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Factors influencing amphibian distributions in Grand Teton National Park and western Wyoming

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Abstract Predicting the distribution of amphibians can be difficult because habitat suitability may depend on a variety of environmental and anthropogenic factors, including water quality of wetlands, geology of watersheds, and presence of invasive pathogens. Previous studies hypothesized that water chemistry may influence the rate of chytrid infection in amphibians where higher conductivity sites may have less infection. We sampled two watersheds in Grand Teton National Park and 3 watersheds adjacent to the park, and measured amphibian presence, chytrid infection, basic water quality, major ion concentrations and geology of the wetland. This is part of a larger project where we are comparing amphibian presence and infection rate among wetlands in the Gros Ventre, Wind River, and Teton Ranges. We sampled watersheds that were predominately limestone, granite or a mixture. Water quality varied among sites with higher conductivity and ion concentrations for limestone watersheds compared to granite watersheds. This report includes preliminary results of amphibian surveys and water quality analyses. Future analyses will relate occupancy rates of amphibians to environmental factors, including water chemistry, geology, and presence of chytrid fungus, as well as comparing detection rates of amphibians with environmental DNA (eDNA) and visual observation surveys.

Introduction

A recent inventory of amphibians in the upper Green River Drainage in western Wyoming revealed that the Wind River Range has surprisingly low amphibian species diversity relative to the rest of the Bridger-Teton National Forest (Estes-Zumpf et al., 2014). Of the four species of amphibians common in the mountains of western Wyoming, the boreal chorus frog (*Pseudacris maculata*) is the only species known to occur throughout much of the Wind River Range. Western (boreal) Toads (*Anaxyrus boreas*) were documented in only two disjunct drainages, tiger salamanders (*Ambystoma mavortium*) were at a few low elevation sites, and no Columbia Spotted Frogs (*Rana luteiventris*) were detected.

Much habitat in the Wind River Range appears suitable for amphibians, which raises the question of why it has such low diversity. Amphibians could be scarce there for several reasons: 1) unsuitable water quality due to bedrock geology (i.e., granite), 2) limited dispersal into the area, 3) a decline or extirpation from the mountain range due to disease (i.e. chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*)) or habitat changes (e.g. water quality), or 4) a combination of these factors. We wanted to compare amphibian presence, infection rate and water chemistry in the Wind River Range to the Teton Range because these mountain share similar geology but also differ in several ways (e.g., land use upwind, land management). Understanding factors influencing the distribution of and potential threats to amphibians in western Wyoming is vital to informing ongoing management

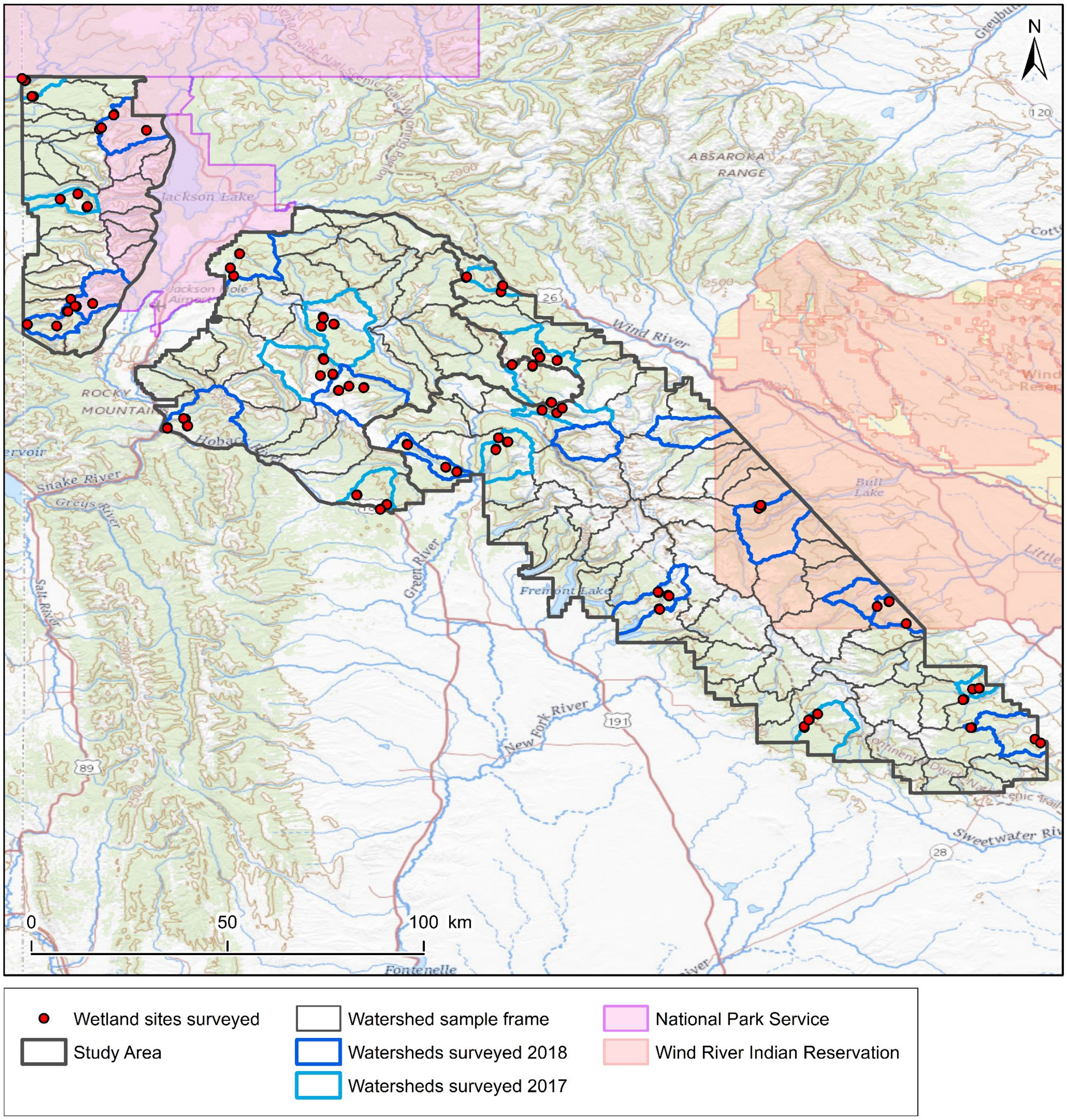

Figure 1. Study area, showing the sampling frame of all watersheds, potential watersheds and wetland sites surveyed in 2017 and 2018.

practices in the region.

