PRE-DRILLING SURVEYS OF AMPHIBIAN AND REPTILE HABITATS IN THE POWDER RIVER BASIN OF WYOMING

YEAR ONE PROJECT REPORT, 2008

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INTRODUCTION

Overview

This report details preliminary results from the first of a three-year study targeting reptiles and amphibians in the Powder River Basin of Wyoming. The Aquatic Task Group (ATG) is an inter-agency and inter-state working group focused on studying and mitigating impacts of energy development in northeastern Wyoming and southeastern Montana. The ATG's Monitoring Plan (ATG, 2006) provided the guidelines and objectives for this project and the Wyoming Natural Diversity Database (WYNDD) developed the survey methods and conducted the field work. The majority of funding for this work was provided by the Buffalo Field Office of the Bureau of Land Management (BFOBLM) with some matching funds from the WYNDD. As outlined in Table 1, approximately 18 species of reptiles and amphibians occur in the Powder River Basin of Wyoming. Most of these species belong to the suite of Great Plains species whose far western range occurs in northeastern Wyoming.

The BFOBLM recently prepared an Environmental Impact Statement (EIS) for coal bed natural gas (CBNG) development in Wyoming and Montana which outlines potential impacts to hydrology, wildlife, and other natural resources in the Powder River Basin (PRB) (BFOBLM, 2003). The EIS identified two primary areas of concern with respect to amphibians and reptiles; (1) changes in water quality and timing, and (2) road mortalities from vehicular traffic. The release of CBNG discharge water into rivers and streams could negatively impact breeding habitat for amphibians by increasing salt concentrations and altering the prey populations and vegetation on which they depend. As energy development increases, so do new roads and vehicular traffic, and this may pose a threat to reptile and amphibians populations, especially as they migrate between breeding, feeding, and hibernation sites. In addition to looking at these questions, the project will establish protocol and collect baseline data for long-term monitoring in order to detect additional impacts from CBNG development.

The Wyoming Game and Fish Department (WGFD) conducted a baseline inventory of reptiles and amphibians (hereafter 'herptiles') along the Powder River and its major tributaries from 2004-2006 (Turner, 2007). The study gave an overview of species distribution, road mortality, and *Batrachochytrium* infection rates along the major rivers and streams within the PRB. Many of the WGFD's survey methods and some of the sampling reaches were incorporated into this project for consistency and long-term monitoring purposes; however the spatial extent of our work is intended to include upland and CBNG reservoir habitats in order to map distribution and study potential impacts across a broader area of the PRB.

Study Area

The Powder River watershed is located in northeastern Wyoming (Figure 1) and has an area of approximately 25,000 km^2 (9,600mi^2). The main stem of the river flows north into Montana where it joins the Yellowstone River and then the Missouri River in South Dakota. Within Wyoming, tributary streams which originate in the Bighorn Mountains to the west generally have perennial flow fed by snowmelt, whereas ephemeral tributaries originating in the plains to the south and east are characterized by short duration flows from rainstorms (Davis et al., 2006). The majority of CBNG development in the PRB is below 1,370 m elevation and as such defines the study area for this project. Topography is characterized by rolling hills and breaks. The climate is semi-arid and land cover is dominated by two ecological systems; Inter-mountain Basins Big Sagebrush Steppe and Northwestern Great Plains Mixedgrass Prairie (USGS, in prep). Riparian vegetation is usually composed of cottonwoods and tall grasses but there are very few natural, lentic wetlands in the Basin.

Over 50% of land ownership in the PRB is private. Livestock grazing dominates land use with limited irrigated agriculture along perennial rivers (Davis et al., 2006). CBNG production has increased greatly in the last 10 years, especially in the eastern half of the PRB where natural gas is most easily recoverable. Gas field development often entails building and maintaining extensive road networks to serve well pads, pipelines, and compression stations. Deep aquifer water is pumped to the surface and discharged into ephemeral drainages or reservoirs in order to release natural gas in trapped coal seams. The aquifer water is usually colder than surface water and often has higher concentrations of sodium bicarbonate and other salts (Davis et al., 2006). For more background information about the potential impacts of water quality and road network changes on herptiles, please see the literature review provided in this document (Appendix A).

Objectives

This project has three overall objectives. The first is to complete a literature review of impacts on herptiles from vehicular traffic and changes in water quality (see Appendix A). The second is to map the general distribution, and characterize the status and habitat of all herptiles occurring in the PRB (below 1,370 m). The final objective is to measure the potential influences of CBNG activities on herptile guilds and suggest possible mitigation measures. This will be achieved through measuring road mortality rates and water quality tolerance levels and establishing a long-term, repeatable monitoring protocol that can be used to track population trajectories and impacts from CBNG activities in the future.

Objective 1: ***Literature Review***

Using gray and peer-review sources, a comprehensive review was completed assessing herptile sensitivity to the types of aquatic and terrestrial changes occurring in the Powder River Basin as a result of CBNG development. The first topic covered is a summary of toxicity and reproductive impacts to amphibians from the major constituents associated with CBNG discharge waters. In addition, articles concerned with increased water flow in historically ephemeral streams in Great Plains ecosystems will be covered. Finally, there exists an extensive body of literature assessing herptile mortality rates resulting from road traffic. These articles will be summarized with respect to the kinds of road network expansion occurring in the PRB.

Objective 2: ***Species Distribution, Status, and Habitat***

The specific locations of survey sites will be determined by land owner access and areas targeted according to Objective 3. Within that framework, however, we will attain as wide coverage of the PRB as possible. Coordinates for all herptofauna observations will be combined with data from the WGFD (Turner, 2007) and WYNDD’s database to produce a distribution map for each species. Overall abundance measures and current literature will be used to categorize species into general groups such as ‘common in PRB’, ‘moderately abundant in PRB’, and ‘rare in PRB’. Qualitative descriptions of habitat by species will be produced by combining standardized variables collected at survey sites (including water quality) and those presented in the literature.

Objective 3: ***Impacts of CBNG Activities***

The impact of CBNG activities on herptiles can only be *roughly estimated* within the framework of this project. A true calculation of impacts would require several years of surveying and a population viability analysis for each species within the context of their changing environment (such a study would be extremely intensive and expensive and is beyond the means of this project). We will attack the question from two angles; current impacts and long-term impacts:

Current Impacts

Road mortality rates and water quality data collected throughout the PRB can be used to make rough predictions about large-scale impacts and possible mitigation measures. Basic water quality and habitat measures will be collected alongside species and reproductive observations in order to delineate ranges of water quality and habitat tolerances for each species. Traffic intensity will be quantified along with mortality rates by species in order to suggest the impacts from increasing networks of roads.

Long-term Impacts

Impacts from roads and water quality degradation may be difficult to detect within the life of this project. In addition, there may be unforeseeable impacts that will only come to light in the future. By conducting surveys at fixed locations and using repeatable methods throughout this project, future surveyors can replicate the effort several years from now and quantify any changes in the abundance of different species. In 2008, a suite of long-term monitoring sites were identified and surveyed including nocturnal routes, roadkill routes, river bank surveys, and water bodies within subwatersheds. There are several metrics that can be used to compare current and future population trends; species presence/absence, number of observations/species/survey, relative abundance, and percent occupancy of sites within watersheds.

METHODS

Nocturnal Surveys

Nocturnal surveys were conducted for anurans (frogs and toads) during the 2008 breeding season. Males of all anuran species in the PRB vocalize under suitable weather conditions to attract females to breeding sites. Calls can be used to identify individuals to species and, depending on environmental conditions, they can be heard up to an estimated 2 km away. Road-based nocturnal surveys are an effective way of measuring species presence across relatively large distances without requiring physical access to adjacent land. Two types of nocturnal surveys were conducted in 2008; a method identical to the WGFD (Turner, 2007), using several of the same routes, and a modified method for covering more distance. Both methods involved a two person crew starting at a fixed location on a public road and driving a predefined distance to each subsequent listening point. All surveys were conducted after dark and detailed weather information was collected at the beginning and end of the survey (wind speed, barometric pressure, relative humidity, cloud cover, and temperature). At each listening point, surveyors got out of the vehicle and listened for a predefined amount of time (see below), recording the coordinates, species, calling intensity, direction and distance to caller(s), and ambient noise. Surveys using the WGFD's methods and routes were along fixed 1.0 mile segments, listening every 0.1 mi for 2 minutes. The modified nocturnal survey method was used along all other routes, listening every 0.5 mi for 3 minutes each.

Water Body and River Surveys

Visual encounter surveys were conducted along the banks of standing and flowing water throughout the PRB. The method primarily targets amphibians and the protocol we used followed the guidelines described in the ATG Monitoring Plan for Amphibians and Reptiles (of which Bryce Maxwell, Montana State Heritage Program, was a major contributor). Two surveyors walked and searched at a consistent rate around the edge of lentic water bodies or on each side of stream channels. In the case of routes along the Powder River, both surveyors were on one side of the river (due to flooding and not being able to cross the river).

At each site, surveyors collected extensive data on habitat, water chemistry, species, and life stage (detailed in field forms in Appendix B). *Batrachochytrium dendrobatidis* (hereafter chytrid) swabs were taken from adult amphibians using sterile equipment. Chytrid fungus has been implicated in amphibian declines around the world, especially in concert with other environmental stressors, and infected animals have been found in the PRB (Turner, 2007). After collecting chytrid swabs, they were dried and frozen until their analysis at Pisces Molecular (Boulder, CO) in the fall of 2008. When dead herptiles were found, they were labeled and preserved in 95% ethanol for analysis at a later time.

Water body surveys were focused within 11 priority watersheds (6^{th}-level Hydrologic Unit Codes (HUCs) throughout the PRB (displayed in Figure 2). Using the guidelines outlined in the ATG Monitoring Plan, water bodies were sampled in order to produce a 'percent occupancy' estimate by species per HUC (the number of water bodies occupied by a species divided by the number surveyed). This measure can be used to monitor changes across time, even if water bodies dry up or new ones are created. HUC selection was not random but based primarily on where cooperative land owners and a high percentage of BLM surface ownership occur.

River surveys were not confined to priority watersheds, but instead were determined by fixed monitoring reaches established by the USGS and WGFD. The USGS has several biotic monitoring sections of the Powder River, Crazy Woman Creek, and Clear Creek where it samples (during most years) for fish, macroinvertebrates, algae, etc. Four of our survey routes overlapped with these sections (three along the Powder River and one along Crazy Woman Creek) in order to pair the herpetological results with other biotic variables. The WGFD surveyed numerous sections along the same major rivers between 2004 and 2006 (Turner, 2007). We selected 12 of these sections to be re-surveyed; 6 along Salt Creek, 3 along Crazy Woman Creek, and three along Clear Creek.

Rock Outcrop Surveys

Opportunistic visual encounter surveys were conducted in 2008 along the south-facing sides of rock outcrops in search of reptile hibernacula. These surveys were conducted where habitat was visible, time permitted, and permission was granted by the land owner. The survey involved searching under and around rocks and organic debris. Species encountered were photographed and the habitat described. This method has been successful in southeastern Montana (Bryce Maxwell, *pers comm.*, 2008) at locating multi-species groups of hibernating snakes, however timing and weather conditions can heavily influence results.

Roadkill and Basking Herptile Surveys

There were three main objectives for conducting surveys for dead and basking herptiles. The first was to test the idea that surveyors could successfully locate herptile roadkill while driving and that 'roadkill hotspots' could be identified with this method in order to help pinpoint potential reptile migration corridors. The second was to try to use these hotspots to locate active hibernacula in the vicinity and identify sections of road that would be good for long-term monitoring. The final objective was to repeat surveys conducted by the WGFD (Turner, 2007) so that mortality rates could be calculated and compared across years.

We conducted opportunistic and fixed surveys to fulfill these objectives. The opportunistic surveys were conducted throughout the PRB while technicians traveled to and from other survey sites. They were instructed to look for herptiles along all roads they traveled, and record dead or basking herptile locations with GPS and photographs, as well as record the type of road (dirt, paved, etc). If two or more roadkill sites were found within 0.25 mi. of each other, a two mile section encompassing the locations was established, and visual encounter surveys were conducted on foot along the entire section a total of three times throughout the summer. In addition, five fixed surveys were conducted using the same road sections and methods used by the WGFD (Turner, 2007) from 2004-2006. These were 1-mile sections of dirt or paved road where one observer walked along each side of the road, recording all dead and basking herptiles.

