Factors affecting the distribution of amphibians in western Wyoming




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Abstract

Effective conservation and management of amphibians requires an understanding of how environmental and anthropogenic factors affect their distributions. Previous inventories of amphibians in western Wyoming suggested the Wind River Range had low species diversity relative to the rest of the region, despite apparently suitable habitat. To understand why, we surveyed amphibians and chytrid fungus (*Batrachochytrium dendrobatidis*; *Bd*) in montane wetlands of western Wyoming using a combination of visual and environmental DNA (eDNA) sampling, and tested hypotheses on factors influencing their occurrence and detectability using hierarchical models. Unique to this study was an interest in the potential influence of bedrock geology on amphibian occurrence through its effects on water quality. Our results suggested water quality, landscape context, and wetland characteristics had the strongest influences on amphibian occupancy. Relationships of occupancy with geology were not apparent for most amphibian species, but may have occurred indirectly through the influence of bedrock on wetland water chemistry. Boreal toads (*Anaxyrus boreas*) were more likely to occupy wetlands with higher calcium concentrations, which were associated with calcite sandstone bedrock, Tiger salamanders (*Ambystoma mavortium*) were more likely to occupy wetlands with warmer water temperatures and higher ion concentrations, Columbia spotted frogs (*Rana luteiventris*) occupied lower elevation sites that received more precipitation, and Boreal chorus frogs (*Pseudacris maculata*) occupied sites without fish in areas with greater forest cover and topographic positions that were more flat or mid-slope relative to valleys. We found no evidence that amphibian occupancy was negatively influenced by atmospheric nitrogen deposition or presence of *Bd*, as measured by eDNA. Visual sampling significantly outperformed eDNA for detecting all amphibian species except boreal chorus frog. Our results highlight the diversity of factors influencing amphibian habitat suitability and the value of collecting water quality data during amphibian surveys.

Introduction

Habitat quality for amphibians is influenced by biotic and abiotic factors, including water quality (Sparling 2010), hydrology (Welch and MacMahon 2005), vegetation (Munger et al. 1998), topography (Murphy et al. 2010), geology (Russell et al. 2010), climate (Sodhi et al. 2008, Walls et al. 2013), predation risk (Klaver et al. 2013, Amburgey et al. 2014), and pathogens (Vredenburg et al. 2010). Amphibians are an important component of biodiversity (Crump 2009) and contribute to ecosystem services (Hocking and Babbit 2014); however, amphibians are declining globally (Stuart et al. 2004) as a result of habitat loss and fragmentation (Cushman 2006), climate change (Corn 2005, Blaustein et al. 2010), disease (Skerratt et al. 2007), and interactions of these and other factors (Kiesecker et al. 2001, Hof et al. 2011). Effective conservation and management of amphibians requires an understanding of where they occur and how environmental and anthropogenic factors interact to affect their distributions.

The distribution and status of amphibians are still being studied in some areas of the western United States and explaining patterns of occurrence can be difficult when historical data are lacking. For example, a recent inventory of amphibians in the upper Green River drainage of western Wyoming revealed that the Wind River Range had 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*; NSS5) is the only species known to occur throughout much of the Wind River Range. Boreal (western) toads (*Anaxyrus boreas*; NSS1) were documented in only 2 disjunct drainages, tiger salamanders (*Ambystoma mavortium*; NSS4) were found at a small number of low elevation sites, and no Columbia spotted frogs (*Rana luteiventris*; NSS3) were detected. Much wetland habitat in the Wind River Range appears suitable based on visual characteristics, raising the question of whether more species occurred there historically or if this area has always lacked amphibian diversity. Amphibians could occur at lower densities in the Wind River Range for several reasons: 1) unsuitable water quality resulting from acidic bedrock geology (i.e., granite) and/or atmospheric deposition, 2) decline or extirpation due to disease (i.e., chytrid fungus *Batrachochytrium dendrobatidis*; hereafter *Bd*), 3) variation in other important characteristics of amphibian habitat (e.g., vegetation, topography, climate), or 4) a combination of these factors.

The Wind River Range differs from other mountain ranges in western Wyoming in a variety of biotic and abiotic characteristics. For example, it is steeper and higher in elevation than the neighboring Gros Ventre Range, which could limit dispersal of amphibians into the area, and receives less precipitation than the Gros Ventre or Teton Ranges, which could make wetlands more ephemeral. Unlike other mountain ranges in the region, the Wind River Range is primarily composed of granite, which weathers slowly causing low concentrations of ions in water (i.e., low conductivity) and little buffering capacity due to low calcium carbonate concentrations. Recent research in Yellowstone National Park found that boreal toads bred in wetlands with higher conductivity and acid neutralizing capacity (Klaver et al. 2013), while other studies found boreal chorus frog occurred in areas with lower conductivity (Browne et al. 2009, Klaver et al.

2013). Low calcium carbonate concentrations could influence habitat suitability for some species because calcium controls the permeability of amphibian skin (Curran and Gill 1962) and is essential to ion transfer across the gills of fish and the skin of amphibians (Hunn 1985; Jeffries and Mills 1990). Waters with higher conductivity could also help amphibians to cope with disrupted osmoregulatory functions of their skin caused by *Bd* (Klaver et al. 2013, Voyles et al. 2007). *Bd* is a leading cause of global amphibian declines (Skerratt et al. 2007) and the fungus is now present throughout much of western Wyoming (Estes-Zumpf et al. 2014). Thus, naturally low calcium concentrations in the Wind River Mountains could reduce habitat suitability by making amphibians more susceptible to stressors such as *Bd*.

Another potential negative influence of granite geology on amphibians is its inability to buffer against changes in pH. The pH of precipitation decreases as a result of sulfur and nitrogen compounds released into the air from burning fossil fuels, causing acid precipitation, commonly known as acid rain. Waters in granitic bedrock have low concentrations of calcium carbonate, which reduces their ability to buffer against acidification. Acid precipitation can result in declines of fish, invertebrates, and some amphibians (e.g., Jeffries and Mills 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 susceptible to acid precipitation from regional and local sources of pollution because the majority of bedrock is granitic, average pH of water is acidic (mean pH = 6; Estes-Zumpf, unpublished data), higher amounts of sulfate are deposited there, and buffering capacity is low (Bruns et al. 1992). While 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 potential interactions with *Bd* because it had not yet been discovered. Optimal pH for *Bd* is slightly acidic (6.0–7.5) and the fungus can tolerate a pH range of 5–10 (Johnson and Speare 2005). Interactions between acidification and *Bd* could, thus, influence the severity of its effects on amphibians.

Goals and objectives

Our goal was to understand the factors affecting the distribution of amphibians in western Wyoming, with an emphasis on potential interactions of geology and *Bd*. To account for other important influences on amphibian habitat, we measured a larger set of water quality variables than most previous studies, and also evaluated habitat characteristics of wetland sites, topography and vegetation of the surrounding landscape, fish presence, and climate. We used probabilistic sampling to select a representative sample of wetland sites, multiple survey methods for amphibians (visual and eDNA sampling) and *Bd* (eDNA and skin swabs), and appropriate analytical techniques to account for biases in sampling and survey methods. 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 these species in western Wyoming. Results from this project can be applied to other mountain ranges across Wyoming and will help refine our understanding of amphibian distributions and habitat selection. Our specific objectives were to:

1) Estimate amphibian species occupancy of wetlands in areas of the Wind River, Gros Ventre, and Teton Mountain Ranges with various types of bedrock geology
2) Measure water quality at wetlands (dissolved oxygen, pH, specific conductivity, and water temperature), cation concentrations (e.g., calcium and potassium), anion concentrations (e.g., nitrogen and sulfur), and acid neutralizing capacity (ANC or alkalinity)
3) Test for *Bd* presence at wetlands using eDNA and amphibian skin swabs
4) Assess the influence of bedrock geology, *Bd*, and water quality on amphibian occurrence
5) Compare detection efficiency of amphibians between visual encounter and eDNA sampling
6) Compare water quality among sites

Methods

Study area and site selection

We conducted our study in the mountains of western Wyoming, USA. We defined our study area as all public lands in the Teton, Gros Ventre, and Wind River mountain ranges managed by the U.S. Forest Service and Grand Teton National Park, and a portion of the Wind River Indian Reservation. We delineated the southern extent of the Teton Range as highway 22, the northern and western extent of the Gros Ventre Range as highways 26 and 191, and extended a straight line from adjacent USFS lands across the Wind River Indian Reservation (Figure 1). To maximize efficiency of access to remote, back-country sites, our sampling design used three levels of spatial clustering: wetland sites within catchments within watersheds.

We divided the study area into watersheds using the 12-digit hydrologic unit code (HUC) boundaries from the National Watershed Boundary Dataset (USGS and NRCS 2013, 2017). We clipped the HUCs to the study area boundary and combined adjacent watersheds with <20 km^2 area. We discarded 14 watersheds with <12 wetlands and 1 watershed with no roads or trails; these were mainly along the crest of the Wind River Range, where access was difficult and we expected little amphibian habitat to be present. This resulted in a total of 116 watersheds in the sampling frame. Based on our projected survey effort, we drew a spatially-balanced, random sample of 27 watersheds from the study area using the Generalized Random Tessellation Stratified (GRTS) sampling algorithm (Stevens and Olsen 2004). We stratified the sample proportionately by the three mountain ranges to ensure it was representative of the study area. To maintain spatial balance, we focused our survey effort on the lowest numbered watersheds in the master sample order, skipping only watersheds that were not accessible due to private land. Additionally, we surveyed three watersheds that were not included in the sample to satisfy objectives of collaborating agencies.

We delineated catchments within each watershed using the National Wetlands Inventory (NWI) dataset (USFWS 2013), excluding riverine wetland classes and wetlands on private land based on the methods of Estes-Zumpf et al. (2014). Within each watershed, we drew a GRTS sample of 3 wetland sites with a four-fold oversample, resulting in a total of 15 potential survey catchments

per watershed. To facilitate access to catchments, we stratified the sample disproportionately by distance to roads or trails to include 2/3 sites <1 km and 1/3 >1 km from a roads or trails. To avoid visiting catchments without amphibian habitat, we evaluated the quality of wetlands visually using multiple years of aerial imagery in GoogleEarth (https://www.google.com/earth), excluding sites that did not have visible open water or wetland vegetation. We digitized the boundaries of catchments using aerial imagery and delineated two discrete wetland sites within each catchment. We surveyed three catchments within each watershed in the order of their GRTS sample ranking, when logistically possible. Given lengthy travel times to remote sites in our study area, it was not always practical to default to the next site in the GRTS sample order when a catchment was determined in the field to be inaccessible or unsuitable habitat (e.g., dry or not a wetland). In these cases, we established a new catchment in suitable amphibian habitat ≤500 m from the randomly selected point. We intensified survey effort of sites with limestone and dolomite bedrock during 2018 after preliminary data suggested they were under-represented in our sample because of inaccessibility of sites in 2017. Due to these deviations from our sampling design necessitated by field logistics, we did not use the inclusion probabilities of the GRTS sample in our analyses. We acknowledge that our scope of inference is, therefore, technically limited to the sites that we surveyed and not the population of sites in our sampling frame. However, we suggest that our study design provided a rigorous and spatially balanced sample that is likely representative of the population of wetland sites in our study area.

Amphibian visual encounter surveys

We conducted visual encounter surveys following standard double-observer protocol (Estes-Zumpf et al. 2014). Both observers recorded the species, number, and life stage of amphibians encountered. We surveyed each site once and used these data to create detection histories for occupancy analysis, indicating if each species was detected by one, both, or neither observer. Additionally, we summed the number of species detected at each site to use as a predictor for *Bd* occurrence (Table 1).

We recorded standard data on survey effort and weather conditions (data sheet included in Appendix B) and used handheld GPS units to track the survey routes of each observer. We created 99% minimum convex polygons around the combined track points of both observers with the package adehabitatHR (v.4.15, https://cran.r-project.org/web/packages/adehabitatHR) in Program R (v.1.5, www.r-project.org). We used these polygons to define the boundaries, and calculate the area and centroids of sites. If track data for one observer was missing we used the data from the other observer to define the site. If polygons overlapped, we divided the overlapping area equally between the two wetland sites in the catchment. We used survey data to estimate predictors for occurrence of amphibians and *Bd* (site area), and detectability of amphibians (year, Julian date, site area; Table 1).

We predicted amphibian occupancy would be higher at larger sites, and *Bd* would be more likely to occur at larger sites with more amphibian species. We predicted detection probability would be higher for earlier dates, smaller sites, and could vary among years.

Wetland site characteristics

We recorded characteristics of wetland habitat (Appendix B) and used these data to estimate variables representing water temperature, stream influence, ephemerality, depth, and fish presence (Table 1). We estimated additional habitat-related variables using remotely-sensed data products (see *Remotely sensed variables* below).

We predicted amphibian occupancy would be higher at more permanent sites without fish, and effects of depth and stream influence would vary among species.

Geology

We collected rock samples from wetlands and tested them with 5% hydrochloric acid as an indication of calcium carbonate in bedrock (i.e., limestone and dolomite, or sandstone with calcite cement). We used these measurements, the Wyoming Geologic map (Green and Drouillard 1994), and the opinion of experts on Wyoming geology (Ramsey Bentley, University of Wyoming, personal communication; Jack Oviatt, Kansas State University, personal communication) to classify bedrock in the study area into four categories: limestone and dolomite, sandstone with calcite cement, acidic (largely granitic), and unknown mix.

We calculated the site-specific watershed for the centroid of each wetland using the "pour-point" method (Parmenter and Melcher 2012) in ArcGIS (v.10. Environmental Systems Research Institute, Redlands, CA). We summarized the bedrock geology type at each wetland centroid and the composition of bedrock geology within the site-specific watershed and larger watershed (12-digit HUC). We used these data to define a categorical variable representing the bedrock geology type at each site, and continuous variables for percentages of limestone and dolomite bedrock, sandstone with calcite cement, and acidic bedrock within site-specific watersheds and HUCs (Table 1).

We predicted amphibian occupancy would be higher for wetlands in watersheds with proportionally more calcium carbonate bedrock (i.e., limestone-dolomite and sandstone with calcite cement) and less acidic (granite) bedrock.

eDNA

We filtered water from wetlands to estimate the presence of amphibian species and *Bd* using eDNA. We used these data as response variables in models of amphibian and *Bd* occurrence. Additionally, we used *Bd* occurrence as a predictor of amphibian occurrence (Table 1).

We collected one liter of water total from three areas within each wetland that appeared to be suitable amphibian habitat (e.g., ~333 mL from 3 microhabitats in each wetland). Using sterile techniques, we filtered the water through 0.45 µm cellulose nitrate filters until the filter clogged, and recorded the volume filtered. Filters were stored in 95% ethanol in microcentrifuge tubes until analysis. All bottles and forceps were soaked in 50% bleach between uses and rinsed three times with deionized water. Bottles were rinsed three times with wetland water before collecting

samples. We filtered negative control ("blank") samples of deionized water before each catchment to monitor for contamination.

All laboratory methods followed Gygli (2017). Primer and probe designs are included in Table 2. We used multiple negative controls at the survey, DNA extraction, and qPCR assay steps to monitor for contamination (Turner et al. 2014; Goldberg et al. 2015). We extracted eDNA filters with a modified Qiagen DNEasy Blood and Tissue Kit (Cat. # 69581; Zero and Murphy 2016). To limit the potential impacts of naturally occurring PCR inhibitors that can be present in eDNA samples, we used the OneStep-96 PCR Inhibitor Removal kit (Zymo Research Cat. #D6030; Caren Goldberg, Washington State University, personal communication). We then determined species presence and absence within each sample with real-time quantitative PCR (Arya et al. 2005; Goldberg et al. 2015) using a Bio-Rad CFX 96 Touch thermocycler (Cat. # 1855195) and Bio-Rad iQ Powermix polymerase (Cat. # 1725849). To limit potential of lab contamination, we set up all qPCR reactions in a "clean" environment inside a Fisher Scientific PCR workstation (Cat. # 103-603-09) that was spatially separated from PCR products and used UV-C and bleach sterilized and dedicated eDNA implements (Goldberg et al. 2016).

We incorporated all study species into two multiplexes (Table 3). Each reaction contained an internal positive control assay to differentiate between failed/inhibited reaction and negative result (Goldberg et al. 2015). Each set of samples ("plate") contained positive controls: a titration (10^{-1} to 10^{-5}) of extracted DNA of each target species of known concentration. Positive controls were used to 1) monitor for reaction failure and 2) estimate DNA concentration for known samples. We ran each sample in triplicate (3 qPCR wells on a single plate). We interpreted 2 or 3 positive results to indicate species presence and 0 positive results to indicate species absence if the internal control amplified. Samples with 1 positive result were assayed again and only considered a positive result if replicated (Goldberg et al. 2015; Strickler et al. 2015). If the samples were inhibited (i.e., internal positive control did not amplify), we reran the assays on that sample considering the first run as no data. In the case where samples were likely inhibited (e.g., internal control did not amplify), we considered the results as no data.

Amphibian skin swabs

During visual encounter surveys, we captured up to 2 individuals per amphibian species and swabbed their skin for presence of *Bd*. Swabs were preserved in ethanol and analyzed for presence of *Bd* DNA by qPCR assay (Pisces Molecular LLC, Boulder, Colorado). We converted these results into a binary variable indicating *Bd* presence-absence for sites where ≥1 individual of any species was swabbed.

To prepare samples for analysis, the liquid in each skin swab sample was mixed by pipetting the liquid up and down repeatedly. The entire volume of each sample was then transferred into individual microfuge tubes. The tubes were spun in a microcentrifuge at 16,000 × G for 3 minutes. Next, the supernatant was drawn off and discarded. Lysis buffer was added to the tubes and any pellet present was resuspended by vortexing. Ten micrograms of carrier DNA was added to the lysis buffer. Total DNA was extracted from all samples using a spin-column DNA

purification procedure. The sample DNAs were assayed for the presence of the *Bd* ribosomal RNA Intervening Transcribed Sequence (ITS) region by 45-cycle PCR amplification using a qPCR assay developed at Pisces and an Agilent AriaMx real-time PCR instrument. The reaction master mix contained a PCR inhibitor resistant Taq polymerase (PerfeCTa Multiplex ToughMix, Quantabio) and a VIC-labeled internal positive control (IPC; Life Technologies) to detect PCR inhibition. The detection sensitivity of this assay is three target sequence molecules (approximately 0.02 zoospore equivalents). Each PCR run included positive DNA and no DNA controls. Positive DNA controls were prepared from a plasmid constructed at Pisces Molecular containing the *B. dendrobatidis* ribosomal RNA Intervening Transcribed Sequence (ITS) region. Serial ten-fold dilutions of this plasmid DNA from 4.2 × 106 to 4.2 × 100 molecules per reaction were used to generate the standard curve. No DNA controls used water in place of template DNA. This reaction remained uncapped during addition of sample DNA to the test reactions and served as a control to detect contaminating DNA in the PCR reagents or carryover of positive DNA during reaction set-up.

Water quality

We measured chemical components of each wetland to estimate the degree to which water chemistry influenced occurrence of amphibians and *Bd*. We measured water temperature, specific conductivity, dissolved oxygen (percent saturation and concentration), and pH using a Professional Plus by Yellow Springs Instruments (YSI) that was calibrated before each backcountry trip (approximately every 3 days for pH, specific conductivity) or each day (dissolved oxygen). We collected water samples to analyze for anions (chloride, nitrate, phosphate, sulfate) and major cations (calcium, sodium, magnesium, potassium) at each site by filtering 20 mL of water with PALL Type A/E filters. We also collected 20 mL of unfiltered water to measure acid neutralizing capacity (ANC). Both ion and ANC samples were kept cool in the field by placing them in snowbanks or cold water at each site and wrapping samples in an insulated jacket, and placed in a cooler with ice when we returned to our vehicles. When we returned to the laboratory, ion samples were stored in a freezer, and ANC samples were stored in a refrigerator and measured within 2 weeks of collection. Cation samples were measured with a PerkinElmer Optical Emission Spectrometer Optima 8300. Anion concentrations were estimated using an Dionex ion chromatograph. Acid neutralizing capacity was measured using an autotitrator and gradually adding 0.02 N H_2SO_4 until the water reached a pH of 4.2. We used these data to estimate predictors representing water quality at wetland sites (Table 1).