Unlike all other mountain ranges in the Bridger-Teton NF, the Wind River Range and Teton Range are primarily composed of granite, which weathers slowly causing water to have low concentrations of ions (i.e., low conductivity) and little buffering capacity due to low calcium carbonate concentrations. Recent research found that Western Toads tend to breed in wetlands with higher conductivity and higher acid neutralizing capacity (ANC; higher buffering capacity) in Yellowstone National Park (Klaver et al., 2013). Waters with higher conductivity could allow amphibians to cope with stressors, including disrupted osmoregulatory function of amphibian skin caused by *Bd* (Klaver et al., 2013; Voyles et al., 2007), a leading cause of global amphibian declines (Skerratt et al., 2007) now present throughout much of the study area (Estes-Zumpf et al., 2014).

Life Stage	Species		
	Boreal Chorus Frog	Columbia Spotted Frog	Tiger Salamander
Adult	57	67	6
Juvenile	15	3	0
Metamorph	62	23	1
Tadpole/Larva	3	502	
TOTAL	137	595	7

Table 1. Total minimum counts of amphibians by species and life stage detected during visual surveys of wetlands in the Teton Range, Wyoming, 2017–2018.

Species	Number of Sub-sites occupied	
	All Life Stages	Evidence of Breeding
Boreal Chorus Frog	4 (14%)	4 (14%)
Columbia Spotted Frog	9 (31%)	3 (10%)
Tiger Salamander	5 (17%)	1 (3%)

Table 2. Number and percentage of wetland sub-sites occupied by amphibian species of all life stages and with evidence of breeding (i.e., presence of eggs, juveniles, metamorphs, or tadpoles/larvae) from surveys in the Teton Range, Wyoming, 2017–2018.

Low calcium carbonate concentrations also could influence habitat suitability. Calcium controls the permeability of amphibian skin (Curran and Gill Jr, 1962) and is essential to ion transfer across the gills of fish and the skin of amphibians (Hunn, 1985; Jeffries et al., 1990). Therefore, naturally low calcium concentrations in the Wind River Mountains could reduce habitat suitability and/or make amphibians more susceptible to additional stressors such as *Bd*.

Waters in granitic bedrock have low concentrations of calcium carbonate and therefore little ability to buffer against acidification. The pH of precipitation decreases as a result of sulfur and nitrogen compounds released into the air from burning fossil fuels. The effects of acid precipitation can result in declines of fish, invertebrates, and some amphibians (Jeffries et al., 1990). The eggs and larvae of many amphibians cannot develop when the pH of water is <5 (Freda, 1986) and these effects can be especially lethal when combined with aluminum, which is more toxic at lower pH (Clark and Lazerte, 1985; Freda et al., 1990). The Wind River Range may be highly susceptible to acid precipitation from regional and local sources of pollution because the bedrock is granite, higher amounts of sulfate are deposited in the Wind River Mountains (Figures 2,3), the mean pH of water is acidic (mean pH = 6; Estes-Zumpf, unpublished data), and the buffering capacity is low (Bruns et al., 1992). Although earlier field studies have not linked acidification with amphibian declines in the western U.S. (Corn et al., 1989; Bradford et al., 1994; Vertucci and Corn, 1996), these studies did not account for the yet undiscovered *Bd*. Optimal pH for *Bd* is 6-7.5 though the fungus can tolerate a pH range of 5-10 (Johnson and Speare, 2005). Interactions between acidification and *Bd* could influence the severity of effects on amphibians.

Because documenting occurrence of *Bd* at each site is a major aspect of this study but, traditionally, could only be detected by swabbing the skin of captured amphibians, we will also be sampling for *Bd* using environment DNA (eDNA) collected from water at sites. Use of eDNA allows researchers to test for the presence of *Bd* even when no amphibians are detected or captured for sampling. Although eDNA is a promising new method for *Bd* detection based on laboratory results, it's effectiveness in the field has been variable and researchers are still trying to investigate environ-

Species	All Life Stages	Evidence of Breeding
Boreal Chorus Frog	1.00	0.88
Columbia Spotted Frog	0.89	1.00
Tiger Salamander	0.70	0.50
AVERAGE	0.85	0.72

Table 3. Simple detection probabilities for amphibian species of all life stages and with evidence of breeding (i.e., presence of eggs, juveniles, metamorphs, or tadpoles/larvae) from double-observer surveys of wetlands in the Teton Range, Wyoming, 2017–2018. Simple detection probability is calculated as the probability that a species is detected by both observers during a survey of a sub-site averaged over all sub-sites.

mental conditions responsible for the varying success of this method (Brittany Mosier and Dr. Larissa Bailey, personal communication; Chestnut et al., 2014). For example, factors such UV radiation and pH may affect DNA degradation rates, and other components of site water may inhibit amplification of DNA from water samples, necessitating chemical "cleanup" procedures. Thus, part of this project will involve an assessment of *Bd* detection using eDNA vs. conventional skin swab methods.