RESULTS & DISCUSSION

Nocturnal Surveys

The primary focus of nocturnal surveys in 2008 was to get wide coverage of the PRB in order to map the range and relative abundance of anuran species. Seventy percent of all observations were gathered using the nocturnal method and all 5 anuran species were detected. Figure 3 shows the start points of the 23 road-based nocturnal surveys conducted from April 22 - July 7. Five of the transects (labeled 'Turner') correspond to routes and methods in used by the WGFD (Turner, 2007). These five transects were repeated 3 times in 2008 to test the use of occupancy modeling. We are considering using occupancy modeling as an alternative and potentially more robust measure of species presence than occupancy rate for long-term comparisons. However, we have yet to perform the trial analyses to assess its utility with the nocturnal dataset. Table 3 shows the results of these 5 repeated surveys to illustrate that different species are detected along the same transect on different nights. Occupancy modeling should produce a level of confidence associated with each species which can be rolled into a more robust comparison across time.

Water Body and River Surveys

Lentic Water Bodies

Forty-six lentic water body surveys were completed in 2008 for a total of 23 survey hours. All were confined to the 11 priority HUCs displayed in Figure 2 (locations are shown as blue dots in Figure 4). The majority of the water bodies sampled were created and maintained by CBNG effluent water and a handful were temporary puddles and stock ponds created by overland flow. Water quality and aquatic vegetation cover varied greatly between sites.

Watershed Results

Table 2 shows the level of effort per survey type dedicated to each priority HUC in 2008. LX Bar Creek received the most field effort (11.6 hours) producing 65 individual observations, representing 7 species. As expected, more survey effort spent in a watershed generally resulted in more species observations. Lower Castle Creek and Salt Creek-Crooked Creek were anomalies yielding no herptile observations after a combined 3.7 hours of surveying. The ATG

Monitoring Plan stipulates surveying all lentic water bodies within priority HUCs so that percent occupancy by species can be calculated at the subwatershed level. Approximately 20% of existing water bodies within HUCs were surveyed in 2008. Although we will attempt to survey additional water bodies within priority HUCs in 2009, we do not foresee being able to survey all of them due to land owner restrictions and the high number of water bodies in some watersheds (e.g. Dry Fork of the Powder River). Occupancy rates at the HUC level were not calculated for this report because there were too few water bodies surveyed to yield meaningful measures at the species level. We estimate a minimum of 50% of water bodies should be surveyed in order to produce reliable results. If we are unable to reach this level with future surveys (due to limited access), we will combine results from adjacent HUCs and calculate occupancy rates at aggregated watershed levels.

<u>Salamander Mortality</u>

One noteworthy result from lentic water body surveys in 2008 was the observation of dead tiger salamander larvae (*Ambystoma tigrinum*) at 12 of the 14 sites where the species was seen. Six of the twelve locations (where specimens were collected) are displayed in Figure 5. Although the cause(s) of death have not been determined yet, we have investigated some possible explanations. Chytrid samples of dead larvae were collected at 6 sites and analyzed at Pisces Molecular, however, all specimens were negative for the virus. Although chytrid fungus does not appear to be a source of mortality, additional histological analyses, if conducted, may find ranavirus or other pathogens to be present in the tissues. Another hypothesis was bacterial infection caused by the *Bacillus* ‘cakes’ that are dispensed into most CBNG reservoirs to prevent mosquito outbreaks. However, consultation with Allan Pessier, Herpetological Toxicologist at the San Diego Zoo, and a review of a number of toxicological studies involving *Bacillus* and amphibians lead us to think that it is not a probable cause. We also plan to speak with CBNG operators to rule out any management practices at the affected reservoirs that might have caused the deaths.

Water quality changes at the affected reservoirs may have played a role in the salamander deaths due to rapidly evaporating water and increasing water temperatures associated with the onset of summer. Table 4 displays water temperature, pH, total dissolved solids, conductivity, and salinity levels collected at the reservoirs where all tiger salamanders were observed. Toxicological studies conducted by the USGS on fathead minnows show 100% mortality of young fish in sodium bicarbonate concentrations of 1.4 mS or greater (Aïda Farag, *pers comm*.). Because sodium bicarbonate is the primary constituent in CBNG effluent measured by conductivity, our data suggest that 10 of the 14 reservoirs were above 1.4 mS. Nevertheless, conductivity levels do not seem to entirely explain the phenomenon since three reservoirs below that level had dead salamanders and one site at 3.0 mS had only live salamanders. We believe that the dead salamander question merits additional investigation in 2009. The tiger salamander is currently a common species in the PRB, but there is concern that CBNG discharge ponds at the landscape scale may be causing a population sink. In 2009, we will follow many of the leads mentioned above as well as conducting a more complete constituent analysis on water collected at any dead salamander sites. We also hope to collaborate with the USGS toxicological group (Aïda Farag, Jackson, WY) to set up a toxicological trial with tiger salamander larvae. Further investigation into the dead salamander larvae question will also involve collaboration with BLM biologists in order to deal with the potentially sensitive issues involving private land owners.

River Surveys

Twenty-two river bank surveys were conducted in 2008 for a total of 18.2 survey hours. Figure 4 shows the locations of the survey reaches in red. Although the method is easily repeatable in future years for long term monitoring, it was not found to be an efficient way to observe herptiles, and weather conditions greatly affected detectability. For that reason, four sites were surveyed a second time under more favorable weather conditions. As with the repeated nocturnal surveys, we will investigate the utility of occupancy modeling to better correct for these detectability differences in future surveys.

Chytrid Infection Results

Twenty-seven of the chytrid samples collected from live amphibians along rivers and lentic water bodies were tested for *Batrachochytrium dendrobatidis* across the Basin. Figure 6 displays the results of the tests. One sample from a northern leopard frog along Wild Horse Creek was highly infected and a batch of eight samples from Woodhouse's toads in the Clear Creek-Powder River confluence was weakly positive for the virus. All other samples were negative yielding an approximate infection rate of 18%; comparable to the 14% infection rate reported by Turner (2007) in the PRB.

Rock Outcrop Surveys

Sixteen rock outcrop surveys representing 4.6 hours of survey time were completed in late April and early May. Figure 7 shows that these surveys were primarily on BLM land in the vicinity of I-90 and the Powder River. We found no hibernating snakes; all observations were of sagebrush lizards except for one short-horned lizard. Low detection of snakes may have been, in part, due to wet and cold weather during the survey period as well as the relatively small number of sites surveyed. In order to access more upland sites owned by the BLM, we plan to work with the BLM in 2009 to obtain permits in order to access roads crossing private land.

Roadkill and Basking Herptile Surveys

Thirteen chance encounters with basking or dead herptiles were recorded in 2008 with one site along Dead Horse Road yielding three dead bullsnakes during one survey and thus warranting two repeat surveys in subsequent weeks. Of the six fixed roadkill surveys conducted on foot, the average mortality rate was 0.75 dead herps/mi. Table 6 displays the road type, species encountered, and status (dead or alive) of all road observations. Thirteen of the eighteen (72%) were of dead animals, presumably killed by vehicles (Figure 8). Seventy-five percent of the mortalities were bullsnakes, however prairie rattlesnakes, snapping turtle, terrestrial gartersnake, Woodhouse's toad, and yellowbelly racer were observed as well. Our results are similar to Turner (2007) who found that bullsnakes, prairie rattlesnakes, and painted turtles were most heavily impacted by traffic collisions.

Although we would like to be able to identify specific areas or geographical features suggesting reptile migration corridors, we have concluded that vehicle-based surveys are not effective at identifying the roadkill 'hotspots' that might be indicators. Future surveyors will continue to look for and record roadkill from vehicles, but more organized surveys will be conducted on foot along fixed sections of well-travelled roads. Distance to water and other prominent geographical features will be recorded at future roadkill sites. We will also work to obtain traffic counters in 2009 to place along survey sections so that mortality rates can be linked to traffic intensity and broader conclusions about basin-wide impacts can be estimated.

Results by Species

2008 was a very successful year for amphibian observations because so much effort was spent on nocturnal and riparian visual encounter surveys. Figure 9 displays the locations of all amphibians seen or heard except for boreal chorus frogs (which were very abundant throughout the basin). Woodhouse's toad was the second most abundant amphibian, occurring along rivers, temporary ponds, and in CBNG reservoirs. Plains spadefoot and Great Basin toads were the least common, occurring primarily in temporary ponds fed by rainstorms. A preliminary habitat analysis (Table 5) suggests that boreal chorus frogs and Woodhouse's toads are most tolerant of high salinity and conductivity levels (Burger Draw has the highest levels of all sample sites). Although reproduction was documented at several sample sites, it was not detected at all the sites where adults were seen. Timing of surveys at some sites, however, may have occurred outside the narrow range of conditions suitable for reproduction, limiting our ability to make inferences about water quality and reproduction.

Although we did observe most reptile species thought to occur in the PRB in 2008, there were relatively few observations (Figure 10 & 11) compared to amphibians. This is partially due to their dispersed and cryptic nature and partially due to less survey effort. As far as overall abundance, we found that bullsnakes and sagebrush lizards were most common with only a handful of observations for all other species. It is possible that other species were more abundant but simply less observable than bullsnakes and sagebrush lizards. There were also very few turtle observations, which we predict will continue to be the case in future years. Turner (2007) spent considerable effort trapping and surveying turtles along the perennial rivers in the Basin and found them to occur almost exclusively in deep backwaters. Since our efforts will continue to concentrate more on upland sites, we do not foresee finding many turtles. In 2009, we plan to focus more attention on roadkill and rock outcrop surveys than in 2008 in order to gather more information about the distribution and habitat of upland reptile species.

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TABLES AND FIGURES

Table 1. Species of herptiles expected to occur in the Powder River Basin of Wyoming (Turner, 2007; Parker and Anderson, 2001.

Common Name	**Scientific Name**	**Found by WGFD 2004-2006**	**Found by WYNDD 2008**
Bullfrog*	*Rana catesbeiana*	Maybe	N
Spiny Softshell	*Apalone spinifera*	Y	N
Milksnake*	*Lampropeltis triangulum*	N	N
Northern Prairie Lizard*	*Sceloporus undulatus garmani*	N	N
Boreal Chorus Frog	*Pseudacris maculata*	Y	Y
Northern Leopard Frog	*Rana pipiens*	Y	Y
Great Plains Toad	*Bufo cognatus*	Y	Y
Woodhouse's Toad	*Bufo woodhousii*	Y	Y
Plains Spadefoot toad	*Spea bombifrons*	Y	Y
Tiger Salamander	*Ambystoma tigrinum*	Y	Y
Short-horned Lizard	*Phrynosoma hernandesi*	Y	Y
Sagebrush Lizard	*Sceloporus graciosus*	Y	Y
Eastern Yellowbelly Racer	*Coluber constrictor flaviventris*	Y	Y
Prairie Rattlesnake	*Crotalus viridis*	Y	Y
Western Hog-nosed Snake	*Heterodon nasicus*	Y	Y
Bullsnake	*Pituophis catenifer sayi*	Y	Y
Terrestrial Garter Snake	*Thamnophis elegans*	Y	Y
Plains Garter Snake	*Thamnophis radix*	Y	Y
Common Garter Snake	*Thamnophis sirtalis*	Y	Y
Snapping Turtle	*Chelydra serpentina*	Y	Y
Painted Turtle	*Chrysemys picta*	Y	Y

* Indicates questionable occurrence in PRB due to lack of supporting documentation from field survey efforts.

Table 2. Survey effort by Hydrologic Unit Code (HUC).

6th Level Hydrologic Unit Code (HUC)	Water Body VES	River/Creek VES	Nocturnal	Rock Outcrop VES	All survey types	# Visual Adult Herp Obs.	# Herp Species
	Minutes of survey time (2-person team)						
Powder River - Big Remington Creek	115	121	0	50	286	34	8
Powder River - Dry Creek	197	0	300	105	602	32	8
Powder River - Burger Draw	214	0	170	60	444	36	8
Powder River - Ivy Creek	115	370	0	0	485	26	7
L X Bar Creek	529	0	170	0	699	65	7
Wild Horse Creek - Cedar Draw	45	265	175	0	485	41	6
Crazy Woman Creek - Keathley Draw	0	161	120	0	281	6	5
Clear Creek - Cabin Creek	0	112	0	0	112	4	3
Dry Fork Powder River - Bullwhacker Creek	96	0	0	0	96	21	3
Salt Creek - Crooked Creek	25	64	0	60	149	0	0
Lower Castle Creek	70	0	0	0	70	0	0
Sites outside targeted HUCs	0	95	1486	30	1611	1	6
# of sites surveyed by type	46	22	34	16	130	-	-
Total number of hours surveyed	23.4	18.2	15.6	4.6	61.8	-	-

Table 3. Nocturnal survey results using WGFD fixed routes (Turner, 2007). Each survey was conducted using the WGFD methods and was repeated 3 times.