We predicted amphibian occupancy would be positively associated with specific conductivity, concentrations of calcium and other ions, acid neutralizing capacity, and higher water temperatures; and negatively associated with concentrations of anions that could result from atmospheric deposition (i.e., nitrate), and lower pH.

Remotely sensed variables

We used spatial data products to estimate environmental variables related to atmospheric nitrogen deposition, climate, landscape context, and habitat. We summarized spatial data within

a 500-m circular radius around the site centroids to capture landscape context of each wetland. All spatial data layers were 30-m^2 resolution, except where noted. To estimate atmospheric deposition, we averaged raster layers of models from the National Atmospheric Deposition Program (NADP; Schwede and Lear 2014) predicting total wet plus dry nitrogen deposition (kg/ha) at a 12-km^2 resolution annually from the from 2000–2016 (the most recent year available). We estimated the average number of frost days per year at each site with a data layer derived from DAYMET data using annual means 1980–1997 (Thornton et al. 1997, Thornton and Running 1999, Thornton et al. 2000). We estimated the average amount of precipitation during the warmest quarter of the year using the bioclim18 data layer from the bioclim dataset (Hijmans et al. 2005). To describe landscape position and topography, we used the 1 arc-second National Elevation Dataset (Gesch et al. 2009) to estimate elevation, aspect as an index ranging from 0 (West) to 2 (East), and topographic position index within a 31-cell focal window as a unit-less index where values above zero indicated hills or ridgetops, values below zero indicated valleys or depressions, and values near zero indicated flat or mid-slope areas (Jenness et al. 2013). We estimated forest cover as the percentage of cells classified as forest vegetation in the LANDFIRE existing vegetation type layer (LANDFIRE 2016). To quantify the amount of wetland habitat in the surrounding area, we estimated the proportion of cells classified as wetland edge using methods developed by Estes-Zumpf et al. (2014).

We predicted amphibian occupancy would be higher at sites with western aspects, lower topographic positions, more wetlands in the surrounding area, more precipitation, and fewer frost days; lower at sites with higher predicted nitrogen deposition; and effects of elevation and forest cover would vary among species.

Data analysis

We tested relationships of environmental variables to amphibian occurrence and detectability separately for each amphibian species using single-season, single-species occupancy models (MacKenzie et al. 2006). Occupancy models are a type of hierarchical model that estimate the probability of an organism (e.g., amphibian species) occurring in a sample unit (e.g., wetland site) and use replicate sampling occasions to account for imperfect probability of detection. Detection histories for occupancy models in our study had three sampling occasions (two occasions from the double-observer visual encounter survey and one occasion from the eDNA sample) that enabled us to estimate separate detection probabilities to compare the efficiency between sampling methods. We adapted the single-season occupancy model for our two-stage cluster sampling design by including nested random intercepts to account for clustering of wetland sites within catchments and catchments within watersheds. Our model was similar to the "bottom-up" model proposed by G. Guillera-Arroita in Kéry and Royle (2015) and implemented by Kroll et al. (2015), where inference is to the smallest unit of a nested sampling design and the non-independence of sample units is accounted for with random effects. We preferred this model to the "multi-scale" occupancy model commonly used to analyze nested study designs because we had multiple levels of clustering, we were interested in making inference to the smallest unit, and model development was simplified by considering predictors for occupancy at only one

scale. Our model treated the occupancy state of the wetland sites to be conditionally independent and made the assumption that detection probability was independent at the site level.

We developed occupancy models using a multi-stage approach in which we 1) selected the best model for detection probability while holding occupancy probability constant at the largest converging sub-global model; 2) reduced the number of candidate predictors for occupancy by comparing all univariate models for occupancy probability using the model for detection probability selected in stage 1; 3) compared all subsets of additive models for occupancy probability including up-to 3 predictors that outranked the null model in stage 2 and were not strongly correlated ($r > 0.60$) or redundant; and 4) selected the single best model or competing models and re-fit them in a Bayesian analysis with nested random intercepts for catchments and watersheds. This hybrid approach (e.g., Tack et al. 2019) enabled us to efficiently reduce a large suite of environmental variables using the proven method of information theoretic model selection (Burnham and Andersen 2004), while taking advantage of the flexibility of Bayesian methods to estimate random effects in hierarchical models (Kéry and Schaub 2011).

We tested relationships of *Bd* occurrence to environmental variables using separate presence-absence datasets from eDNA and amphibian skin swab samples. We used binomial generalized linear mixed models (GLMMs) to relate the response of *Bd* presence-absence to environmental variables we predicted would influence occurrence of the pathogen. Due the small number of detections, we evaluated only models with one environmental predictor and nested random intercepts for catchments and watersheds. We summarized water quality data using ANOVA with Tukey's Honestly Significant Difference (HSD) for multiple comparisons. We also used ANOVA for post-hoc tests of differences in environmental variables from competitive occupancy models by geology class and mountain range.

We performed all analyses using the R statistical language. We fit GLMMs with package lme4 (v.1.1-15, https://cran.r-project.org/web/packages/lme4). We assessed fit of GLMMs using adjusted R^2 metric described by Nakagawa and Schielzeth (2013), in which conditional R^2 is a measure of the variance explained by the fixed-effects and marginal R^2 of the variance explained by the entire model. We fit frequentist occupancy models using Program MARK (White and Burnham 1999) with the RMark interface (v.2.2.6, https://cran.r-project.org/web/packages/RMark), and Bayesian occupancy models using the software JAGS (Plummer 2003) with the jagsUI interface (v.1.5.0, https://cran.r-project.org/web/packages/jagsUI). We used vague priors for all parameters in Bayesian analyses, following the recommendations of Northrup and Gerber (2018), except for the mean intercept for occupancy of Columbia spotted frog and tiger salamander that required a weakly informative prior to approximate estimates from frequentists models. We ran Bayesian models for 100,000 iterations, with 3 chains, thinned by 10, discarded 10,000 iterations as burn-in, and made inference from the resulting 30,000 posterior samples. We assessed model convergence with trace plots and the Gelman-Rubin statistic ($\hat{r}$), with $\hat{r} < 1.1$ indicating convergence (Gelman and Rubin 1992). We assessed fit of Bayesian models using posterior predictive checks and interpreted Bayesian *p*-values near 0.5 to indicate that models fit the data well and values between 0.05–0.95 as adequate (Kéry and Royle 2015). Additionally, we calculated the lack-of-

fit ratio ($\hat{c}$) and interpreted values of $\hat{c}$ near 1 as providing no evidence of over-dispersion and $\hat{c}$ < 4 as adequate.

We compared models in an information theoretic framework using the small sample size variant of Akaike's Information Criterion (AIC_c). We interpreted competitive models as those that were within 2 AIC_c of the top-ranked model and did not include uninformative parameters (Arnold 2010). We included uninformative models in summary tables, but excluded them from calculations of model weight. We estimated 95% confidence intervals or Bayesian credible intervals (CI) of coefficients and predictions for GLMM with a bootstrap procedure (Bolker 2019), frequentist occupancy models with the profile method (White and Burnham 1999), and Bayesian occupancy models using quantiles of the posterior distribution (Kéry and Schaub 2011). We interpreted predictors to be statistically significant if their coefficient CI did not overlap zero and selected "best models" that ranked competitively and had significant relationships for all predictors. If predictors that were significant in a frequentist occupancy model were not significant in the final Bayesian model, we interpreted this as evidence that the importance of the predictor had been inflated by not accounting for spatial correlation in the frequentist model and selected a different best model. If a model initially selected as the best model had poor fit, but a smaller, nested version of the model had adequate fit, we selected the simpler model as the final best model.

Results

We surveyed a total of 137 wetland sites located within 69 catchments and 23 watersheds, including 72 sites visited from 20 June–9 August 2017 and 65 sites from 6 July–13 August 2018. We skipped one watershed in the sample order due to inaccessibility of private land and added a total of 4 watersheds that were not selected randomly within Grand Teton National Park and the Wind River Indian Reservation.

Amphibian surveys

We detected all five of our focal amphibian species with visual and eDNA surveys: boreal chorus frog, boreal toad, Columbia spotted frog, tiger salamander, and Northern leopard frog (*Lithobates pipiens*; Table 5). Amphibian diversity varied among sites, with 1 species at 48 sites, 2 species at 26 sites, 3 species at 6 sites, and none at 57 sites. Boreal chorus frog was the most common species, detected at 55 sites, followed by tiger salamander at 28 sites, Columbia spotted frog at 26 sites, boreal toad at 14 sites, and Northern leopard frog at 5 sites.

Visual encounter

Visual surveys averaged 39 minutes per observer per site (SD: 21 min, range: 8–128 min). Sites with evidence of breeding from visual observations (tadpoles, metamorphs, or juveniles) comprised 31 sites for boreal chorus frog, 12 for tiger salamander, 8 for Columbia spotted frog, 8 for boreal toad, and 1 for Northern leopard frog.

eDNA

We filtered water for eDNA analysis at all wetlands where we conducted visual surveys. We filtered an average of 414 ml of water per site (SD: 241 ml, range: 55–1080 ml). We extracted DNA from 135 wetlands, 66 negative controls, and 2 samples were lost. All extraction negatives and PCR negatives were negative. We had some contamination in negative controls (6 of 36 in 2017 and 2 of 30 in 2018). However, contamination resulted in the loss of relatively few data because we ran controls at almost every site and contamination was mostly for species that were not detected at affected sites. In 2018, internal controls failed to amplify in 6 of 6 PCRs for 14 samples, indicating potential of PCR inhibition. This resulted in the loss of data for 11 samples that were inhibited for both multiplexes, 2 that were inhibited for 1 multiplex, and 1 that was partially inhibited for 1 multiplex.

Compared to visual surveys, we detected species at fewer sites with eDNA sampling. This was due in part to missing eDNA data from contaminated and inhibited samples. However, comparing only sites with data from both methods, visual surveys still had substantially more detections (Table 6): boreal chorus frog was detected at 21 sites by visual survey only, 11 sites by eDNA only, and 20 sites by both methods. Boreal toad was detected at 12 sites by visual survey only, 1 site by eDNA only, and 0 sites by both methods. Columbia spotted frog was detected at 20 sites by visual survey only, 1 site by eDNA only, and 2 sites by both methods. Tiger salamander was detected at 17 sites by visual survey only, 4 sites by eDNA only, and 5 sites by both methods. The only species detected more frequently by eDNA than visuals surveys was Northern leopard frog, which was detected at 1 site by visual survey only, 3 sites by eDNA only, and 0 sites by both methods.

Bd

We collected 57 swabs from four amphibian species at 47 wetlands. Of these, 32 swabs (56%) from 21 wetlands (45%) tested positive for *Bd* (Table 7). We also sampled *Bd* via eDNA in water at all wetlands. *Bd* eDNA samples successfully amplified for 123 wetlands and 15 (12%) tested positive for *Bd*. We expected higher detection rates of *Bd* from skin swabs than eDNA sampling because swabbing requires presence of amphibians, while some sites sampled for eDNA likely did not support any amphibians. Nonetheless, a comparison of the 44 sites with both eDNA and skin swab data showed that *Bd* was detected by both methods at only 4 sites, by swabs only at 15 sites, by eDNA only a 0 sites, and neither method at the remaining 25 sites (Table 8). The probability of *Bd* presence from eDNA was not strongly related to any of the environmental variables we evaluated. Although three models ranked slightly higher than the null model (intercept and spatial random-effects structure only; Table A1), the environmental variables in these models were not significant and explained little variation in the response. They predicted *Bd* eDNA was more likely to occur at wetland sites with a higher percentage of acidic bedrock in the larger watershed (β = –0.53, CI: –1.66 to 0.03; R^2 marginal: 0.08, R^2 conditional: 0.08), more frost days (β = 0.48, CI: –0.12 to 1.36; R^2 marginal: 0.06, R^2 conditional: 0.06), and more wetland habitat in the surrounding landscape (β = 0.39, CI: –0.15 to 1.13; R^2 marginal: 0.05, R^2 conditional: 0.05). We selected the null model as the best model (R^2 conditional: 0.19). This

model predicted an average occurrence probability for *Bd* of 0.09 (CI: 0.00–0.17) across all wetlands.

The probability of *Bd* presence from amphibian skin swabs was related to several environmental variables. Model-selection uncertainty was high, with 2 models < $2\Delta AIC_c$ and 5 models < 4 ΔAIC_c (Table A2). The two competitive models suggested probability of *Bd* occurrence from skin swabs increased with the percentage of limestone and dolomite bedrock in the watershed (β = 1.07, CI: 0.43–9.00; R^2 marginal: 0.24, R^2 conditional: 0.38) and the average amount of precipitation in the warmest quarter (β = 1.04, CI: 0.39–8.37; R^2 marginal: 0.24, R^2 conditional: 0.28). Predictors in lower-ranked models suggested weak relationships with other variables that were not significant. These results should be interpreted with caution because they included only sites where amphibians occurred and were successfully captured to collect skin swabs, and was thus not a random sample of wetlands in the study area.

Amphibian occupancy

Boreal chorus frog

The model for detection probability of boreal chorus frog selected in stage 1 had strong support (88% model weight) and indicated detection varied among years (Table A3). Models in which detection of boreal chorus frogs varied between visual and eDNA survey methods had no support. In stage 2, we retained 9 predictors for occupancy probability that ranked above the null model (Table A4). These included variables related to presence of fish, water quality, landscape context, and climate, but no variables representing geology or presence of *Bd*. In stage 3, we evaluated 93 models that were additive combinations of up-to 3 predictors for occupancy selected in stage 2 and the detection model selected in stage 1 (Table A5). Three competitive models with 69% of model weight all included negative relationships of occupancy probability with fish presence and positive relationships with forest cover. The top-ranked model also included a significant positive relationship with topographic position index. Other competitive models included additional predictors that were not significant, and lower ranked models were combinations of the same variables with low model weights indicating little support. Accordingly, we selected the top-ranked model as the best model to estimate with random effects in stage 4.

The best model for boreal chorus frog suggested detection probability was lower (β = –2.02, CI: –2.88 to –1.11) in 2018 (p = 0.21, CI: 0.11–0.36) than 2017 (p = 0.66, CI: 0.56–0.75; Figure 11). Occupancy probability was lower at sites with fish (β = –2.84, CI: –6.62 to –2.67), and higher at sites with more forest cover in the surrounding landscape (β = 1.62, CI: 0.24–3.67) and topographic positions that were more flat or mid-slope relative to valleys or depressions (β = 1.49, CI: 0.05–3.56). Assuming average forest cover and topographic position, sites with fish had an average occupancy probability of 0.20 (CI: 0.01–0.67), compared to a higher probability of 0.69 (CI: 0.30–0.96) for sites without fish. The standard deviations of the random intercepts suggested variation at both levels of clustering that was greater among sites within catchments

(SD = 2.74) than catchments within watersheds (SD = 1.15). Posterior predictive checks suggested good model fit (Bayesian $p = 0.46$, $\hat{c} = 1.05$).

Boreal toad

The model for detection probability of boreal toad selected in stage 1 had strong support (82% model weight) and suggested detection varied between visual and eDNA survey methods (Table A6). In stage 2, we retained 11 predictors for occupancy probability that ranked above the null model (Table A7). These included variables related to geology, water quality, climate, wetland characteristics, and time, but no variables representing landscape context or presence of *Bd*. In stage 3, we evaluated 56 models that were additive combinations of up-to 2 predictors for occupancy selected in stage 2 and the detection model selected in stage 1 (Table A8). The top-ranked model had strong support (81% model weight) and suggested a positive relationship of occupancy with the concentration of calcium in wetlands and a negative relationship with the percentage of limestone and dolomite bedrock in the site-specific watershed. The next five ranked models, though not technically competitive, comprised 17% of model weight and all contained a positive relationship of occupancy with the percentage of sandstone bedrock with calcite cement in the site-specific watershed. Because the negative relationship with limestone bedrock in the top-ranked model was counter to our predictions, we also included the second-ranked model to support our interpretation that the calcium at sites with boreal toads came from sandstone with calcite cement, and the negative correlation with limestone was driven by factors other than water chemistry. We selected the top-ranked model as the best model and also estimated the second-ranked model in stage 4.

The best model for boreal toad suggested detection probability was lower ($\beta = -3.40$, CI: –6.57 to –1.33) for eDNA ($p = 0.05$, CI: 0.00–0.17) than visual surveys ($p = 0.46$, CI: 0.25–0.70; Figure 12). Occupancy probability had a positive relationship with the concentration of calcium in wetlands ($\beta = 4.23$, CI: 1.39–8.68), and a negative relationship with the percentage of limestone and dolomite bedrock in the site-specific watershed ($\beta = -4.03$, CI: –8.26 to –1.14). Assuming average calcium concentration and limestone bedrock, overall occupancy probability for boreal toad was very low: 0.02 (CI: 0.00–0.12). The standard deviations of the random intercepts suggested variation at both levels of clustering that was greater among sites within catchments (SD = 2.15) than catchments within watersheds (SD = 1.22). Posterior predictive checks suggested adequate model fit (Bayesian $p = 0.36$), but provided evidence for over-dispersion ($\hat{c} = 4.88$).

Columbia spotted frog

The top-ranked model for detection probability of Columbia spotted frog in stage 1 had moderately strong support (65% model weight) and indicated detection varied between visual and eDNA survey methods, and was lower for larger sites (Table A9). However, we chose to include only survey method in the detection model after final models including wetland area had poor fit. In stage 2, we retained 15 predictors for occupancy probability that ranked above the null model (Table A10). These included variables related to geology, water quality, climate,

wetland characteristics, landscape context, and presence of *Bd*. In stage 3, we evaluated 107 models that were additive combinations of up-to 2 predictors for occupancy selected in stage 2 and the detection model selected in stage 1 (Table A11). The top-ranked model had moderate support (64% model weight) and suggested a negative relationship of occupancy with elevation and a higher occupancy probability for sites where *Bd* was detected by eDNA sampling. However, accounting for uncertainty in the final Bayesian analysis reduced the significance of the *Bd* term by expanding its 95% CI to include 0, suggesting the relationship was driven by a small number of sites clustered in the same watersheds or catchments. Thus, we selected the second-ranked model as the best model (Figure 13). After removing weight from all models including the variable for *Bd* occurrence, the second-ranked model had moderately strong support (62% model weight). All lower ranked models with >0% weight also included a negative relationship with elevation combined with other predictors that were not significant.

The second-ranked model for Columbia spotted frog suggested detection probability was lower (β = –3.29, CI: –4.90 to –1.91) for eDNA (p = 0.12, CI: 0.03–0.28) than visual surveys (p = 0.75, CI: 0.58–0.88; Figure 13). Occupancy probability was negatively related to elevation (β = –1.64, CI: –3.73 to –0.29) and positively related to the amount of precipitation in the warmest quarter (β = 1.75, CI: 0.27 to 4.2). Assuming average elevation and precipitation, mean occupancy probability was 0.16 (CI: 0.12–0.31). The standard deviations of the random intercepts suggested variation among sites within catchments (SD = 2.89) was greater than catchments within watersheds (SD = 2.10). Posterior predictive checks showed good model fit (Bayesian p = 0.43, $\hat{c}$ = 1.67).

Tiger salamander

The model for detection probability of tiger salamander selected in stage 1 had strong support (91% model weight) and indicated detection varied between visual and eDNA survey methods (Table A12). In stage 2, we retained 21 predictors for occupancy probability that ranked above the null model (Table A13). These included variables related to geology, water quality, wetland characteristics, and landscape context, but no variables representing climate or presence of *Bd*. In stage 3, we evaluated 189 models that were additive combinations of up-to 2 predictors for occupancy selected in stage 2 and the detection model selected in stage 1 (Table A14). Although there was greater model-selection uncertainty for tiger salamander than other species, models that included a positive relationship of occupancy with water temperature had 92% of total model weight. Four competitive models with 57% of total model weight each consisted of water temperature paired with another variable: specific conductivity, depth, wetland edge proportion, and chloride.

The top-ranked competitive model for tiger salamander (16.2% model weight) suggested detection probability was lower (β = –1.14, CI: –2.12 to –0.25) for eDNA (p = 0.27, CI: 0.12–0.46) than visual surveys (p = 0.53, CI: 0.34–0.71; Figure 14). Occupancy probability increased with water temperature (β = 2.53, CI: 0.93–6.86) and specific conductivity (β = 1.27, CI: 0.03–3.65). Assuming average water temperature and specific conductivity, mean occupancy probability was 0.17 (CI: 0.12–0.34). The standard deviations of the random intercepts suggested

variation among sites within catchments (SD = 2.16) was greater than catchments within watersheds (SD = 1.08). Posterior predictive checks suggested good model fit (Bayesian p = 0.40, $\hat{c}$ = 1.07).