Our overall project will evaluate the relative influence of bedrock geology and water quality on amphibian species distributions. We will investigate potential interactions between *Bd* and water quality at sites with contrasting bedrock geology. Understanding the influence of background factors such as bedrock geology on the distribution of amphibians is an essential first step in designing management plans for this suite of species. Results from this project can be applied to other mountain ranges across Wyoming and, importantly, will refine our perception of amphibian habitat especially in areas that otherwise appear suitable based solely on above-ground characteristics.

Methods

During spring and summer of 2017–2018, we conducted surveys in 5 watersheds in the Teton Range, including 3 on National Forest lands (Moose Creek, North Leigh Creek-Spring Creek, Upper Falls River-Winegar Creek) and 2 within Grand Teton National Park (Granite Creek and Berry Creek; Figures S1-5). These areas were selected as part of a larger spatially-balanced random sample of modified 12-digit hydrologic unit code watersheds (HUCs; U.S. Geological Survey, 2013) in the Teton, Gros Ventre, and Wind River Ranges (Figure 1). Within each watershed, we sampled 3 wetland sites, each consisting of 2 sub-sites. Wetland sites were selected from a spatially-balanced random sample within each watershed from the National Wetlands Inventory (NWI US Fish and Wildlife Service, 2015). One wetland site had only a single sub-site, resulting in a total of 29 sub-sites sampled. At each sub-site, we conducted a visual encounter survey (VES) for amphibians using a simultaneous double observer protocol (Corn et al., 2005; Estes-Zumpf et al., 2014), filtered water for eDNA, recorded habitat characteristics, made water quality measurements, and collected water samples for ion analysis. When amphibians were detected, we attempted to collect skin swabs from at least two individuals of each species. Swabs were analyzed for presence of the DNA of *Bd* using a quantitative polymerase chain reaction (qPCR) assay.

Preliminary Results

Amphibian surveys

We surveyed amphibians at a total of 15 wetland sites in 5 watersheds (Figures S1-5). The average area of sub-sites was 0.61 ha (range: 0.03–5.14 ha) and the duration of VES averaged 39 min (range: 8 min—1 hr 52 min) per observer. We detected three species of amphibians: boreal chorus frog (*Pseudacris maculata*), Columbia spotted frog (*Rana luteiventris*), and tiger salamander (*Ambystoma mavortium*; Figures S1-5). Of 29 sub-sites surveyed, 1 had all three species of amphibians, 6 had 2 species, 8 had 1 species, and 14 had 0 species. Columbia spotted frogs were the most abundant species (Table 1) and occupied the largest percentage of sub-sites (31%; Table 2). Boreal chorus frogs occupied fewer sub-sites (14%; Table 2) than Columbia spotted frogs, but were detected as adults in similar numbers (Table 1). Tiger salamanders were detected at slightly more sub-sites than boreal chorus frogs (Table 2), but were less abundant overall (Table 1).

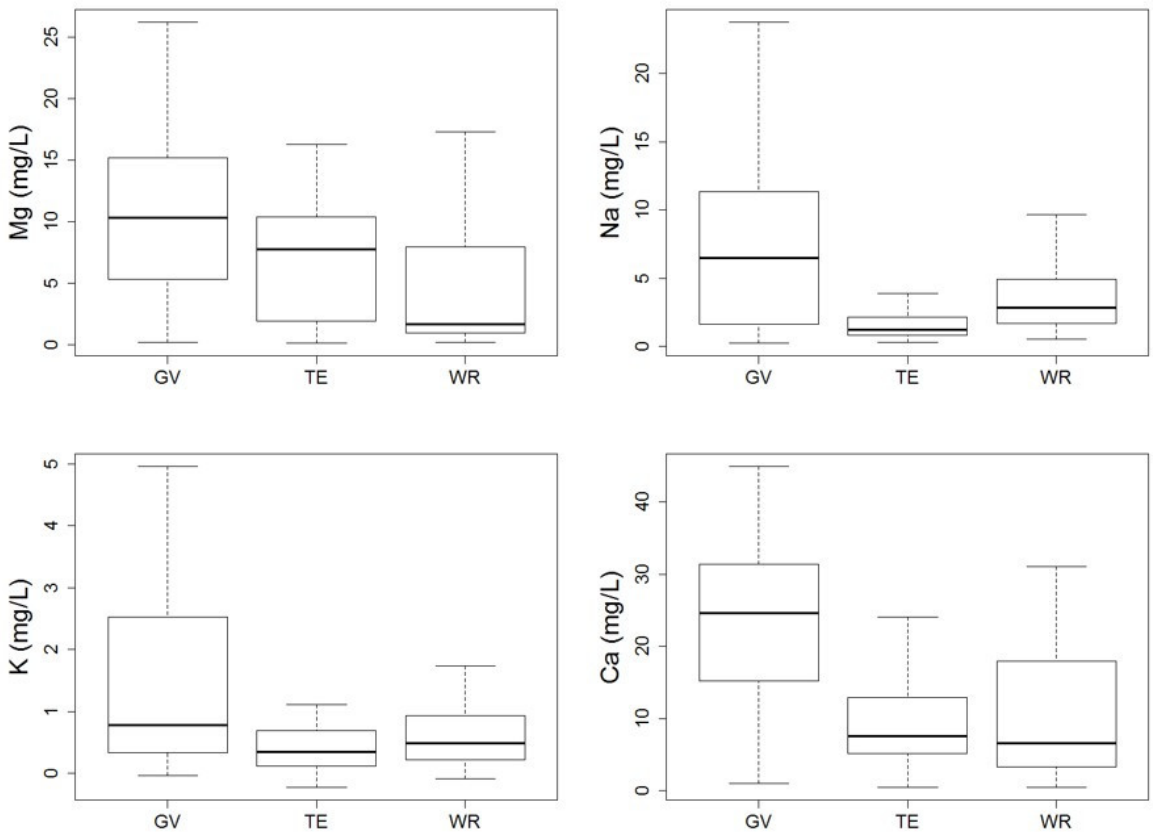

Figure 2. The concentration of A.) magnesium, B.) sodium, C.) potassium and D.) calcium in wetlands in the Gros Ventre (GV), Teton (TE) and Wind River (WR) mountains of Wyoming.

