



Greater Yellowstone Network Amphibian Monitoring

2012-2013 Biennial Status Report

Natural Resource Report NPS/GRYN/NRR—2016/1140



ON THE COVER

Beaver dam on a side channel of Thorofare Creek, Yellowstone National Park, July 2012. Western toad breeding was documented in this beaver pond complex for the first time in 2012.
NPS Photo.

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Authors

Andrew Ray

National Park Service
Greater Yellowstone Network
2327 University Way, Suite 2
Bozeman, MT 59715

Debra Patla

Northern Rockies Conservation Cooperative
PO Box 2705
Jackson, WY 83001

Editing and Design

Tani Hubbard

Northern Rockies Conservation Cooperative
National Park Service
Greater Yellowstone Network
2327 University Way, Suite 2
Bozeman, MT 59715

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Executive Summary

The national parks (Grand Teton National Park, John D. Rockefeller, Jr. Memorial Parkway, and Yellowstone National Park) within the Greater Yellowstone Ecosystem provide an opportunity to monitor wetlands and amphibians within a relatively intact ecosystem and at spatial and temporal scales that offer valuable insights on the status of regional wetland flooding and drying patterns, amphibian population dynamics, and the wetland habitat features associated with amphibian use. The Greater Yellowstone Network amphibian monitoring program is the only long-term wetland and amphibian monitoring program in the Greater Yellowstone Ecosystem that annually revisits a large number of randomly selected wetlands across the region. The goal of this program is to estimate occupancy rates for the reproductive component of native amphibian species and incorporate the dynamics of wetland sites that provide potential breeding habitat. These annual measures of amphibian occurrence and wetland flooding status also contribute to local, regional, and national level trends analysis in amphibian populations. This work, when combined with historical data, will provide managers and the public with information on a class of native fauna and on wetland characteristics that are important for understanding long-term trends or changes in wetland resources.

This report describes amphibian monitoring efforts and results from the years 2012 and 2013. In both years, we conducted surveys in 31 catchments (sampling units) selected for long-term monitoring. All species targeted for monitoring were found. There were no plains spadefoot (*Spea bombifrons*), or northern leopard frogs (*Lithobates pipiens*) and no non-native American bullfrogs (*Lithobates catesbeianus*) detected at monitoring sites.

Occupancy rates varied by species and year. Boreal chorus frogs (*Pseudacris maculata*) had the highest occupancy rates in both years. Longer term analyses (2006-2012) also indicate annual changes in occupancy. Further monitoring and analysis will be needed to interpret how amphibian occupancy in the Greater Yellowstone Ecosystem is changing through time and to fully understand the drivers of this change.

Using Daymet gridded data sets, the average annual temperature of monitoring sites (averaged across all sites) was 1.7 times higher in 2012 than 2013. Total and April to June precipitation levels (averaged across all sites) were also higher in 2012 than 2013. Despite these differences, the percentage of dry wetlands was similar in 2012 and 2013.

Supplemental western toad (*Anaxyrus boreas*) monitoring occurs informally and opportunistically to augment knowledge on the status of amphibians in all three park units. Although toad breeding sites within the selected catchments are scarce, previous projects and incidental encounters have identified approximately 40 widespread toad breeding areas since 2000. In 2012 and 2013, we documented toad breeding at 18 of 23 and seven of 11 toad areas visited, respectively.

An increase in beaver activity in two catchments, one in each park unit, created additional habitat for breeding amphibians in 2012 and 2013. The effect of the new habitat contributed to an increase in the number of western toad breeding sites (although still a small number) by 2013, and the creation of an amphibian 'hotspot', with all four species breeding at one site.

In 2013, we worked with Dr. Caren Goldberg, Washington State University, to develop molecular markers for the four widespread species of amphibians present within Grand Teton and Yellowstone national parks. A pilot sampling effort in 2013, showed that environmental DNA (eDNA) survey method detections were similar to visual encounter surveys of the same wetlands. However, eDNA of target species was detected in some wetlands where visual surveys failed to detect the species. In other instances, eDNA was not detected despite visual

observations from field surveyors. The Greater Yellowstone Network will continue to work with cooperators to better understand how eDNA methods may be used to complement our monitoring and provide new monitoring tools to strengthen our understanding of amphibians in this region.

Finally, long-term and continued observations of amphibian mortality indicate that lethal disease is present in the parks' amphibian populations; ranavirus is widespread, as is the parasitic fungus causing chytridiomycosis . Columbia spotted frog (*Rana luteiventris*) and western toad mortality events, presumably related to amphibian disease, were found in 2012 and 2013. Our amphibian monitoring will continue to record and collect specimens that will be provided to Yellowstone's Wildlife Health Program or U.S. Geological Survey (USGS) for review and examination.

Acknowledgments

Funding for this program was provided by the National Park Service Greater Yellowstone Inventory and Monitoring Network (GRYN) and the U.S. Geological Survey (USGS). Field work was administered through the Northern Rockies Conservation Cooperative (NRCC), Jackson, Wyoming, and sampling dates and assignments were coordinated with GRYN. We appreciate and value our ongoing partnership with NRCC. At GRYN, Rob Daley provided database management and technical support, and Cathie Jean and Kristin Legg accomplished budgeting and program planning. USGS provided technical support for data collection and database assistance; special thanks to Blake Hossack. A warm thank you to the field crews of 2012 and 2013: Andrew Bulla, Janene Colby, Mary Greenblatt, Jaime Hazard, Dennis Kaleta, Scot Martin, Chris Olsen, Dillon Oslegar, and Daniella Reagan. USGS lent needed field crew support; thanks to Maddy Cochrane, Cayley Fautot-Daniels, and Leah Schwartz. Andrea Litt with Montana State University worked with GRYN to provide an internship opportunity for Bryan Cossairt through the Montana Institute on Ecosystems. Char and Dave Corkran traveled from Portland, Oregon, to assist with monitoring as they have for nearly every year since 1996; we are grateful for their expertise and support. The GRYN whitebark pine monitoring coordinator, Erin Shanahan, assisted by lending field biologists Tyson Roth and McLean Worsham, who accomplished amphibian monitoring in the most remote and challenging catchment. We thank Karin Bergum, Sarah Haas, Mary Levandowski, and Chad Whaley for their reviews of this report and William Gould for his continuing modelling support and guidance. At Grand Teton National Park, we thank Linda Franklin for permits, and Sue Consolo-Murphy for her continued support of the monitoring and Chad Whaley for providing information on western toad sightings. At Yellowstone National Park, we thank Christie Hendrix and Stacey Sigler for permit and logistics assistance, John Treanor and Yellowstone's Wildlife Health Program for their assistance with investigations of amphibian disease, the backcountry office for camping permits and advice, Resource Management (especially Eric Reinertson), and district rangers for their cooperation.

Introduction

Concerns about amphibians have escalated over the past three decades since population declines have become apparent in diverse areas around the world (Collins and Storfer 2003; Wake and Vredenburg 2008). Systematic examinations have revealed that in some regions, including North America, declines began in the middle of the twentieth century and rates in some regions accelerated in the 1990s (Houlahan et al. 2000; Alford et al. 2001). Worldwide, 32% of amphibian species are now threatened with extinction and 43% exhibit some form of population decrease (Stuart et al. 2004). Amidst declines in global biodiversity, amphibian population losses are particularly alarming because they are occurring not only where habitat has been lost, but also in protected areas that are relatively free from anthropogenic impacts (Adams et al. 2013). The major causes of amphibian decline include land use changes that result in habitat loss and degradation, diseases, climate change, environmental pollution, commercial exploitation, increases in ultraviolet-B irradiation due to ozone pollution, and invasive species (Collins and Storfer 2003; Hof et al. 2011). These threats may act singularly or interact at local and regional scales (Hof et al. 2011 and sources therein).

In the Greater Yellowstone Ecosystem, several of these threats are known to be present. Amphibian declines on the Northern Range in Yellowstone National Park have been attributed to decreasing annual precipitation and increasing summer temperatures. Combined, these conditions led to a loss of surface water and critical wetland breeding habitat (McMenamin et al. 2008). Lethal outbreaks of amphibian disease have also been detected within Yellowstone and Grand Teton national parks (Corn 2007), including ranavirus (viruses of the Iridoviridae group that affect frogs and other ectotherms) and chytridiomycosis (a lethal skin disease caused by the chytrid fungus *Batrachochytrium dendrobatidis*). Ranavirus is geographically widespread in the Greater Yellowstone Ecosystem, and associated with many of the mortality events documented by field crews since 2000 (Patla

et al. 2016). Introduced and non-native species including fish and American bullfrogs (*Lithobates catesbeianus*) are also of potential concern due to their well-documented effects on native amphibians.

Grand Teton and Yellowstone national parks have relatively few amphibian species compared to more temperate or subtropical regions in the U.S. (see Tuberville et al. 2005 for a summary of amphibian richness of southeastern national parks). Three amphibian species are considered common in suitable habitat in these parks (Koch and Peterson 1995): western tiger salamanders (*Ambystoma mavortium*), boreal chorus frog (*Pseudacris maculata*), and Columbia spotted frog (*Rana luteiventris*). Western toads (*Anaxyrus boreas*) are thought to be less widespread and less common than in the 1950s (Koch and Peterson 1995; Figure 1). The plains spadefoot (*Spea bombifrons*) have been documented just a few times in Yellowstone's history including recent breeding activity in the Lower Geyser Basin (Schneider et al. 2015). The northern leopard frog (*Lithobates pipiens*) has had only one confirmed sighting in Grand Teton National Park since the 1950s (Koch and Peterson 1995). One non-native species, the American bullfrog, was introduced in the 1950s or earlier and still occurs in Grand Teton National Park at Kelly Warm Springs and associated irrigation ditches.