The second-ranked competitive model (15.9% model weight) included the same relationships of detection probability with survey method and occupancy with water temperature, and higher occupancy probability for wetlands >1m deep (β = 2.08, CI: 0.34–4.29; Figure 14). Assuming average water temperature, wetlands with >1m depth had an average occupancy probability of 0.39 (CI: 0.10–0.86), compared to 0.08 (CI: 0.05–0.17) for sites with <1 m depth. The third-ranked competitive model (14.8% model weight) included the same relationships of detection probability with survey method and occupancy with water temperature, and higher occupancy probability for sites with a lower proportion of wetland edge in the surrounding landscape (β = –2.22, CI: –5.33 to –0.45; Figure 14). The fourth-ranked competitive model (10.3% model weight) included the same relationships of detection probability with survey method and occupancy with water temperature, and greater occupancy probability for wetlands with higher concentrations of chloride, although this relationship was only marginally significant in the final Bayesian analysis (β = 1.32, CI: –0.01 to 4.04; Figure 14).

Northern leopard frog

We did not have sufficient data to analyze occupancy and detectability of Northern leopard frog.

Water chemistry

Results from analysis of water samples suggested the concentration of ions was highest in the Gros Ventre Range. Nitrate concentrations were high (mean = 0.03, SD = 0.09) among all ranges (ANOVA, F = 0.2, p = 0.81), and varied by watershed (F = 2.1, p = 0.03; Figure 16). Sulfate concentrations in the Gros Ventre Range were higher than in the Teton Range (F = 4.2, p = 0.02; Tukey HSD, p = 0.015; Figure 16). The Gros Ventre Range had a large proportion of gypsum bedrock ($MgSO_4$), which may explain the higher sulfate concentrations observed. Similarly, chloride concentrations were highest in the Gros Ventre Range (F = 13.3, p < 0.0001; Tukey HSD, p < 0.0003; Figure 16). Phosphate concentrations were often below detection limit and did not vary among ranges (F = 1.0, p = 0.33; Figure 16). Calcium (F = 9.0, p = 0.0002; Tukey HSD, p < 0.004; Figure 17A), sodium (F= 7.9, p = 0.0007; HSD, p < 0.004; Figure 17B), magnesium (F = 13.3, p < 0.0001; Tukey HSD, p < 0.008), and potassium concentrations (F = 9.6, p = 0.0001; Tukey HSD, p < 0.005 were highest in the Gros Ventre Range (Figure 17). Finally, acid neutralizing capacity (i.e., alkalinity) was also highest in the Gros Ventre Range (F = 13.6, p < 0.0001; Tukey HSD, p < 0.008, Figure 17).

Discussion

Factors affecting amphibian occupancy

Our results suggested water quality, landscape context, and wetland characteristics were the strongest influences on amphibian occupancy of montane wetlands in western Wyoming. Relationships of occupancy with geology were not apparent for most amphibian species, but may have occurred indirectly through the influence of bedrock on wetland water chemistry. Contrary to our predictions, we found no evidence that amphibian occupancy was negatively influenced by nitrogen deposition or presence of *Bd*, as measured by eDNA. Our results were consistent with other studies that found amphibian occurrence was most strongly influenced by water quality (Browne et al. 2009, Klaver et al. 2013), and habitat characteristics at both site and landscape scales (Gould et al. 2012, Browne et al. 2009), and was not strongly influenced by atmospheric deposition (Pierce 1993, Bradford et al. 1994, Vertucci and Corn 1996). While there is no doubt that *Bd* has caused extirpation and extinction of amphibian species globally (Skerratt et al 2007, Lips 2016), our results were consistent with previous evidence from Wyoming (Murphy et al. 2009) and elsewhere in North America (Longcore et al. 2007) that some amphibian populations are currently persisting with infection.

Relative occupancy rates of amphibian species in our study were comparable to others from the region: boreal chorus frog was the most common species and had the highest average occupancy rate, while Columbia spotted frog and Tiger Salamander were moderately common, and boreal toad and Northern leopard frog were rare (Gould et al. 2012, Gygli et al. 2017, Klaver et al. 2013). We did not make direct comparisons of occupancy rates among studies because they depend on the definition of sample units.

Water quality

Boreal toads are known to breed in wetlands with higher conductivity (Klaver et al. 2013 and reference therein), which may support resistance to infection (Hawk 2000). Our results indicate boreal toads occupied wetland sites with higher concentrations of dissolved calcium, which is a component of conductivity. These variables were highly correlated in our study ($r = 0.81$), but calcium concentration was a better predictor of boreal toad occupancy than specific conductivity, which is a more general measure that includes all ions. It is unknown which mineral ions drove associations of amphibians with conductivity reported in previous studies (Klaver et al. 2013 and reference therein), but our finding that boreal toads occupied sites with more dissolved calcium is consistent with their results and with evidence for the importance of calcium to physiological functions of amphibians (Curran and Gill 1962, Hunn 1985, Jeffries and Mills 1990).

Tiger salamander occupancy in our study area was higher at sites with warmer water temperatures and higher concentrations of ions. The latter relationship appeared in competitive models as positive associations with specific conductivity and chloride, corresponding with another study that found higher tiger salamander abundance in wetlands with more chloride (Brodman et al. 2003). Specific conductivity was also strongly correlated with other variables

related to water chemistry, including acid neutralizing capacity, and concentrations of calcium and magnesium.

We did not find negative associations of boreal chorus frogs with higher conductivity reported in previous studies (Browne et al. 2009, Klaver et al. 2013); instead, our results suggested landscape context was a stronger influence on occupancy for that species. Nonetheless, the commonness of boreal chorus in the Wind River Range documented in our study and others (Estes-Zumpf et al. 2014) could be related in part to their ability to live in waters with low conductivity.

We did not find evidence that atmospheric nitrogen deposition had a negative influence on amphibian occurrence. We predicted the effects of deposition would be greatest in the Wind River Range, due to limited buffering capacity of granitic bedrock. Contrary to our predictions, we found concentrations of nitrate and sulfate did not differ significantly by mountain range or bedrock type, sulfate concentration in the Wind River Range was intermediate compared to the other ranges, and predicted nitrogen deposition was higher in the Teton Range than the Wind River and Gros Ventre Ranges. Although nitrogen deposition predictions from the NADP model were highly correlated with the amount of precipitation in the warmest quarter ($r = 0.81$), we found Columbia spotted frog occupancy was positively related to precipitation, which was the opposite of our prediction for nitrogen deposition. Deposition may have the largest effect during the spring snowmelt period when pH drops to its lowest level during the year (Křeček et al. 2019); however, we did not measure water chemistry that early in the season because wetlands were inaccessible. Thus, our measurements may not have captured the potential effects of low pH during snowmelt on amphibians.

Landscape context

Amphibian occupancy of wetland sites was influenced by the vegetation and topography of the surrounding landscape. Boreal chorus frogs were more likely to occupy wetlands in areas with greater forest cover and topographic positions that were more flat or mid-slope relative to valleys or depressions. Canopy cover in forested areas can provide favorable conditions for amphibian breeding (Schiesari 2006) and other studies also found associations of boreal chorus frog with forest cover and other habitat characteristics were strongest at broader "landscape" scales (Browne et al. 2009). Although boreal chorus frog selected wetlands in forested areas of our montane study area, this species is a habitat generalist that occurs across a wide range of forested and non-forested habitats in Wyoming.

Columbia spotted frogs occupied lower elevation sites in our study area. In northeastern Oregon, Columbia spotted frogs produced more and larger egg masses at higher elevations (Bull and Hayes 2000); however, that study was conducted in an area where lower elevation habitats were more arid. By contrast, lower elevations in our high-mountain study area were associated with larger river systems and extensive riparian habitats that may have provided more of the oxbows, pools, ponds, and hiding cover known to be used by this Columbia spotted frogs (Munger et al. 1998).

Contrary to a study from Yellowstone National Park (YNP) that documented the importance of wetland connectivity to tiger salamander occupancy (Gould et al. 2012), we found this species occupied sites in areas with less wetland habitat in the surrounding landscape. This result may be explained by the inclusion in our study area of National Forest lands managed for cattle grazing, where tiger salamanders used isolated stock ponds and other agricultural water impoundments that do not occur in YNP.

Wetland habitat

Habitat characteristics of wetland sites influenced occupancy for boreal chorus frog and Columbia spotted frog. Consistent with other studies, we found sites with fish were less likely to be occupied by boreal chorus frogs (Klaver et al. 2013, Amburgey et al. 2014). We interpret this result to suggest predation limited the distribution of boreal chorus frogs, but it is also possible that wetland characteristics favorable to fish were negatively correlated with other aspects of suitable habitat for boreal chorus frogs. We did not find negative effects of fish presence reported in previous studies of Columbia spotted frog (Bull and Hayes 2000, Welch and MacMahon 2005) and tiger salamander (Maurer et al. 2014); however, many individuals of these species detected in our surveys were adults, which are less susceptible to predation than earlier life-stages. Columbia spotted frog occupied wetlands that received more precipitation during the warmest quarter of the year. Greater precipitation may be associated with permanence of wetland sites used by Columbia spotted frogs; for example, this species occupied sites in Utah that had less annual change in size (Welch and MacMahon 2005).

Geology

Occupancy was not directly related to geology for most amphibian species. The only geology variable included in a competitive model suggested that, contrary to our predictions, boreal toads occupied wetlands with a lower percentage of limestone-dolomite bedrock in their site-specific watersheds. Only 4 sites where boreal toads were detected had any limestone or dolomite bedrock in their watersheds and 2 of those sites had only 1%. We speculate that this correlation resulted from the lack of suitable amphibian habitat in areas with limestone-dolomite bedrock, which tended to occur as cliffs and outcrops in steep, high-elevation terrain, but additional analyses including more topographic variables would be necessary to confirm this hypothesis. We are aware of only one other study that directly tested hypotheses on the influence of bedrock geology on amphibian occurrence; however, the focus of that study was on the effects of the parent material on substrate and gradient of mountain streams, not water chemistry (Russell et al. 2004).

Although evidence for direct effects of geology on amphibian occupancy was limited in our study, some environmental variables included in top occupancy models differed significantly among bedrock geology types, suggesting the possibility of indirect effects. For example, the positive influence of dissolved calcium on boreal toad occupancy was likely influenced by sandstone bedrock with calcite cement, which had significantly higher calcium concentrations and specific conductivity than other geology classes (Figure B2). This relationship was further

supported by five models ranking below the best model that were not technically competitive, but all contained a positive relationship of occupancy with the percentage of sandstone bedrock with calcite cement in the site-specific watershed. For tiger salamander, specific conductivity varied significantly among bedrock types, but conductivity measurements were a better predictor of occupancy than geology variables. Similarly, topographic position index was lower for wetlands on limestone/dolomite bedrock than other geology types, but we found boreal chorus frog occupancy was better predicted by the topographic index than by geology type. Without more detailed field data on geology and hydrology, we cannot know whether the superior predictive performance of our water quality measurements compared to geology variables reflected actual ecological relationships or was the result of the geological mapping we used to classify sites. Regardless of the somewhat coarse nature of our geology classifications, we would expect field measurements of water chemistry to be better predictors of amphibian habitat because they are the end product of numerous influences, including but not limited to bedrock geology.

Mountain ranges

Amphibian distributions are known to vary among the mountain ranges of western Wyoming (Estes-Zumpf et al. 2014) and our results provide insight in the environmental variables behind these differences (Figure B1). Boreal chorus frog occupancy was lowest in the Tetons, compared to the Gros Ventre and Wind River ranges. This pattern was driven by more sites with fish and lower topographic position indexes in the Tetons, as forest cover did not vary among mountain ranges. Boreal toad occupancy was highest in the Gros Ventre and adjacent northwestern portion of the Wind River range. These differences were explained by higher concentrations of calcium and the percentage of sandstone bedrock with calcite cement in watersheds of the Gros Ventre Mountains. The percentage of limestone bedrock in watersheds in the Tetons was significantly higher than the Wind River Range, but the Gros Ventre Range was intermediate; the negative relationship of boreal toad occupancy to limestone bedrock was driven in part by the fact that we had only one detection of that species in the Tetons. Greater occupancy of Columbia spotted frog in the Teton and Gros Ventre ranges was related to significantly higher elevations of wetland sites in the Wind River Range, as well as significant differences in the amount of precipitation during the warmest quarter among all mountain ranges, with the Tetons receiving the most and the Wind Rivers the least. Tiger salamanders occurred throughout the study area and water temperature, the strongest predictor of occupancy for that species, did not vary significantly among mountain ranges. Other variables in competitive models for tiger salamander occupancy varied among mountain ranges, including higher specific conductivity and chloride concentrations, which explained the greater number of sites with that species in the Gros Ventre range.

Factors affecting Bd occurrence

Our results were inconclusive on factors affecting the distribution of *Bd*. None of the environmental variables we tested were strong predictors of *Bd* occurrence based on eDNA sampling. We found weakly lower probability that *Bd* eDNA occurred at wetlands in watersheds

with more acidic bedrock, but this relationship was not significant. A study across temperate North America that included samples from western Wyoming found *Bd* eDNA was more likely to occur at lower elevations (Chestnut et al. 2014); however, our entire study area was near the upper range of elevation included in that study and we did not find a relationship of *Bd* with elevation. Our results should be interpreted with caution because few eDNA samples tested positive for *Bd* even when we detected the infection in amphibian skin swabs from the same sites, raising concerns about the effectiveness of our eDNA protocol (see 'Skins swabs vs. eDNA sampling of *Bd'* below).

Analysis of data from amphibian skin swabs suggested *Bd* occurred at wetlands in watersheds with a greater proportion of limestone bedrock and sites that received more precipitation during the warmest quarter. The latter result is consistent with evidence that incidence of *Bd* in amphibian populations was associated with wetter conditions across the western hemisphere (Ron 2005) and that infection intensity was greater in wetter conditions within a portion of our study area in Grand Teton National Park (Murphy et al. 2009). Additionally, we found that watersheds of sites with at least one *Bd*-positive swab had a higher average percentage of limestone bedrock (28% limestone), compared to sites with only *Bd*-negative swabs (9% limestone). A study of the broader Rocky Mountain region found that *Bd* occurred in areas with warmer daily maximum temperatures (Muths et al. 2008), while a study in Oregon and northern California failed to find support for any hypotheses on *Bd* occurrence (Adams 2010). Taken together, these studies suggest *Bd* is associated with warm, wet conditions at a continental scale, while factors influencing its distribution at regional and local scales are variable and remain poorly understood.

Our results on *Bd* should be interpreted with caution for several reasons. First, our data could include false absences because we collected fewer replicates of skin swabs and water samples for eDNA than some other studies. For example, we collected an average of 1.4 swabs (min: 1, max: 4) at sites where we captured amphibians, while Murphy et al. (2009) recommend collecting ~20 swabs/site to infer absence of *Bd*. We collected a single water sample for eDNA at each wetland, aggregated from 3 locations within the site, while Chestnut et al. (2014) suggest 4–5 samples/site were necessary to infer absence using eDNA. Collecting only one water sample for eDNA was not a concern for amphibians because our analytical approach allowed us to compare detection efficiency of eDNA with visual surveys, but replicate water samples could have enabled us to estimate detection-adjusted occupancy rates for *Bd*. Second, we were unable to collect *Bd* samples from all sites: eDNA analysis used the subset of sites (N = 123) without missing data due to contamination or failed amplification, while the skin swab analysis used only sites at which we successfully detected, captured, and swabbed amphibians (N = 44). Finally, our skin swab results may have been biased by effort: we collected more swabs and detected more species at sites where *Bd* was detected (mean: 1.62 swabs, 1.76 species), compared to sites where *Bd* was not detected (mean: 1.15 swabs, 1.46 species). We recommend future studies on *Bd* collect more skin swabs and water samples for eDNA per site, and use modeling methods that enable estimation of detection probabilities.

Comparison of sampling techniques

Visual vs. eDNA sampling of amphibians

Compared to visual surveys, we detected amphibian species at fewer sites with eDNA sampling. Model-based estimates confirmed that detection probability was significantly lower for eDNA than visual surveys of all species except boreal chorus frog. We were not able to model detection rates for Northern leopard frog due to small sample sizes, but it was the only species detected at more sites by eDNA (N = 3) than visual surveys (N = 1). Despite substantially lower detection probabilities, eDNA sampling increased the total number of detections for all species, including 1 site where boreal toad was detected by eDNA only. Gygli (2017) had better agreement between eDNA and visual methods than our study and found that detection by eDNA out-performed visual surveys for tiger salamander. Similar to our study, they found detection rates of boreal toad and boreal chorus frog were lower by eDNA than visual surveys (Gygli 2017). Combining survey methods enabled us to maximize the number of detections for rare species of conservation concern, but it is not clear whether the added effort and expense of eDNA sampling was justified. Unfortunately, results of our study and others (Gygli 2017) suggest the performance of eDNA relative to visual sampling was poorest for boreal toad, the species with highest level of conservation concern among our focal group. Low detection rates for boreal toad with eDNA may be influenced by habitat use and behavior of this species, which spends less time in the water as adults than the other amphibians in our study.

Other studies have suggested increasing detection efficiency of eDNA sampling by collecting temporal or spatial replicates of water samples (Chestnut et al. 2014). We did not collect temporal replicate samples because we made only one visit to each remote back-country site. We recommend future studies consider repeat sampling to quantify seasonal changes in detectability, but acknowledge this would increase the cost of projects considerably. Although we could have collected replicate water samples on our single visit to each site, cumulative detection probabilities (p^*)[1] calculated using average detection probabilities for eDNA from our occupancy analysis suggested a large number of additional water samples would be necessary to attain a reasonable threshold of detectability for most species. To achieve a cumulative detection probability of 0.90 using only eDNA sampling would have required 44 water samples for boreal toad, 18 water samples for Columbia spotted frog, 8 water samples for tiger salamander, and 3 for boreal chorus frog. Nonetheless, supplementing double-observer visual surveys with additional water samples could have increased detection probabilities for rare species. Cumulative detection probabilities with 2 visual surveys (double-observer method) and 1 water sample were already >0.90 for boreal chorus frog ($p^* = 0.96$) and Columbia spotted frog ($p^* = 0.95$). Adding 2 more replicate water samples for tiger salamander could have increased p^* from 0.84 to 0.91, but would only have increased p^* for boreal toad from 0.72 to 0.75.

Given our relatively small sample sizes, the complexity of our detection models was limited by our objective to compare eDNA and visual survey methods. Previous studies have found

[1] $p^* = 1–(1–p)^n$, where p is detection probability on a single occasion and n is the number of occasions.

influences of time of day, day of year, observer skill, volume of water filtered, and other factors on detection probabilities of amphibians (Estes-Zumpf et al. 2017, Gygli et al. 2017). We were limited in the number of variables we could include and chose to include only variables that were applicable to both methods. For example, we did not include the volume of water filtered for eDNA as a covariate for detection probability because it does not have plausible effect on results from a visual survey. Likewise, we did not include the duration of visual surveys as a covariate for detection probability because it does not have a plausible effect on the results of an eDNA sample. Separate analyses by survey method could account for this issue (e.g., Gygli 2017), but would negate the value of combining data from both methods. Larger sample sizes could also support more complex models for detection and occupancy, but were not an option for our study, due in part to the effort allocated to eDNA sampling. Other disadvantages of eDNA sampling included missing data from contaminated and inhibited samples, uncertainty in the definition of the site sampled, and lack of information on the breeding status of amphibians that are acquired with visual surveys.

Skins swabs vs. eDNA sampling of *Bd*

We expected higher detection rates of *Bd* from skin swabs than eDNA sampling because swabbing requires presence of amphibians, while some sites sampled for eDNA likely did not support any amphibians. Nonetheless, a comparison of the sites with both eDNA and skin swab data suggested that eDNA was not a reliable method to detect *Bd* at sites where it was present on amphibian skin: *Bd* was detected only by swabs at 15 sites, by both methods at 4 sites, and only by eDNA a 0 sites. We acknowledge that eDNA and skin swab samples measure different parts of the sample space, so perfect correlation in unlikely; however, the poor agreement between the methods is problematic for researchers interested in selecting a single, reliable method to quantify occurrence of *Bd*. Sampling *Bd* with eDNA has the clear advantage of being able to detect the fungus at sites where amphibians are not detected or captured, which included 11 sites in our study. However, our results suggest further research and development of sampling methods is necessary before eDNA can be considered a reliable substitute for skin swabbing.