WYNDD Route Name	Bill's Survey number	Date	Survey min	Species Detected*
NOC008	B4	19-May-08	40	none
NOC008	B4	13-Jun-08	37	BCF, WOT
NOC008	B4	3-Jul-08	47	none
NOC014	B23	3-Jun-08	38	BCF
NOC014	B23	23-Jun-08	46	BCF
NOC014	B23	2-Jul-08	40	none
NOC015	B8	3-Jun-08	45	BCF
NOC015	B8	23-Jun-08	35	BCF
NOC015	B8	2-Jul-08	40	PST
NOC022	B11	13-Jun-08	34	BCF
NOC022	B11	24-Jun-08	42	none
NOC022	B11	7-Jul-08	39	none
NOC027	B22	24-Jun-08	37	BCF
NOC027	B22	7-Jul-08	35	BCF
NOC027	B22	16-Jul-08	40	BCF

* BCF = Boreal chorus frog; WOT = Woodhouse’s toad; PST = Plains spadefoot toad

Table 4. Habitat and water quality parameters at all water bodies where tiger salamanders were found.

Max water depth	% Emergent Veg	Water Temp	Water pH	TDS	Conductivity	Salinity	status
1-2 M	1-25	24.9 C	7.40	0.1333 g/L	0.206 mS	0.1 ppt	alive
> 2 M	1-25	23.1 C	7.45	0.379 g/L	0.584 mS	0.3 ppt	dead & alive
> 2 M	1-25	27.2 C	7.69	0.577 g/L	0.889 mS	0.4 ppt	dead
> 2 M	1-25	21.8 C	7.48	0.890 g/L	1.288 mS	0.7 ppt	dead
> 2 M	0	14.1 C	7.25	1.201 g/L	1.845 mS	0.9 ppt	dead & alive
> 2 M	1-25	23.3 C	7.50	1.236 g/L	1.899 mS	1.0 ppt	dead
> 2 M	0	25.1 C	7.53	1.233 g/L	1.910 mS	1.0 ppt	dead
> 2 M	0	23.3 C	7.52	1.721 g/L	2.645 mS	1.4 ppt	dead
> 2 M	0	27.1 C	7.49	1.890 g/L	2.91 mS	1.5 ppt	dead
> 2 M	0	23.1 C	7.48	1.904 g/L	2.925 mS	1.5 ppt	dead
> 2 M	1-25	29.4 C	7.67	1.943 g/L	2.984 mS	1.5 ppt	dead
> 2 M	1-25	24.0 C	7.50	1.965 g/L	3.027 mS	1.6 ppt	dead & alive
> 2 M	1-25	28.2 C	7.63	1.968 g/L	3.029 mS	1.6 ppt	alive
> 2 M	0	26.2 C	7.25	3.88 g/L	5.96 mS	3.2 ppt	dead

Table 5. Preliminary habitat and water quality parameters for aquatic species surveyed in 2008.

Species	Habitat Type	# of sites	% Aquatic Vegetation	Water Dammed?	Water Temp C	Water pH	Total Dissolved Solids (g/L)	Conductivity (mS)	Salinity (ppt)
Tiger Salamander	Reservoir	14	0-25	Y,N	14- 29	7.3- 7.7	0.13-1.97	0.20-3.03	0.1- 1.6
Boreal Chorus Frog	Stream channel, reservoir, ephemeral channel, marsh, puddle	9	0-100	Y,N	13-30	7.1-7.9	0.10- 6.17	0.10-9.53	0.1- 5.3
Northern Leopard Frog	Stream channel, reservoir	12	0-50	Y,N	14-30	7.1-7.9	0.13-2.19	0.20-3.38	0.1-1.8
Woodhouse's Toad	Stream channel, reservoir, ephemeral channel	19	0-100	Y,N	12- 31	7.1- 7.8	0.08- 6.17	0.12- 9.53	0.1- 5.3
Plains Spadefoot Toad	Reservoir	2	1-50	N	25-30	7.4-7.9	0.13	0.20-0.21	0.1
Great Plains Toad	Reservoir	1	1-25	N	25	7.4	0.13	0.21	0.1
Snapping Turtle	Reservoir		1-25	N	24	7.5	1.9	3.03	1.6
Painted Turtle	Reservoir, ephemeral channel	1	1-25	Y	26- 27	7.3- 7.5	2.92- 3.01	-	1.5

Table 6. Dead and basking herptiles found during road surveys in 2008.

Survey Type	**Road Type**	**Area**	**Date**	**Species**	**Dead or Alive**	**Mortality Rate (#** ***dead herps/mi*****)**
Turner-fixed	graded, major dirt	Fixed-B_road1	8-Jul-08	-	-	0
Turner-fixed	minor dirt	Fixed-B_road2	9-Jul-08	-	-	0
Turner-fixed	paved, minor	Fixed-B_road3	9-Jul-08	1 Bullsnake	Dead	1
Turner-fixed	state highway	Fixed-B_road5	11-Jul-08	1 Prairie Rattlesnake	Dead	1
Turner-fixed	state highway	Fixed-B_road6	11-Jul-08	1 Bullsnake	Dead	1
Chance	state highway	Kaycee area	8-Jun-08	1 Bullsnake	Dead	-
Chance	paved, major	near Buffalo	11-Jul-08	1 Snapping Turtle	Alive	-
Chance	graded, major dirt	Dead Horse Area	19-May-08	3 Bullsnakes	Dead	1.5
Chance	graded, major dirt	Dead Horse Area	19-May-08	1 Terrestrial Gartersnake	Dead	-
Chance	graded, major dirt	Lower PR Road	20-May-08	1 Woodhouse's Toad	Alive	-
Chance	graded, major dirt	Schoonover Road	10-Jun-08	1 Bullsnake	Dead	-
Chance	graded, major dirt	Dead Horse Area	23-Jun-08	1 Bullsnake	Dead	-
Chance	graded, major dirt	Tipperary Road	8-Jul-08	1 Bullsnake	Dead	-
Chance	graded, major dirt	Kaycee area	15-Jul-08	1 Prairie Rattlesnake	Dead	-
Chance	paved, minor	Dead Horse Area	6-May-08	1 Bullsnake	Alive	-
Chance	minor dirt	Dry Creek	1-May-08	1 Bullsnake	Dead	-
Chance	minor dirt	Spotted Horse Ck.	28-May-08	1 Bullsnake	Alive	-
Chance	minor dirt	Spotted Horse Ck.	30-Jun-08	1 Eastern Yellowbelly Racer	Alive	-

Figure 1. Study Area: Overview of the Powder River Basin and major tributaries.

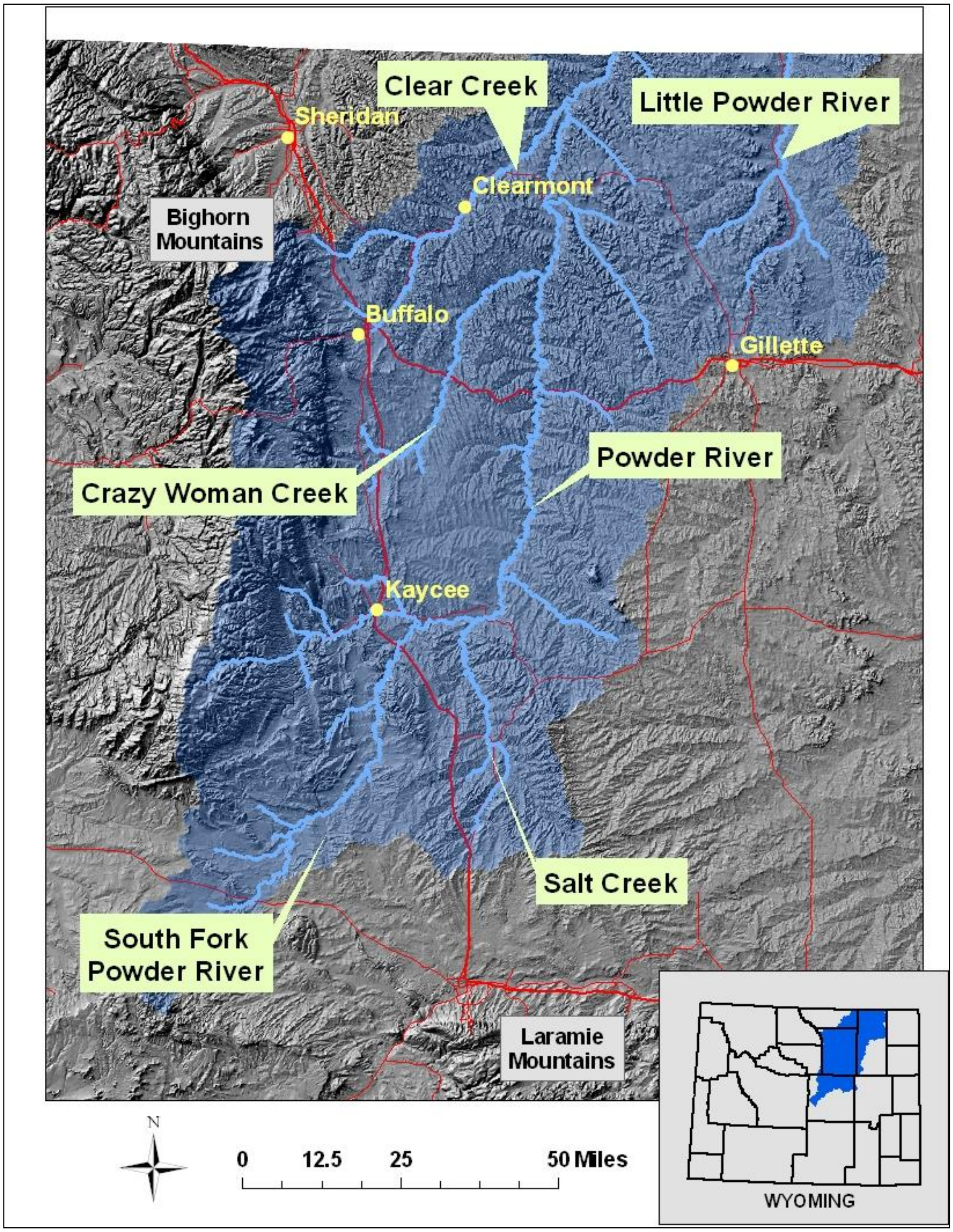


Figure 2. Priority Hydrologic Units surveyed in 2008.

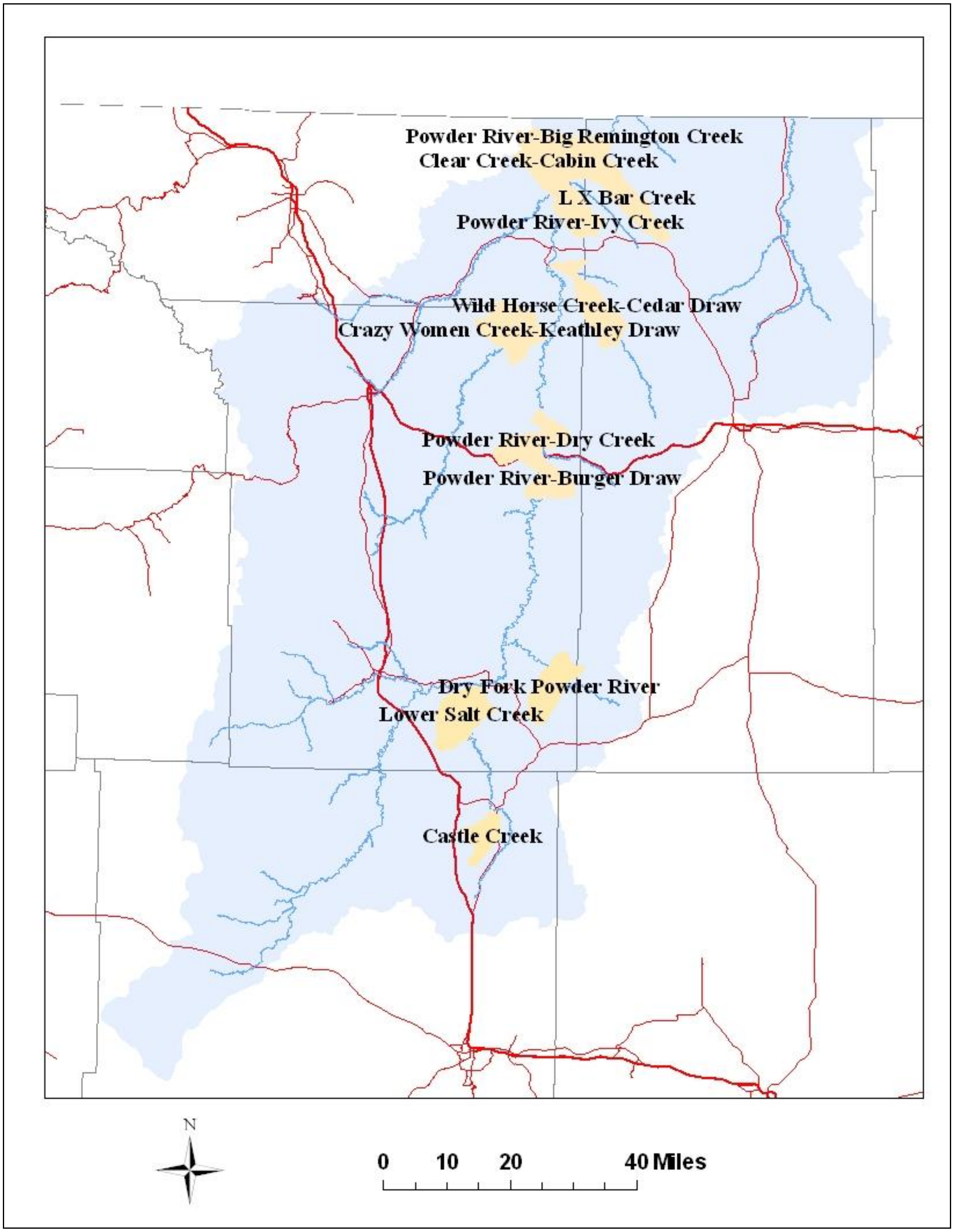


Figure 3. Nocturnal survey transects.