The Greater Yellowstone Network, as part of the National Park Service's Inventory and Monitoring Program, has identified amphibians in Grand Teton and Yellowstone national parks as a vital sign for long-term monitoring (Jean et al. 2005). Long-term monitoring of amphibian populations provides an opportunity to observe trends that may not be apparent at local scales or in areas with more direct human influences on habitat quality. Starting in 2001, and building on efforts to assess amphibians in the Greater Yellowstone Ecosystem since the 1980s (Koch and Peterson 1995), the NPS collaborated with the United States Geologic Survey (USGS) and Idaho State University (ISU) to develop this amphibian monitoring program. The USGS



Figure 1. Widespread native amphibians of Grand Teton and Yellowstone national parks include Western tiger salamanders (*Ambystoma mavortium*; upper left), western toads (*Anaxyrus boreas*; lower left), boreal chorus frogs (*Pseudacris maculata*; upper right), and Columbia spotted frogs (*Rana luteiventris*; lower right). Plains spadefoot (*Spea bombifrons*) has been documented by Yellowstone National Park scientists in recent years, but the species is not believed to be widespread across the parks. Northern leopard frogs (*Lithobates pipiens*) have not been documented in any park unit for over two decades. NPS Photos.

Amphibian Research Monitoring Initiative (ARMI; <http://armi.usgs.gov/>) designated Grand Teton and Yellowstone national parks as part of the Great Divide Transect, a system of amphibian monitoring and research projects extending from Glacier National Park to Rocky Mountain National Park (Corn et al. 2005a,b; Hossack et al. 2015). After adopting the ARMI conceptual approach for long-term monitoring, the Greater Yellowstone Network prepared a comprehensive protocol (Bennetts et al. 2013) that details the approach, rationale, and methods of using repeated surveys of wetlands within small watersheds (referred to as catchments) to monitor amphibian occupancy. An evaluation of these methods and description of the

approach was also described in Gould et al. (2012).

The current objectives of this monitoring are to:

1. Estimate the proportion of catchments and sites used for breeding by each native amphibian species annually to consider whether the rate and direction of use may be changing over time.
2. Determine the number of sites within catchments that are potentially suitable for amphibian breeding annually (i.e., have standing water during the breeding season).
3. For western toads, estimate the propor-

tion of previously identified breeding areas that are used annually and estimate the rate at which their use is changing over time.

The third objective is intended to supplement Objective 1; site-level occupancy rates are presumed to be too low to enable reliable estimation of rates of change with inference to the entire study area, given the rarity of toads relative to other amphibian species. Objective 3 is not currently being implemented due to inadequate development of analytic and sampling methods needed to detect trends in toad populations. Given that, this objective is currently not attainable with the available funding. However, we do present observations from supplemental toad monitoring of historic and newly identified toad breeding areas outside our sample frame that are visited opportunistically (see methods).

Since the first year of fully implementing the protocol in 2006, adaptive changes have occurred. Funding reductions have led us to

prioritize objectives and seek out strategies for collaboration to increase efficiency. One such strategy has been to combine medium and high quality catchments (see methods); a recent analysis revealed no difference in the occupancy levels of species in these catchments (Gould et al. 2012). In addition, events occasionally prevent surveys of some catchments; for example, in 2012 a catchment was not visited because of a known pair of trumpeter swans (*Cygnus buccinator*) using Grebe Lake. Catchments that become unavailable (such as the Grebe Lake catchment [Y1317] in Yellowstone National Park) due to administrative closures can be replaced by others in order to maintain the desired sampling effort, but only if we have foreknowledge and adequate time to plan field work in the replacements.

The primary purpose of this report is to summarize the amphibian-related monitoring activities that occurred in 2012 and 2013.

Methods

Study Area

Our study area is composed of Yellowstone and Grand Teton national parks, including the John D. Rockefeller, Jr. Memorial Parkway. Hereafter, references to Grand Teton National Park include John D. Rockefeller, Jr. Memorial Parkway. The sample frame consists of the portions of these parks containing shallow, semi-permanent, permanent, or seasonally flooded emergent wetlands. The primary sampling unit—catchments—are small watershed units that were defined by topography as they relate to the flow and collection of water (Bennetts et al. 2013).

Sampling Scheme

Our sampling frame and methods are described in detail elsewhere (see Bennetts et al. 2013). Briefly, after determining that the focal parks contained 3,370 catchments with potentially suitable wetlands (from a total of 4,385 total catchments covering the parks), we selected a stratified random sample. Catchments were initially stratified based on amount of mapped wetland habitat described on U.S. Fish and Wildlife Service National Wetland Inventory maps (Cowardin et al. 1979). High quality catchments (135 catchments) were those that contained > 4 ha of permanent and semi-permanent wetlands and > 2 ha seasonally flooded wetlands. Medium quality catchments (990 catchments) contained > 0 ha of permanent and semi-permanent wetlands, and at least 1 ha of seasonally flooded wetlands. Low quality catchments constitute all the remaining catchments in the parks containing some permanent or seasonal wetlands; this class is overwhelmingly the largest stratum (2,245 catchments in total). Previous analyses (Gould et al. 2012) revealed similar occupancy and extinction and colonization rates in high and medium quality catchments; therefore, we combined these habitat types for this analysis. Moreover, Gould et al. (2012) found the low quality habitat stratum provided little additional information on amphibian occupancy, and thus excluded from monitoring and analysis in 2012 and 2013.

To offer reasonable spatial representation of the sample frame, monitored catchments were selected from five major drainages (subbasins; Figures 2 and 3). In the subbasin stratification, we recognized that major park drainages differ with respect to geology, topography, precipitation patterns, and the existence or influence of large lakes or rivers. To further diversify spatial representation in Grand Teton National Park, the Snake River Headwaters subbasin was further divided into two approximately equal zones, north and south. This division resulted in a total of six subbasins in our sample frame (Grand Teton North, Grand Teton South, Madison-Gallatin, Northern Range, Snake-Henry's, and Yellowstone; Figures 2 and 3).

In 2012 and 2013, we sampled 31 catchments representing all subbasins (Table 1). Sampling within these catchments supports inference to a total 1,125 catchments or approximately one-third of the initial sample frame (catchments with wetlands) and just 23% of the total number of catchments covering the parks.

Amphibian Surveys

Sites within the selected catchments were visited once per season. We attempted to time the surveys to occur within the optimal period for finding the larvae of all four species. However, timing is constrained by a variety of factors including administrative closures by the parks for human safety or wildlife protection, river crossings, and field crew availability.

Survey procedures are detailed in the protocol (Bennetts et al. 2013). In brief, two-person field crews visit all potential amphibian breeding sites within the boundaries of the selected catchments. Visual encounter surveys are conducted at all suitable wetlands, following standard methodology (Thoms et al. 1997). Visual encounter surveys require walking wetland perimeters and transects within wadeable portions of the wetland and using long-handled dip-nets to sweep the water for amphibian larvae (Figure 4). To determine the presence of breeding populations, we searched for the following life stages: eggs,

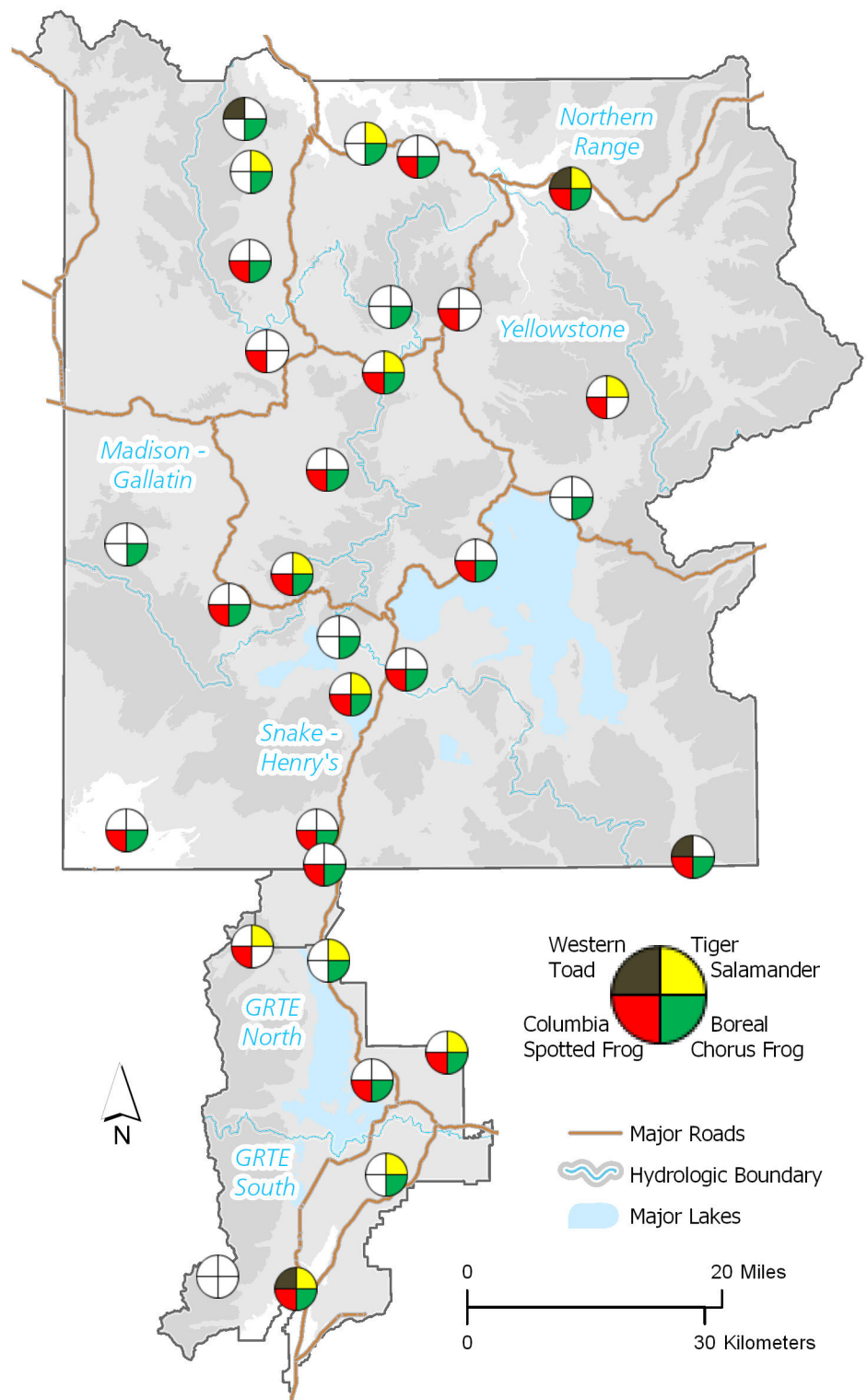


Figure 2. Map showing the locations of catchments sampled during 2012 and the species detected within each catchment. In 2012, a total of 31 catchments were sampled. Pies show which species, if any, were detected through shading. Yellow shading in the upper right sector indicates western tiger salamanders, green shading in the lower right sector indicates boreal chorus frogs, red shading in the lower left sector indicates Columbia spotted frogs, and black in the upper left sector indicates western toads were present. Elevation ranges are shown as white (< 2000 m), light grey (2000-2500 m), or dark grey (> 2500 m). The six subbasins are separated with blue lines and indicated with labeling.