Limitations

Our study was exploratory in nature and the ecological relationships identified are correlations that do not imply causation. The method of model selection was designed to reduce the number of variables and identify those with the strongest relationships; however, measurement of variables at different scales using different methods could have advantaged some predictors. For example, we did not find strong relationships of amphibian occupancy with nitrogen deposition, but this variable was measured at a coarser scale than others (12 km^2 vs. 30 m^2). Likewise, our model sets included a mix of field measurements (e.g., water quality) with variables derived from remote sensing (e.g., forest cover) and other techniques (e.g., geology mapping). These challenges are inherent to many contemporary ecological studies and we have done our best to address them transparently in the methods and discussion.

To avoid creating unnecessary stratification in our sample, we sampled watersheds randomly across the study area, stratified proportionally by mountain range. This enabled us to evaluate the influence of all variables across the study area, rather than being limited by having to account in all analyses for disproportionate pre-stratification by geology type or mountain range. A drawback of this random sampling approach was that it resulted in fewer limestone-dolomite sites than granitic sites in our sample, which may have limited our power to detect differences among geology classes. We felt this was an acceptable tradeoff, as the alternative of pre-stratifying by geology type would have limited our power to evaluate the importance of other factors in the absence of strong effect of geology. Additionally, we found no evidence of bias from sampling more sites near roads and trails by testing the distance to road/trail as a variable in exploratory analyses; however, our results could still include unknown biases from other departures from our random sampling scheme necessitated by field logistics.

Recommendations for future research

We found that amphibian occupancy of wetlands was related to concentrations of specific ions. Knowledge of the mechanisms by which water quality affects amphibians could be improved if other studies adopted our approach of measuring more water quality variables in the field and collecting water samples for laboratory analysis of cations and anions. We would be especially interested in sampling water from sites in Yellowstone National Park where boreal toad breeding is associated with higher conductivity to understand the specific cations involved and the potential influence of geology.

We acknowledge that eDNA sampling is a relatively new technique that has already proven its value in studies of diverse taxa and systems (Bohmann et al. 2014, Rees et al. 2014). However, we found that it did not substantially improve our sampling of amphibians and *Bd* in the montane wetland habitats of western Wyoming. Future studies should consider modifications to eDNA sampling protocols (e.g., more replicates, greater water volumes, different filter pore sizes) that could improve detection efficiency. From the perspective of practitioners in the field, we felt the amount of effort and expense dedicated to eDNA sampling outweighed the resulting increase in amphibian detections. Additionally, the comparisons with skin swab data inspired concerns about the efficacy of detecting *Bd* with eDNA sampling given what we felt was a reasonable level of effort in our study. Additionally, we note that traditional visual encounter surveys provide information on breeding status, body condition, and disease prevalence that may be important to answer scientific questions and inform management decisions.

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Tables

Table 1. Predictors for analyses of amphibian and *Bd* occurrence.

Category	Variable name	Description	Units	Scale	Source
Water quality	*waterTemp*	Water temperature	°C	Wetland site	Field data
	spc	Specific conductivity	µS/cm		
	pH	pH	pH		
	anc	Acid neutralizing capacity	meq/L		Water samples
	calcium	Calcium ion concentration	mg/L		
	magnesium	Magnesium ion concentration			
	potassium	Potassium ion concentration			
	chloride	Chloride anion concentration			
	nitrate	Nitrate anion concentration			
	phosphate	Phosphate anion concentration			
	sulfate	Sulfate anion concentration			
	nDeposition	Total (wet + dry) Nitrogen deposition	kg/ha	12-km^2	Spatial data (Schwede and Lear 2014)
Geology	*geologyClass*	Mapped geology type (4 levels)	Categorical	Wetland site	Digital geologic map of Wyoming (Green and Drouillard 1994)
	limeWatershed	Limestone and dolomite bedrock	%	Site-specific watershed	
	acidWatershed	Granite and other acidic bedrock			
	calWatershed	Sandstone bedrock with calcite cement			
	limeHuc	Limestone and dolomite bedrock		12-digit HUC	
	acidHuc	Granite and other acidic bedrock			
	calHuc	Sandstone bedrock with calcite cement			
Site	*stream*	Stream influence (yes or no)	Categorical	Wetland site	Field data
	ephemeral	Ephemeral wetland (yes or no)	Categorical		
	areaHa	Wetland size from survey tracks	ha		
	depth	Depth (>1m of <1m)	Categorical		
	fish	Fish detected (yes or no)	Categorical		
	nSpecies	Number of amphibians species detected	Count		Field data (used in *Bd* models only)

(Continued next page)

Category	Variable name	Description	Units	Scale	Source
Landscape	*elevation*	Elevation	m	500 m radius	Spatial data (Gesch et al. 2009)
	aspect	Aspect	Index: 0 (West) to 2 (East)		
	tpi	Topographic position index	Index: >0 (hills or ridgetops), <0 (valleys or depressions), ~0 (flat or mid-slope)		
	mtnRange	Mountain Range	Categorical	Wetland site	From study design
	forest	Forest vegetation cover	%	500 m radius	Spatial data (LANDFIRE 2016)
	wetlandEdge	Wetland edge density			Spatial data (Estes-Zumpf et al. 2014)
Climate	*frostFree*	Frost-free period	days		Spatial data (Thornton et al. 2000)
	precip	Precipitation of warmest quarter	mm		Spatial data (Hijmans et al. 2005)
Chytrid fungus	*eDnaBd*	Occurrence of *Bd* from eDNA	categorical	Wetland site	Water samples
Time	*year*	Year	years		Field data
	julian	Julian day	days		
Survey method	*method*	Method	categorical	Survey	

Table 2. Primer and probe designs for all amphibian species. Probe designs read as (PROBE COLOR) SEQUENCCE (QUENCHER). Note wobble bases in tiger salamander probe design. Several primers that are unpublished assays developed by Dr. Caren Goldberg at Washington State University have been redacted from the report until publication.

Species	**Type**	**Sequence (5'->3')**	**Reference**
P. maculate Boreal chorus frog	Forward	AATCCCATTCCACGCCTACTAC	Zero (2014)
	Reverse	ATAAAGCTAAAAGAACGGCAAAGC	
	Probe	(FAM) CATACAAGGACGCTTTT (MGB)	
A. mavortium Tiger salamander	Forward	GGCAGATAGTTGGATGCACGATAG	Goldberg et. al (2018)
	Reverse	ACTACCTCTTGTCCTGGTTTTCCT	
	Probe	(CalRed610) pdCApdUAApdUApdUGpdUpdUGpdCpdCA pdCGpdCpdUApdCpdU (BHQ2)	
L. sylvaticus Wood frog	Forward	Unpublished	C. Goldberg, unpublished
	Reverse	Unpublished	
	Probe	(CY5) unpublished (MGB)	
A. boreas Boreal toad	Forward	Unpublished	C. Goldberg, unpublished
	Reverse	unpublished	
	Probe	(Cy5)unpublished (MGB)	
L. pipiens Northern leopoard frog	Forward	Unpublished	C. Goldberg, unpublished
	Reverse	Unpublished	
	Probe	(Q705) unpublished (BHQ2)	

Table 3. Multiplex and reaction setup for all qPCR analyses of eDNA samples. Note that *L. pipens* is present in both multiplexes.

Multiplex	Species/reaction component	Concentration (Volume)	Color (Quencher)	Reference
1	*P. maculata*	.4 uM probe/primers (.75 ul)	FAM (MGB)	Zero et al. in prep
	A. mavortium	4 uM probe/primers (.75 ul)	CalRed 610 (BHQ2)	Zero et al. in prep
	A. boreas	4 uM probe/primers (.75 ul)	Cy5 (MGB)	C. Goldberg, pers. comm.
	L. pipiens	4 uM probe/primers (.75 ul)	Quasar 705 (BHQ2)	Zero et al. in prep
	Internal control assay	10X (.75 ul)	VIC (MGB)	Qiagen Cat. # 211354
	Internal control DNA	10X (.75 ul)	-	Qiagen Cat. # 211354
	iQ Powermix polymerase	2X (7.5 ul)	-	Bio-Rad Cat. # 1725849
	Sample DNA	- (3 ul)	-	-
2	*B. dendrobaditis*	4 uM probe/primers (.75 ul)	FAM (MGB)	Boyle et al. 2004
	R. luteiventris	4 uM probe/primers (.75 ul)	CalRed 610 (BHQ2)	C. Goldberg, pers. comm.
	L. sylvaticus	4 uM probe/primers (.75 ul)	Cy5 (MGB)	C. Goldberg, pers. comm.
	L. pipiens	4 uM probe/primers (.75 ul)	Quasar 705 (BHQ2)	Zero et al. in prep
	Internal control assay	10X (.75 ul)	VIC (MGB)	Qiagen Cat. # 211354
	Internal control DNA	10X (.75 ul)	-	Qiagen Cat. # 211354
	iQ Powermix polymerase	2X (7.5 ul)	-	Bio-Rad Cat. # 1725849
	Sample DNA	- (3 ul)	-	-

Table 4. Thermocycler conditions (CFX 96, Bio-Rad Cat. # 1855195). All samples across Multiplexes 1 and 2 were amplified under these conditions.

Stage	Temperature (°C)	Time	# Cycles
Polymerase activation	95°	2 min	1
Denaturation	94°	1 min	50
Annealing & elongation	60°	1 min	50
Melt curve	65°-95° (+ 0.5° /cycle)	1 min	30

Table 5. Minimum counts of amphibians by species and life stage. Counts per life stage are from visual surveys and eDNA row shows number of sites with detections. Tadpole counts used lower bound of estimated ranges.

Life stage	Species				
	Boreal Chorus Frog	Boreal Toad	Columbia Spotted Frog	Northern Leopard Frog	Tiger Salamander
Adult	120	14	96	132	48
Juvenile	32	6	10	0	2
Metamorph	256	23	69	0	119
Larva/Tadpole	885	601	1004	25	29
Eggs	29	1	0	0	0
eDNA	31	1	3	3	9

Table 6. Comparison of detection efficiency for amphibians using double-observer visual encounter surveys (visual) and environmental DNA (eDNA) sampling methods. Matrices show the number of sites where individuals of each species were detected (+) or not detected (–) by each survey method. Sample sizes (N) for comparisons exclude sites with missing data (NA) for eDNA.

Boreal chorus frog

N = 115		Visual	
		–	+
eDNA	–	63	21
	+	11	20
	NA	19	3

Boreal toad

N = 123		Visual	
		–	+
eDNA	–	110	12
	+	1	0
	NA	13	1

Columbia spotted frog

N = 124		Visual	
		–	+
eDNA	–	101	20
	+	1	2
	NA	10	3

Northern leopard frog

N = 122		Visual	
		–	+
eDNA	–	118	1
	+	3	0
	NA	14	1

Tiger salamander

N = 122		Visual	
		–	+
eDNA	–	96	17
	+	4	5
	NA	13	2

Table 7. Number of skin swabs testing positive and negative for chytrid fungus (*B. dendrobatidis*; *Bd*) by amphibian species.

***Bd* Swab Result**	**Species**			
	Boreal Chorus Frog	**Boreal Toad**	**Columbia Spotted Frog**	**Tiger Salamander**
Positive	5	1	14	12
Negative	14	7	2	2
Total	19	8	16	14

Table 8. Comparison of eDNA and skin swabs for detection of amphibian chytrid fungus (*B. dendrobatidis*; *Bd*). Sample sizes (N) for comparisons exclude sites where skin swabs were not collected (NA).

B. dendrobatidis

N = 44		**eDNA**	
		–	+
Skin swab	–	25	0
	+	15	4
	NA	68	11

Figures

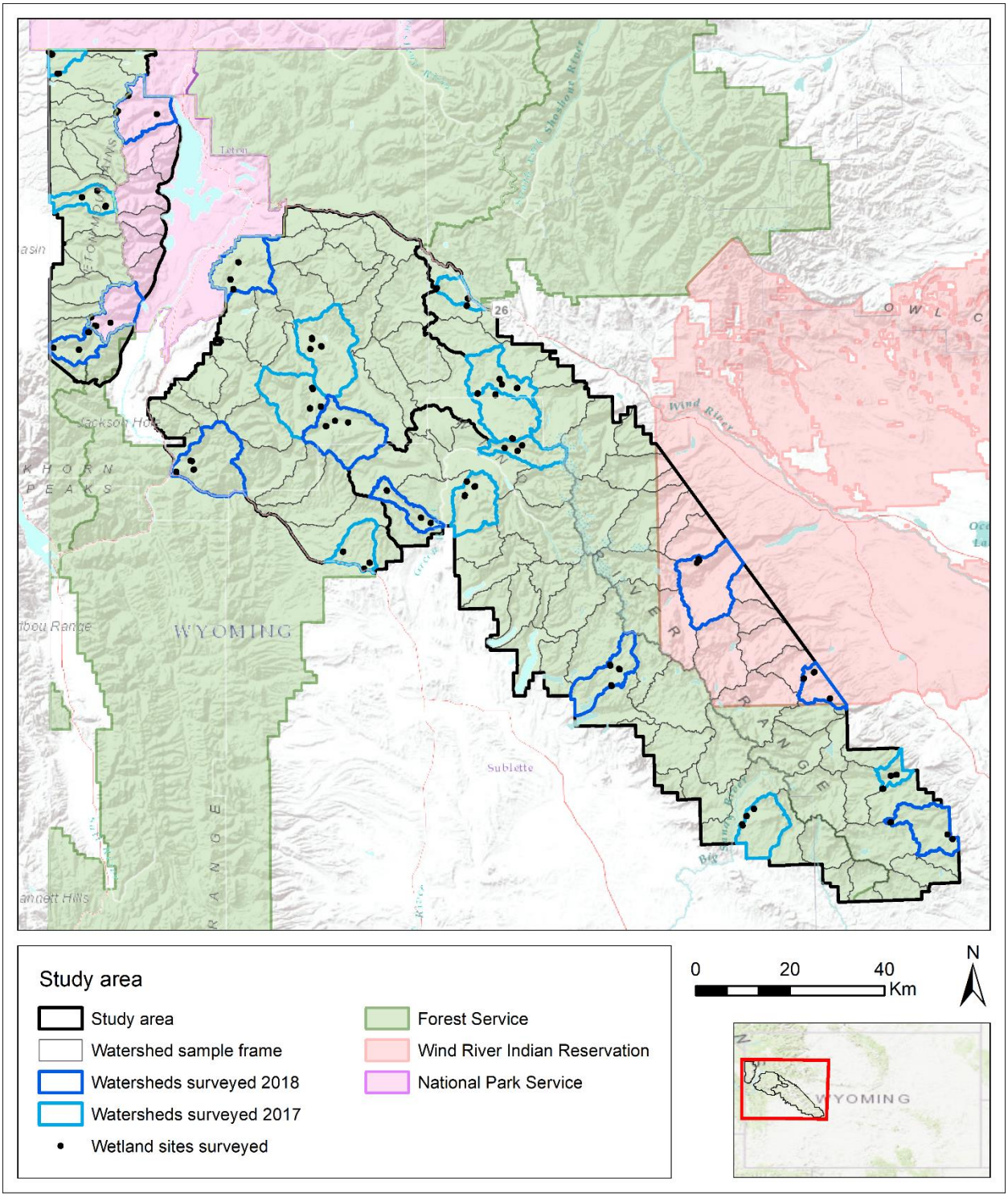


Figure 1. Study area. Bold black lines define the study area boundary and show boundaries between the Teton, Gros Ventre, and Wind River ranges.

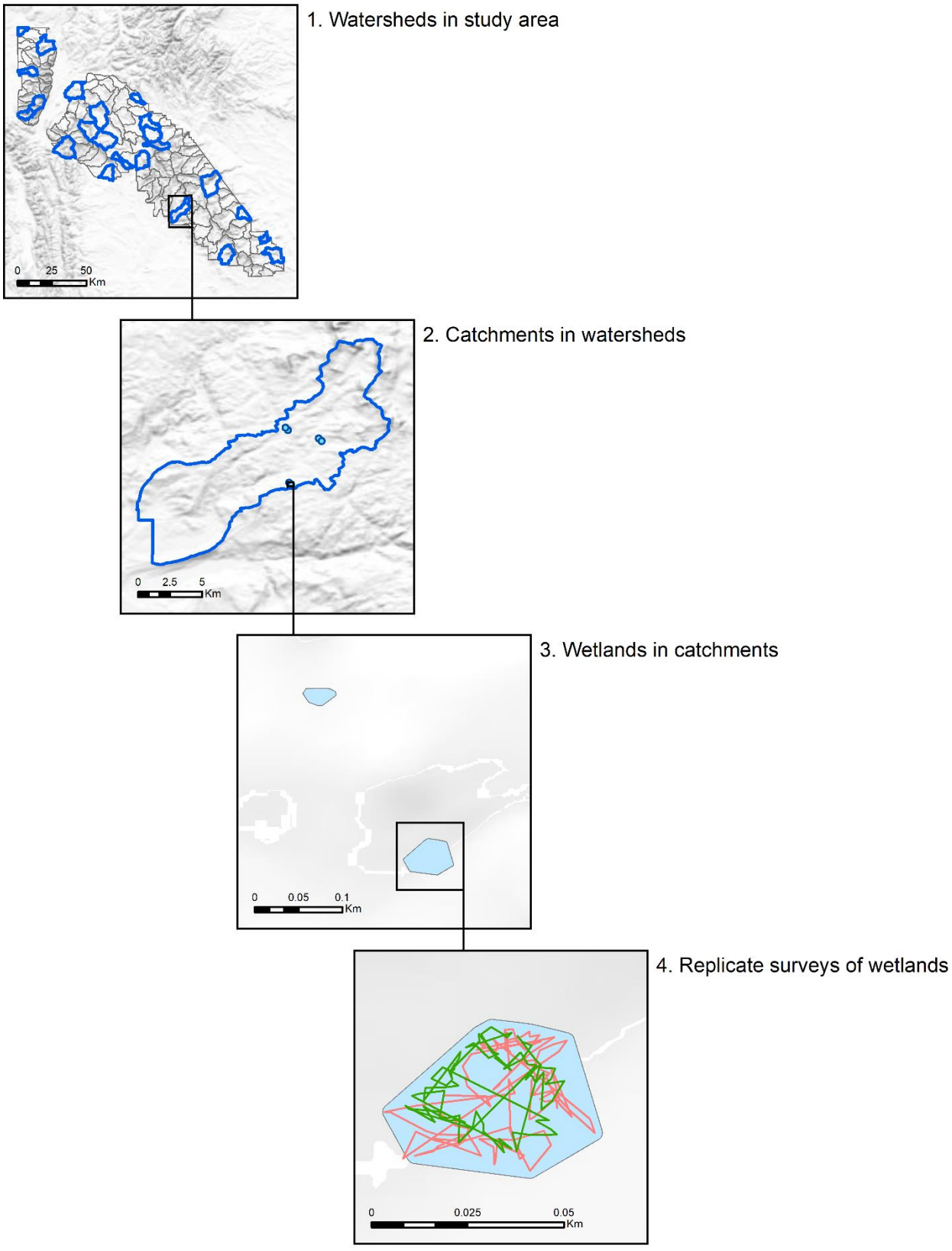


Figure 2. Nested sampling design for amphibians in western Wyoming.