Simple detection probability (calculated as the probability that a species is detected by both observers during a survey of a sub-site averaged over all sub-sites) averaged 0.85 across all species and life stages, and was higher for occupancy by any life stage than occupancy with evidence of breeding (Table 3). Our results suggest that tiger salamanders were more difficult to detect than frog species, especially in their larval stage. The counts, naive occupancy rates, and simple detection probabilities presented here are preliminary summaries of visual encounter survey data; future analyses will provide robust estimates of detection probabilities and detection-adjusted occupancy rates that include eDNA data.

We collected skin swabs from 26 individual amphibians at 11 sub-sites to test for presence of *Bd* (Figures S1-5). A total of 11 of 26 samples tested positive for *Bd*, including 0 of 6 samples from boreal chorus frogs, 8 of 15 samples from Columbia spotted frogs, and 3 of 5 samples from tiger salamanders. *Bd*-positive skin swabs from Columbia spotted frogs were collected at both sub-sites of one wetland site in North Leigh Creek (Figure S4) and one wetland in Moose Creek (Figure S5), and one sub-site of a wetland in Berry Creek (Figure S1). Positive results for tiger salamanders came from the other sub-site of the same wetland in Berry Creek, as well as one sub-site of a wetland in Upper Falls River-Winegar Creek (Figure S2). Thus, *Bd*-positive amphibians were detected in all watersheds where skin swabs were collected (no swabs were collected in Granite Creek).

Results from lab analyses of eDNA samples for amphibians and *Bd* were not available in time for this report.

Water quality

Magnesium, sodium, potassium and calcium concentrations in the Teton Mountains were lower than those

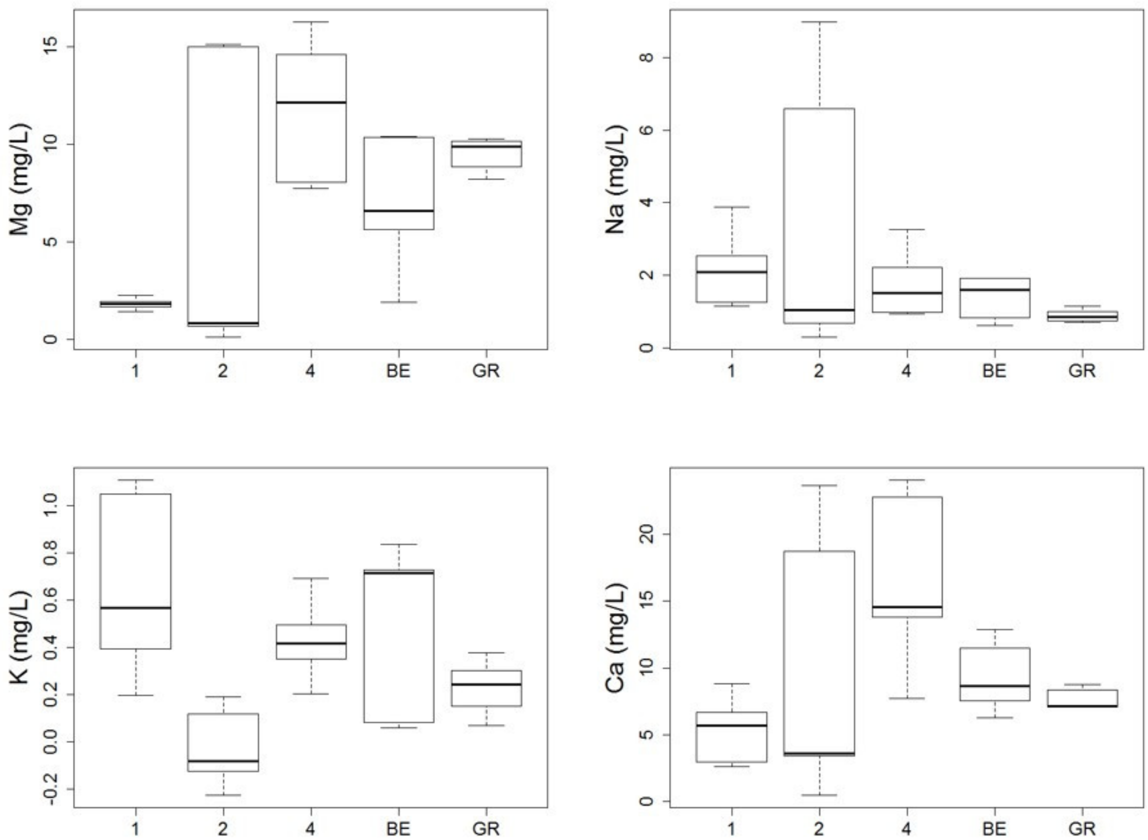

Figure 3. The concentrations of magnesium (Mg), sodium (Na), potassium (K) and calcium (Ca) in wetlands in five watersheds of the Teton Mountain Range. We sampled Upper Falls River-Winegar Creek (1), North Leigh Creek-Spring Creek (2), Moose Creek-Trail Creek (4), Berry (BE) and Granite (GR) watersheds.

measured in the Gros Ventre Mountains (Figure 2). Many watersheds in the Gros Ventre Mountain had a high percentage of limestone which erodes faster likely increasing cation concentrations. Conversely, cation concentrations in the Teton and Wind River Mountains usually did not differ. Most watersheds in the Teton Mountains were predominately granite, and wetlands in the Wind River Mountains were also dominated by granite. Calcium had the highest concentration in all mountain ranges followed by magnesium, sodium and potassium. Cation concentrations in the Teton Mountains varied among watersheds (Figure 3). Berry and Granite Creek watersheds generally had ion concentrations that were intermediate to ion concentrations of watersheds on the west side of the Tetons. Water with higher calcium concentration can resist changes in pH. The acid neutralizing capacity of watersheds in the Teton Mountains was lower than that of watersheds in the Gros Ventre Mountains and similar to that of watersheds in the Wind River Mountains (Figure 4, left). Acid neutralizing capacity was highest in the Berry, Granite and Moose-Trail Creek watersheds in the Teton Mountains (Figure 4, right).