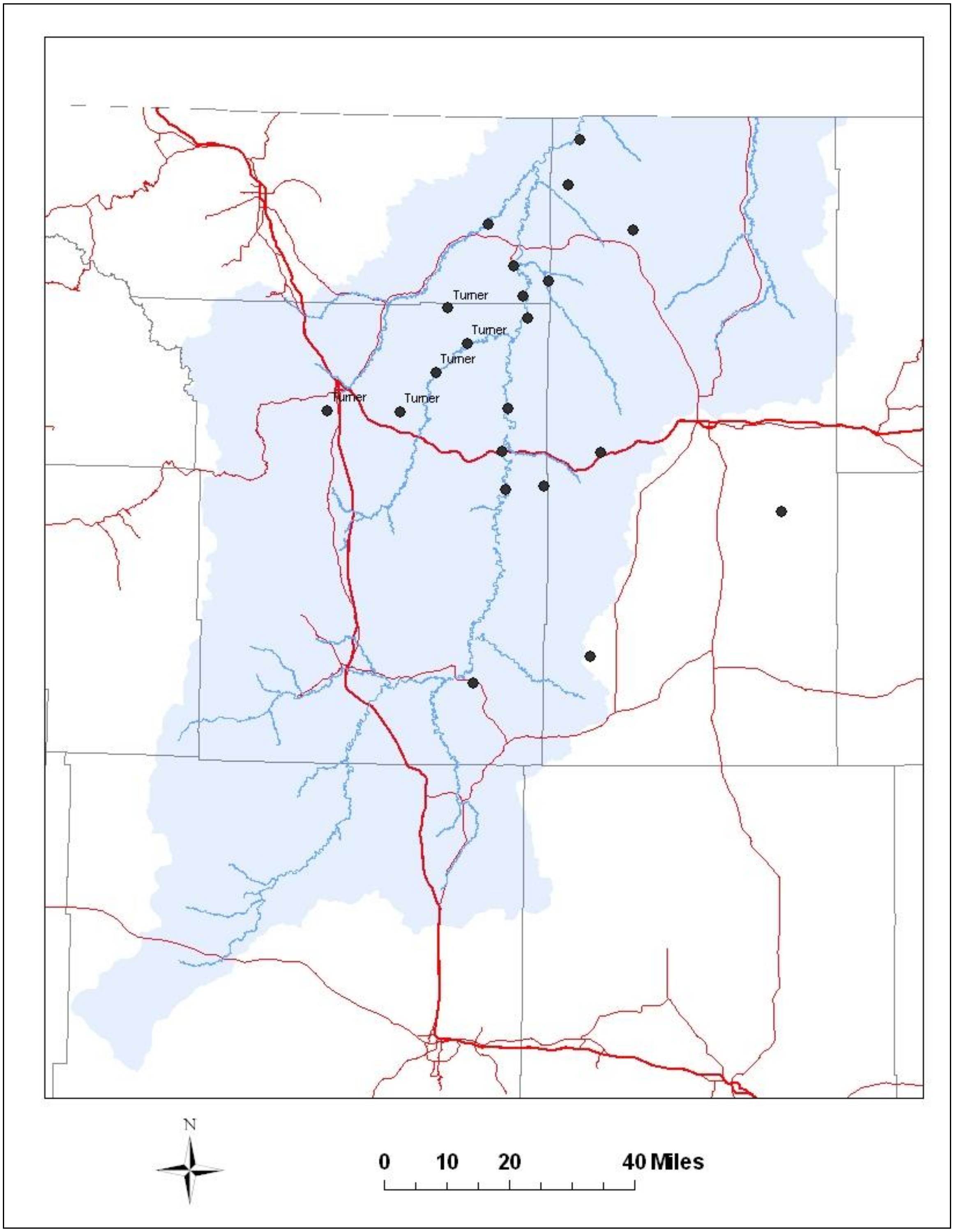


Figure 4. Water body and river visual encounter survey sites.

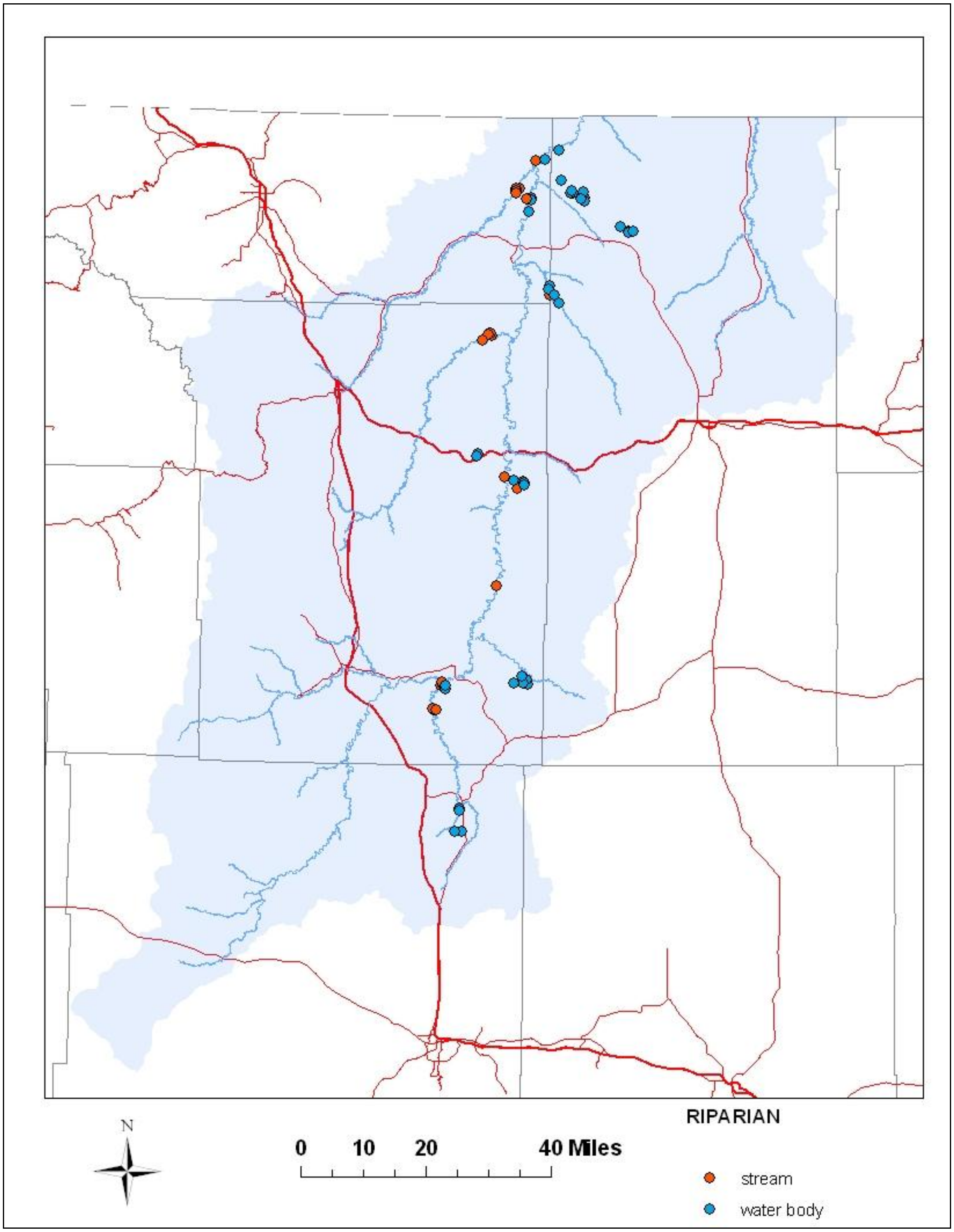


Figure 5. Water bodies where dead salamanders were collected.

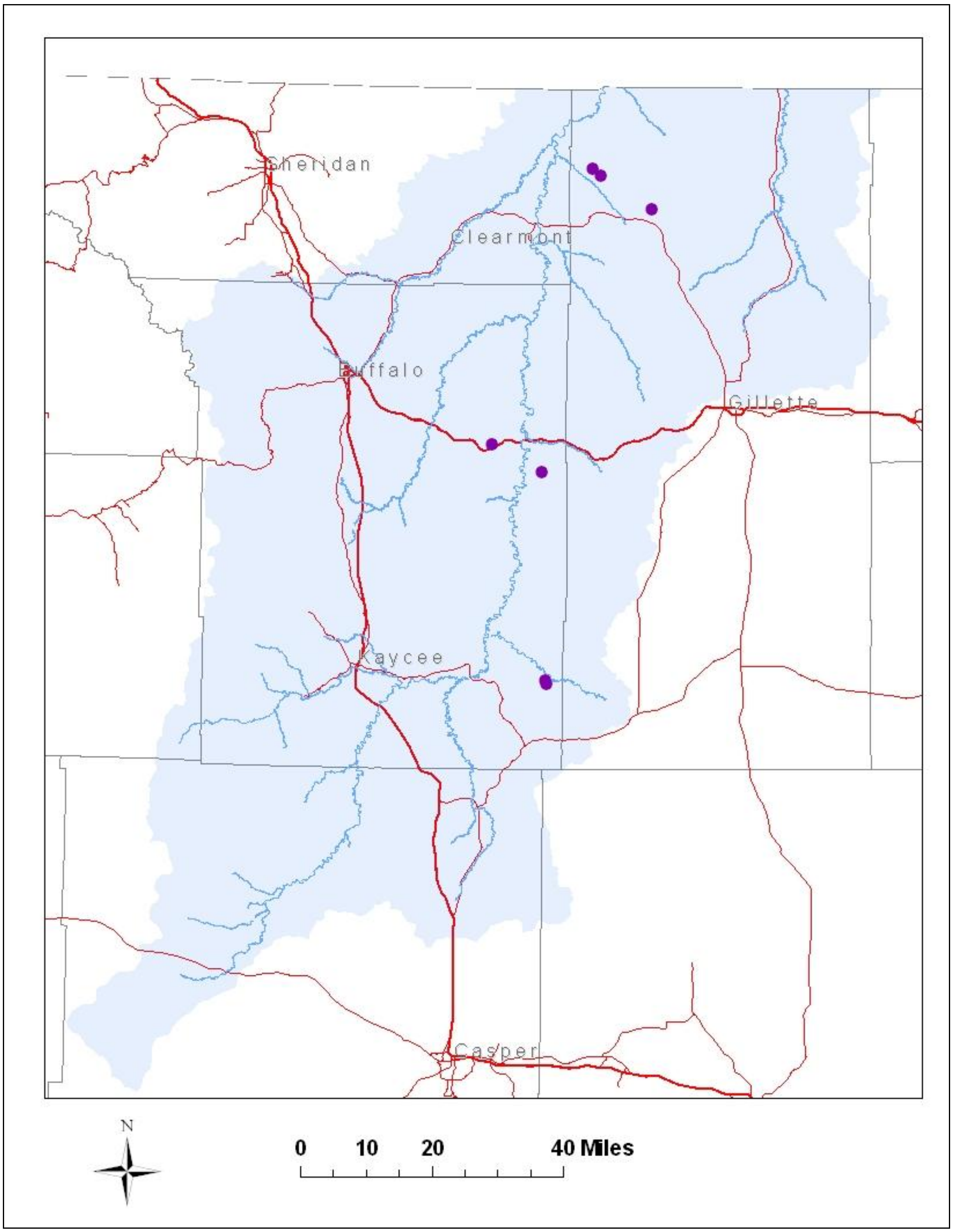


Figure 6. *Batrachochytrium dendrobatidis* (chytrid fungus) infection results.