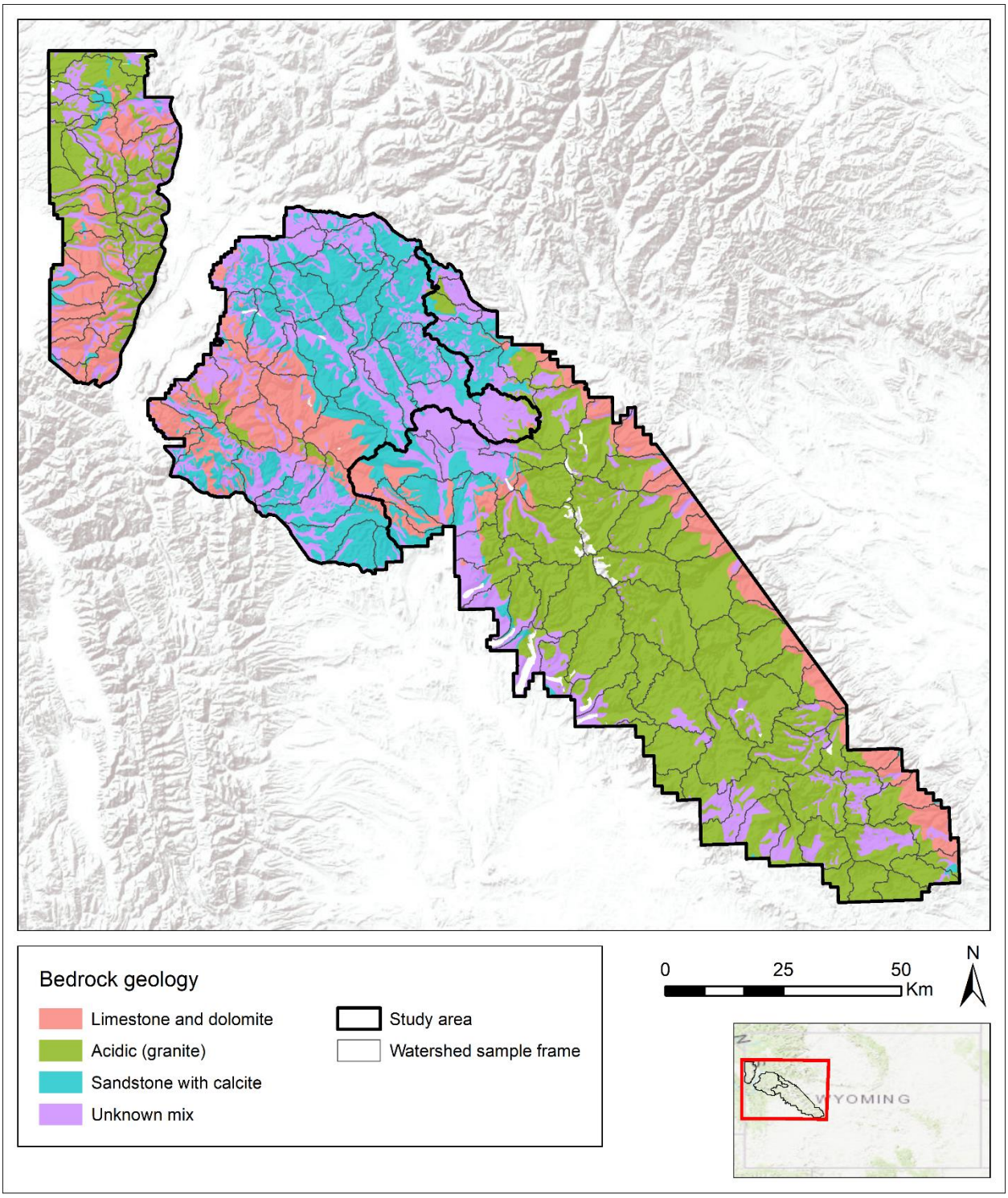


Figure 3. Study area geology.

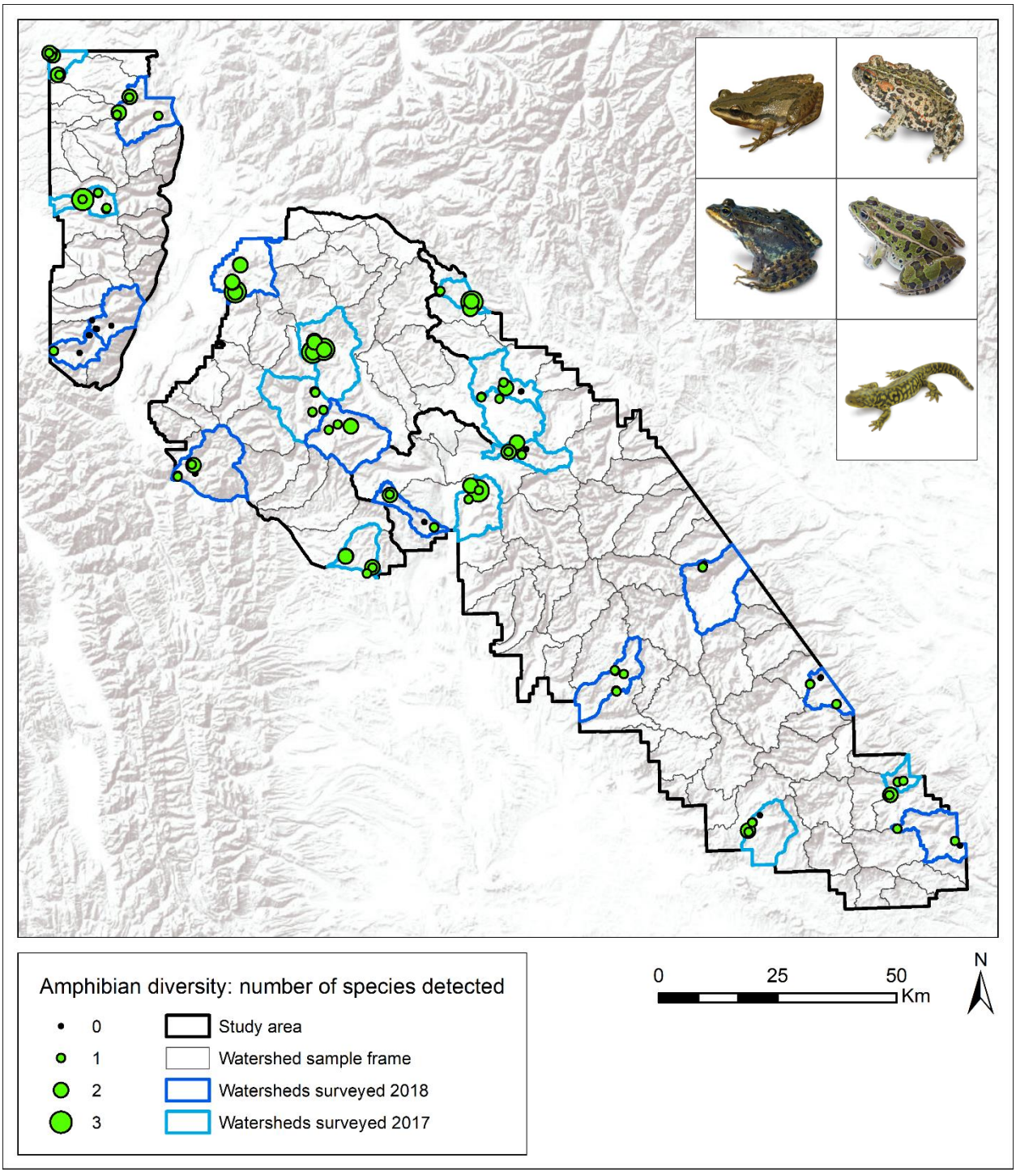


Figure 4. Number of amphibian species detected at wetland sites.

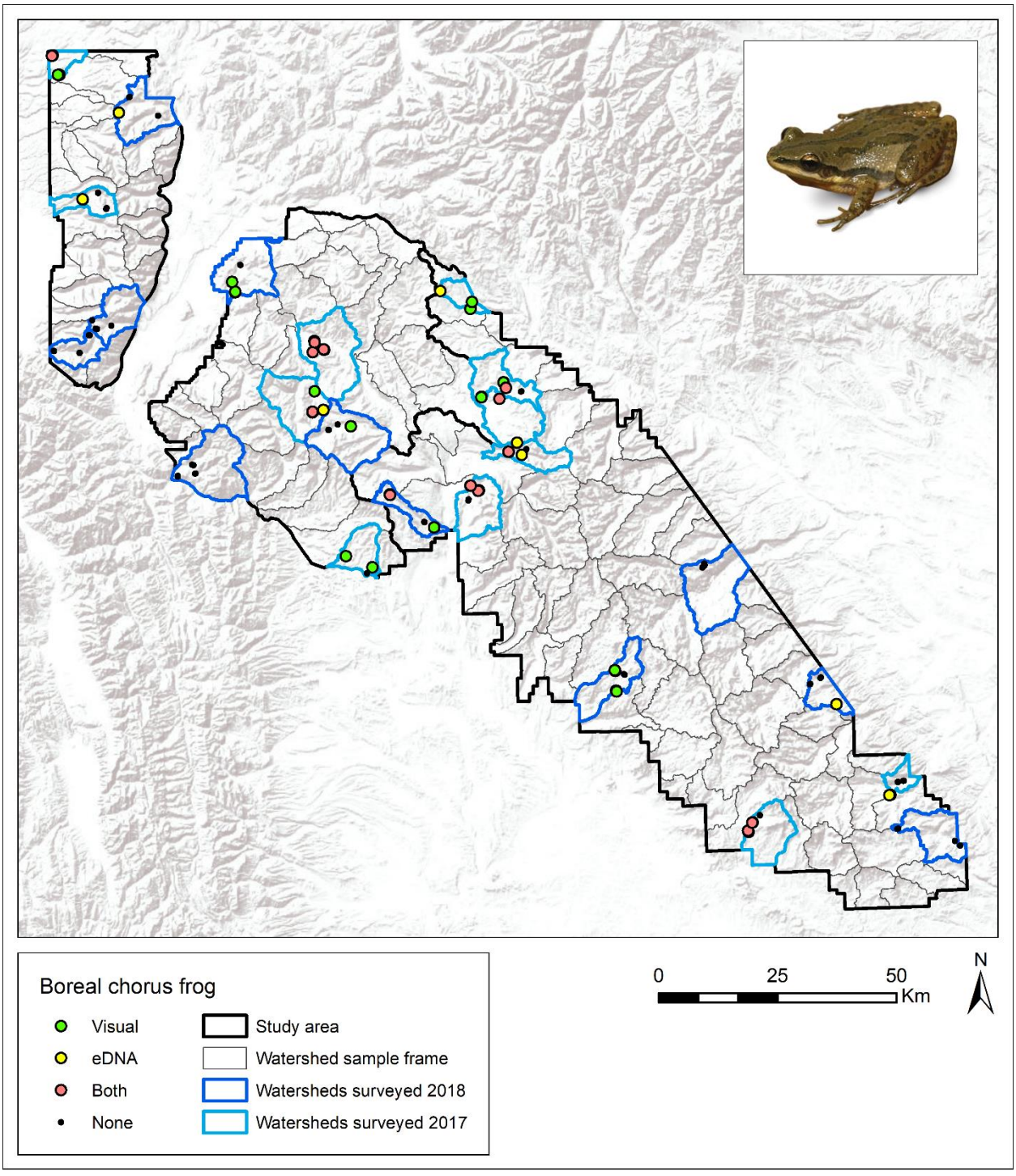


Figure 5. Wetland sites where boreal chorus frogs were detected by visual surveys (green), eDNA sampling (yellow), both methods (red), or not detected (black). Inset photo by Alberta Conservation Association.

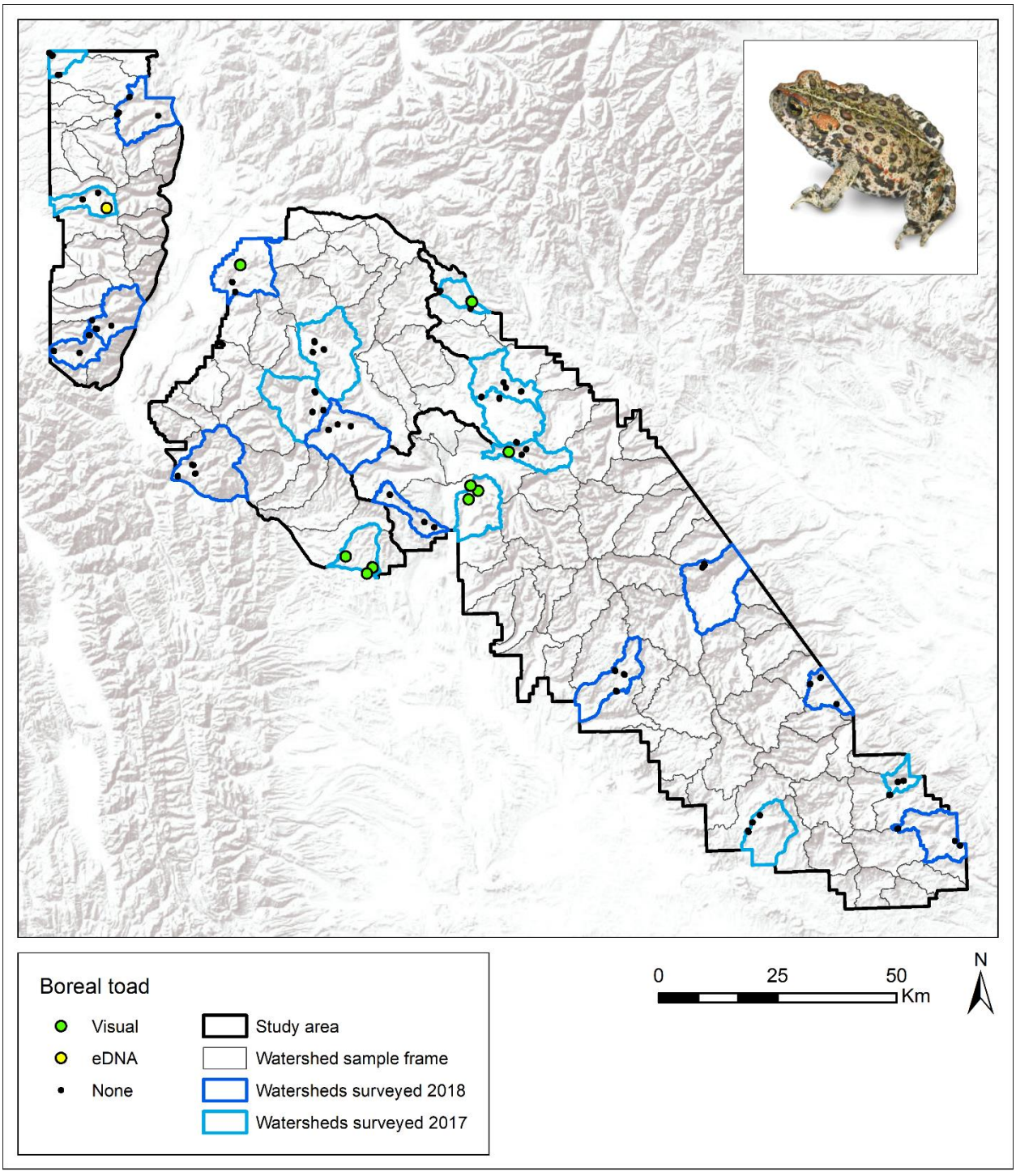


Figure 6. Wetland sites where boreal toads were detected by visual surveys (green), eDNA sampling (yellow), or not detected (black). Inset photo by Alberta Conservation Association.

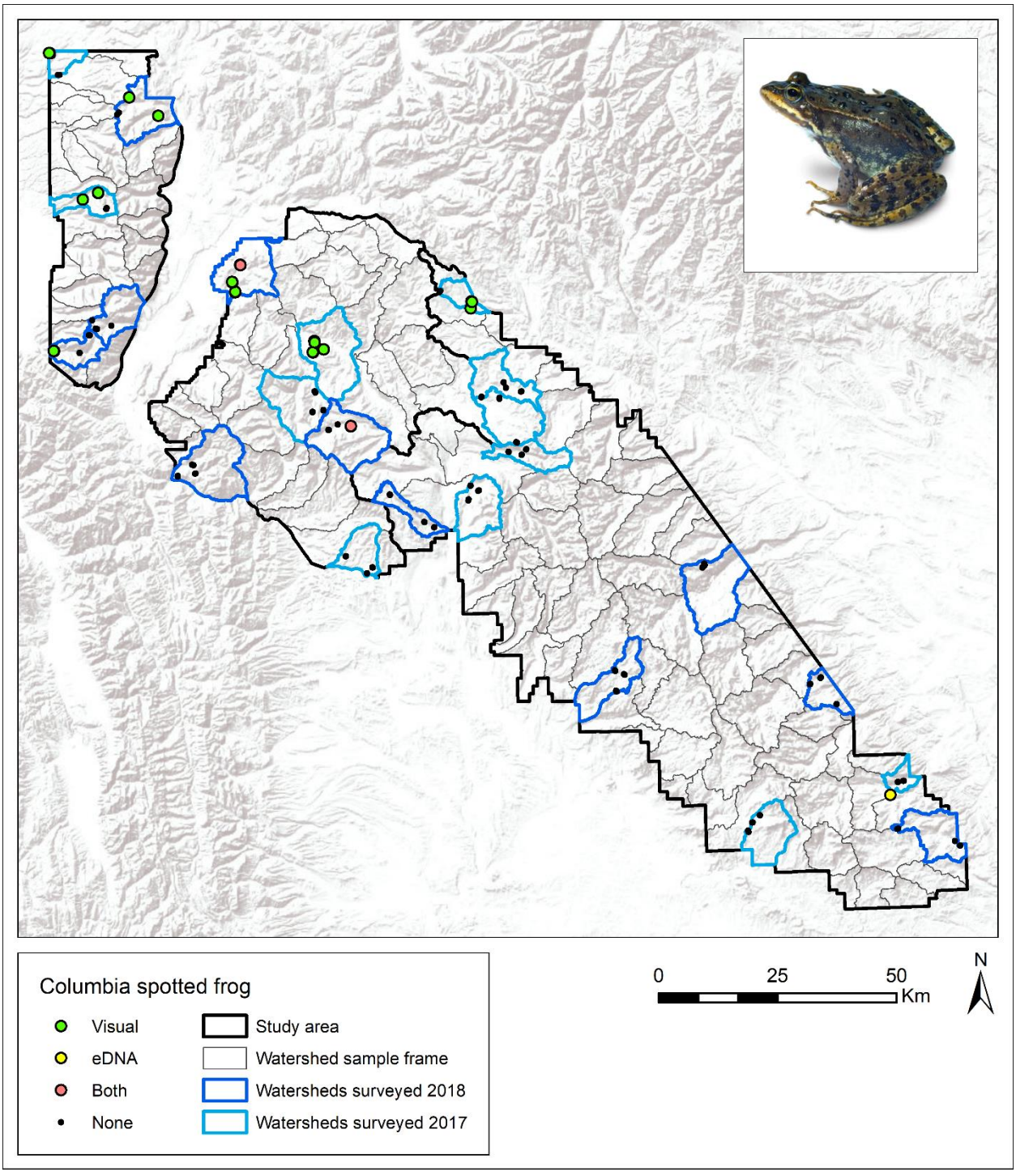


Figure 7. Wetland sites where Columbia spotted frogs were detected by visual surveys (green), eDNA sampling (yellow), both methods (red), or not detected (black). Inset photo by Alberta Conservation Association.