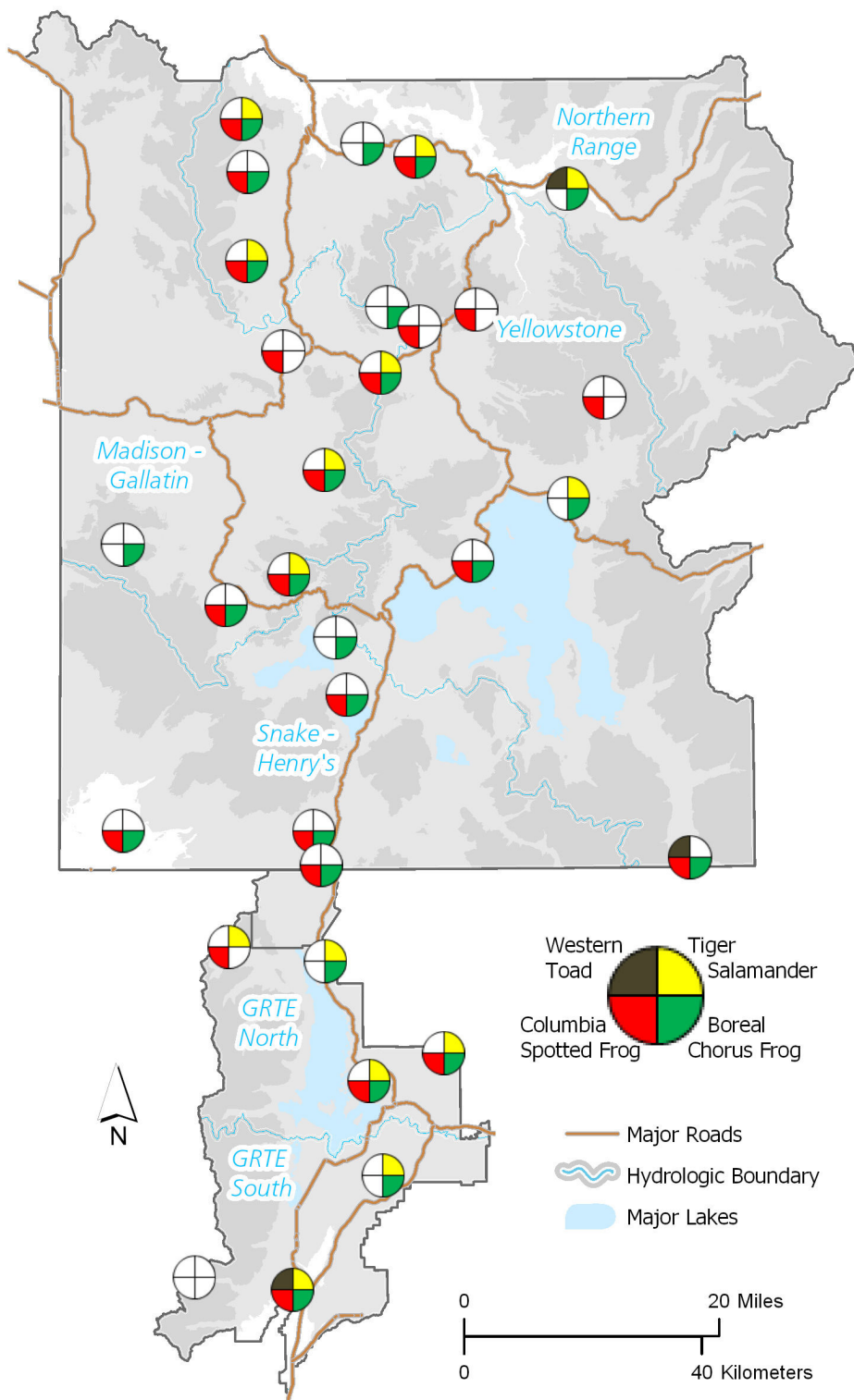


Figure 3. Map showing the locations of catchments sampled during 2013 and the species detected within each catchment. In 2013, a total of 31 catchments were sampled. Pies show which species, if any, were detected through shading. Yellow shading in the upper right sector indicates western tiger salamanders, green shading in the lower right sector indicates boreal chorus frogs, red shading in the lower left sector indicates Columbia spotted frogs, and black in the upper left sector indicates western toads were present. Elevation ranges are shown as white (< 2000 m), light grey (2000-2500 m), or dark grey (> 2500 m). The six subbasins are indicated with blue lines and indicated with labeling.

Table 1. Number of catchments per habitat class (see methods) and subbasin selected for long-term monitoring. Thirty-one catchments were sampled in 2012 and 2013 in the high and medium quality classes. Low quality catchments (not listed here) were dropped from monitoring after 2010.

Year	Habitat Class ¹	Subbasins						Total Catchments
		Northern Range	Yellowstone	Madison-Gallatin	Snake-Henry's Fork	Grand Teton-North ²	Grand Teton-South	
2012	High	3	3	3	3	1	1	14
	Medium	3	3	3	3	3 ³	2	17
	Total	6	6	6	6	4	3	31
2013	High	3	2	4	3	1	1	14
	Medium	3	3	3	3	3 ³	2	17
	Total	6	5	7	6	4	3	31

¹Based on the amount and type of wetlands in each catchment identified by the National Wetland Inventory.

²Grand Teton is in the Snake Basin; it was divided into north and south zones to achieve better spatial representation.

³Indicates one of the catchments included as 'medium' quality was originally described as 'low' quality.



Figure 4. Surveyors sampling shallow wetlands using visual encounter surveys in Grand Teton National Park catchment G4817 on July 2, 2013 (top photo), and Yellowstone National Park catchment Y1439 on June 27, 2013 (bottom photo). NPS photos.

larvae, or recent metamorphs. Each field crew member surveyed the site independently (dual observer method) to provide data on species detectability.

At each site we confirmed or recorded (for new sites) site coordinates and documented time spent searching, species observed (life stages, number of adult and juveniles, and categorical estimates of larvae and metamorph numbers), weather, water temperatures, and habitat conditions (Bennetts et al. 2013). Sites were documented with drawings on the initial visits and updated as necessary. Photo points were established on the initial visit and photos were re-taken each year from the same location. Data were recorded electronically using personal digital assistant devices and backed up on internal secure digital memory cards and abbreviated field log sheets. The personal digital assistant devices were uploaded directly into a Microsoft Access database. The relational database, containing multiple tables, is standardized for use in the Rocky Mountain Region by USGS-ARMI.

In 2012, surveys of catchments began on 2 June and ended by 1 August. In 2013, surveys began on 17 June and ended by 29 July. Several two-person field crews worked through the season each year. In addition, the USGS-ARMI, Montana State University, and trained volunteers provided assistance on a small number of catchments. In 2012 and 2013, the Greater Yellowstone Network whitebark pine field crew trained for amphibian monitoring and assisted in the completion of one large catchment in a remote area of Yellowstone National Park.

Potential Breeding Sites Within Catchments

To address Objective 2, we annually track the number of wetlands within the target catchments that are potentially suitable for amphibian breeding following guidelines outlined in the protocol (Bennetts et al. 2013). Snowpack of the preceding winter and weather conditions of spring and early summer strongly influence the amount and persistence of surface water in shallow wetlands and thus, the number of suitable breeding sites. By tracking the number of sites with standing water (i.e.,

flooded) and dry sites within the selected catchments annually, our long-term monitoring program will help identify to what extent habitat loss due to drought or climate change may affect the occurrence of amphibians and other wetland dependent species (Ray et al. 2015).

Western Toad Supplemental Monitoring

Western toad breeding sites outside the catchments are monitored informally and opportunistically to supplement information on the status of amphibians in the parks. Although toad breeding sites within the selected catchments are scarce, previous projects and incidental encounters have identified approximately 40 widespread toad breeding areas since 2000. “Toad breeding areas” are defined as single isolated sites or multiple sites within 500 m of each other, where toad eggs, larvae, or recent metamorphs have been found or reported previously.

In 2012 and 2013, a subset of the previously-identified toad breeding areas was surveyed by an experienced volunteer team who has worked on amphibian monitoring in Yellow-



NPS Photo

Adult female western toad (*Anaxyrus boreas*). Adult toads use wetlands, beaver ponds, lakes, and stream backwaters for breeding. In late summer (July and August), adults are more frequently found in terrestrial habitats. In the Greater Yellowstone Ecosystem, female toads can move long distances from breeding sites (1 to 2 km; 0.6 to 1.2 miles). Toads are considered critically imperiled by the Wyoming Natural Diversity Database.

stone since 1996 (Char and Dave Corkran), and by other trained personnel when time was available. Sites were selected for convenience and access rather than randomly. Most of the remote previously-identified toad areas (those requiring multiple days of travel, N=12) have not been checked since the early 2000s (see Appendix I in Patla and Jean 2010). Overall, our survey methods were consistent

with catchment monitoring described above and consisted of visual encounter surveys and dip-netting. When multiple observers were engaged, they usually worked together to cover the area, rather than independently surveying the same areas (as in the formal catchment monitoring). Collected data include date and time, weather conditions, limited habitat data, amphibian species detected (life stages and number counted), site drawings, and photographs. Data are recorded on paper forms and compiled in a database at the end of the season.

Documenting Beaver Activity within Catchments

Beaver (*Castor canadensis*) are increasingly recognized for their effects on wetlands and riparian habitats (Rossell et al. 2005) and their importance to amphibians (Karraker and Gibbs 2009; Hossack et al. 2015). Specifically, beaver damming and flooding activities can extend hydroperiods, expand wetlands, increase connectivity among sites, and improve habitat complexity. Because of these benefits, field crews document any evidence of beaver activity (e.g., fresh willow cuttings, new dam under construction, or abandoned dams; Figure 5) encountered during surveys (Bennetts et al. 2013). We annually track the number of wetlands affected by beavers and record use by breeding amphibians pre- and post-beaver activity.

