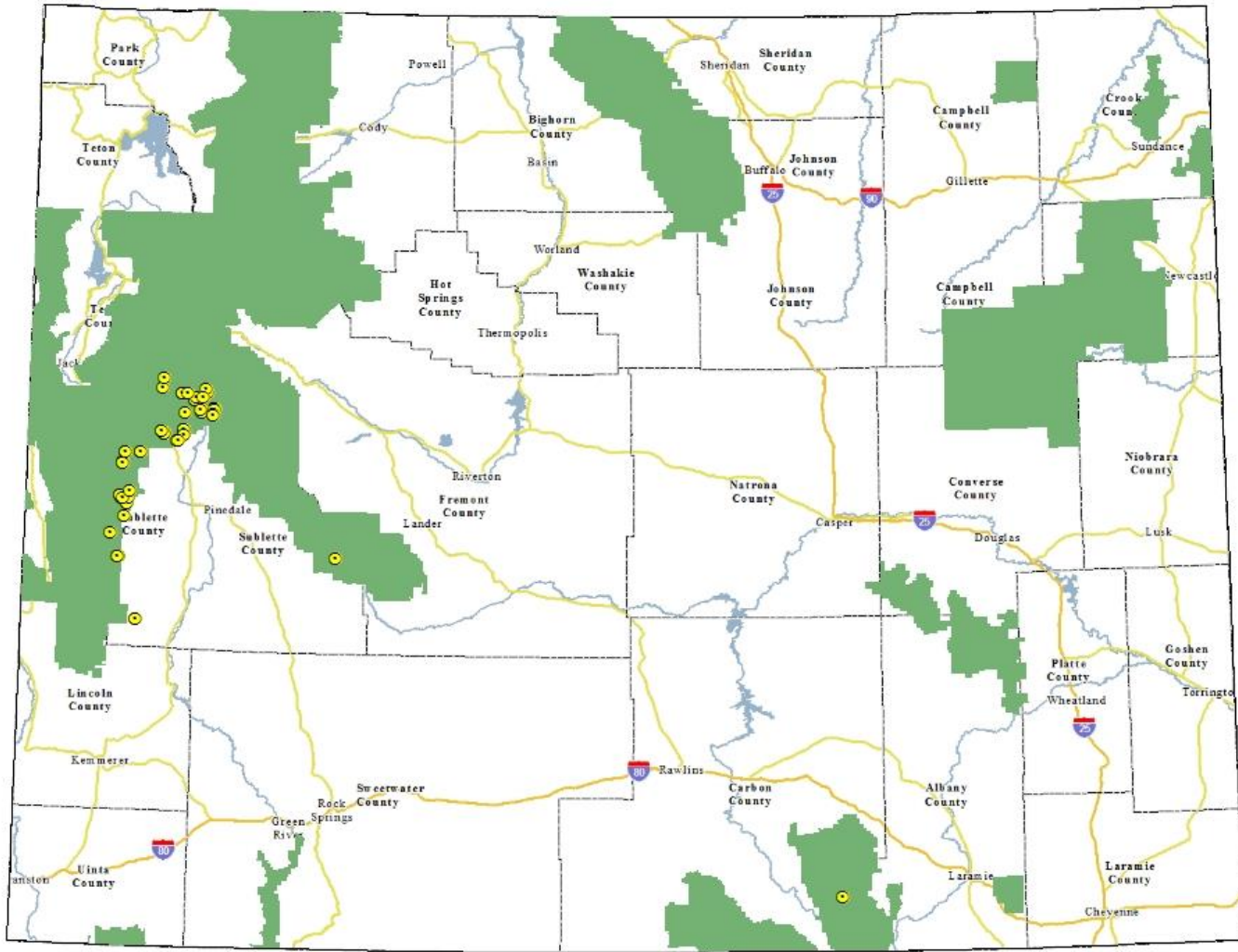




**Figure 15.** Spatial distribution of amphibian chytrid fungus that tested positive (red circles) or negative (black circles) for the disease. Samples were taken from boreal toads, Columbia Spotted Frogs, Boreal Chorus Frogs, or Tiger Salamanders from 2012-2014 in the Bridger-Teton National Forest and upper Green River drainage.