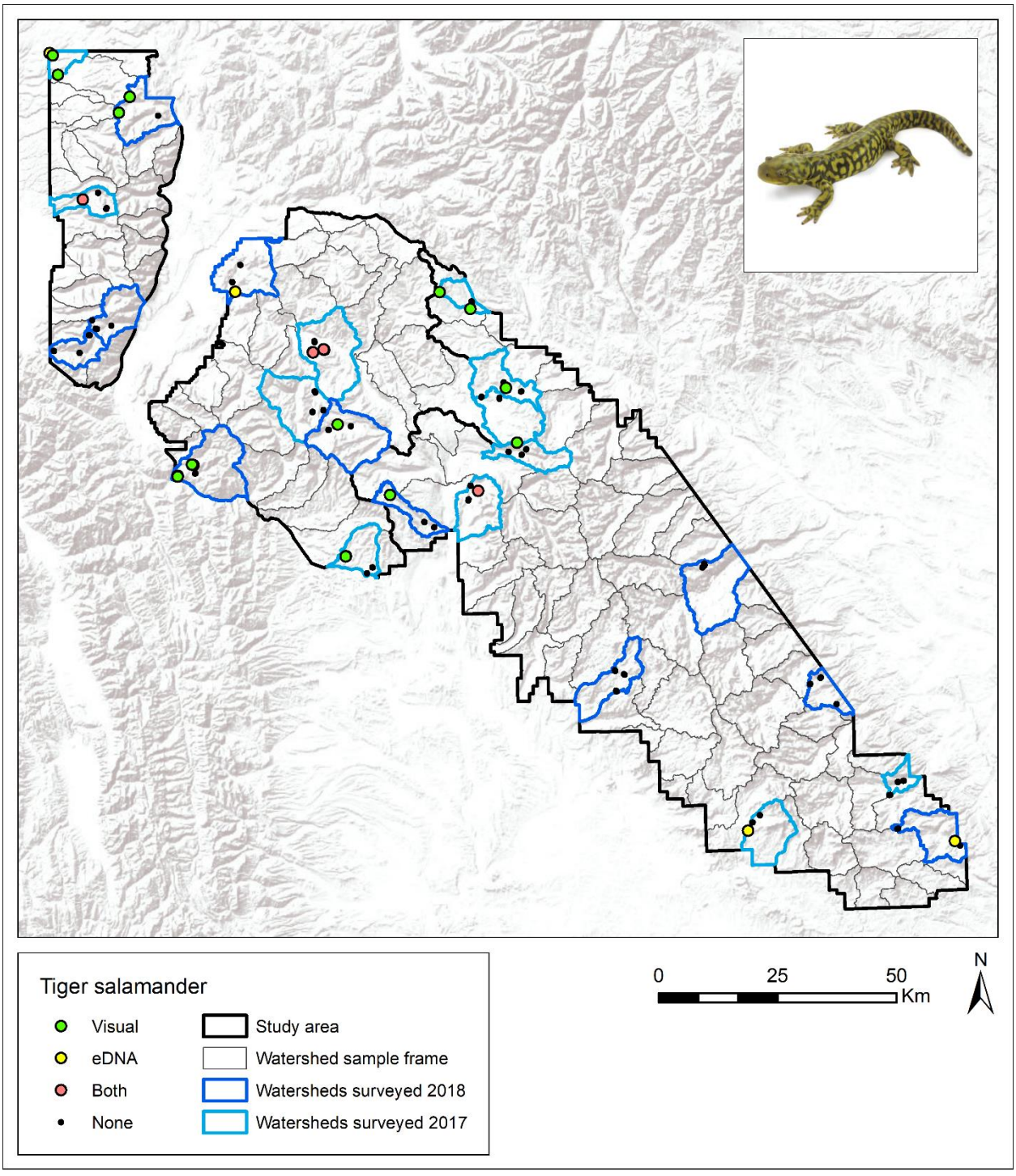


Figure 8. Wetland sites where tiger salamanders were detected by visual surveys (green), eDNA sampling (yellow), both methods (red), or not detected (black). Inset photo by Alberta Conservation Association.

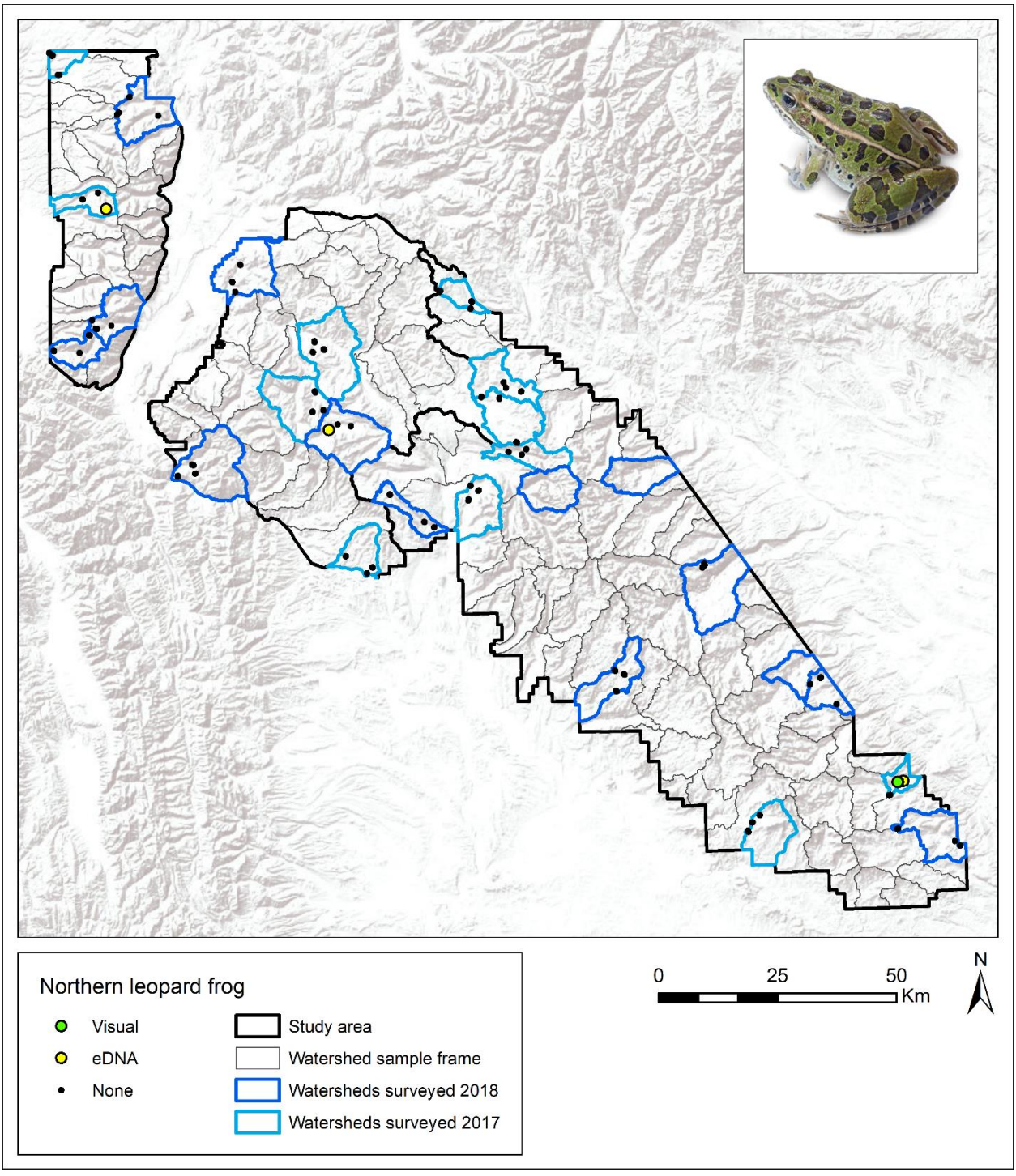


Figure 9. Wetland sites where Northern leopard frogs were detected by visual surveys (green), eDNA sampling (yellow), both methods (red), or not detected (black). Inset photo by Alberta Conservation Association.

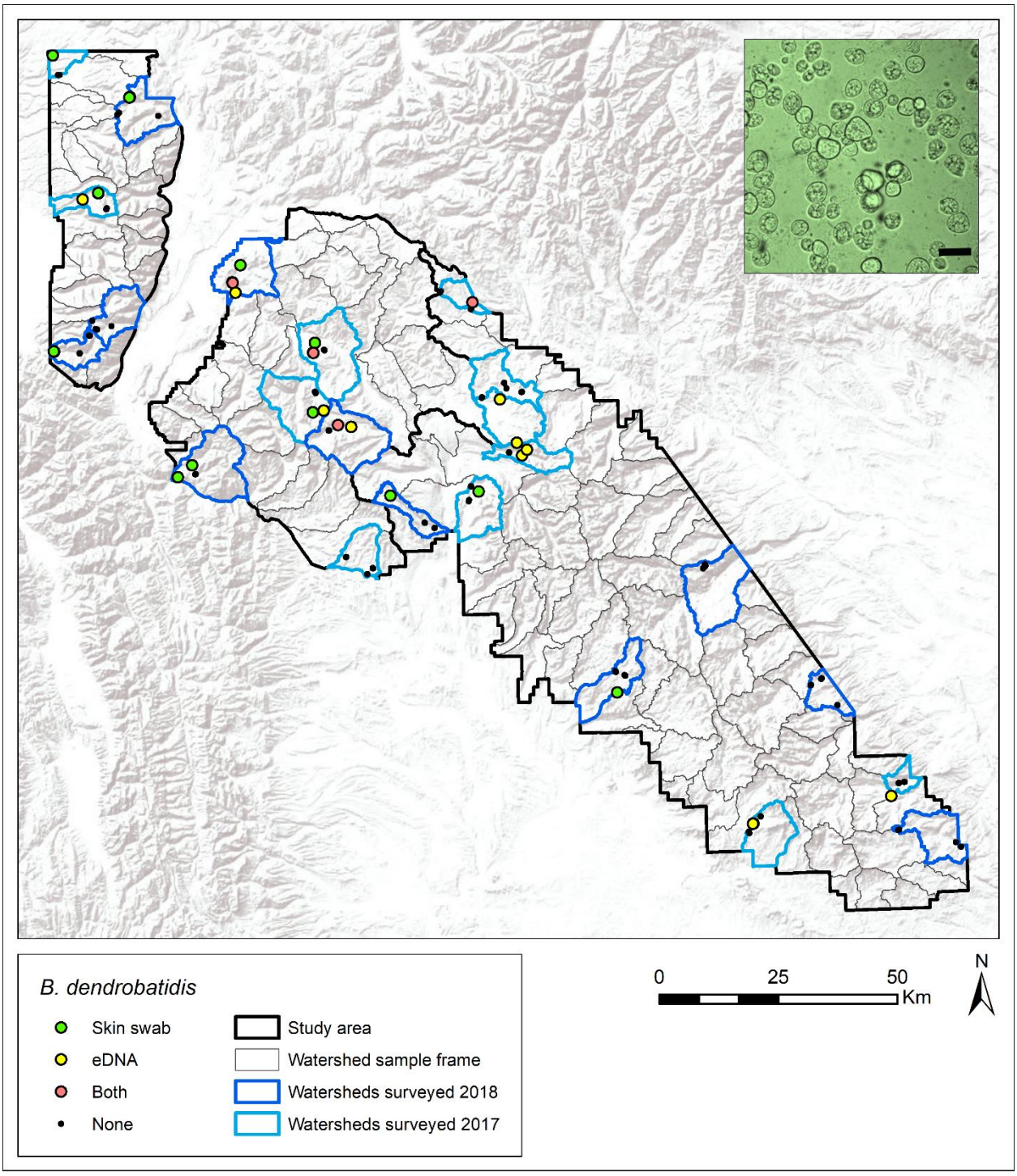


Figure 10. Wetland sites where amphibian chytrid fungus (*B. dendrobatidis*) was detected by amphibian skin swabs (green), eDNA sampling (yellow), both methods (red), or not detected (black). Skin swab data were limited to sites where amphibians were captured, and eDNA data were missing for some sites due to failed amplification and contamination. Inset photo from Voyles et al. (2012).

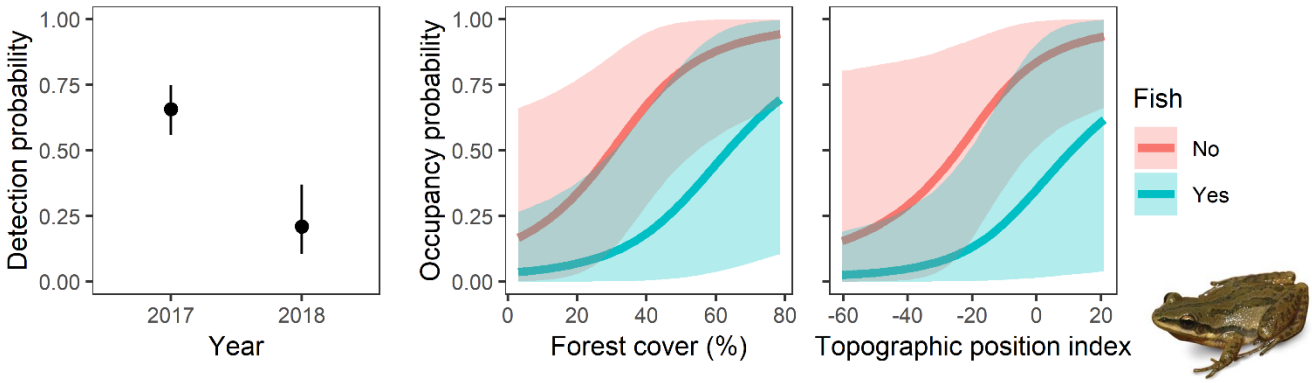


Figure 11. Detection and occupancy probabilities of boreal chorus frog as functions of environmental variables from the best model. Detection probability was higher in 2017 than 2018 (left). Occupancy probability was higher at sites without fish (center and right), increased with the percentage of forest cover in the surrounding landscape (center), and increased as topographic position index changed from valleys or depressions (negative values) to relatively flat or mid-slope areas (0) and hills or ridgetops (positive values; right).

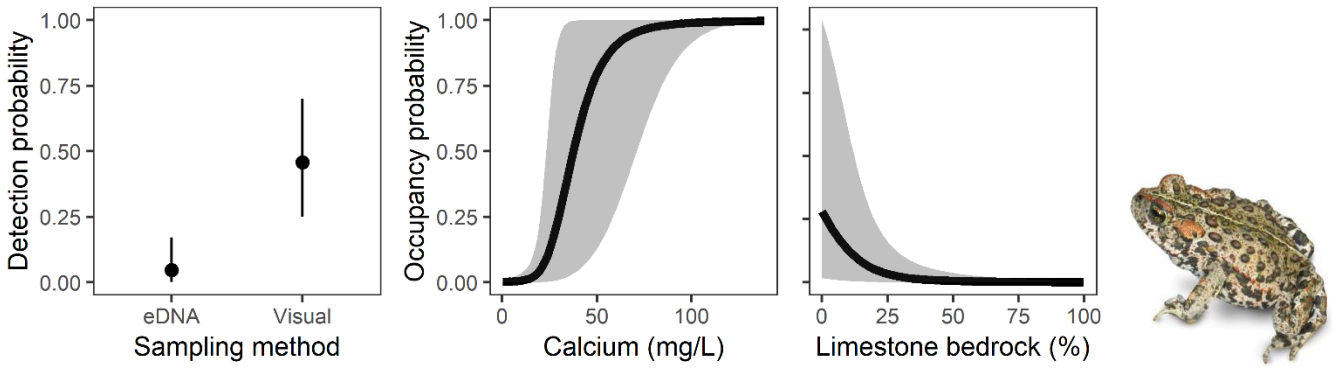


Figure 12. Detection and occupancy probabilities of boreal toad as functions of environmental variables from the best model. Detection probability was higher for visual encounter surveys than eDNA sampling in both models (left). Occupancy probability was greater at wetlands with higher calcium concentrations (center) and less limestone bedrock in the site-specific watershed (right).

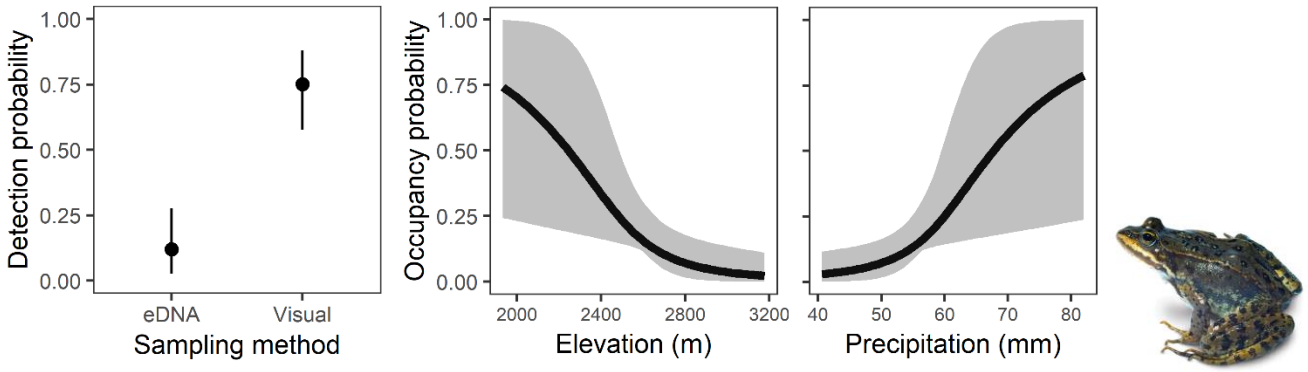
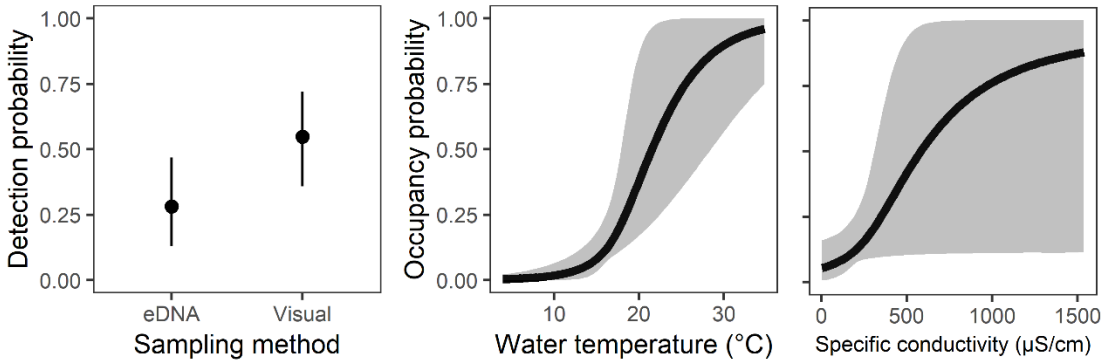
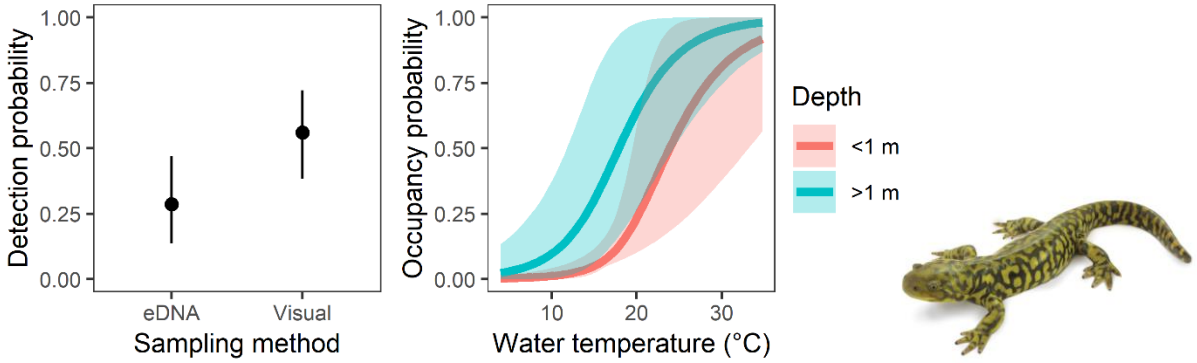


Figure 13. Detection and occupancy probabilities of Columbia spotted frogs as functions of environmental variables. Detection probability was higher for visual encounter surveys than eDNA sampling in all models (left), and occupancy probability was higher at sites with lower elevation (center) and greater precipitation in the warmest quarter of the year (right).