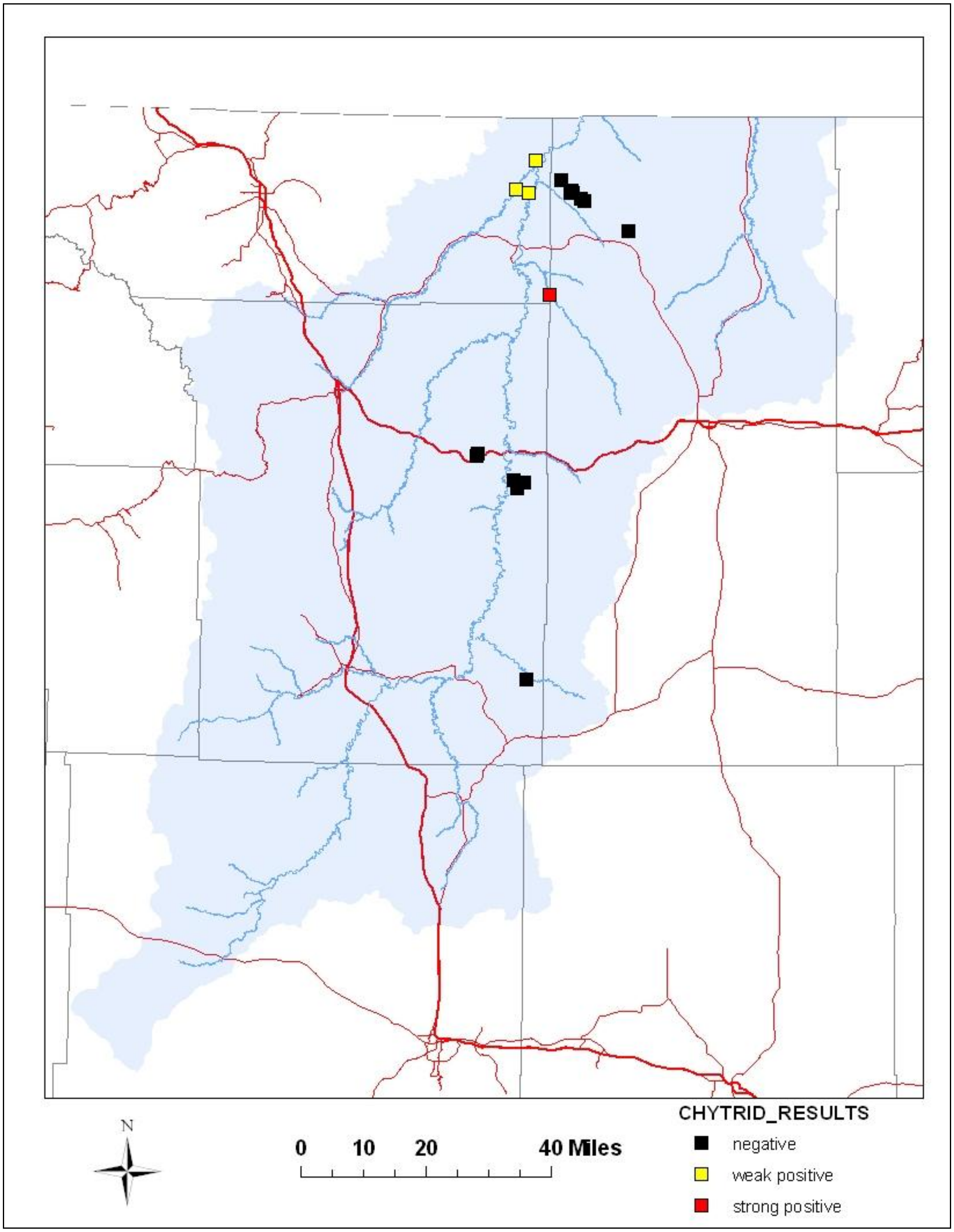


Figure 7. Rocky outcrop visual encounter survey sites.

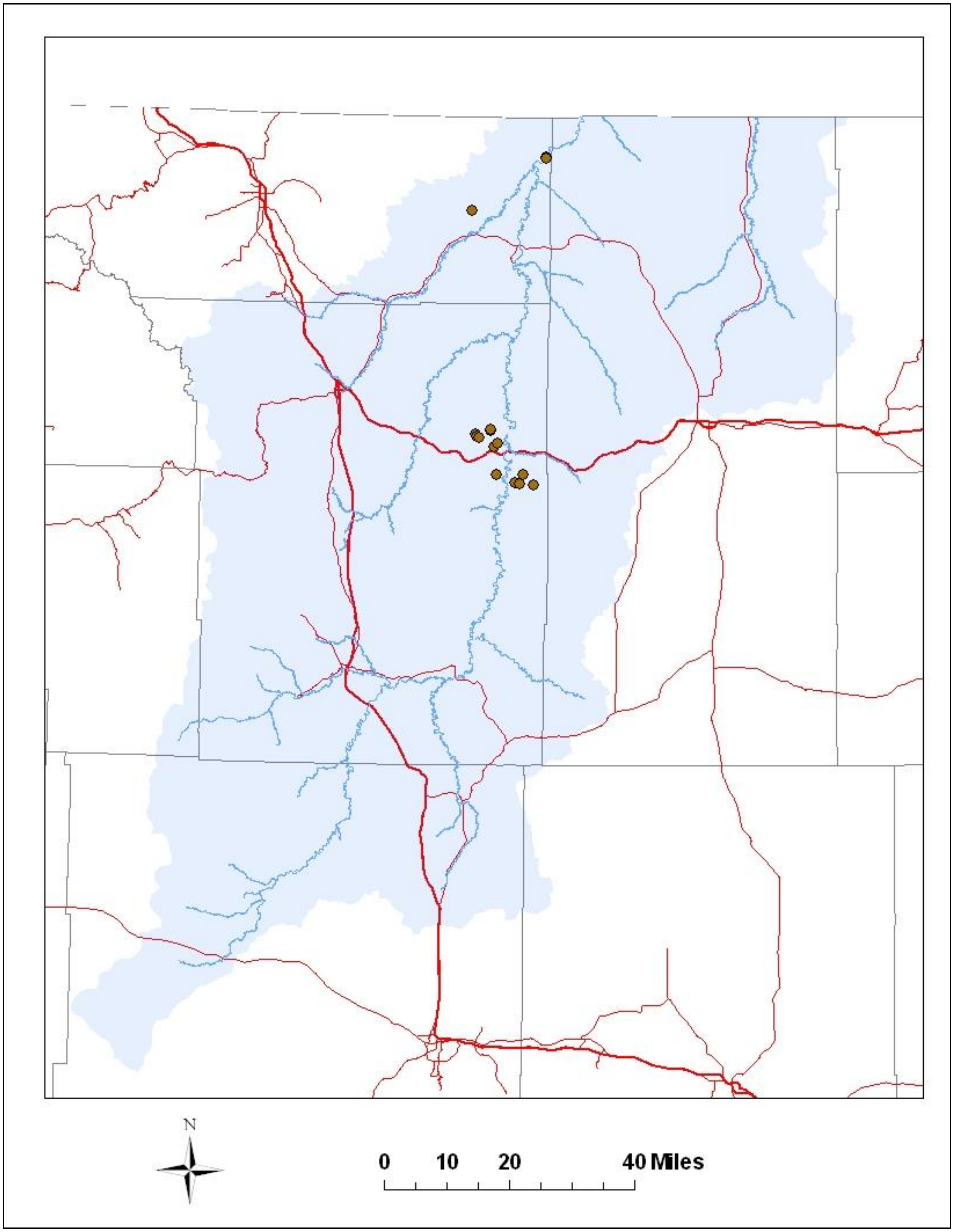


Figure 8. Roadkill and basking herp sites.

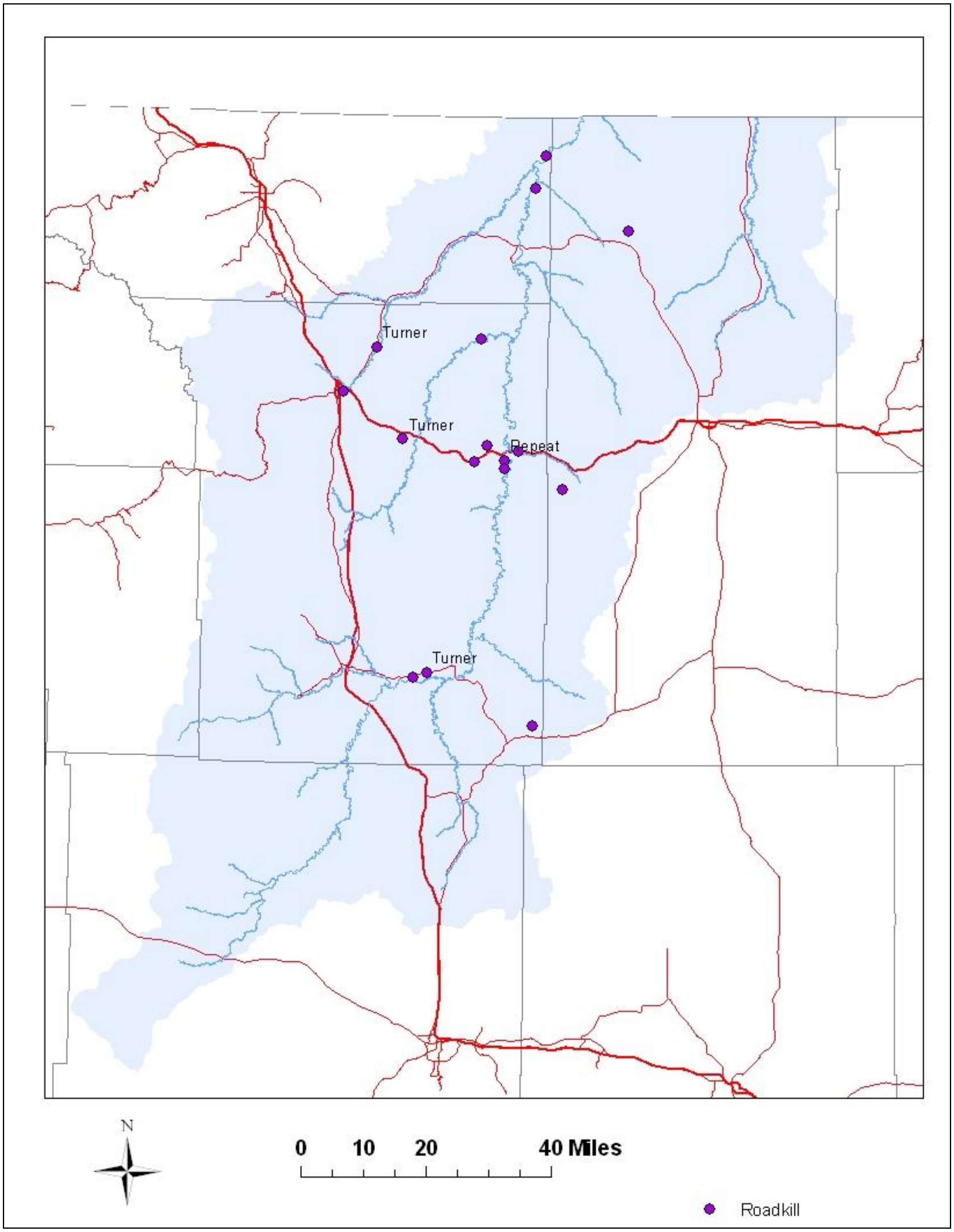


Figure 9. Amphibian observations by species.