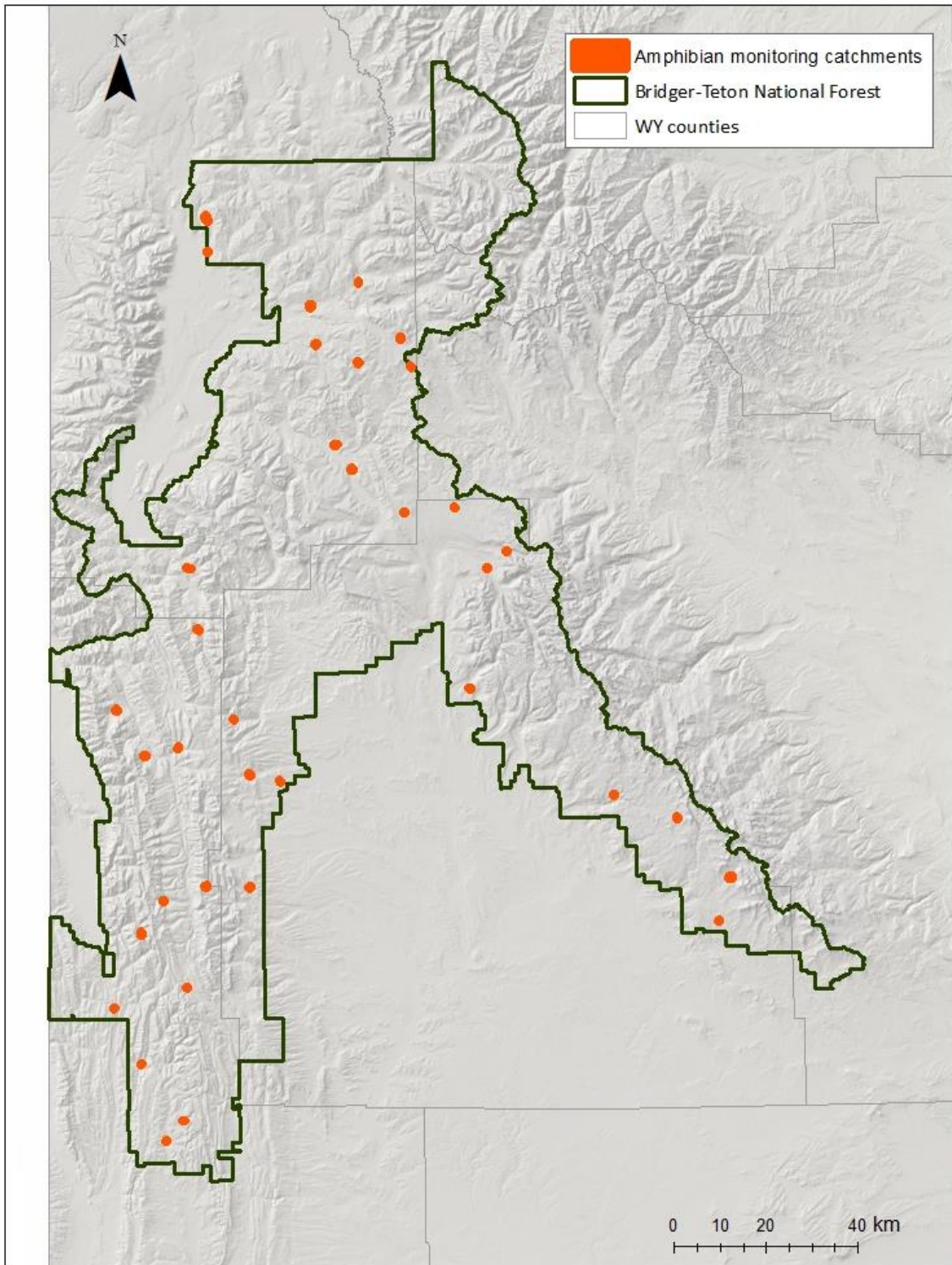


Wyoming Boreal Toad Genetic Samples (n=68) from 2012-2013



Map by WYNDD - 30 Sept 2014

Figure 16. Map showing the location of 68 boreal toad genetic samples collected from 2012-2014.