Conclusions

The preliminary data presented here is part of a larger project comparing amphibian presence and infection rates across wetlands in the Gros Ventre, Wind River and Teton Ranges. We sampled watersheds that were predominately limestone, granite or a mixture of the two. Water quality varied among sites and mountain ranges with higher conductivity and ion concentrations for limestone watersheds compared to granite watersheds. We detected three species of amphibians: boreal chorus frogs (*Pseu-*

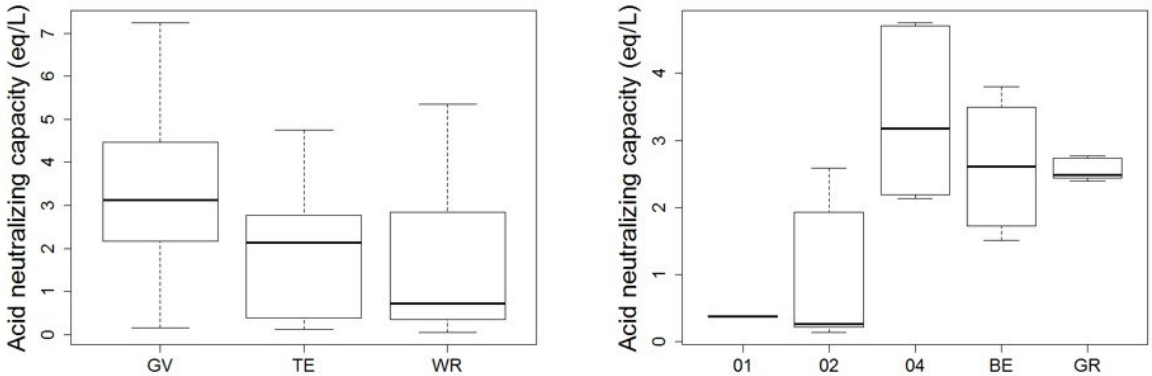

Figure 4. The acid neutralizing capacity measured in wetlands of the Gros Ventre (GV), Teton (TE) and Wind River (WR) mountains (left), and in wetlands in five watersheds of the Teton Mountain Range (right). We sampled Upper Falls River-Winegar Creek (1), North Leigh Creek-Spring Creek (2), Moose Creek-Trail Creek (4), Berry (BE) and Granite (GR) watersheds.

dacris maculate; 14% of sub-sites), Columbia spotted frogs (*Rana luteiventris*; 31%), and tiger salamanders (*Ambystoma mavortium*; 17%). Of 29 sub-sites surveyed, 1 had all three species of amphibians, 6 had 2 species, 8 had 1 species, and 14 had 0 species.

Future Work

Future analyses will relate occupancy rates of amphibians to environmental factors, including water chemistry, geology, and presence of chytrid fungus, as well as comparing detection rates of amphibians with environmental DNA (eDNA) and visual observation surveys.