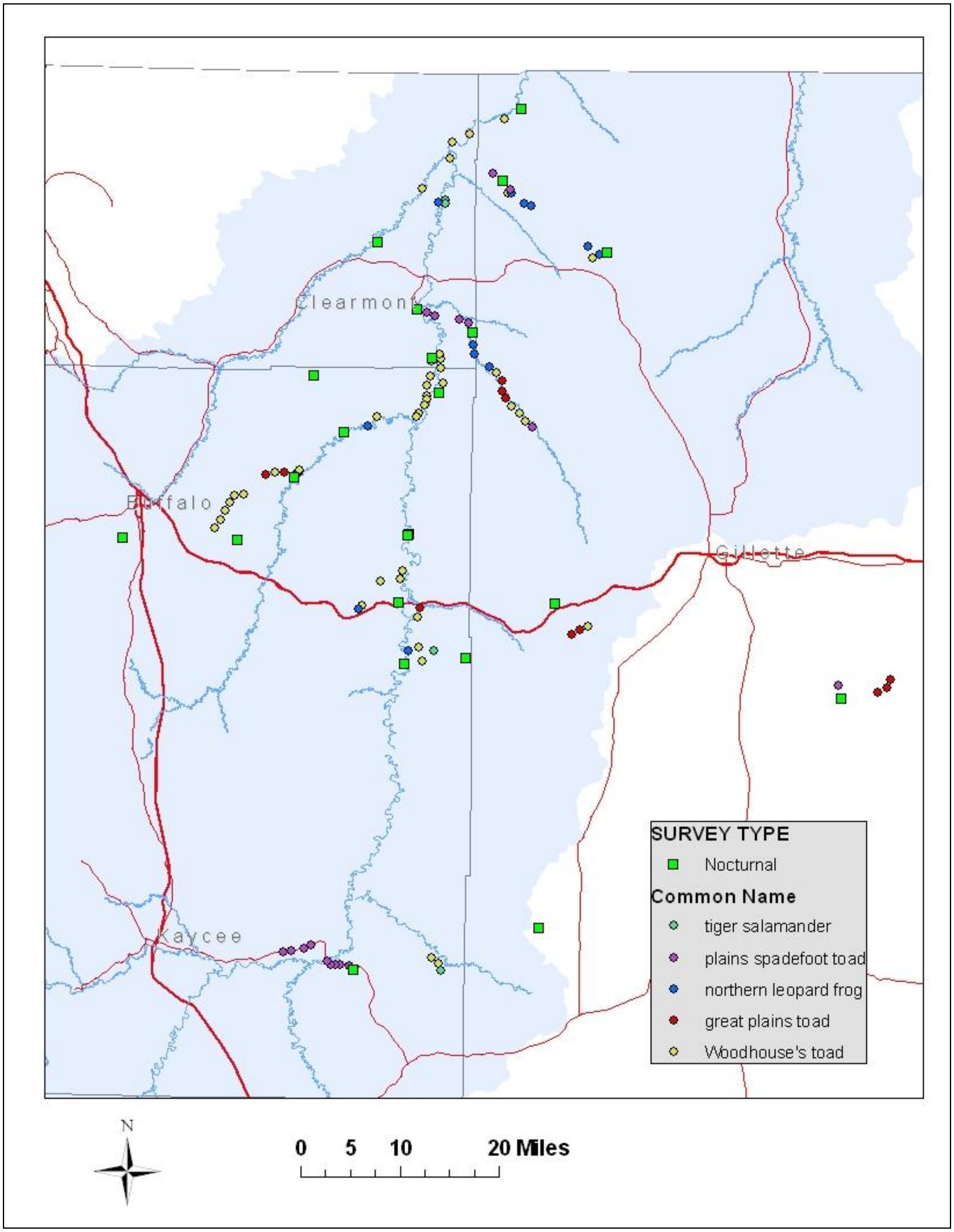


Figure 10. Snake observations by species.

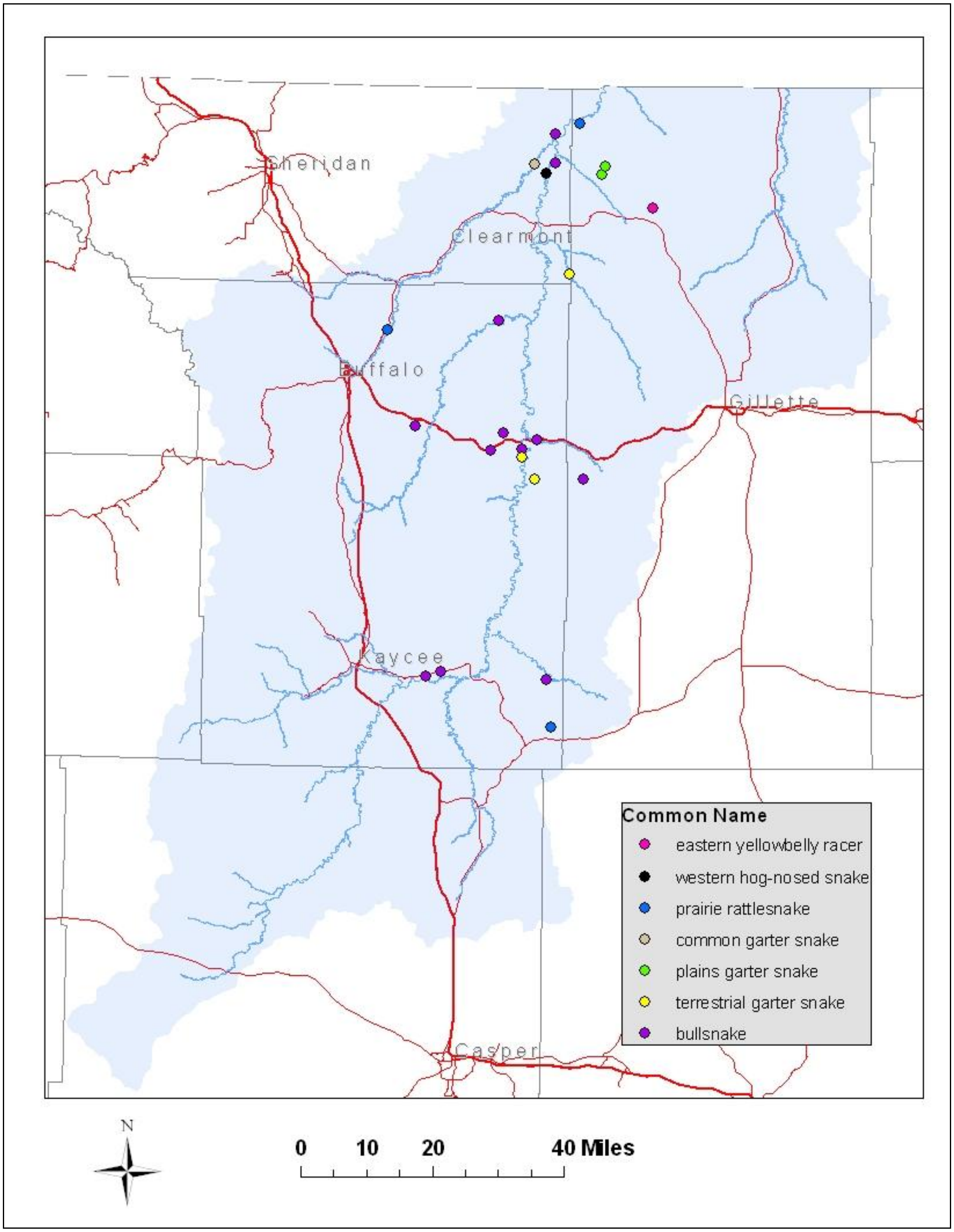
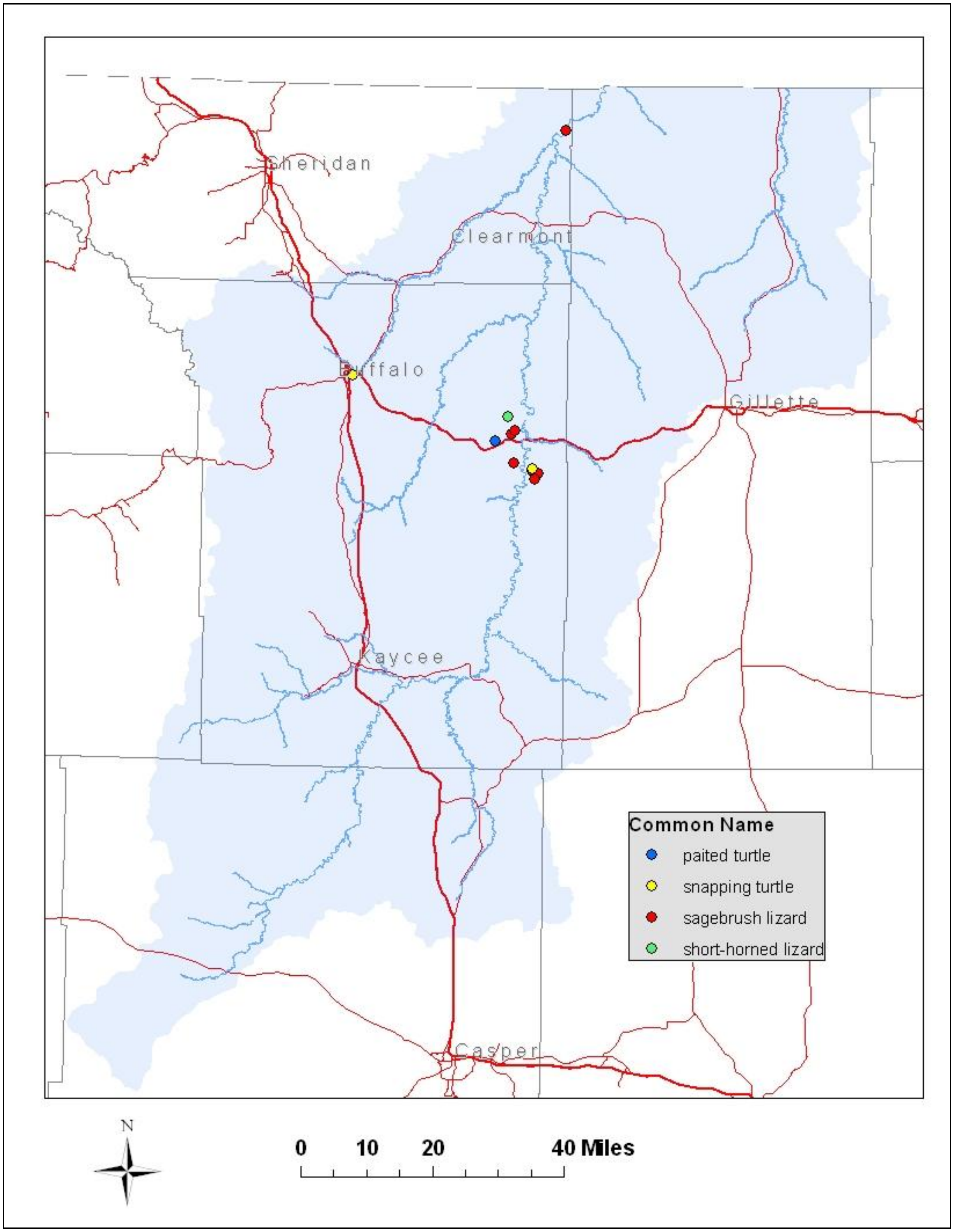


Figure 11. Lizard and turtle observations by species.



APPENDIX A – Literature Review

Changes in both aquatic and terrestrial habitats associated with CBNG development potentially could impact reptile and amphibian populations in the Powder River Basin (PRB), Wyoming. The degree to which these changes could influence herptiles, however, likely will vary across species and across sites due to differences in water chemistry and proximity to important habitat, such as breeding sites and hibernacula.

Amphibians are thought to be particularly sensitive to water quality because they spend a significant portion of their lives in water, and because of the thin skin of adults and larvae and the highly permeable membrane of eggs. CBNG product water tends to be saline, have low concentrations of dissolved oxygen, and contain heavy metals (Rice et al. 2000, McBeth et al. 2003, Wang and Yang 2008). Both the quality and quantity of CBNG product water can vary substantially within basins depending on the depth of the coalbed and the surrounding geology (Bryner 2003). In the PRB, CBNG product waters tend to increase in salinity, total dissolved solids (TDS), and alkalinity from south to north and from east to west (Rice et al. 2000, Wang and Yang 2008).

Amphibians have poor osmoregulatory abilities and can be negatively impacted by changes in water salinity (Boutilier et al. 1992). The response of amphibians to increased salinity tends to be species-specific and vary across developmental stages. In general, however, increased saline concentrations can result in increased mortality and occurrence of morphological abnormalities of embryos (Gomez-Mestre and Tejedo 2003), decreased growth rates, lower weight at metamorphosis, delayed metamorphosis, and increased mortality in larvae (Beebee 1985, Christy and Dickman 2002, Gomez-Mestre et al. 2004, Chinathamby et al. 2006). Viertel (1999) determined that adult *Rana temporaria* selected spawning sites with lower conductivity and salinity values. At the population level, the probability of occupancy of six tadpole species at wetlands in southeastern Australia was inversely related to salinity (Smith et al. 2007). However, Smith et al. (2007) documented among-species differences in salinity tolerance. Within a species, adaptation to local conditions can influence salinity tolerance of amphibians (Gomez-Mestre and Tejedo 2003).

Ammonia is a potential toxicant found in CBNG product waters and was found to be elevated in CBNG discharge waters relative to many surface waters in the PRB (Johnson 2007). Although elevated ammonia levels can result in decreased survival, increased prevalence of deformities, and slower growth rate in anuran embryos and tadpoles, these effects tend to occur only at relatively high concentrations (>0.6 mg/L; Diamond et al. 1993, Jofre and Karasov 1999). Furthermore, Johnson (2007) suggested that ammonia toxicity in CBNG product water in the PRB was mitigated by biochemical interactions with surface waters after discharge.

Although pH levels in CBNG product water may differ from surface water, Davis et al. (2006) suggested that pH levels for CBNG product water in the PRB (Rice et al. 2000) are within the optimal range for fish productivity. Davis et al. (2006) caution, however, that because pH of product water increases as the water reacts with soils and the atmosphere after discharge (McBeth et al. 2003, Patz et al. 2004), predicting the effect of pH fluctuations on fish is difficult. Similar difficulties exist in predicting the effect of pH on amphibians, especially since fluctuating pH levels may influence developmental rates in larvae, and characteristics of the natal habitat could determine the response of larvae to changes in pH and other abiotic factors (Gerlanc and Kaufman 2005).