Development and Preliminary Evaluation of eDNA Markers

DNA-based amphibian detection tools are currently used in a variety of field settings; adoption of these approaches is based largely on the understanding that environmental DNA (eDNA) monitoring is potentially more sensitive than traditional monitoring approaches (Pilliod et al. 2013; Rees et al. 2014; Goldberg et al. 2015). As a result, eDNA monitoring may facilitate detection of organisms present at low densities. Plus, eDNA sampling approaches require little more than dipping sterilized sample bottles into a body of water and, once developed, the processing costs are relatively modest (Rees et al. 2014). As a result, amphibian monitoring programs are using eDNA to aid in the detection of amphibians (Goldberg et al. 2011; Pilliod et al.



Figure 5. Beaver dam and associated pond in the Cygnet South catchment (G4817; top photo). Evidence of beaver activity was documented in 2012 when this site was first visited. The dam was larger in 2013 and the pond grew from an estimated 1,120 m² in 2012 to an estimated area of 2,715 m² in 2013. Photo of beaver dam from 2 July 2013. Beaver lodge present in the Pacific Creek catchment (G4698; bottom photo). Evidence of beaver has been documented at this site since 2006. Photo of beaver lodge from 11 July 2012. NPS Photos.

2013). In addition, recent expansions of the use of eDNA surveillance demonstrated that eDNA can be used to simultaneously detect multiple species from a single water sample (Thomsen et al. 2012). Finally, this emerging detection tool monitors recent (not just active) use of habitats by pond-breeding amphibians and therefore may have the desired benefit of extending (by weeks to a month) the sampling window for pond-breeding amphibians beyond the period that visual encounter surveys typically allow (Rees et al. 2014). Currently, eDNA cannot distinguish between breeding activity and wetland use by juvenile and adult amphibians for purposes other than breeding.

To initiate a preliminary assessment of eDNA and to begin considering how it might be incorporated into the Greater Yellowstone Network's monitoring program, we worked with Dr. Caren Goldberg (Washington State University) to develop species-specific molecular markers for all four widely distributed amphibian species. To maximize species specificity (i.e., to minimize a false-presence), Dr. Goldberg compared developed molecular markers to all available sequence data including those of closely related species and non-target species common to wetlands in other parts of the Western U.S. Validation of these markers was completed in the lab by testing them on a variety of DNA samples including heterospecifics and conspecifics found in other parts of the species' ranges.

To test if developed markers were able to detect the four species of amphibians in the field, we collected approximately 1 L of surface water (4 x 250 mL samples) from four different locations within each of the five wetlands located within the following long-term monitoring catchments (Y379 and Y4170) in Yellowstone National Park. We also completed four independent visual encounter surveys (using two observers on two different dates) of these same wetlands. These visual surveys provided independent observations of species and relative measures of the abundance of eggs, larvae, metamorphs, juveniles, and adults for each species. Because these sites have multiple years of monitoring information, we have species lists and estimates of abundance from two observers dating back



NPS Photo

Janene Colby (left) and Scot Martin (right), members of the 2013 field crew in Grand Teton National Park's Cygnet South catchment (G4817).

to the mid 2000s. These current and historic observations may help us to make inferences about the minimum number of individuals required for eDNA detection in the field. Detailed methods of our validation, quality control, and extraction techniques are available in Goldberg et al. (2013).

Amphibian Disease Surveillance

Incidental disease observations from monitoring surveys and associated work have been reported since 2000 in Grand Teton National Park and Yellowstone National Park (see Patla et al. 2016). In 2012 and 2013, and consistent with previous years, field crews recorded amphibian mortalities during visual encounter and dip-netting surveys and collected some specimens for diagnostic examinations. Here we summarize annual amphibian mortality observations (see Patla et al. 2016 for additional information).

Data Analysis

Catchment and Site Occupancy

Occupancy modeling, also known as proportion of area occupied, provides a statistical framework for assessing changes in species occurrence (MacKenzie et al. 2002; Royle and Nichols 2003; MacKenzie et al. 2006). The approach has become widely accepted



Two adult Columbia spotted frogs (*Rana luteiventris*) basking amongst sedges and other emergent vegetation. Spotted frogs are found in wetlands, beaver ponds, ponds, lakes, and, less commonly, in flowing water. Breeding sites are typically permanent wetlands and shallow ponds.

for reliable estimates of species occupancy (MacKenzie et al. 2006). Information from repeated observations at sample units is used to estimate detectability and adjust occupancy rates for imperfect detection (failure to observe a species that is actually present). Occupancy models also allow for analysis of covariates potentially affecting occupancy (e.g., habitat characteristics such as wetland depth and vegetation or seasonal weather conditions), and covariates affecting detectability (e.g., overcast sky).

Occupancy in this monitoring program is assessed at two levels: catchments (small watershed units containing multiple potential amphibian breeding sites) and sites (individual ponds or wetlands within the catchments). The catchment level is intended to approximate the “breeding population” identified by ARMI as the feasible target for monitoring amphibian population trends in national parks. Occupancy at this level was selected as the major vehicle for meeting the goal of determining if amphibians are declining, stable, or increasing in Grand Teton and Yellowstone national parks. Occupancy at the site level is a finer-scale measure that allows one to investigate the importance of site-specific and survey-specific variables affecting occu-

pancy and detection probability. Interpretation of site occupancy differs from catchment occupancy since the number of suitable sites within each catchment varies annually and the total number of sites (unlike catchments) in the sample frame is unknown. New multi-state occupancy approaches may provide opportunities to explicitly model how site-level dynamics and three occupancy states (wetland flooded and occupied, wetland flooded and unoccupied, and wetland dry and unoccupied; see Martin et al. 2009) affect occupancy estimates at the site level.

For occupancy rates at the catchment level, we combined the results of surveys in all wetland sites within the catchment boundaries. This is a coarse scale; occurrence of the species in a qualifying life stage (egg, tadpole, or recent metamorph) at one wetland site is sufficient to indicate that the catchment is occupied. The “observed rate” is the proportion of catchments in which the species was observed; it assumes that non-detection is the same as absence. The “occupancy” rate entails an estimate of the rate at which non-detections of a species are due to failure to find it rather than true absence. Thus, estimated occupancy rates are typically higher than the observed (naïve) rates if detection is less than 100%.

Site occupancy depicts the proportion of sites surveyed (suitable breeding sites) in which reproductive life stages (eggs, larvae, or recent metamorphs) of the species occurred. This is a finer-scale measure than catchment occupancy, and offers a larger sample size. A substantial reduction in wetland sites containing breeding occurrence within catchments could occur but this loss would not be noticed if only catchment-level occupancy was considered (Gould et al. 2012).

For this report, we conducted a provisional analysis of occupancy at the catchment and site levels for each species using the software program Presence (Hines 2006). In Presence, we used the basic single-season model, and we did not attempt to model covariates potentially affecting occupancy and detectability. Analysis of occupancy at the catchment and site levels in the Greater Yellowstone Ecosystem for the years 2006-2009 and 2002-

2011 have been formally evaluated using extinction and colonization rates and covariates to estimate changes in occupancy (Gould et al. 2012 and Hossack et al. 2015) and the 2006-2012 analysis has been submitted for publication (Ray et al., unpublished material). Please see the aforementioned analysis for a more comprehensive review of the analytical techniques used. Those techniques are not presented in this biennial report.

Potential Breeding Site Data Analysis

Central to understanding amphibian occupancy dynamics is the assessment of how wetlands change from year to year. Methods for combining occupancy and wetland flooding status to determine how amphibian occupancy changes as a function of available wetlands are becoming increasingly important to understand how changes in wetland state

influence amphibian occupancy estimates (McKenzie et al. 2006; Gould 2010). Currently, we track and report on the number and percentage of sites that are potentially suitable for amphibian breeding each year. In addition, we are beginning to use gridded climate data sets (see Thornton et al. 2014) to help characterize which climate drivers (i.e., temperature, precipitation, and evapotranspiration) influence wetlands flooding dynamics and, ultimately, occupancy dynamics (see Ray et al. 2015; Ray et al., unpublished material). The Greater Yellowstone Network provides climate data to parks through the Climate Analyzer website at www.climate-analyzer.org in formats that follow the Rocky Mountain Climate Protocol (RMCWG 2010). This website allows parks to explore weather and climate data at stations of interest.

Results

Catchments Sampled and Potentially Suitable Breeding Sites Surveyed

In 2012 and 2013, we sampled a total of 31 catchments per year; 7 in Grand Teton National Park and 24 in Yellowstone National Park (Table 2). All catchments sampled were initially characterized as high and medium quality habitat strata except for catchment G3504 in Grand Teton National Park, which was originally described as low quality. This catchment meets the criteria for inclusion as a medium quality catchment described in our protocol (Bennetts et al. 2013)¹.

Within these catchments, we visited 327 wetlands in 2012; 235 of these sites were flooded and thus surveyed for amphibian presence (Table 2). Site visits determined 74 wetlands in 2012 were dry. It took approximately 71 field crew days (two people working about 10 hours is one crew day) to accomplish the work.

In 2013, we visited 315 sites from 31 catchments. Of these, 224 wetland sites were flooded and thus surveyed for amphibian presence (Table 2). Site visits determined 85 wetlands were dry in 2013. Due to a management closure for nesting swans, we only partially surveyed one catchment (2 sites and < 1% of available habitat) in the Yellowstone subbasin. Data from this catchment are not included in this summary due to the limited effort. It took approximately 58 crew days (two people working a 10 hour day), plus an additional 12 days with 3 or more people to accomplish the work.

During 2012 and 2013 surveys, we found active breeding (identified by the presence of eggs, larvae, or recent metamorphs) of all four widely distributed species in both parks each year (Table 2; Figures 2 and 3). No northern



Some of the 2013 field crew members on Crystal Bench (catchment Y615) in Yellowstone National Park's Northern Range. Crew members from left to right are Tyson Roth, Dillon Oslegar, Chris Olsen, Andy Ray, Deb Patla, Andrew Bulla, and Jaime Hazzard.

leopard frogs, spadefoots, or American bullfrogs were found during our surveys.

Amphibian Occupancy and Detectability

Catchment Occupancy

Catchment-level estimated occupancy rates in 2012 ranged from 0.19 (19%) for western toads to 0.84 (84%) for boreal chorus frogs (Table 3). Detection rates were unusually low for toads at 0.41 (41%); toad larvae were seen by only one observer in three of the four catchments where toads were detected. Detection rates were high for chorus frogs and spotted frogs (> 90%; Table 3).