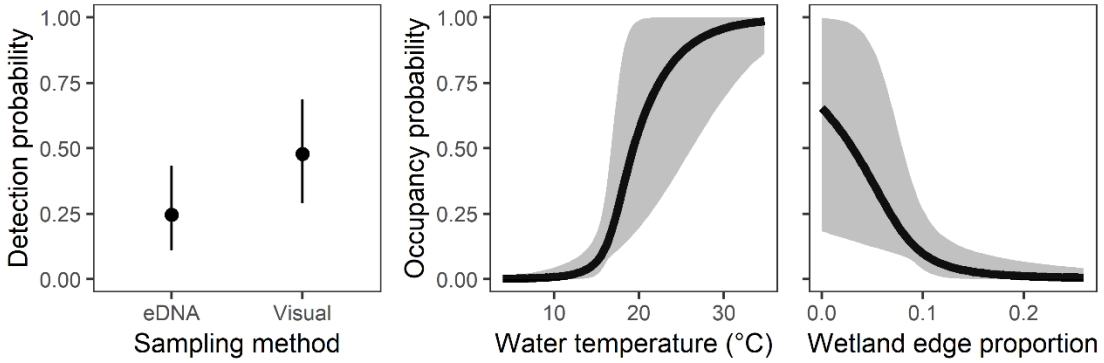
Model 1



Model 2



Model 3



Model 4

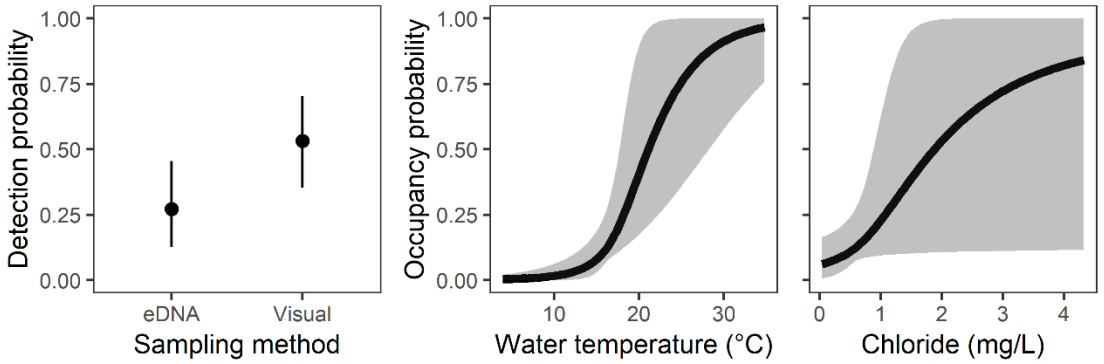


Figure 14. Detection and occupancy probabilities of tiger salamanders as functions of environmental variables from four competitive models. Detection probability was higher for visual encounter surveys than eDNA sampling in all models (left column). Occupancy probability was higher at sites with warmer water in all models (center column). Other predictors in competitive models suggested occupancy increased with specific conductivity (model 1), water depth > 1m (model 2), sites with less wetland habitat in the surrounding area (model 3), and more chloride (model 4).

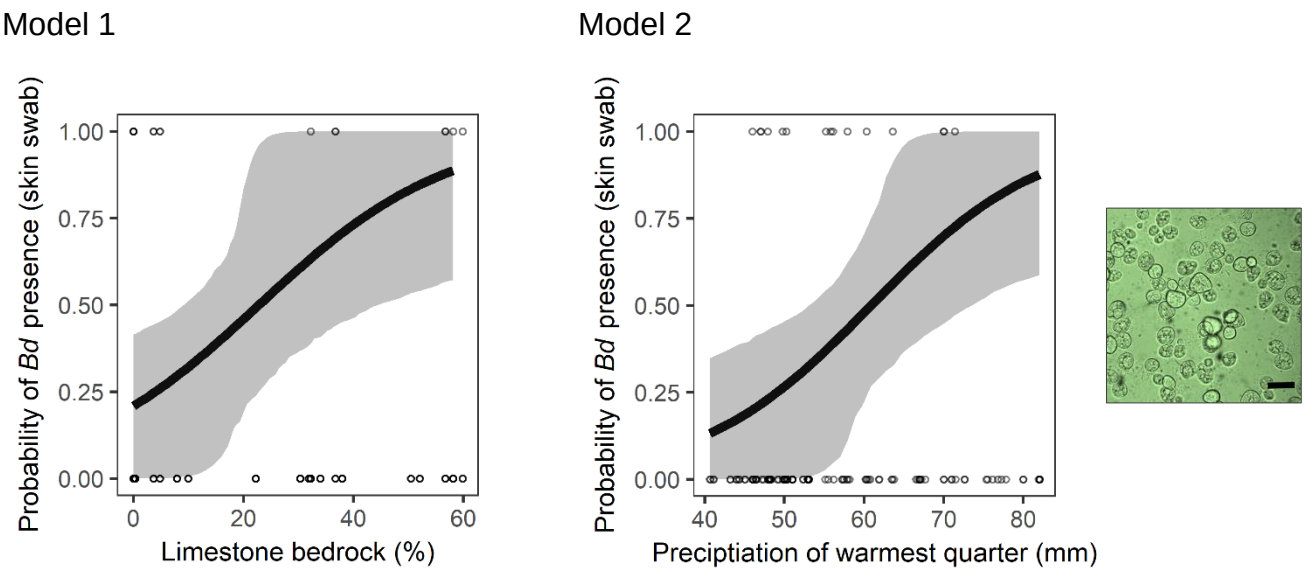


Figure 15. Environmental variables predicting occurrence of chytrid fungus (*B. dendrobatidis*; *Bd*) in amphibian skin swabs from two competitive models. Probability of *Bd* occurrence from skin swabs increased with the percentage of limestone and dolomite bedrock in the watershed (model 1) and the average amount of precipitation in the warmest quarter (model 2).

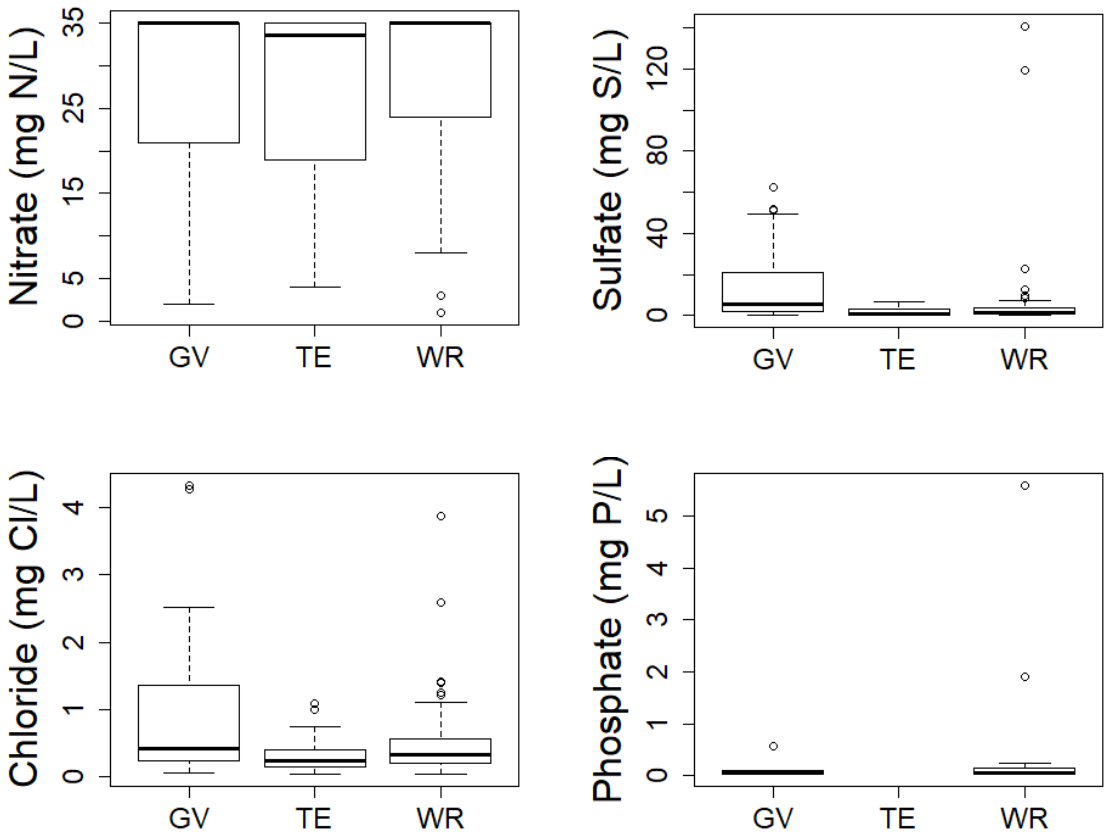


Figure 16. Concentrations of the anions nitrate (upper left), sulfate (upper right), chloride (lower left) and phosphate (lower right) in the Gros Ventre (GV), Teton (TE) and Wind River (WR) Ranges. The bold line is the median value, the box represents the 25th and 75th quartiles, the whiskers are 1.5 times the interquartile range, and the circles are outlier values.

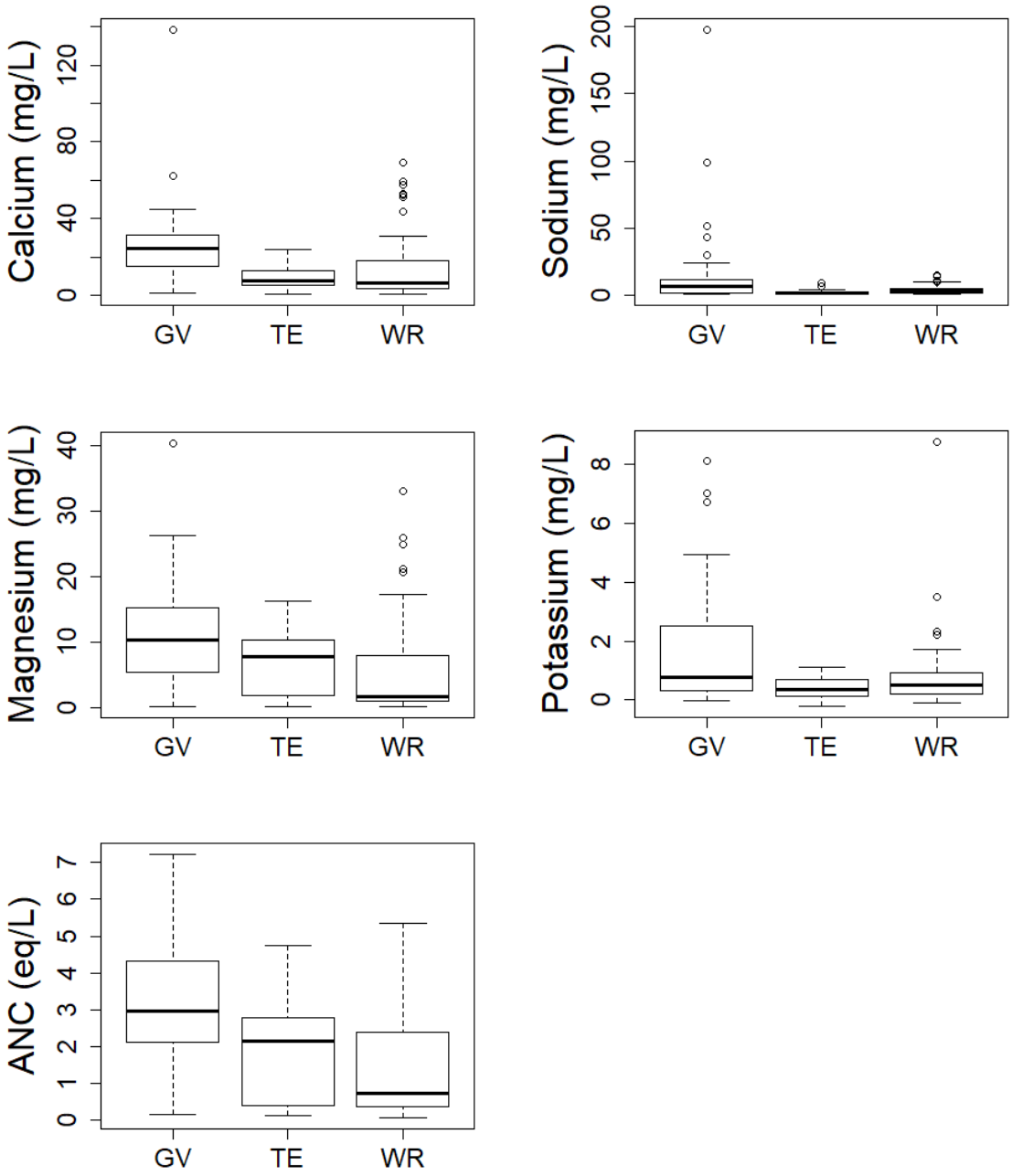


Figure 17. Concentrations of the cations calcium (upper left), sodium (upper right), magnesium (middle left) and potassium (middle right), and acid neutralizing capacity (ANC; lower left) in the Gros Ventre (GV), Teton (TE) and Wind River (WR) Ranges. The bold line is the median value, the box represents the 25th and 75th quartiles, the whiskers are 1.5 times the interquartile range, and the circles are outlier values.

Appendix A: Model Selection Results

Bd

Table A1. Model selection table for eDNA *Bd*. All models included nested random effects to account for clustering of sites within watersheds and wetlands within sites (1|watershed/site). Variable names are defined in table 1.

Variable	**K**	**AIC_c**	**ΔAIC_c**	**w_i**	**-2*LL**
acidHuc	4	96.70	0.00	0.29	-44.18
frostDays	4	97.04	0.34	0.25	-44.35
wetlandEdge	4	97.17	0.47	0.23	-44.42
1	3	97.24	0.55	0.22	-45.52
year	4	97.39	0.69	NA	-44.52
waterTemp	4	97.44	0.74	NA	-44.55
mtnRange	5	97.52	0.83	NA	-43.51
potassium	4	97.75	1.05	NA	-44.70
calcium	4	97.93	1.24	NA	-44.80
magnesium	4	98.01	1.31	NA	-44.84
calWatershed	4	98.43	1.73	NA	-45.05
areaHa	4	98.47	1.77	NA	-45.07
elev	4	98.57	1.87	NA	-45.12
spc	4	98.75	2.05	NA	-45.20
anc	4	98.88	2.18	NA	-45.27
ephemeral	4	98.94	2.24	NA	-45.30
phosphate	4	99.00	2.30	NA	-45.33
nDeposition	4	99.01	2.31	NA	-45.33
pH	4	99.01	2.31	NA	-45.33
chloride	4	99.09	2.39	NA	-45.37
limeWatershed	4	99.12	2.43	NA	-45.39
fish	4	99.12	2.43	NA	-45.39
calHuc	4	99.20	2.51	NA	-45.43
limeHuc	4	99.24	2.54	NA	-45.45
forest	4	99.31	2.62	NA	-45.49
aspect	4	99.32	2.62	NA	-45.49
nSpecies	4	99.32	2.62	NA	-45.49
depth	4	99.34	2.64	NA	-45.50
stream	4	99.35	2.65	NA	-45.50
tpi	4	99.36	2.66	NA	-45.51
acidWatershed	4	99.37	2.68	NA	-45.52
precip	4	99.38	2.68	NA	-45.52
nitrate	4	99.38	2.68	NA	-45.52
julian	4	99.38	2.68	NA	-45.52
sulfate	4	99.38	2.68	NA	-45.52
geologyClass	6	99.96	3.26	NA	-43.62

Table A2. Model selection table for *Bd* occurrence in amphibian skin swabs.

Variable	**K**	**AIC$_c$**	**ΔAIC$_c$**	**w_i**	**-2*LL**
limeHuc	4	60.08	0.00	0.38	-25.53
precip	4	60.58	0.50	0.30	-25.78
tpi	4	62.56	2.48	0.11	-26.77
year	4	63.64	3.56	0.06	-27.31
anc	4	64.14	4.06	0.05	-27.56
limeWatershed	4	64.68	4.60	0.04	-27.83
phosphate	4	64.87	4.79	0.03	-27.92
1	3	65.93	5.85	0.02	-29.66
nSpecies	4	66.11	6.03	NA	-28.54
sulfate	4	66.34	6.26	NA	-28.66
nDeposition	4	66.37	6.29	NA	-28.67
wetlandEdge	4	66.76	6.68	NA	-28.87
chloride	4	67.24	7.16	NA	-29.11
julian	4	67.30	7.22	NA	-29.14
calWatershed	4	67.31	7.23	NA	-29.14
acidHuc	4	67.53	7.45	NA	-29.25
geologyClass	6	67.64	7.56	NA	-26.68
elev	4	67.74	7.66	NA	-29.36
potassium	4	67.85	7.77	NA	-29.41
frostDays	4	67.88	7.80	NA	-29.43
depth	4	67.92	7.84	NA	-29.45
pH	4	67.99	7.91	NA	-29.48
acidWatershed	4	68.00	7.92	NA	-29.49
calcium	4	68.01	7.93	NA	-29.49
stream	4	68.06	7.99	NA	-29.52
fish	4	68.10	8.02	NA	-29.54
nitrate	4	68.28	8.20	NA	-29.63
aspect	4	68.29	8.21	NA	-29.63
forest	4	68.30	8.22	NA	-29.64
spc	4	68.32	8.25	NA	-29.65
waterTemp	4	68.33	8.25	NA	-29.65
areaHa	4	68.34	8.26	NA	-29.66
calHuc	4	68.35	8.27	NA	-29.66
magnesium	4	68.35	8.27	NA	-29.66
ephemeral	4	68.35	8.27	NA	-29.66
mtnRange	5	69.20	9.12	NA	-28.81

Boreal chorus frog

Table A3. Stage 1 model selection results for boreal chorus frog. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
Year	sub-global	19	368.73	0.00	0.92	324.24
method + year	sub-global	20	370.96	2.22	NA	323.71
mtnRange	sub-global	20	374.87	6.14	0.04	327.63
1	sub-global	18	375.15	6.42	0.04	333.35
Julian	sub-global	19	375.90	7.17	NA	331.40
method	sub-global	19	377.37	8.64	NA	332.87
method + mtnRange	sub-global	21	377.53	8.80	NA	327.50
areaHa	sub-global	19	377.84	9.11	NA	333.34
method + julian	sub-global	20	378.14	9.41	NA	330.90
method + areaHa	sub-global	20	380.10	11.37	NA	332.86

Table A4. Stage 2 model selection results for boreal chorus frog. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
year	*fish*	4	352.67	0.00	0.73	211.18
year	*tpi*	4	356.44	3.77	0.11	348.13
year	*waterTemp*	4	357.14	4.48	0.08	348.84
year	*forest*	4	360.12	7.45	0.02	351.82
year	*nDeposition*	4	360.41	7.74	0.02	352.10
year	*nitrate*	4	360.48	7.81	0.01	352.18
year	*potassium*	4	361.00	8.33	0.01	352.70
year	*frostDays*	4	361.05	8.38	0.01	352.74
year	1	3	361.25	8.58	0.01	221.88
year	*limeHuc*	4	361.41	8.74	NA	353.11
year	*stream*	4	361.47	8.81	NA	219.98
year	*aspect*	4	361.49	8.82	NA	353.19
year	*phosphate*	4	361.71	9.04	NA	353.41
year	*year*	4	361.87	9.20	NA	220.38
year	*calcium*	4	362.46	9.79	NA	354.15
year	*acidWatershed*	4	362.59	9.92	NA	354.29
year	*wetlandEdge*	4	362.73	10.06	NA	354.43
year	*precip*	4	362.82	10.16	NA	354.52
year	*acidHuc*	4	362.84	10.17	NA	354.54
year	*julian*	4	362.85	10.18	NA	354.54
year	*limeWatershed*	4	362.86	10.19	NA	354.56
year	*chloride*	4	363.12	10.45	NA	354.82
year	*anc*	4	363.16	10.49	NA	354.85
year	*elev*	4	363.17	10.50	NA	354.87
year	*eDnaBd*	4	363.17	10.51	NA	221.68
year	*sulfate*	4	363.18	10.51	NA	354.87
year	*depth*	4	363.18	10.51	NA	221.69
year	*spc*	4	363.19	10.52	NA	354.88
year	*ephemeral*	4	363.26	10.59	NA	221.77
year	*calHuc*	4	363.31	10.64	NA	355.00

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
year	*calWatershed*	4	363.33	10.66	NA	355.02
year	*areaHa*	4	363.33	10.66	NA	355.03
year	*magnesium*	4	363.34	10.67	NA	355.04
year	*pH*	4	363.36	10.69	NA	355.06
year	*mtnRange*	5	363.74	11.07	NA	220.10
year	*geologyClass*	6	366.84	14.17	NA	221.01