Because the concentrations of heavy metals and trace elements in CBNG product water vary between wells, it is also difficult to predict the effect of these factors on amphibians. Exposure to heavy metals has been found to decrease survival and growth rate in tadpoles (Lefcort et al. 1998). Lefcort et al. (1998) found that exposure of tadpoles to some heavy metals (medium and high levels of zinc and lead), and to soil from a superfund site containing a number of heavy metals, caused delayed fright response to predators, potentially increasing susceptibility to predation. Horne and Dunson (1995) determined that acute and chronic exposures to aluminum and copper resulted in reduced survival of Jefferson salamanders (*Ambystoma jeffersonianum*) and wood frogs (*Rana sylvatica*). Aluminum toxicity in amphibians tends to vary across species and developmental stages and is influenced by pH and dissolved oxygen levels in the water (Freda 1991). Experiments conducted by Lefcorte et al. (1998) also determined that metals presented together were toxic to amphibians at lower doses than single metals.

Water temperature has also been shown to affect growth and development in amphibians (Smith-Gill and Berven 1979, Hayes et al. 1993, Olsson and Uller 2002). Thermal tolerance varies across species and across developmental stages within species (see Denver 1997, Cupp 1980). Cupp (1980) found that critical thermal maxima were highest for anuran larvae, decreased during metamorphosis, and usually increased in adults. Survival and growth of embryos is also influenced by temperature, and the range of temperatures under which embryos develop normally is often limited (Moore 1939, Duellman and Trueb 1994).

Turbidity of CBNG product water likely varies between wells and may be more or less turbid than the surface waters into which they are being discharged. Thus, the impact of turbidity on amphibians may vary across the PRB. Relative abundance of green frog (*Rana clamitans*) and American bullfrog (*Rana catesbeiana*) tadpoles was lower in wetlands that were higher in conductivity and turbidity and lower in dissolved oxygen (Schmutzer 2008). In addition, potential impacts of turbidity on fish assemblages may change the suite of predatory fish in streams receiving CBNG product water (see Davis et al. 2006), possibly increasing predation on amphibians.

Because many of the streams into which CBNG product water is discharged in the PRB are ephemeral or intermittent, increased stream flow is expected. While this may benefit some herptiles by increasing the amount of aquatic habitat available, the discharge of CBNG product water into ephemeral and intermittent streams has the potential to increase soil erosion in these channels, resulting in sedimentation of the streams and loss of aquatic and riparian habitat (Allen et al. 1999, Thoman and Niezgoda 2008). Amphibians that rely on streambeds with coarse substrates for food, cover, or reproduction may be adversely affected by increased sedimentation. For example, experiments revealed decreased growth and development in *Litoria spenceri* tadpoles with increasing sedimentation (Gillespie 2002). In the Pacific Northwest, increased stream sedimentation caused by logging and road constructions resulted in decreased abundance of stream amphibians (Corn and Bury 1989, Welsh and Ollivier 1998). By calculating stable channel capacity estimates for each area, however, the release of CBNG product water could be managed to minimize erosion (Thoman and Niezgoda 2008).

The construction of road networks associated with CBNG natural gas development may also pose a threat to reptiles and amphibians. Rates of direct mortality can be high, especially as animals move between breeding, summering, or wintering sites (Ashley and Robinson 1996, Pope et al. 2000, Carr and Fahrig 2001, Aresco 2005). Herptiles also may be attracted to roads, using heat from the road surface to increase their body temperature (Sullivan 1981, Rosen and Lowe 1994). Concern over the impacts of road mortality on populations of reptiles and amphibians is increasing (Wright 2006). Road mortality has been associated with significant declines in local anuran and turtle densities (Fahrig et al. 1995, Pope et al. 2000, Carr and Fahrig 2001, Gibbs and Steen 2005) and is believed to substantially decrease snake populations (Rosen and Lowe 1994). Using population viability analysis, Row et al. (2007) calculated that road mortality was sufficient to increase the probability of extinction of a local populations of black ratsnakes (*Elaphe obsolete*) from 7.3% to 99% in Ontario, Canada. Altered population structure and sex-ratios also can result from road mortality due to a greater propensity for a certain sex or age-class to exhibit seasonal movements (Marchand and Litvaitis 2004, Steen and Gibbs 2004, Gibbs and Steen 2005). In addition to direct mortality, roads have also been shown to negatively affect some snake populations by modifying or inhibiting individual movements, especially in smaller species, (Shrine et al. 2004, Andrews and Gibbons 2005) and, potentially, fragmenting habitat (Andrews and Gibbons 2005).

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APPENDIX B – Data Field Forms

Nocturnal Calling Route Data Sheet

Date_________________________ Observers _______________________________________

Route Name________________________

Start Time______________________ End Time _________________________
Route Starting GPS Coordinates : Latitude (X)___________________ Longitude (Y)____________________
Route Ending GPS Coordinates: Latitude (X)____________________ Longitude (Y)

____
Time since rain: ___________days
Barometric Pressure: Start___________ End____________
Relative Humidity: Start___________ End____________
Weather Code[1]: Start___________ End____________
Wind Speed: Start___________ End____________
Temperature: Start___________ End____________

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

Nocturnal Call Surveying Protocol and Code Keys

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

Turner 2007 method (to be used along "B" routes)
Begin survey at the predefined location. Get out of vehicle and record all starting location and weather information on the datasheet. Listen and record for 2 min. Stop every 0.1 mi along the route and listen for 2 minutes for a total of twenty survey minutes. Record all ending information on datasheet.

Modified WYNDD 2008 methods (to be used on all other routes)
Same as above except stops are every .5 miles, listening for 3 minutes.

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

Equipment List

GPS + extra batteries
Maps
Recording of species' calls
Datasheets
Weather collecting device (i.e. Kestral)
Compass

CODES

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

Species Code Key:			
	BCF: Boreal Chorus Frog	**BF**: Bullfrog	**NLF**: Northern Leopard Frog
	GPT: Great Plains Toad	**WHT**: Woodhouse's Toad	**PSFT**: Plains Spadefoot Toad

Road Survey Data Sheet

Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	
Observation #:	Observer(s):	Date:	Time:	Habitat Description:
UTM Zone:	UTM East:	UTM North:	GPS waypoint #:	
Species:	Dead or Alive	Picture #:	Specimen Collected? Y N	

Repeat Roadkill Survey

Original Observation Numbers:	Survey Site Name:	Habitat Description:	
Repeat Survey Number (1,2, or 3):	Observer(s):	Date:	Time:
UTM zone:	UTM East Start:	UTM North Start:	GPS waypoint Start:
	UTM East End:	UTM North End:	GPS waypoint End:
Species:	Dead or Alive	Picture #:	Specimen collected: Y N
Species:	Dead or Alive	Picture #:	Specimen collected: Y N
Species:	Dead or Alive	Picture #:	Specimen collected: Y N
Species:	Dead or Alive	Picture #:	Specimen collected: Y N

Original Observation Numbers:	Survey Site Name:	Habitat Description:	
Repeat Survey Number (1,2, or 3):	Observer(s):	Date:	Time:
UTM zone:	UTM East Start:	UTM North Start:	GPS waypoint Start:
	UTM East End:	UTM North End:	GPS waypoint End:
Species:	Dead or Alive	Picture #:	Specimen collected: Y N
Species:	Dead or Alive	Picture #:	Specimen collected: Y N
Species:	Dead or Alive	Picture #:	Specimen collected: Y N
Species:	Dead or Alive	Picture #:	Specimen collected: Y N

Original Observation Numbers:	Survey Site Name:	Habitat Description:	
Repeat Survey Number (1,2, or 3):	Observer(s):	Date:	Time:
UTM zone:	UTM East Start:	UTM North Start:	GPS waypoint Start:
	UTM East End:	UTM North End:	GPS waypoint End:
Species:	Dead or Alive	Picture #:	Specimen collected: Y N
Species:	Dead or Alive	Picture #:	Specimen collected: Y N
Species:	Dead or Alive	Picture #:	Specimen collected: Y N
Species:	Dead or Alive	Picture #:	Specimen collected: Y N

Reptile VES / Hibernaculum Site Survey Form

Search Area Name:		Date:	Time:	Observer(s):
Aspect of slope:	Picture of Habitat:	Habitat Description:		
UTM zone:	Initial UTM East:	Initial UTM North:		Initial GPS waypoint:
	Ending UTM East:	Ending UTM North:		Ending GPS waypoint:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:

Search Area Name:		Date:	Time:	Observer(s):
Aspect of slope:	Picture of Habitat:	Habitat Description:		
UTM zone:	Initial UTM East:	Initial UTM North:		Initial GPS waypoint:
	Ending UTM East:	Ending UTM North:		Ending GPS waypoint:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:
Species	Number found	Picture #:	Dead or Alive	Habitat:

I. ROAD SURVEYS

all season

Overview: Dead and basking reptiles and amphibians will be recorded when seen on or near roads. There are two purposes of this opportunistic survey. The first is to help identify potential migration corridors from winter hibernacula to summer territories. Two or more dead or basking reptiles within 0.25 mi of each other will serve to guide ground searches early in the season within nearby rock outcroppings for communal hibernacula. Second, by recording the locations of dead herptiles along roads throughout the summer, mortality 'hotspots' will help guide protocol for standardized 'roadkill' surveys in 2009. The information will also help us provide management and mitigation recommendations to the ATG.

Protocol: Driving speed should not exceed what is needed to see dead or basking animals. Identify and GPS the locations of all dead and basking reptiles and amphibians while driving to and from other field sites. Concentrations of observations (2 or more individuals within 0.25mi.) will **trigger 3 things**.

1. COMPLETION OF ROAD SURVEY DATA SHEET

2. REPTILE VISUAL ENCOUNTER SURVEYS (see description below)

Potential habitat within about 1 mile of roadkill/basking 'hotspots' should be surveyed ASAP (latest: June 15). **Use 'Reptile VES' datasheet-see below**

3. REPEAT ROADKILL SURVEYS

Roadkill/basking 'hotspots' that are identified should be returned to and carefully canvassed (+2 mile of road on each end) 2 more times during the season. **Use Repeat Roadkill Survey Data Sheet.**

II. REPTILE VES

April 21-June 15

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

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

Riparian Visual Encounter Data Sheet

Locality Information

Date	Observer(s)	Land Owner	Site Selection: Predetermined Opportunistic

HUC Name		UTM Zone:	UTM East:	UTM North:
HUC Number	Site Name			

Species Information

Amphibian Species		No. Egg Masses		Number Larvae	≤10 ≤100 ≤1000 ≤10K >10K		
Time at first detection	E L M J A	Number Juveniles		Number Adults		Chytrid samples Taken?	
		Vouchers collected?		Picture Number		If breeding with fish is cover present?	Y N
Amphibian Species		No. Egg Masses		Number Larvae	≤10 ≤100 ≤1000 ≤10K >10K		
Time at first detection	E L M J A	Number Juveniles		Number Adults		Chytrid samples Taken?	
		Vouchers collected?		Picture Number		If breeding with fish is cover present?	Y N
Amphibian Species		No. Egg Masses		Number Larvae	≤10 ≤100 ≤1000 ≤10K >10K		
Time at first detection	E L M J A	Number Juveniles		Number Adults		Chytrid samples Taken?	
		Vouchers collected?		Picture Number		If breeding with fish is cover present?	Y N
Amphibian Species		No. Egg Masses		Number Larvae	≤10 ≤100 ≤1000 ≤10K >10K		
Time at first detection	E L M J A	Number Juveniles		Number Adults		Chytrid samples Taken?	
		Vouchers collected?		Picture Number		If breeding with fish is cover present?	Y N

Reptile Species		Time at first detection	E J A	Number Individuals		SVL in CM		Tissue Number		Voucher Number	
Reptile Species		Time at first detection	E J A	Number Individuals		SVL in CM		Tissue Number		Voucher Number	

Reptile Species		Time at first detection	E J A	Number Individuals		SVL in CM		Tissue Number		Voucher Number	
Reptile Species		Time at first detection	E J A	Number Individuals		SVL in CM		Tissue Number		Voucher Number	

Water Quality

Water Temp °C	Water pH	TDS	Conductivity	Salinity	Grab Sample Taken? Y N	Grab Sample #:
General site description and notes:						

Site Map for Riparian Visual Encounter Surveys

Grid Scale:

N↑

***** Indicate the following locations on the map: **T** = temperature, **G** = GPS reading, **C** = clinometer reading, and **P→** = photo locations and directions of photos. Indicate area with emergent vegetation with cross-hatching and indicate a 2-meter depth contour with a dashed line.