In 2013, catchment occupancy rates ranged from 0.10 (10%) for western toads to 0.81 (81%) for boreal chorus frogs (Table 4). Detection rates were lowest for tiger salaman-

¹Catchment G3504 includes a semi-permanent wetland that was misclassified by wetland mapping inventory, and provides similar or more wetland habitat than is contained in some medium quality catchments. Therefore, this catchment was retained after 2010 and is included in annual monitoring of medium and high quality catchments.

Table 2. Visitation and detection results of 2012 and 2013 amphibian monitoring. Total numbers of catchments and sites (i.e., wetlands) surveyed per subbasin, and the number of catchments and sites containing active breeding for each amphibian species and for any species.

Year	Subbasin	Catchments Surveyed		Western Tiger Salamander		Western Toad		Boreal Chorus Frog		Columbian Spotted Frog		Any Species Breeding	
		Catchments	Sites	Catchments	Sites	Catchments	Sites	Catchments	Sites	Catchments	Sites	Catchments	Sites
2012	Northern Range	6	27	3	6	2	2	6	13	3	4	6	19
	Yellowstone	6	46	1	2	1	1	4	22	5	14	6	29
	Madison-Gallatin	6	52	1	1	0	0	5	24	4	9	6	29
	Snake-Henry's Fork	6	47	2	2	0	0	6	19	5	15	6	26
	Grand Teton	7	63	5	10	1	1	5	18	4	11	6	26
	TOTAL	31	235	12	21	4	4	26	96	21	53	30	129
2013	Northern Range	6	29	4	6	1	1	6	15	4	6	6	16
	Yellowstone	5	41	1	1	1	4	3	12	4	10	5	21
	Madison-Gallatin	7	55	2	3	0	0	5	27	5	16	7	39
	Snake-Henry's Fork	6	39	1	1	0	0	6	21	5	11	6	24
	Grand Teton	7	60	6	12	1	4	5	24	4	19	6	37
	TOTAL	31	224	14	23	3	9	25	99	22	62	30	137

Table 3. Catchment observed (naïve), occupancy (adjusted), and detection rates with standard errors from 2012. Estimates are for all catchments surveyed, N = 31 catchments.

Species	Observed Rate	Occupancy Rate	Standard Error, Occupancy	Detection Rate	Standard Error, Detection
Western Tired Salamander	0.38	0.41	0.09	0.77	0.10
Boreal Chorus Frog	0.84	0.84	0.07	0.92	0.04
Columbia Spotted Frog	0.68	0.68	0.08	0.92	0.04
Western Toad	0.13	0.19	0.13	0.41	0.27

Table 4. Catchment observed (naïve), occupancy (adjusted), and detection rates with standard errors from 2013. Estimates are for all catchments surveyed, N = 31 catchments.

Species	Observed Rate	Occupancy Rate	Standard Error, Occupancy	Detection Rate	Standard Error, Detection
Western Tired Salamander	0.45	0.49	0.10	0.73	0.11
Boreal Chorus Frog	0.81	0.81	0.07	0.98	0.02
Columbia Spotted Frog	0.71	0.72	0.08	0.87	0.06
Western Toad	0.10	0.10	0.05	1.00	0.00

ders in 2013 (73%), but consistent with detection rates for 2012 (77%). Detection rates were 100% for western toads; toads were detected by both observers in all three of the catchments when they were observed (Table 4).

Basic catchment-level occupancy rates since 2006 in the high and medium quality habitat strata within our sample frame are shown in Figure 6. Sampling has been fairly consistent in these wetland-rich catchments (Patla and Jean 2010; Patla and Legg 2012), but caution should be exercised when interpreting occupancy estimates at this coarse scale. Gould et al. (2012) noted that there could be a reduction in the number of wetlands with breeding (within catchments), but this loss may not be detected if only catchment-level occupancy rates were considered.

Site Occupancy

In 2012, one or more species of breeding amphibians (eggs, larvae, or recent metamorphs) were detected at 55% of the sites surveyed (129 active breeding sites within the set of 235 sites surveyed; Table 2). In 2013, there was an increase in the proportion of sites where we detected breeding amphibians; 61% of the sites surveyed (137 of 224

sites) were observed to host one or more species.

Site occupancy in 2012 ranged from 0.01 (1%) for western toads, to 0.41 (41%) for chorus frogs (Table 5). Due to the low number of observations (only 4 detections out of 235 surveys) site detection rates were not estimable for western toads. Detection rates were estimated as 63% for tiger salamanders and $\geq 85\%$ for spotted frogs and chorus frogs. In 2013, site occupancy estimates ranged from 0.04 (4%) for western toads to 0.45 (45%) for chorus frogs (Table 6). Site detection rates were lowest for tiger salamanders in 2013 (72%), and $> 77\%$ for the other three species. For all species, site occupancy levels in 2013 were similar to or above estimates from 2012. Detection rates were similar across years.

Potential Breeding Sites

In 2012, we visited 327 sites and conducted surveys at 235 potential amphibian breeding sites. In 2013, we visited 315 sites and conducted surveys at 224 sites (Tables 2 and 7).

The percent of available wetlands (i.e., those potentially suitable for breeding) that were dry or too shallow varies annually (Table 7). In 2012 and 2013, we observed the highest

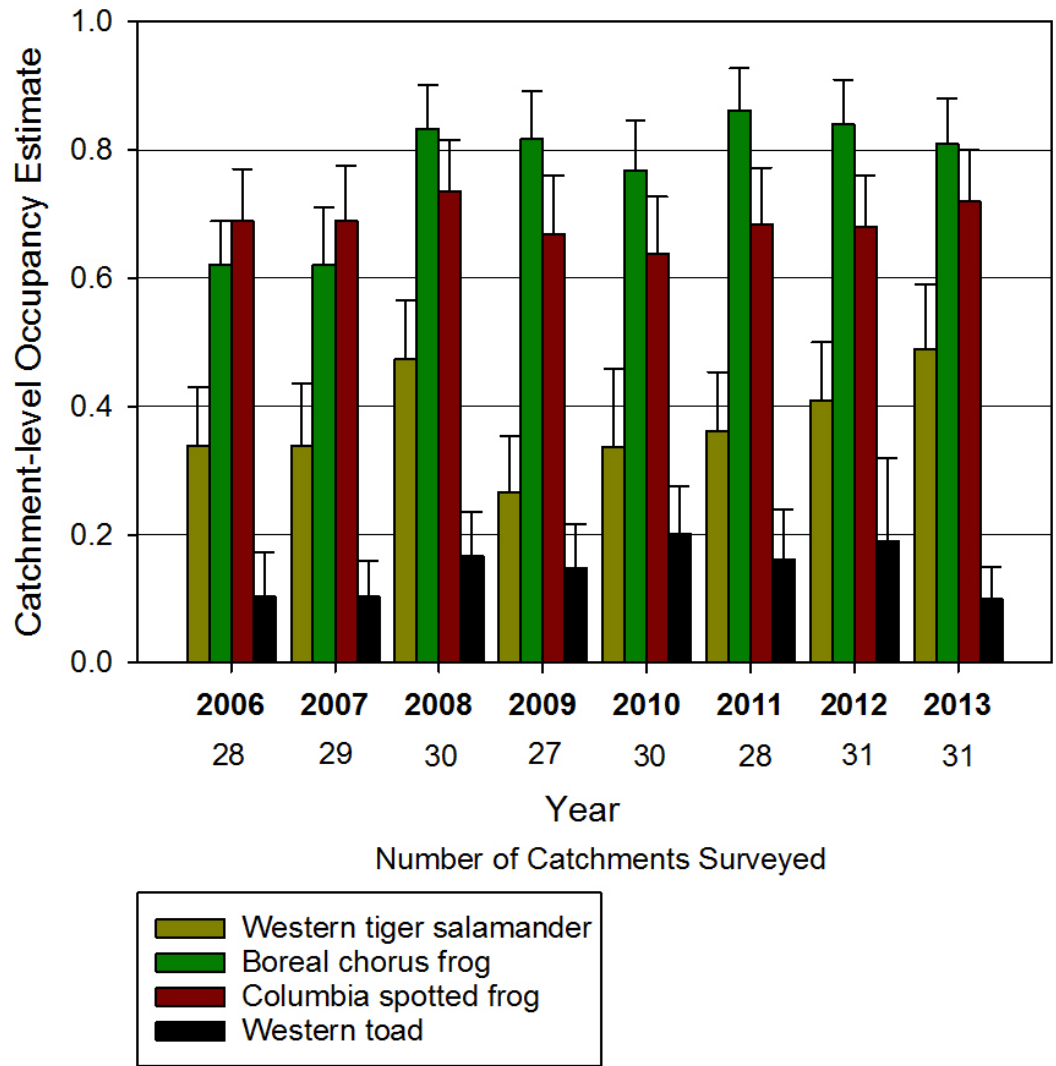


Figure 6. Occupancy rates estimated for high and medium quality habitat catchments by species from 2006-2013, with standard errors. The number of catchments surveyed per year is shown beneath the year labels. This graph should not be interpreted as a trend analysis across years.

Table 5. Site observed (naïve), occupancy (adjusted), and detection rates with standard errors for 2012.

Species	Observed Rate	Occupancy Rate	Standard Error, Occupancy	Detection Rate	Standard Error, Detection
Western Tired Salamander	0.09	0.10	0.02	0.63	0.10
Boreal Chorus Frog	0.41	0.41	0.03	0.89	0.02
Columbia Spotted Frog	0.23	0.23	0.03	0.85	0.04
Western Toad	0.01	--	--	--	--

'--' indicates an insufficient number of detections for occupancy and detection estimation

Table 6. Site observed (naïve), occupancy (adjusted), and detection rates with standard errors for 2013.