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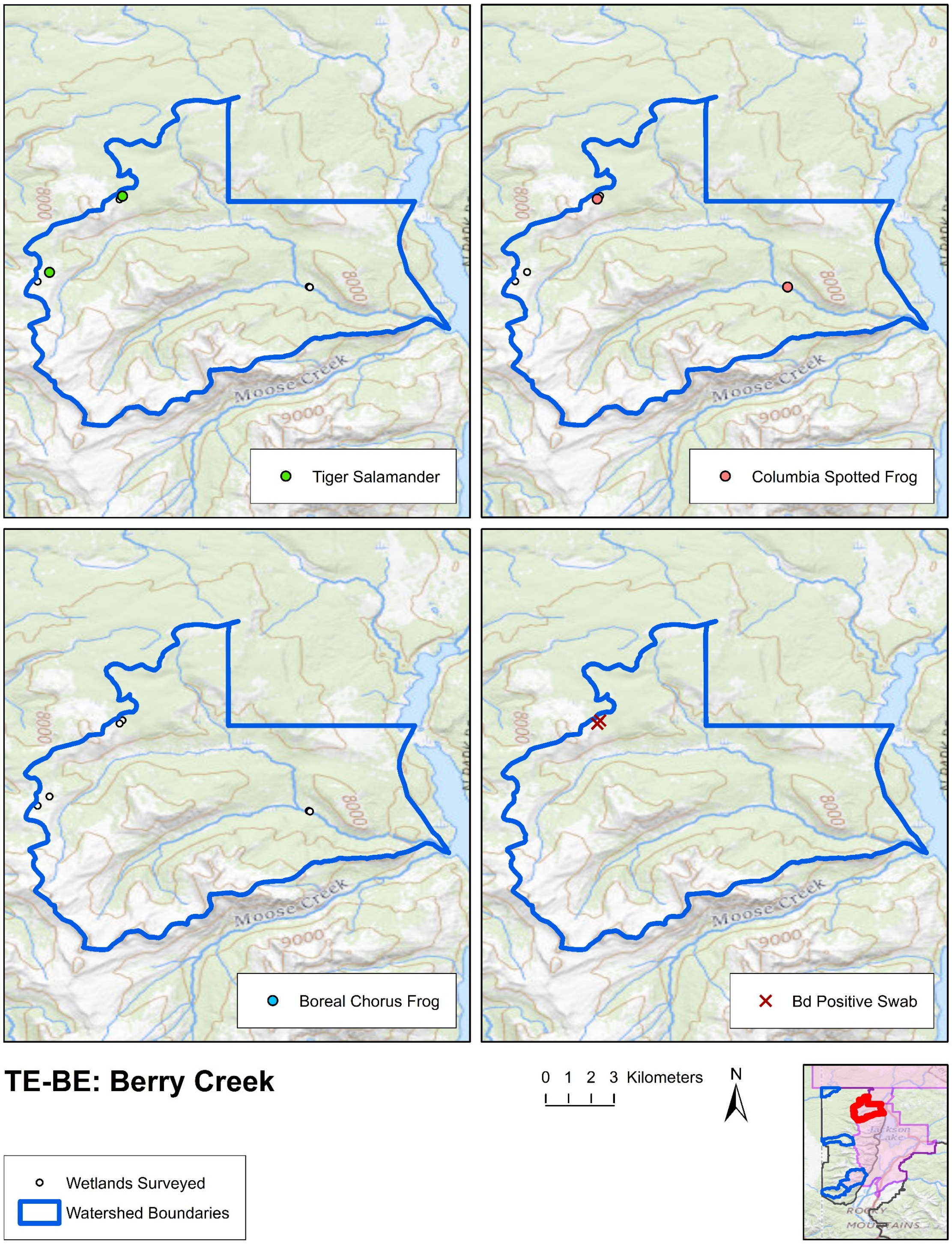
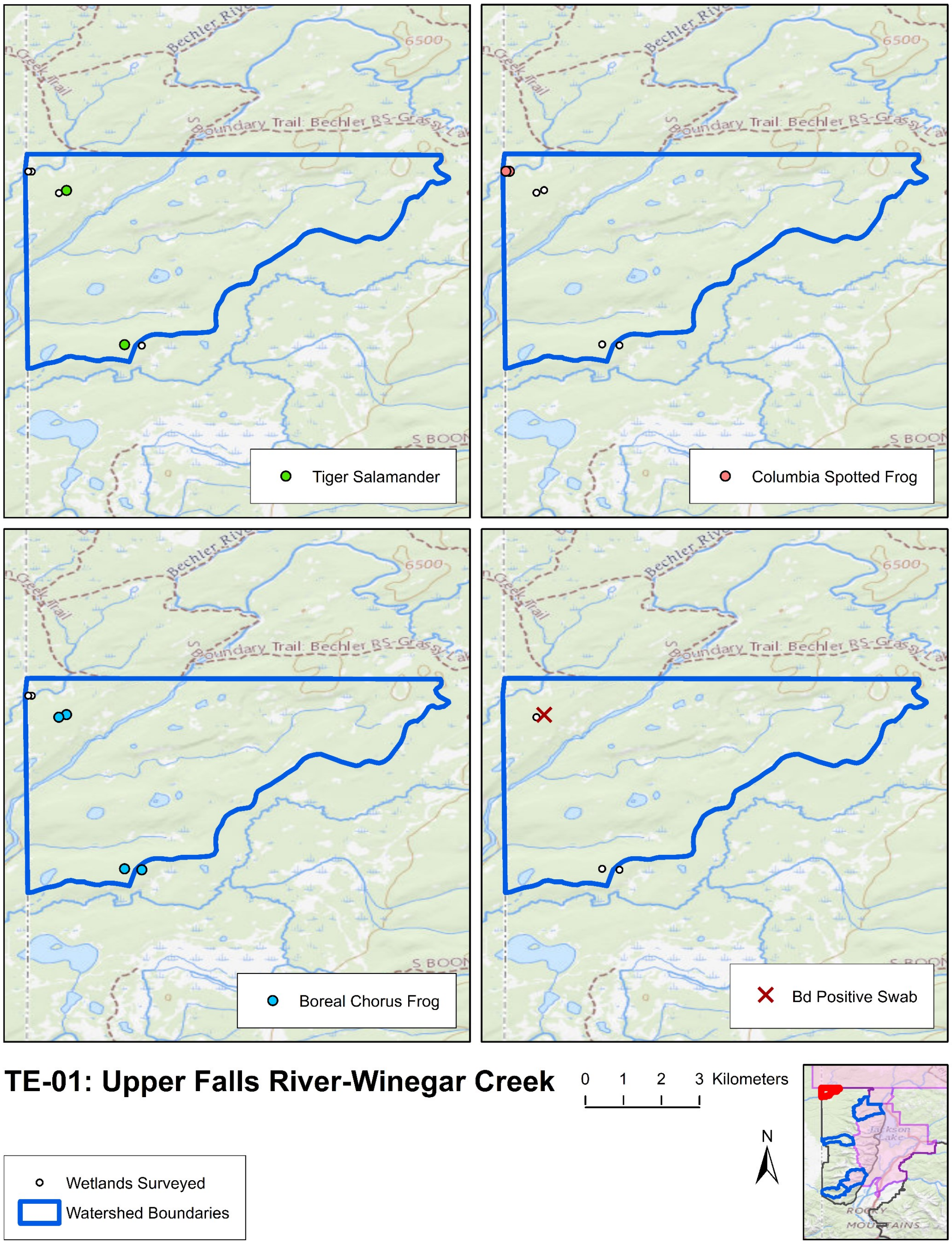
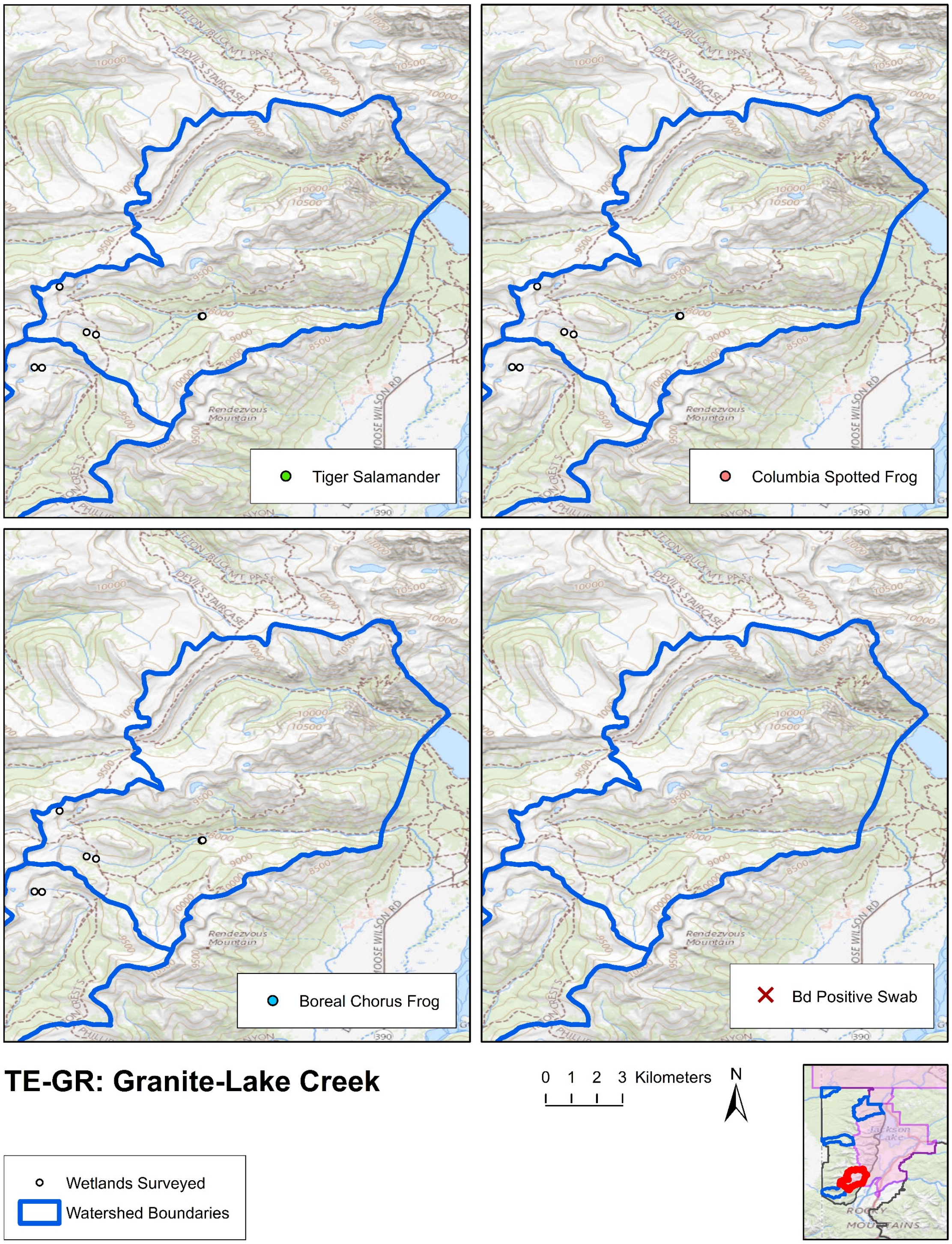