Other Notes:

Definitions of Variables for Riparian Visual Encounter Data Sheet

Locality Information

Date: Use MM-DD-YY format (e.g. 5/12/00 for May 12 of 2000).
Observers: List names or initials of individuals involved with survey of this site and circle the name of the recorder.
Land Owner: Use abbreviation of the government agency responsible for managing the land you surveyed. (e.g. USFS, BLM). If private land was surveyed list the owner's full name to indicate that you did not trespass.
Site Selection: If the site was determined before entering the field, circle "Predetermined"; if the site was found while in the field, circle "Opportunistic".
HUC Number and Name: The sample number of the 6^{th} level HUC in one of the nine sample strata defined for western Montana.
Site Number and Name: The number pre-assigned to the water body within each 6^{th} level HUC. If the water body was not pre-assigned a number because it was not on topographic maps or aerial photos then assign it a sequential number and draw it on the topo map.
UTM Zone: Universal Transverse Mercator zone recorded on the topographic map. Use NAD 27 as the map and GPS datum.
UTM East: Universal Transverse Mercator easting coordinate in meters as recorded on the topographic map or GPS receiver. Be sure to note any major differences between UTM coordinates on the map and those on the GPS receiver.
UTM North: Universal Transverse Mercator northing coordinate in meters as recorded on the topographic map or GPS receiver. Be sure to note any major differences between UTM coordinates on the map and those on the GPS receiver.

Habitat Information

Begin Time: List the time the survey began in 24-hour format.
End Time: List the time the survey ended in 24-hour format.
Total Person Minutes of Search: Record the total person minutes the site was searched (e.g. if one person surveys for 15 minutes and another surveys for 30 minutes, but takes 5 minutes to measure a specimen the total person minutes is 40 minutes).
Camera and Photo Number(s) / Description (s): Identify the camera and the number of the photo as viewed on the camera's view screen and a description of the contents of the photograph. Take photos of all portions of the site and anything else that may be of interest (e.g., areas with fish versus areas with amphibians).
Site Dry: Circle whether the site was dry or not at the time of the survey.
Site Origin: Circle whether the site within a channel, a holding pond, or other.
Water Permanence: Circle whether the site contains water throughout the entire year (Permanent), or contains water for only a portion of the year (Temporary).
Water Connectedness: Circle if water body has permanent connection to flowing water (Permanent), is connected to flowing water for a temporary period each year (Temporary), or is never connected to flowing waters or other water bodies (Isolated).
Habitat Type: Circle the appropriate habitat type of the site being surveyed. If site is multi-pooled water information does not need to be gathered for every pool, but you may wish to record this information on the map. If breeding activity is limited to one pool at a multi-pooled site water information should be recorded for this pool and this should be noted in the comments.
Weather: Circle weather condition during survey.
Wind: Circle wind condition during survey (> 20 mph winds should be classified as strong).
Air Temp: Record air temperature at chest height in the shade. Record temperature in Celsius. °C = (°F – 32)/1.8
Color: Circle whether the water is clear or stained a tea or rust color from organic acids.
Turbidity: Circle whether water is clear or cloudy.
Max Depth: Circle the category corresponding to the maximum depth of the water body.
Percent of Site > 2 M: Circle the percentage of the site with water depth greater than 2 meters deep.
Percentage of the Site at ≤ 50 cm Depth: Circle the appropriate percentage.
Percentage of Site with Emergent Veg: Circle the percentage of the entire site with emergent vegetation.

Rank Emergent Veg Species in Order of Abundance: Record the rank order of abundance in front of the 3 most prevalent emergent vegetation species. If the vegetation present is "other" indicate what it is.
Approximate Area with Emergent Veg (M^2): The approximate area of the site that contains emergent vegetation.
Site Length: The length of the longest dimension of the standing water body.
Site Width: The width of the second longest dimension of the standing water body.
Percentage of Site with Larval Activity: Circle the percentage of the site where amphibian larvae were observed.
Percentage of Site Searched: Circle the percentage of the site surveyed.
Primary Substrate: Circle the substrate that covers the majority of the bottom of the site.
Grazing Impact: Circle the appropriate grazing category defined as follows: no grazing noted in the vicinity of the site; grazing noted in the vicinity of the site, but no major impacts to wetland structure or water quality; heavy structural impacts to site (e.g.,vegetation destroyed creating bare ground, hummocks, pugging, or altered hydroregime); heavy structural impacts and water quality impacted due to animal waste; and water quality impacted due to animal waste.
Water Dammed/Diverted: Circle whether or not water has been dammed or diverted at the site.
North Shoreline Characteristics: Circle whether shallows and emergent vegetation are present or absent on the north shoreline.
Other Human Impacts or Modifications: Briefly describe if, how, and when the site has been altered by human activities. If the site has not been altered record none for not altered. If multiple anthropogenic impacts exist document all of these using the back of the data sheet if necessary and qualify approximate timing of impact (e.g., recent versus historic).
Fish Detected?: Circle whether or not fish were detected.
Fish Species if Identified: List the fish species identified.

Species Information

For each species record the first two letters of the scientific genus and species names for all amphibian and reptile species found at the site (e.g., BUBO for *Bufo boreas*). Record the total number of person minutes of survey required before each life history stage of each species was encountered beside the E (egg), L (larvae), M (metamorph), J (juvenile), or A (adult). Record the number or category of number of each of the specified life history and/or size classes. For amphibians indicate whether they have bred in the same water body where fish are present, and if they have, indicate whether there is protective cover (e.g., extensive shallows with emergent vegetation, a log barrier, talus). Record the tissue number or range of tissue numbers for tissue samples collected (see tissue collection protocols). If the animal was swabbed in preparation for testing the animal for chytrid infection indicate the chytrid sample number in the Tissue Number field. Record the preliminary museum voucher specimen number for voucher specimens collected (see voucher specimen collection protocols).

Water Quality

Water Temp: Record water temperature where larvae or egg masses are observed or at 2cm depth 1 meter from the margin of the water body. Record temperature in Celsius. °C = (°F – 32)/1.8
Water pH: Record water pH at the same location water temperature was recorded.
TDS: Record the amount of total dissolved solids measured at the site
Conductivity: Record the conductivity of the water at the site
Salinity: Record the salinity of the water at the site
Grab Sample Taken? Indicate whether or not a grab sample was taken
Grab Sample Number: Record the number of the grab sample if one was taken
General Notes: Record any notes about the sampling or the site for future reference

Site Map for Riparian Visual Encounter Surveys

General: Include a rough sketch of the site including the shape of the site and the shape and spatial relations of surrounding biotic and abiotic features. Indicate the area covered with emergent vegetation with cross-hatching. Indicate a 2-meter depth contour for the water body with a dashed line. Indicate the location where the water temperature was taken, the location where the GPS position was taken, the location where clinometer readings for southern exposure were taken, and the location of any photographs with an arrow indicating the direction in which

the photo(s) were taken. Make sure that the orientation of the sketch (i.e. the north arrow) corresponds to the orientation of the site.
Grid Scale: Indicate the approximate scale of the grid lines relative to the site sketched in meters.
Other Notes: Include any other notes of interest in this space. Examples: (1) areas of highest larval density; (2) thoughts on why a species may not have been detected at a site; (3) problems associated with the survey of the site (e.g., dangerous boggy conditions); (4) If a site was dry would it support reproduction during wetter years.
Southern Exposure: From a site on along the northern shoreline that would most likely to be used as an oviposition or larval rearing area (e.g., shallow waters with emergent vegetation in the NW corner of the water body) record the degree inclination from your position to the skyline (e.g., mountain or solid tree line) at each of the eight compass bearings listed. Note that the compass bearings are true north so you will need to adjust your compass according to the map being used to correct for the deviation from magnetic north (15 to 19.5 degrees in western Montana).

Appendix 8. Detection of (*Batrachochytrium dendrobatidis*), the Chytrid Fungus Associated with Global Amphibian Declines, in Montana Amphibians

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In order to identify potential causes of declines in the northern leopard frog (*Rana pipiens*) and western toad (*Bufo boreas*) which have been noted since the 1980s and assess the risk posed to other amphibian species whose status is uncertain, we submitted 98 tissue samples gathered from 8 amphibian species across Montana for PCR based identification of the chytrid fungus (*Batrachochytrium dendrobatidis*). This chytrid fungus has been associated with declines, extirpations, and losses of numerous amphibian populations and entire species around the globe over the last 2 decades. Tissue samples from 30 museum voucher specimens of 3 species collected in the Flathead Valley in the 1970s, prior to amphibian declines in the area, were all negative for *B. dendrobatidis*. However, 4 species and 26 of 68 tissue samples gathered during inventory work across the state since 1998 tested positive for *B. dendrobatidis*. In light of its association with other amphibian declines, *B. dendrobatidis*, acting alone or synergistically with other stressors, is a potential cause of the declines observed and should be regarded as an ongoing threat to Montana amphibians. In order to prevent additional spread of this fungal pathogen personnel working in either lentic or lotic systems should thoroughly rinse and decontaminate all equipment with 10% bleach between (1) any sites where dead, dying, or ill amphibians are encountered, (2) sites located in different local watersheds or definitive clusters of sites, (3) all breeding sites of sensitive species separated by more than 1 kilometer.

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

When to Decontaminate

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

What to Decontaminate

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

Washing and Decontamination Procedures (separate issues)

Washing - Once surveys are completed at a site or watershed scrub and rinse all equipment to remove any lingering mud. In general it is a good idea to do this between all sites if possible.

Decontamination - Prepare a mixture of 10% bleach by putting 4 ounces of bleach (half cup) in one gallon of clean water in a waterproof tub or bucket that can be carried in your vehicle between watersheds or sites. Use a fresh bottle of bleach each field season for this. Also in order to ensure that concentrations remain around 10%, a new bleach mixture should be made on a regular basis. If

the solution of disinfectant becomes cloudy or brown with mud, silt, and vegetation, it should be discarded and a fresh solution made. Diluted bleach solutions should also be discarded after decontaminating equipment from any site where dead, dying, or ill animals are encountered. When discarding used bleach pour it out at least 30-40 meters away from water.
After rinsing equipment dip and thoroughly scrub individual items in the container of 10% bleach. An alternative approach for remote sites and where carrying a tub of bleach is impractical is to spray rinsed equipment with a concentrated (25-30%) bleach solution out of a large spray bottle and then let equipment dry between sites.
Do not rinse bleached equipment between sites. Instead allow the bleach to remain on the equipment to ensure that all fungal pathogens are killed. Most bleach will evaporate between sites so the amount of bleach introduced at the next site should be quickly diluted.

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

PRESERVING SPECIMENS

Collecting Chytrid Samples
Two swabs should be taken from the underbelly of each adult anuran handled and stored in a separate, labeled vial. Turn the individual over and run the swab along its underside several times, being careful not to make personal contact with the cotton tip. Clip the end of the swab directly into a sterilized vial and seal (no solution in the vial). Label the vial with the s**ite name, species, date and swab number** (swab1, swab 2) with a fine-tipped sharpie**.** Indicate on datasheet how many sampled were taken, by species. Do not swab more than 4 individuals/sp. at each site. **Store the vials in a cool place** for the rest of the survey, in a cooler with ice when you return to the vehicle and freeze ASAP at the trailer.

Collecting Tadpoles
When you find tadpole masses, collect two individuals and place in a whirlpack. Preserve in ethanol. Label the whirlpack with the site name and date and indicated that a voucher tadpole was taken in the datasheet.

For Dead Amphibians or Amphibians with Limb Deformities
If a mass mortality event is encountered place newly dead animals on ice and freeze them as soon as possible. If freezing is not possible then preserve the animal in ethanol. Send animals collected during mass mortality events to a qualified pathologist. If individuals with severe or multiple limb deformities are encountered (most likely seen in metamorphs) collect the individual alive and keep it in a cooler, or in plastic container that contains cold water and moss or other wet vegetation. Return the individual to Bryce Maxell alive so that the specimen can be shipped to Pieter Johnson who is studying limb deformities resulting from trematode parasites. If the logistics do not allow the animal to be held alive process the individual as you would a regular museum voucher specimen.

Slugs, Snails, and Millipedes
Millipedes can be placed directly into vials containing 70% ethanol.
Slugs and snails should be drowned in vials full of warm (not hot) water containing menthol crystals so that all air is excluded. Drowning in this solution causes them to relax and extend morphological features such as tentacles for purposes of identification. No more than two animals of each species should be “relaxed” together and “relaxation” should occur in 6-24 hours. After death any mucus exuded should be gently brushed off and the animal should be placed in 95% ethanol for 24-48 hours. Any remaining mucus should then be brushed/washed off and a dissecting pin should be used to perforate the animal along its length so that ethanol will penetrate the body. Animals can then be placed in 70% ethanol for long-term storage so that they can be used as museum vouchers and a source of tissue for genetic analyses. Slugs and snails can also be preserved and stored in 10% buffered formalin over the long-term, but treatment of tissues with formalin limits the ability to extract DNA from tissues so this is less desirable.