Species	Observed Rate	Occupancy Rate	Standard Error, Occupancy	Detection Rate	Standard Error, Detection
Western Tired Salamander	0.10	0.11	0.02	0.72	0.08
Boreal Chorus Frog	0.44	0.45	0.03	0.88	0.03
Columbia Spotted Frog	0.28	0.29	0.03	0.77	0.05
Western Toad	0.04	0.04	0.01	0.94	0.06

Table 7. The number of wetland sites that might support amphibian breeding surveyed by year. Also listed are the number of wetlands visited, but not surveyed due to being dry, too shallow, or for other reasons (e.g., inundated by flowing water or hazardous). The percentage of sites that were dry or too shallow is provided (calculated as the number of sites that are dry or too shallow in any given year/[sites that are surveyed + sites dry or too shallow + sites not surveyed for other reasons]). To consider the effects of seasonal precipitation and annual temperatures on wetland dynamics, we also present the average (averaged across monitored wetlands using Daymet gridded data) April to June precipitation totals (in mm) and average temperature (°C) for each year.

Year	Sites Surveyed	Not Surveyed (dry or too shallow)	Not Surveyed (other reasons)	Percent Sites Dry or Too Shallow	April to June Region-wide Precipitation estimates (mm)	Average Annual Region-wide Temperature (°C)
2006	262	73	11	21%	173	2.5
2007	221	157	6	41%	111	3.2
2008	356	29	8	7%	225	1.7
2009	307	22	6	7%	309	1.6
2010	292	48	12	14%	310	2.0
2011	255	11	3	4%	357	1.4
2012	235	74	3	24%	181	3.1
2013	224	85	2	27%	153	1.8

amounts of dry or almost-dry sites (24 and 27%, respectively) since the record high in our time series of 2007 (41%). Dry wetland conditions likely reflect the influence of current and antecedent weather conditions (e.g., spring/early summer precipitation, previous winter snowpack, and air temperatures that influence evapotranspiration). Using Daymet gridded data sets (Thornton et al. 2014), we estimated average annual temperatures (averaged across all wetland monitoring sites) and seasonal and annual precipitation for both years. In 2012, average estimated annual temperatures were 3.1°C (37.5°F), second only to 2007—the hottest year in our monitoring record (Ray et al. 2015; Table 7). Importantly, these temperatures were over 1.7 times higher than average annual temperatures in 2013. Annual precipitation totals (averaged across all monitored wetlands) indicate that, on av-

erage, both total precipitation (not shown in Table 7) and April to June precipitation were higher in 2012 (832 mm [32.8 inches] and 181 mm [7.1 inches], respectively) than in 2013 (675 mm [26.6 inches] and 153 mm [6.0 inches]). Moreover, 2012 followed a year with large snowpacks and high amounts of precipitation (regional average total precipitation for monitored wetlands in 2011 was 945 mm [37 inches]). It is likely that the high moisture levels in 2011 combined with the spring moisture in 2012 helped maintain surface water in seasonal wetlands, despite the warmer temperatures that could contribute to wetland desiccation. Thus, the percentage of dry wetlands recorded for the relatively wetter and warmer year of 2012 turned out to be roughly similar to the drier, cooler year of 2013.

These results are consistent with a recent analysis that shows climate drivers (e.g., runoff, precipitation, and temperature) are strongly associated with annual wetland drying patterns. Between 2006 and 2013, April to June runoff estimates for this region best explain variation in the percentage of dry wetlands annually. Moreover, wetlands within catchments in the Yellowstone, Madison-Gallatin, and Snake-Henry's subbasins appear to be most sensitive to changes in both runoff and precipitation (Wilmoth et al. 2015). Examples of sites that have changed their availability for amphibian breeding over years of the monitoring project due to changes in flooding conditions are shown in Figure 7.

Western Toad Supplemental Monitoring

As of 2013, there were 41 previously-identified toad breeding areas (some with multiple sites) across our study area; these are in addition to toad breeding sites found in the formally monitored catchments.

In 2012, we surveyed 23 known toad breeding areas (outside the monitored catchments), some containing multiple breeding sites: Grand Teton National Park (7 areas); John D. Rockefeller, Jr. Memorial Parkway (1 area); and Yellowstone National Park (15 areas). We found toad tadpoles at 18 of the 23 toad areas checked in 2012 (Appendix A). This included five new areas where toad breeding was verified or discovered in 2012; three new toad areas in Grand Teton National Park and two new areas in Yellowstone National Park. Mass mortality of toad tadpoles (65-100 dead) was found at three areas in 2012, two in the Pacific Creek drainage of Grand Teton National Park, and one at Gibbon Meadows in Yellowstone National Park.

In 2013, fewer resources were available for supplemental toad monitoring. We conducted surveys in 11 toad areas: Grand Teton National Park (1 area); John D. Rockefeller, Jr. Memorial Parkway (1 area); and Yellowstone National Park (9 areas). Toad tadpoles were found at 7 of the 11 areas (Appendix B). No mortality events were encountered at toad areas in 2013.

Beaver Activity within Catchments

Beavers are rare in our Yellowstone National Park sampling areas; only two of the 24 catchments contained beaver-impounded wetlands in 2012-2013. Beavers are more widespread in Grand Teton National Park, with beaver impoundments identified in four of the seven catchments in 2012-2013. In one of these seven catchments (upper Death Canyon), beavers have long been absent, but their influence persists due to an old dam.

In 2012, the importance of beavers was illustrated by the first detection of western toad breeding since monitoring began in two catchments where beavers had recently built dams; at one site in a catchment in Yellowstone National Park (Thorofare-Snake River confluence area) and at one site in Grand Teton National Park (Sawmill Ponds area; Figure 8). In 2013, we witnessed continued beaver activity and site expansion in these two catchments accompanied by increases in amphibian breeding sites. The Thorofare catchment (Y4339) hosted western toad breeding at a total of four sites in 2013, including two beaver ponds newly created since 2012. The Sawmill Ponds catchment (G4783) also hosted toad breeding at four sites, including one newly created site. The new or expanded beaver sites in these two catchments more than doubled the number of western toad breeding sites found in the monitoring project study area, from a total of four sites in 2012 to nine sites in 2013 (Table 2). The newly expanded wetland in the Sawmill Ponds area not only supported toad tadpoles not observed there in earlier monitoring years, it also hosted larvae of the three other amphibian species in 2013, thus presenting a rare amphibian "hotspot" (Ray et al. 2014).

Evaluating eDNA: 2013

In 2013, Dr. Goldberg and colleagues developed and evaluated primer and probe sets for each of the four widespread amphibians (Table 8). In brief, primer and probe sets were designed using sequences available in GenBank:

(<http://www.ncbi.nlm.nih.gov/genbank/>).

Before employing primers/probes, they were



June 28, 2012

Site Y1980-18, Nez Perce Drainage



June 19, 2013

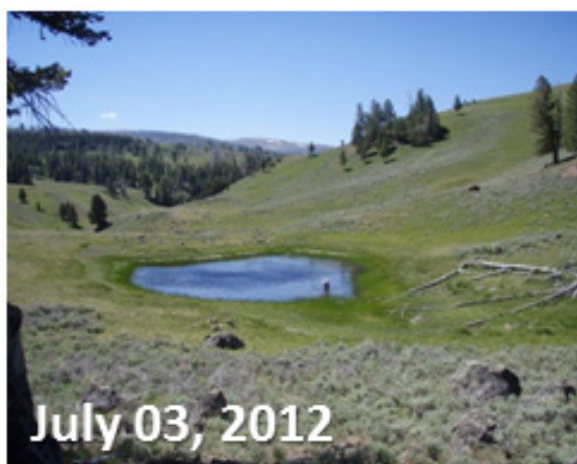


July 12, 2012

Site Y3272-2, Southern Yellowstone



July 02, 2013



July 03, 2012

Site Y4007-3, Northern Range Yellowstone



July 02, 2013

Figure 7. A photographic comparison of three seasonal wetland sites monitored in 2012 and 2013 (and previous years). Y1980-18 was dry in 2007, but flooded in 2012 and 2013. Y3272-2 was dry in 2007 and 2010, nearly dry in 2012 (about 16 m²), and flooded in 2013 (about 100 m²). Y4007-3 was dry in 2006, 2007, 2010, and 2013 and flooded in 2012 (about 700 m²). Conditions in 2012 were warmer with more precipitation than in 2013. In 2012 and 2013, 24% and 27% of visited wetlands were dry respectively. NPS Photos.



Figure 8. Sawmill Ponds catchment (G4783) has experienced changes in amphibian breeding habitat due to increased beaver activity. Site G4783-5 is shown on June 15, 2010 (left photo) prior to beaver damming. In 2010, pond dimensions were 35 x 12 m and maximum depth was < 0.5 m deep. The only species detected in 2010 was the Columbia spotted frog. The photo on the right shows this same wetland on June 12, 2012 flooded by a beaver dam. This site expanded to a size of 170 x 50 m with an estimated depth between 0.5 and 1 m. In 2012, western toad breeding was documented here for the first time since monitoring began in 2006. All four native amphibians were found breeding at this site in 2013. NPS Photos.

Table 8. Primer and probe sequences developed and validated for four species of amphibians found in Grand Teton and Yellowstone national parks.

Species	Primer	Probe
Western tiger salamander	Forward: GGCAGATAGTTGGATGCACGATAG Reverse: ACTACCTCTTGTCTGGTTTTCTCT	ROX-CATAATATGTTGCCACGCTACT-BHQPlus
Boreal chorus frog*	Forward: AATCCCATTCCACGCTACTAC Reverse: ATAAAGCTAAAAGAACGGCAAAGC	6FAM-CATACAAGGACGCTTTT-MGB
Columbia spotted frog	Forward: CCGCTCAATCCCCAATAAACTA Reverse: GAGCGAAGTTTGGAGGTGTGA	6FAM-TCAATCCTAATCCTCTCTCTGA-MGB
Western toad	Forward: GGACATACATGTATAATCGAGCATTC Reverse: CGTGAAACGTWGTCTTGATGA	NED- CATGCATATCATTTCCA-MGB

*Primer/probe information for boreal chorus frogs from Zero (2014).

tested positive on ≥ 10 DNA extracts from voucher samples of each species from the region. In addition, developed primers/probes also tested negative for each of the other four widely distributed species in Greater Yellowstone Network parks, as well as, the non-native American bullfrog (*Lithobates catesbeianus*) found in Grand Teton National Park (Goldberg 2014).

The assay for boreal chorus frogs was developed by Victoria Zero and Melanie Murphy at the University of Wyoming. Tests for Columbia spotted frogs and western toads were developed under agreement 12-JV-11221633-112 with the U.S. Forest Service Rocky Mountain Research Station. The test for tiger salamanders was developed under

Department of Defense Environmental Security Technology Certification Program Project RC-201204 (Goldberg 2014).