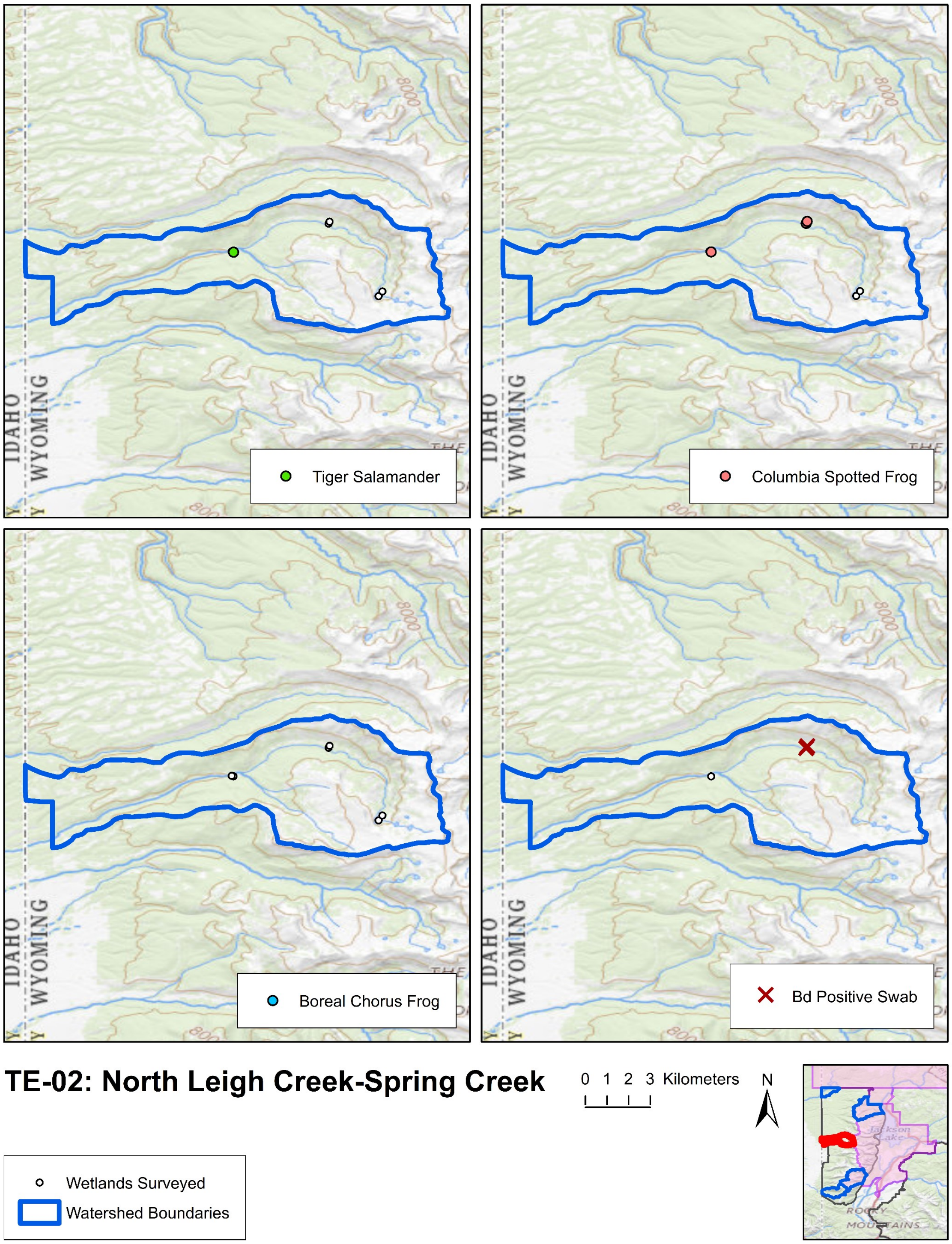
Supplementary Figure 1. Results of amphibian visual encounter surveys and skin swabs collected in the Berry Creek watershed of the Teton Range, Wyoming.