**Figure 17.** Spatial distribution of 36 newly established long-term amphibian monitoring catchments in the Bridger-Teton National Forest.

## **Appendix A:**

Example of catchment overview pages and site-specific datasheets provided for amphibian surveys at long-term monitoring catchments

Example: Catchment Overview

**Rocky Mountain Amphibian Project**

**Catchment Name:** BT54\_Strawberry Creek

<b>Survey Date:</b>	
<b>Surveyor Names:</b>	

**Please survey between:** June 20 - August 10

\*Please check all catchment and site information. Fill in any blank fields and note any changes that should be made in the appropriate field (e.g. changes in Access Point location or Difficulty ranking).

**Management Unit:** Bridger-Teton National Forest

**County:** Sublette

**Accessibility:** All Vehicles?

**State:** WY

**Access/Parking Waypoint:** BT54\_AP

**Difficulty:** Easy

**Datum:** NAD83 Zone 12

**Hiking time from Access Point:** 8 min.

**Easting:** 584715

**Elevation (ft):** 9052

**Northing:** 4811622

**Directions to Access Point:**

Take Hwy352 north of Cora and follow onto forest where it becomes FR600. Just past the Kendall Guard Station, take the left fork to stay on FR600. Stay on FR600 past Mosquito Lake for ~12 miles.

Turn right onto FR640. After 0.5 miles, turn right to Access Point (BT54\_AP). Hike 1/4 mile SW from AP to catchment

**Catchment Description:**

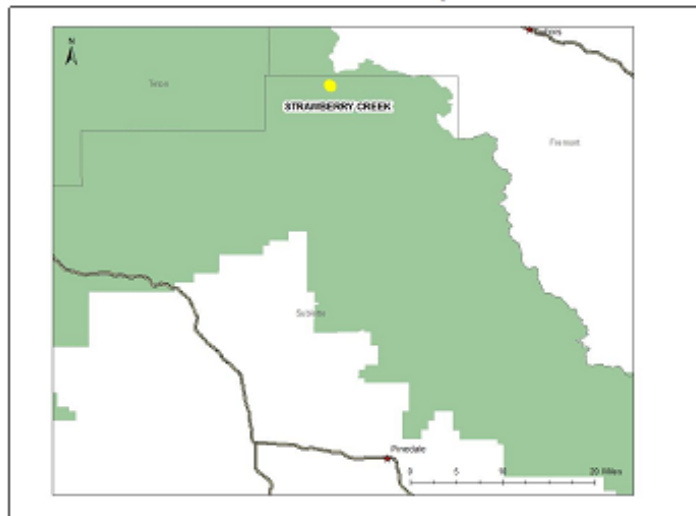
Catchment is a collection of pools and bogs in wet meadows.

**Additional Notes:**

Please note which sites (if any) are dry.