Our preliminary sampling in 2013 indicated DNA from three of four widely distributed species was detected in at least one of five wetlands surveyed. No amphibian DNA was identified in equipment, field, or travel blanks (Table 9). Using eDNA sampling methods, western tiger salamanders were detected in three of five wetlands, chorus frogs were detected in two of five wetlands, and Columbia spotted frogs were detected in a single wetland (Tables 9 and 10).

Detection rates for eDNA surveys also varied by wetland. In three wetlands, a species was detected in only one of four water samples.

In wetland 18 in catchment Y4170 (Figure 9) north of Cygnet Lakes, Columbia spotted frogs were detected in all four water samples (Table 9).

Comparing detection results across the three surveys, we see reasonably good agreement between eDNA results and the combined (June/July and August) visual encounter survey results (Table 10). Environmental DNA methods detected tiger salamanders in catchment Y379 site 20 (Y379-20) in Yellowstone's Northern Range. Similarly, eDNA surveys detected chorus frogs in Y379-2 that were not documented in either visual encounter survey conducted that same summer. Despite not detecting salamanders in site Y379-20 in either of the 2013 visual surveys, salamanders were detected using visual encounter survey techniques in previous years (2011 and 2012). In Y379-2, boreal chorus frogs were not detected during either survey date using visual encounter survey techniques in 2013, but this species has been detected annually in surveys of this site between 2006 and 2012. Historical records suggest that these species may have been present in 2013, but visual encounter surveys failed to detect them.

Chorus frog DNA was not detected in water samples collected from sites Y4170-10 or Y379-71. Using visual encounter survey

techniques in 2013, chorus frogs were detected at site Y4170-10 in modest numbers (18 tadpoles and 21 tadpoles for observer 1 and 2, respectively) during the June 17 surveys and this species has also been detected at this site in previous years (2006 - 2008, 2011-2013). Only five chorus frog tadpoles were observed in visual encounter surveys in the June 20, 2013 surveys at site Y379-71; DNA from chorus frogs was not detected in four water samples collected approximately six weeks later on August 7, 2013. Chorus frogs have been detected from visual surveys in this wetland in other years (2007-2013), although abundance has been consistently low (< 10 individuals observed in most years).

Amphibian Disease Surveillance

In 2012 and 2013, we observed mortality of amphibians (10 to > 100 animals) at six different locations: four sites in Grand Teton and two in Yellowstone National Park. The individuals observed included larvae, recent metamorphs, and juvenile or adult western toads and Columbia spotted frogs. Specimens from three sites were collected and submitted to Yellowstone or to the USGS National Wildlife Health Center. Cause of death was not formally determined or provided for these mortality events. Ranavirus has been previously diagnosed in amphibian mortality



Figure 9. Wetland 18 in catchment Y4170 (Site Y4170-18) on July 9, 2013. Visual encounter surveys on this date detected Columbia spotted frog larvae and adults, as well as boreal chorus frog larvae. In August, eDNA sampling also found both species. In 2013, larvae of both species were abundant. Spotted frogs have been detected at this site annually since 2006, and boreal chorus frogs have been detected in all years except 2009. NPS Photo.

ties in widespread areas in the parks since 2000 (Patla et al. 2016). Specimens at three of the die-off sites in 2012-2013 exhibited symptoms associated with ranavirus (bleeding un-

der the skin and bloating). The fungus that causes the amphibian disease chytridiomycosis has also been detected in wetlands across the region (Patla et al. 2016).

Table 9. Environmental DNA detection results from four 250 mL water samples analyzed for each species. The results of equipment, field, and travel blanks are also shown. Below, 0 = not detected, positive = detected. CSF is Columbia spotted frog, WTS is western tiger salamander, WT is western toad, and BCF is boreal chorus frog.

Sample Description	CSF	WTS	WT	BCF
Equipment Blank	0	0	0	0
Equipment Blank	0	0	0	0
Y379-20-5 (Field Blank)	0	0	0	0
Y379-20-1	0	0	0	0
Y379-20-2	0	0	0	0
Y379-20-3	0	positive	0	0
Y379-20-4	0	0	0	0
Travel Blank	0	0	0	0
Y379-71-1	0	0	0	0
Y379-71-2	0	0	0	0
Y379-71-3	0	positive	0	0
Y379-71-5 (Field Blank)	0	0	0	0
Y379-71-4	0	positive	0	0
Y379-2-3	0	0	0	0
Y379-2-5 (Field Blank)	0	0	0	0
Y379-2-4	0	positive	0	positive
Y379-2-2	0	positive	0	0
Y379-2-1	0	positive	0	0
Y4170-10-4	0	0	0	0
Y4170-10-2	0	0	0	0
Y4170-10-3	0	0	0	0
Y4170-10-1	0	0	0	0
Y4170-10-5 (Field Blank)	0	0	0	0
Y4170-18-5 (Field Blank)	0	0	0	0
Y4170-18-3	positive	0	0	0
Y4170-18-2	positive	0	0	0
Y4170-18-4	positive	0	0	positive
Y4170-18-1	positive	0	0	0

Table 10. Comparison of detection results from field surveys and from water samples that were later extracted to capture environmental DNA (eDNA). Detection results for eDNA samples are shown on the left. For eDNA surveys, detection is positive if any of four 250 mL water samples analyzed tested positive for a species (see Table 9). Detection results for visual encounter surveys (center and right) are summarized for two dates (June/July 2013 and August 2013) and using two independent observers. For visual surveys, detection was positive if one or both observers detected a species. CSF is Columbia spotted frog, WTS is western tiger salamander, WT is western toad, and BCF is boreal chorus frog.

Sample Location	August eDNA results (4 samples)				August Visual Surveys (2 observers)				June/July Visual Surveys (2 observers)			
	CSF	WTS	WT	BCF	CSF	WTS	WT	BCF	CSF	WTS	WT	BCF
Y379-20	0	Positive	0	0	0*	0*	0*	0*	0	0	0	0
Y379-71	0	Positive	0	0	0	Positive	0	0	0	0	0	Positive
Y379-2	0	Positive	0	Positive	0	Positive	0	0	0	Positive	0	0
Y4170-10	0	0	0	0	0	0	0	0	0	0	0	Positive
Y4170-18	Positive	0	0	Positive	Positive	0	0	0	Positive	0	0	Positive

*Note, the entire perimeter of this site was not surveyed in the August 2013 visual encounter survey.

Discussion

Wetland and amphibian monitoring in Grand Teton and Yellowstone national parks in 2012 and 2013 added to a multi-year dataset. This dataset on wetland flooding status and amphibian breeding occupancy is helping to produce a deeper understanding of wetland dynamics and amphibian breeding occupancy status and trends in relatively well protected portions of the Greater Yellowstone Ecosystem for four widespread amphibian species. Occupancy estimates have varied by year and by species. In both 2012 and 2013, boreal chorus frogs had the highest catchment- and site-level occupancy rates. Western toads had the lowest catchment- and site-level occupancy rates; approximately 1% of monitored wetlands contained toad breeding in 2012. These species, as well as Columbia spotted frogs and western tiger salamanders, exhibit annual changes in both catchment- and site-level occupancy that are likely influenced by annual and seasonal weather patterns. Given projections for this region indicate warmer temperatures, further monitoring will be needed to interpret how amphibian occupancy is changing through time and to fully understand the drivers of this change.

Overall, amphibian detection rates were similar across years but varied by species. Catchment-level detection rates exhibited higher among-species variation than detection rates estimated at the site level. Across years and at both catchment and site-levels, tiger salamanders had the lowest detection rates. This finding, consistent with previous years, indicates crews are more likely to fail in finding tiger salamanders than the other amphibian species.

The percentage of dry wetlands were similar in 2012 (24% dry) and 2013 (27% dry). The 2012 and 2013 field seasons had more dry wetlands than all but one year (2007; 41% dry) in the dataset. Moreover, the percentage of dry wetlands in 2012 and 2013 far exceeds conditions observed in 2011 when only 4% of monitored wetlands were dry. In 2012 and 2013, we used Daymet gridded data sets to consider whether average annual temperature of monitoring sites (averaged across all

sites) and seasonal and annual precipitation totals also varied across years. We found that 2012 was a relatively warm and dry year, while 2013 was much cooler but even drier. Importantly, 2013 was the second driest in our time series. Given the probability that wetland drying is negatively associated with April to June precipitation (Wilmoth et al. 2015), these results support claims that changes in precipitation (or precipitation to evaporation ratios) could disproportionately affect wetlands and wetland dependent species in this region (McMenamin et al. 2008).

Supplemental monitoring of toads confirmed the presence of toad breeding in five new areas in 2012. Three new toad areas were visited in Grand Teton National Park and two new areas were visited in Yellowstone National Park. Toad mortality events were also identified in three toad areas in 2012; two in Grand Teton National Park, and one at Gibbon Meadows in Yellowstone. In 2013, fewer supplemental surveys were conducted; however, breeding was documented in seven of 11 toad areas visited. Supplemental toad monitoring will continue on an informal and opportunistic basis to supplement information on the status of toads in all three park units.

An increase in beaver activity in two catchments, one in each of the two park units, created additional habitat that was colonized by breeding amphibians in 2012 and 2013. The effect of the new habitat was a dramatic increase in the number of western toad breeding sites (although still a small number) by 2013, and the creation of an amphibian 'hotspot', with all four species breeding at one site. Annual monitoring allows us the opportunity to document not only the response of amphibians to new beaver impoundments, but also the dynamics involved with beaver presence, disappearance, or absence. A recent publication, which included data from this monitoring program, describes the strong positive influence of beavers on amphibian occupancy and colonization in four national parks in the U.S. Rocky Mountains during 2002–2011, including Yellowstone and Grand Teton national parks (Hossack et al. 2015). This find-

ing has important management implications for the value of maintaining and encouraging beaver populations in park units across the northern Rockies.