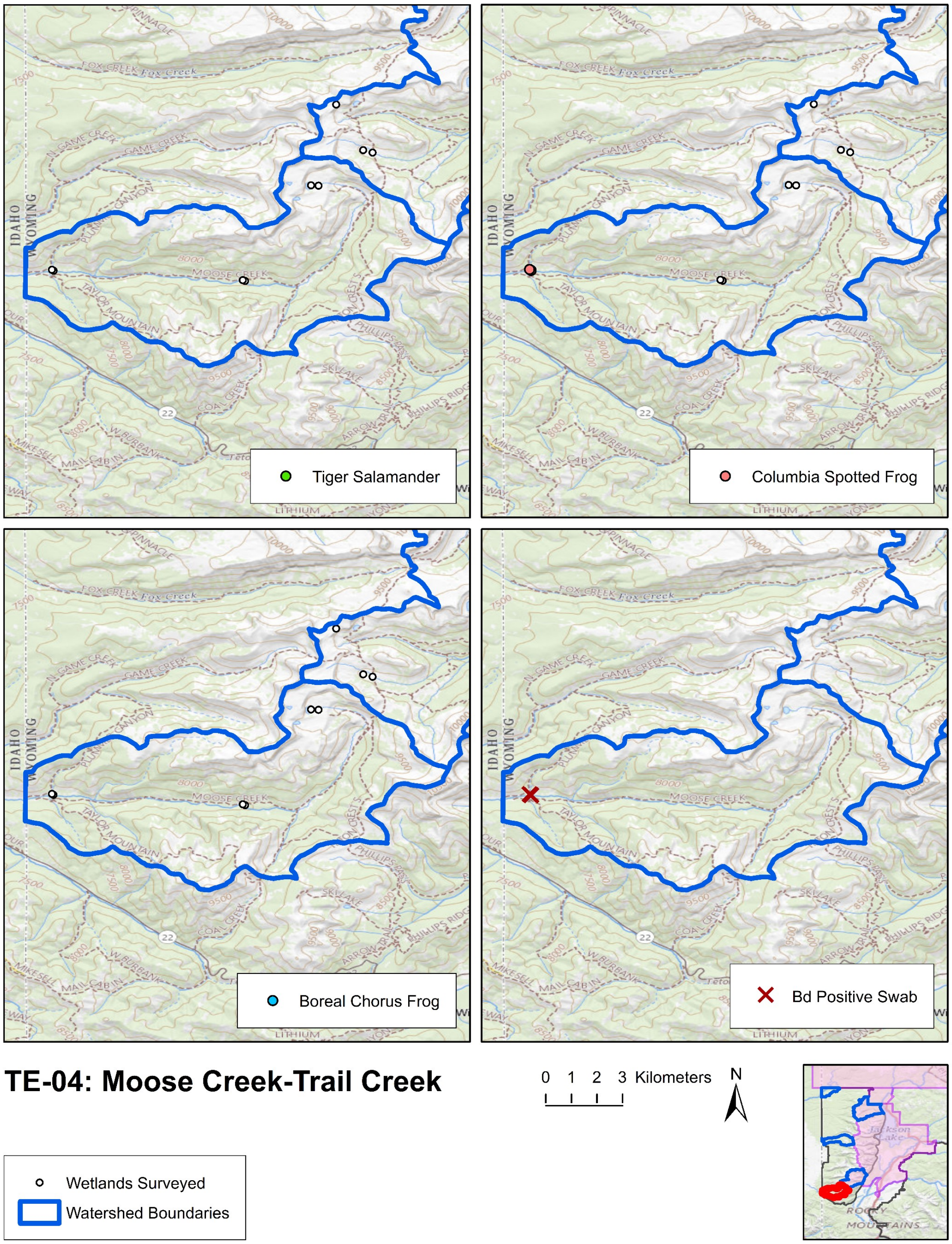
Supplementary Figure 2. Results of amphibian visual encounter surveys and skin swabs collected in the Upper Falls River-Winegar Creek watershed of the Teton Range, Wyoming.



Supplementary Figure 3. Results of amphibian visual encounter surveys and skin swabs collected in the Granite Creek watershed of the Teton Range, Wyoming.



Supplementary Figure 4. Results of amphibian visual encounter surveys and skin swabs collected in the North Leigh Creek-Spring Creek watershed of the Teton Range, Wyoming.



Supplementary Figure 5. Results of amphibian visual encounter surveys and skin swabs collected in the Moose Creek-Trail Creek watershed of the Teton Range, Wyoming.