**Survey notes, updates, or recommendations for Project Managers**

**Catchment Locator Map**

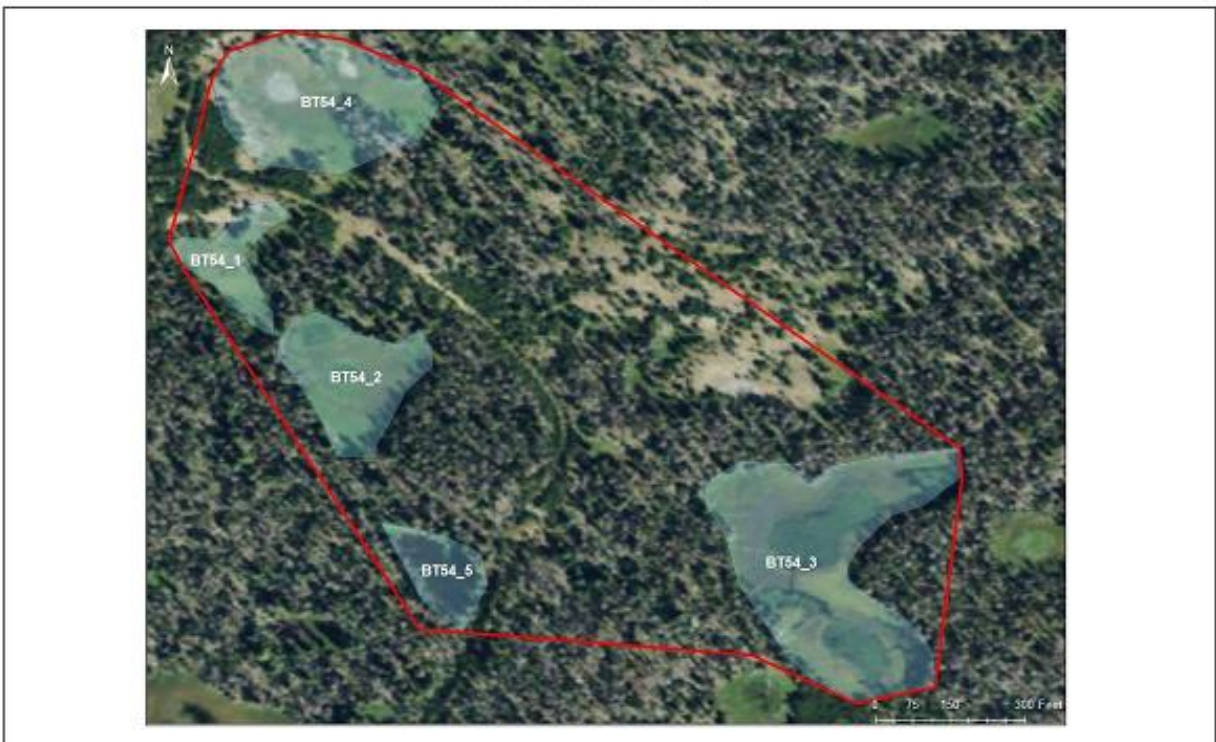




**Catchment Map with Access Point**



**Catchment Detail Map**



Example: Site-specific datasheet (surveyor 1)

### Rocky Mountain Amphibian Project

**Site: BT54\_1**

**Catchment:** BT54\_Strawberry Creek

**Coordinates**

Datum: NAD83 Zone 12

**Start Waypt:** BT54\_1Start  
 UTM Easting: 584431  
 UTM Northing: 4811259

**End Waypt:** BT54\_1End  
 UTM Easting: 584431  
 UTM Northing: 4811178

**Photo Waypt.:** BT54\_1End  
**Photo Direction:** NW

**Photo Easting:** 584431  
**Photo Northing:** 4811178

\*Note in Survey  
 Comments if  
 coordinates  
 differed and why

Approx. survey time (min.): 15

Start Time  
 End Time

Photo #

Who's camera was used?

Survey Date:	
Surveyor Names:	
Sufficient water present for survey	Yes No

**Survey Condition** (circle appropriate conditions or fill in blank)

Air Temp	F or C?	Wind	Calm	Light	Moderate	Strong	Cloud Cover
Water Temp	F or C?	Precipitation	None	Rain	Snow	Hail	0-25% 25-75% 75-100%
Survey Comment							

**Site Conditions** (circle appropriate conditions or fill in blank)

Waterbody Type	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
Water color	Clear Stained	Water Turbidity	Clear	Cloudy	< 3 ft > 3 ft
Manmade?	Yes No	Permanence	Permanent	Intermittent	Fish Present? Yes No
Primary Substrate	silt/mud	sand	cobble	boulder/rock	Fish Species:
Evidence of Cattle Grazing	None	Light	Heavy Veg	Heavy Shoreline	Heavy Veg & Shoreline
% of shoreline with emergent vegetation (top of plant sticks out of water)	0%	1-25%	25-50%	50-75%	75-100%
Site Note					

**Species Detected**

**Survey 1**

Surveyor Name

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

Enter data at [www.toadtrackers.org](http://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 amphibians, especially egg masses or 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



Example: Site-specific datasheet (surveyor 2)

### Rocky Mountain Amphibian Project

Site: BT54\_1

Catchment: BT54\_Strawberry Creek

Survey Date:	
Start Time	
End Time	

**Species Detected**

Survey 2

Surveyor Name	
---------------	--

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

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](http://www.toadtrackers.org)

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