In 2013, we began piloting the use of eDNA methods to detect amphibian presence at some of the monitoring sites. Using five monitoring sites in 2013, DNA-based survey method detections were roughly similar to visual encounter surveys of the same wetlands. Although eDNA methods provided more detections than visual encounter surveys at two sites, success of eDNA surveys was not 100%. Species detected using visual surveys at two sites identified species not detected with eDNA. Elsewhere, Pilliod et al. (2013) found eDNA methods had slightly higher detection rates than traditional visual survey methods for stream amphibians. Other researchers have noted eDNA methods have detected aquatic organisms where traditional methods failed (Rees et al. 2014). Although we found eDNA methods identified species presence not detected from four independent visual surveys of wetlands, results were not consistent across all sites. In our case, validation of species detections (from eDNA) can be considered along with the multi-year detection histories of these same sites. Improvements in field and laboratory techniques for eDNA monitoring are advancing rapidly (Goldberg et al. 2015), but continued work will be needed to better understand the utility of eDNA within our monitoring program.

Observations and diagnoses of amphibian mortalities indicate disease outbreaks have occurred in the Greater Yellowstone Ecosystem since 2000 (see Patla et al. 2016). Ranavirus has been diagnosed at widespread locations, and the fungus causing chytridiomycosis has been documented throughout the Greater Yellowstone Ecosystem. In 2012 and 2013, our monitoring crews identified

dead or dying Columbia spotted frogs and western toads at six locations. We will continue to record mortalities and collect specimens in support of Yellowstone's Wildlife Health Program.

This monitoring effort continues to generate interest by state and federal agencies. For example, the Wyoming Natural Diversity Database has adopted the Greater Yellowstone Network's monitoring protocol for amphibian monitoring efforts on other federal lands in Wyoming. Additionally, the U.S. Environmental Protection Agency and other state agencies have communicated their interest in understanding how climate patterns influence relatively undisturbed wetlands in the northern Rockies. Our collaborative wetland amphibian monitoring efforts have served as the foundation for several scholarly publications that increase visibility of this monitoring program (see Hossack et al. 2015, Ray et al. 2015, and Ray et al. 2014). Other scholarly publications are currently in review (Ray et al., unpublished material) or actively under development.

Wetland and amphibian monitoring will continue in Grand Teton and Yellowstone national parks as part of the Greater Yellowstone Network's annual vital sign monitoring. Moving forward, the Greater Yellowstone Network will seek to engage park staff with opportunities to participate in field activities or in the combined examination and analyses of multi-year data sets. In addition, we will continue to evaluate novel monitoring techniques (e.g., eDNA methods) and more sophisticated analytical techniques (e.g., multi-state occupancy modelling) to strengthen our understanding of wetland and amphibian dynamics in the Greater Yellowstone Ecosystem and beyond.

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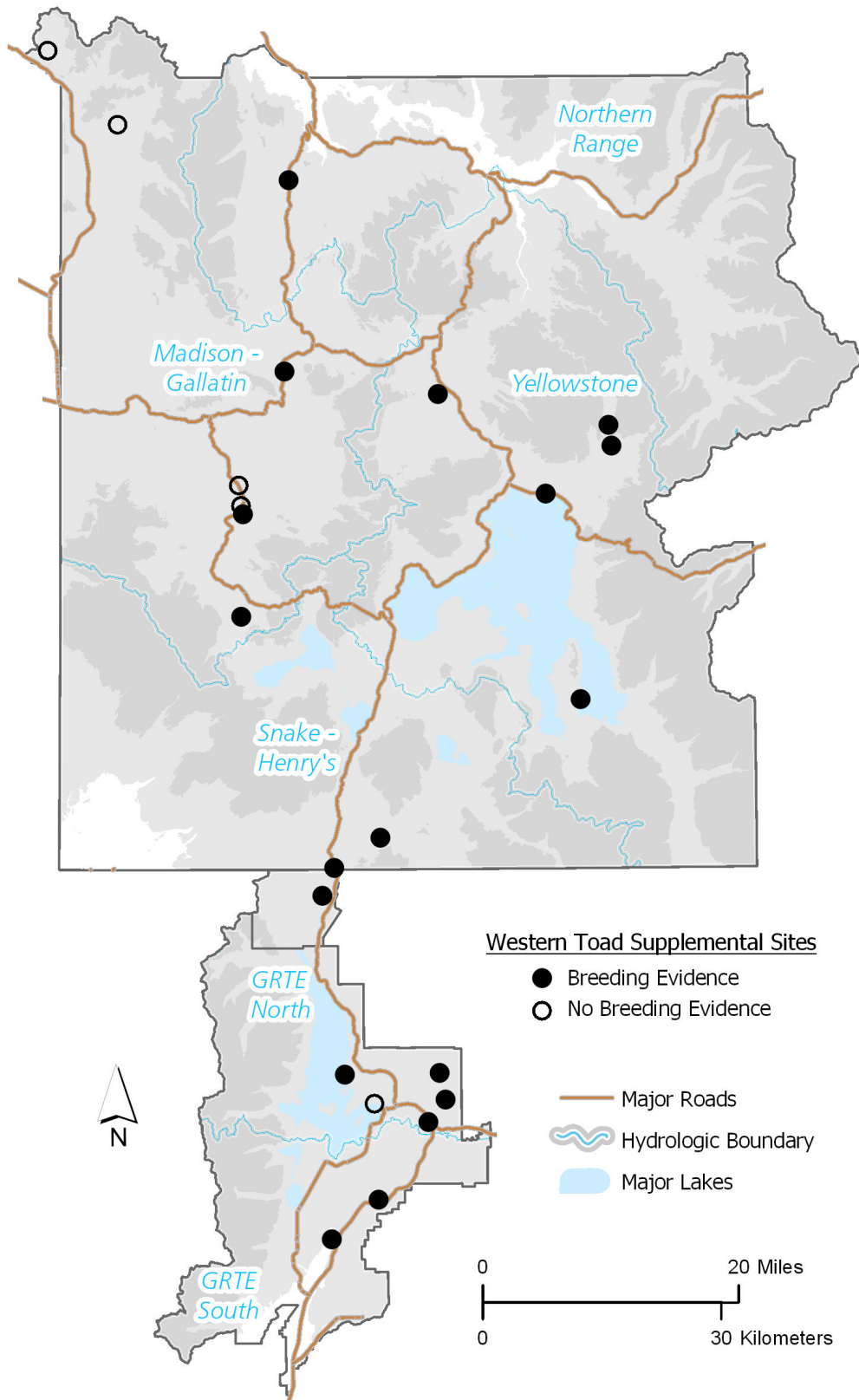
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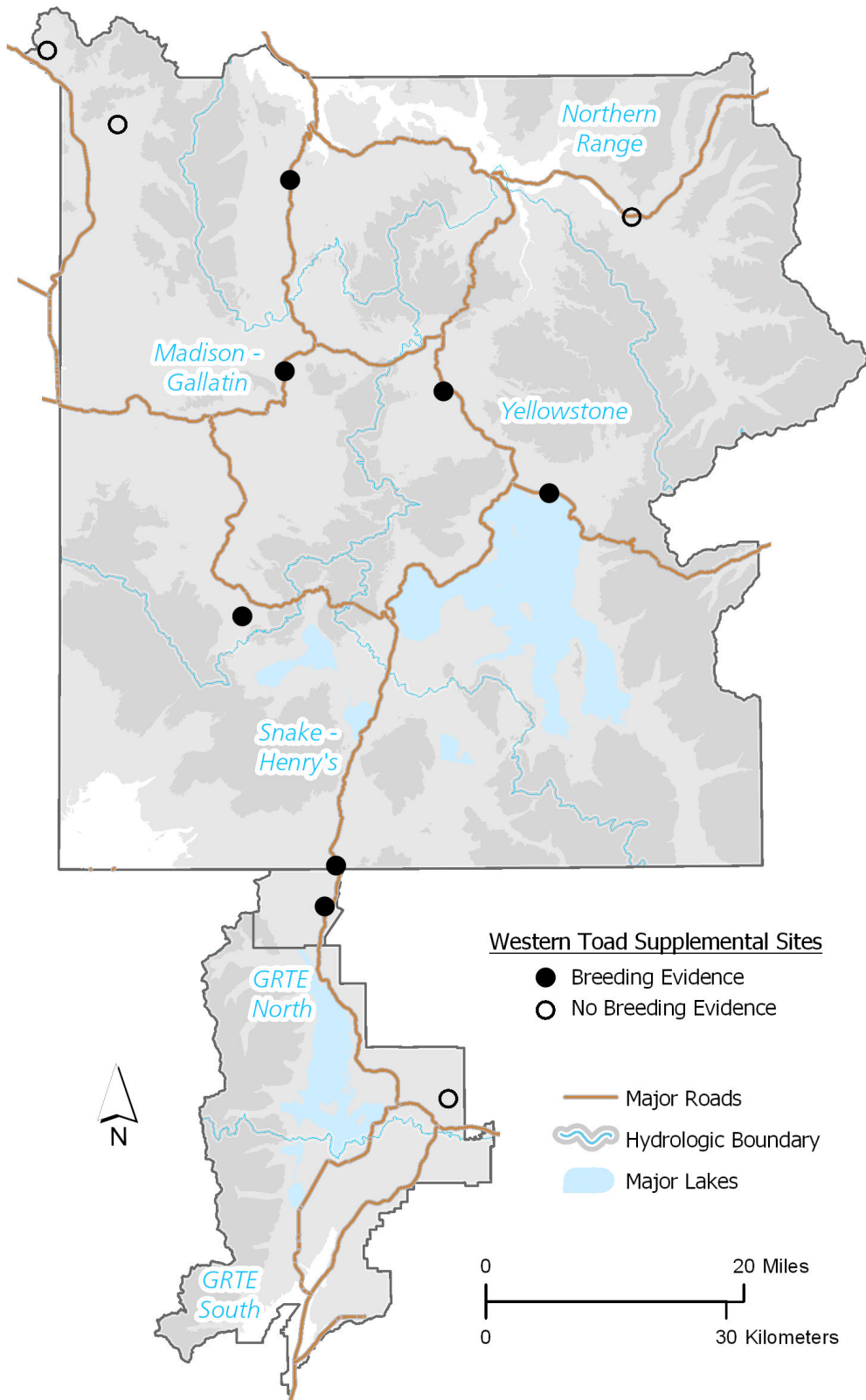
Appendix A

Western toad supplemental monitoring in 2012 included surveys of 23 known toad breeding areas (outside the monitored catchments).



Appendix B

Western toad supplemental monitoring in 2013 included surveys of 11 known toad breeding areas (outside the monitored catchments).



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