Table A5. Stage 3 model selection results for boreal chorus frog. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
year	*fish + tpi + forest*	6	345.45	0.00	0.32	332.80
year	*fish + forest + frostDays*	6	346.53	1.09	0.19	333.89
year	*fish + forest + nDeposition*	6	346.57	1.12	0.18	333.92
year	*fish + forest*	5	348.16	2.71	0.08	337.70
year	*fish + waterTemp + forest*	6	348.20	2.75	NA	335.56
year	*fish + forest + potassium*	6	349.46	4.01	NA	336.82
year	*fish + forest + nitrate*	6	349.57	4.12	NA	336.92
year	*fish + waterTemp + nDeposition*	6	349.73	4.28	0.04	337.08
year	*waterTemp + forest + frostDays*	6	350.17	4.72	0.03	337.52
year	*fish + waterTemp + frostDays*	6	350.69	5.24	0.02	338.04
year	*fish + tpi*	5	350.80	5.35	0.02	340.34
year	*tpi + forest + frostDays*	6	350.86	5.41	0.02	338.21
year	*fish + tpi + nDeposition*	6	350.89	5.44	NA	338.25
year	*fish + tpi + frostDays*	6	351.02	5.57	NA	338.37
year	*fish + nDeposition*	5	351.41	5.97	0.02	340.96
year	*fish + tpi + waterTemp*	6	351.53	6.08	NA	338.88
year	*fish + nDeposition + nitrate*	6	351.86	6.41	NA	339.21
year	*fish + waterTemp*	5	351.92	6.47	0.01	341.46
year	*fish + tpi + nitrate*	6	352.48	7.03	NA	339.84
year	*fish + tpi + potassium*	6	352.65	7.20	NA	340.00
year	*fish*	4	352.67	7.22	0.01	344.37
year	*waterTemp + nDeposition + frostDays*	6	353.01	7.57	0.01	340.37
year	*fish + nDeposition + frostDays*	6	353.05	7.60	NA	340.40
year	*tpi + waterTemp + frostDays*	6	353.11	7.66	0.01	340.46
year	*tpi + waterTemp + forest*	6	353.17	7.72	0.01	340.52
year	*fish + waterTemp + nitrate*	6	353.40	7.95	NA	340.75
year	*fish + frostDays*	5	353.50	8.05	NA	343.04
year	*fish + nDeposition + potassium*	6	353.58	8.13	NA	340.93
year	*fish + nitrate*	5	353.64	8.19	NA	343.18
year	*tpi + forest*	5	353.74	8.29	0.01	343.28
year	*fish + waterTemp + potassium*	6	353.84	8.39	NA	341.19
year	*waterTemp + frostDays*	5	353.97	8.52	0.00	343.52
year	*fish + nitrate + frostDays*	6	354.02	8.57	NA	341.37
year	*fish + potassium*	5	354.19	8.74	NA	343.73
year	*waterTemp + forest + nDeposition*	6	354.27	8.82	0.00	341.63
year	*tpi + forest + potassium*	6	354.28	8.84	NA	341.64
year	*waterTemp + potassium + frostDays*	6	354.47	9.02	NA	341.82
year	*tpi + forest + nDeposition*	6	354.49	9.04	NA	341.84
year	*fish + potassium + frostDays*	6	354.68	9.23	NA	342.03
year	*waterTemp + nitrate + frostDays*	6	354.74	9.30	NA	342.10

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
year	*tpi + forest + nitrate*	6	355.17	9.72	NA	342.53
year	*fish + nitrate + potassium*	6	355.18	9.73	NA	342.54
year	*waterTemp + nDeposition*	5	355.34	9.89	0.00	344.89
year	*tpi + waterTemp*	5	355.51	10.06	0.00	345.05
year	*forest + potassium + frostDays*	6	355.53	10.08	0.00	342.89
year	*waterTemp + nDeposition + nitrate*	6	355.56	10.11	NA	342.91
year	*tpi + waterTemp + nDeposition*	6	355.67	10.22	NA	343.02
year	*tpi + frostDays*	5	355.71	10.26	0.00	345.25
year	*tpi + potassium + frostDays*	6	356.09	10.64	NA	343.45
year	*waterTemp + forest*	5	356.14	10.69	0.00	345.68
year	*tpi*	4	356.44	10.99	0.00	348.13
year	*tpi + nitrate + frostDays*	6	356.60	11.15	NA	343.95
year	*tpi + nDeposition + frostDays*	6	356.88	11.43	NA	344.24
year	*forest + nitrate + frostDays*	6	357.00	11.55	0.00	344.35
year	*tpi + waterTemp + nitrate*	6	357.04	11.59	NA	344.39
year	*tpi + nDeposition*	5	357.04	11.60	NA	346.59
year	*waterTemp + forest + potassium*	6	357.08	11.63	NA	344.43
year	*tpi + waterTemp + potassium*	6	357.08	11.63	NA	344.43
year	*waterTemp*	4	357.14	11.70	0.00	348.84
year	*waterTemp + forest + nitrate*	6	357.17	11.72	NA	344.52
year	*tpi + potassium*	5	357.32	11.87	NA	346.86
year	*waterTemp + nDeposition + potassium*	6	357.44	11.99	NA	344.79
year	*tpi + nitrate*	5	357.50	12.06	NA	347.05
year	*tpi + nDeposition + nitrate*	6	357.69	12.24	NA	345.05
year	*waterTemp + nitrate*	5	357.86	12.42	NA	347.41
year	*forest + frostDays*	5	358.15	12.70	0.00	347.69
year	*forest + nDeposition + nitrate*	6	358.18	12.73	0.00	345.53
year	*forest + nDeposition + frostDays*	6	358.28	12.83	NA	345.64
year	*waterTemp + potassium*	5	358.30	12.85	NA	347.84
year	*tpi + nitrate + potassium*	6	358.42	12.98	NA	345.78
year	*tpi + nDeposition + potassium*	6	358.65	13.20	NA	346.01
year	*nitrate + potassium + frostDays*	6	358.85	13.40	0.00	346.20
year	*nDeposition + nitrate*	5	358.93	13.48	0.00	348.47
year	*waterTemp + nitrate + potassium*	6	359.05	13.60	NA	346.40
year	*forest + nitrate + potassium*	6	359.24	13.80	0.00	346.60
year	*forest + nDeposition*	5	359.44	13.99	0.00	348.98
year	*nDeposition + nitrate + frostDays*	6	359.45	14.00	NA	346.81
year	*forest + potassium*	5	359.56	14.11	0.00	349.10
year	*forest + nitrate*	5	359.58	14.13	0.00	349.12
year	*nitrate + frostDays*	5	359.88	14.43	0.00	349.42
year	*potassium + frostDays*	5	359.92	14.47	0.00	349.46
year	*forest*	4	360.12	14.67	0.00	351.82
year	*nDeposition + nitrate + potassium*	6	360.32	14.87	NA	347.67
year	*forest + nDeposition + potassium*	6	360.36	14.91	NA	347.71
year	*nitrate + potassium*	5	360.36	14.92	0.00	349.91
year	*nDeposition*	4	360.41	14.96	0.00	352.10
year	*nitrate*	4	360.48	15.03	0.00	352.18
year	*nDeposition + frostDays*	5	361.00	15.55	NA	350.54
year	*potassium*	4	361.00	15.55	0.00	352.70
year	*frostDays*	4	361.05	15.60	0.00	352.74
year	1	3	361.25	15.80	0.00	355.07
year	*nDeposition + potassium + frostDays*	6	361.44	15.99	NA	348.79
year	*nDeposition + potassium*	5	361.53	16.09	NA	351.08

Boreal toad

Table A6. Stage 1 model selection results for boreal toad. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	sub-global	11	112.64	0.00	0.82	88.53
method + year	sub-global	12	115.01	2.38	NA	88.50
method + areaHa	sub-global	12	115.03	2.40	NA	88.52
1	sub-global	10	115.74	3.10	0.18	93.99
year	sub-global	11	117.47	4.83	NA	93.36
areaHa	sub-global	11	118.10	5.46	NA	93.99
method + mtnRange	sub-global	13	118.47	5.84	NA	89.51
mtnRange	sub-global	12	120.41	7.77	NA	93.89
method	sub-global	11	112.64	0.00	0.82	88.53
method + year	sub-global	12	115.01	2.38	NA	88.50

Table A7. Stage 2 model selection results for boreal toad. Variable names are defined in table 1.

Parameter		K	AIC*c*	ΔAIC*c*	w_i	-2*LL
p	**ψ**					
method	*calWatershed*	4	117.20	0.00	0.89	108.90
method	*calcium*	4	121.78	4.58	0.09	113.48
method	*limeWatershed*	4	127.42	10.21	0.01	119.11
method	*year*	4	127.44	10.24	0.01	58.18
method	*calHuc*	4	130.27	13.06	0.00	121.97
method	*precip*	4	130.71	13.50	0.00	122.40
method	*geologyClass*	6	130.95	13.74	0.00	57.34
method	*acidWatershed*	4	131.13	13.93	0.00	122.83
method	*nDeposition*	4	131.15	13.95	0.00	122.85
method	*stream*	4	131.86	14.66	0.00	62.60
method	*limeHuc*	4	132.42	15.21	0.00	124.11
method	1	3	132.95	15.74	0.00	65.81
method	*julian*	4	133.14	15.94	NA	124.84
method	*acidHuc*	4	133.70	16.50	NA	125.40
method	*elev*	4	133.70	16.50	NA	125.40
method	*spc*	4	133.90	16.70	NA	125.60
method	*magnesium*	4	133.91	16.70	NA	125.60
method	*aspect*	4	134.53	17.33	NA	126.23
method	*anc*	4	134.56	17.36	NA	126.26
method	*pH*	4	134.59	17.38	NA	126.28
method	*nitrate*	4	134.62	17.41	NA	126.32
method	*mtnRange*	5	134.64	17.43	NA	63.22
method	*tpi*	4	134.68	17.47	NA	126.37
method	*wetlandEdge*	4	134.75	17.54	NA	126.45
method	*chloride*	4	134.75	17.55	NA	126.45
method	*phosphate*	4	134.86	17.66	NA	126.56
method	*potassium*	4	134.87	17.67	NA	126.57
method	*depth*	4	134.89	17.68	NA	65.63
method	*ephemeral*	4	134.89	17.69	NA	65.63
method	*fish*	4	134.91	17.70	NA	65.64
method	*sulfate*	4	134.95	17.75	NA	126.65
method	*eDnaBd*	4	134.97	17.77	NA	65.71

Parameter		K	AIC*c*	ΔAIC*c*	w_i	-2*LL
p	**ψ**					
method	*areaHa*	4	135.02	17.81	NA	126.71
method	*waterTemp*	4	135.03	17.82	NA	126.73
method	*frostDays*	4	135.03	17.83	NA	126.73
method	*forest*	4	135.04	17.83	NA	126.74

Table A8. Stage 3 model selection results for boreal toad. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	*calcium + limeWatershed*	5	109.62	0.00	0.81	99.16
method	*calWatershed + stream*	5	114.60	4.97	0.07	104.14
method	*calWatershed + limeWatershed*	5	116.18	6.56	0.03	105.72
method	*calWatershed + year*	5	116.33	6.71	0.03	105.87
method	*calWatershed + calcium*	5	116.60	6.98	0.02	106.14
method	*calWatershed*	4	117.20	7.58	0.02	108.90
method	*calWatershed + precip*	5	118.65	9.03	NA	108.19
method	*calcium + limeHuc*	5	118.87	9.24	0.01	108.41
method	*calcium + year*	5	118.93	9.31	0.01	108.47
method	*calWatershed + calHuc*	5	119.01	9.39	NA	108.55
method	*calWatershed + limeHuc*	5	119.05	9.43	NA	108.59
method	*calWatershed + nDeposition*	5	119.17	9.55	NA	108.71
method	*calWatershed + acidWatershed*	5	119.19	9.57	NA	108.74
method	*calcium + stream*	5	120.43	10.81	0.00	109.97
method	*limeWatershed + acidWatershed*	5	121.72	12.10	0.00	111.26
method	*calcium*	4	121.78	12.16	0.00	113.48
method	*calcium + calHuc*	5	123.14	13.52	NA	112.69
method	*calcium + precip*	5	123.54	13.92	NA	113.08
method	*calcium + acidWatershed*	5	123.91	14.29	NA	113.45
method	*calcium + nDeposition*	5	123.93	14.31	NA	113.47
method	*limeWatershed + stream*	5	124.27	14.65	0.00	113.81
method	*year + nDeposition*	5	125.18	15.56	0.00	114.72
method	*year + stream*	5	125.27	15.65	0.00	114.81
method	*year + acidWatershed*	5	125.49	15.87	0.00	115.03
method	*limeWatershed + calHuc*	5	125.97	16.35	0.00	115.51
method	*limeWatershed + nDeposition*	5	126.18	16.55	0.00	115.72
method	*limeWatershed + year*	5	126.36	16.74	0.00	115.90
method	*year + calHuc*	5	126.78	17.15	0.00	116.32
method	*year + precip*	5	126.87	17.25	0.00	116.41
method	*limeWatershed + precip*	5	127.17	17.55	0.00	116.72
method	*limeWatershed*	4	127.42	17.79	0.00	119.11
method	*year*	4	127.44	17.82	0.00	119.14
method	*calHuc + stream*	5	128.37	18.75	0.00	117.92
method	*acidWatershed + limeHuc*	5	128.97	19.34	0.00	118.51
method	*year + limeHuc*	5	129.21	19.59	NA	118.75
method	*year + geologyClass*	7	129.36	19.74	NA	114.49
method	*precip + acidWatershed*	5	129.69	20.07	0.00	119.23
method	*geologyClass + stream*	7	129.81	20.19	0.00	114.94
method	*acidWatershed + stream*	5	129.93	20.31	0.00	119.48
method	*calHuc + precip*	5	130.11	20.49	0.00	119.66
method	*calHuc*	4	130.27	20.65	0.00	121.97
method	*calHuc + nDeposition*	5	130.36	20.74	NA	119.90
method	*nDeposition + stream*	5	130.43	20.81	0.00	119.97

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	*limeHuc + stream*	5	130.70	21.08	0.00	120.25
method	*precip*	4	130.71	21.08	0.00	122.40
method	*precip + stream*	5	130.74	21.12	NA	120.28
method	*nDeposition + limeHuc*	5	130.79	21.17	0.00	120.34
method	*acidWatershed + nDeposition*	5	130.84	21.22	0.00	120.38
method	*calHuc + limeHuc*	5	130.93	21.31	NA	120.47
method	*geologyClass*	6	130.95	21.32	0.00	118.30
method	*acidWatershed*	4	131.13	21.51	0.00	122.83
method	*nDeposition*	4	131.15	21.53	0.00	122.85
method	*precip + limeHuc*	5	131.74	22.12	NA	121.28
method	*stream*	4	131.86	22.24	0.00	123.56
method	*limeHuc*	4	132.42	22.79	0.00	124.11
method	1	3	132.95	23.33	0.00	126.77

Columbia spotted frog

Table A9. Stage 1 model selection results for Columbia spotted frog. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
P	**ψ**					
method + areaHa	sub-global	14	160.99	0.00	0.65	129.55
method + year	sub-global	14	163.20	2.21	0.22	131.76
method	sub-global	13	164.13	3.14	0.14	135.17
method + mtnRange	sub-global	15	164.81	3.83	NA	130.85
method + julian	sub-global	14	166.54	5.55	NA	135.10
1	sub-global	12	187.16	26.17	0.00	160.64
Year	sub-global	13	188.15	27.16	NA	159.19
mtnRange	sub-global	14	188.90	27.92	NA	157.46
areaHa	sub-global	13	189.26	28.27	NA	160.30
Julian	sub-global	13	189.60	28.61	NA	160.64

Table A10. Stage 2 model selection results for Columbia spotted frog. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
P	**ψ**					
method	*elev*	4	188.60	0.00	0.94	180.29
method	*mtnRange*	5	195.20	6.61	0.03	118.24
method	*precip*	4	196.62	8.02	0.02	188.31
method	*areaHa*	4	201.23	12.63	0.00	192.93
method	*eDnaBd*	4	202.11	13.51	0.00	127.30
method	*frostDays*	4	202.90	14.31	0.00	194.60
method	*acidWatershed*	4	202.96	14.36	0.00	194.66
method	*depth*	4	204.40	15.80	0.00	129.59
method	*potassium*	4	204.46	15.87	0.00	196.16
method	*geologyClass*	6	204.53	15.93	0.00	125.38
method	*phosphate*	4	205.16	16.57	0.00	196.86
method	*pH*	4	205.33	16.73	0.00	197.02
method	*chloride*	4	205.56	16.97	0.00	197.26
method	*forest*	4	205.59	16.99	0.00	197.29
method	*acidHuc*	4	205.75	17.15	0.00	197.44
method	1	3	205.99	17.39	0.00	133.30
method	*nitrate*	4	206.11	17.51	NA	197.80
method	*limeWatershed*	4	206.32	17.72	NA	198.02
method	*tpi*	4	206.55	17.96	NA	198.25
method	*julian*	4	207.21	18.61	NA	198.90
method	*spc*	4	207.25	18.65	NA	198.95
method	*anc*	4	207.25	18.66	NA	198.95
method	*waterTemp*	4	207.39	18.80	NA	199.09
method	*calHuc*	4	207.40	18.80	NA	199.10
method	*limeHuc*	4	207.76	19.17	NA	199.46
method	*calcium*	4	207.78	19.19	NA	199.48
method	*nDep*	4	207.79	19.19	NA	199.49
method	*stream*	4	207.90	19.30	NA	133.09
method	*fish*	4	207.97	19.38	NA	133.16
method	*sulfate*	4	208.02	19.42	NA	199.71

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
P	**ψ**					
method	*magnesium*	4	208.03	19.43	NA	199.73
method	*ephemeral*	4	208.05	19.45	NA	133.24
method	*calWatershed*	4	208.09	19.49	NA	199.78
method	*wetEdge*	4	208.09	19.49	NA	199.79
method	*year*	4	208.09	19.49	NA	133.28
method	*aspect*	4	208.09	19.50	NA	199.79

Table A11. Stage 3 model selection results for Columbia spotted frog. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	*elev + eDnaBd*	5	180.48	0.00	0.64	170.02
method	*elev + precip*	5	182.59	2.11	0.22	172.13
method	*elev + acidHuc*	5	185.54	5.06	0.05	175.08
method	*elev + mtnRange*	6	187.51	7.03	0.02	174.86
method	*elev + phosphate*	5	187.88	7.40	0.02	177.43
method	*elev + acidWatershed*	5	187.90	7.42	0.02	177.44
method	*Elev*	4	188.60	8.11	0.01	180.29
method	*elev + areaHa*	5	188.74	8.26	NA	178.28
method	*elev + forestPct*	5	188.79	8.31	NA	178.33
method	*elev + depth*	5	189.90	9.42	NA	179.45
method	*elev + potassium*	5	190.01	9.52	NA	179.55
method	*elev + pH*	5	190.04	9.56	NA	179.58
method	*mtnRange + eDnaBd*	6	190.70	10.22	0.00	178.06
method	*elev + chloride*	5	190.74	10.25	NA	180.28
method	*mtnRange + geologyClass*	8	191.05	10.56	0.00	173.92
method	*mtnRange + phosphate*	6	191.23	10.75	0.00	178.58
method	*precip + eDnaBd*	5	191.29	10.80	0.00	180.83
method	*precip + potassium*	5	191.70	11.22	0.00	181.24
method	*precip + acidWatershed*	5	192.06	11.58	0.00	181.60
method	*mtnRange + areaHa*	6	192.37	11.89	0.00	179.72
method	*mtnRange + forestPct*	6	192.72	12.23	0.00	180.07
method	*precip + chloride*	5	193.28	12.80	0.00	182.82
method	*precip + areaHa*	5	193.55	13.07	0.00	183.09
method	*areaHa + eDnaBd*	5	193.90	13.42	0.00	183.44
method	*precip + phosphate*	5	193.90	13.42	0.00	183.44
method	*mtnRange + precip*	6	193.97	13.49	0.00	181.33
method	*mtnRange + potassium*	6	194.48	14.00	0.00	181.84
method	*mtnRange + frostDays*	6	194.49	14.01	0.00	181.84
method	*precip + acidHuc*	5	194.52	14.04	0.00	184.06
method	*precip + depth*	5	194.60	14.12	0.00	184.14
method	*mtnRange + depth*	6	195.00	14.52	0.00	182.36
method	*mtnRange + acidWatershed*	6	195.10	14.62	0.00	182.45
method	*mtnRange*	5	195.20	14.72	0.00	184.75
method	*precip + frostDays*	5	195.62	15.13	0.00	185.16
method	*precip + forestPct*	5	195.74	15.26	0.00	185.28
method	*mtnRange + chloride*	6	195.88	15.40	NA	183.23
method	*areaHa + depth*	5	196.30	15.81	0.00	185.84
method	*eDnaBd + frostDays*	5	196.32	15.84	0.00	185.86
method	*precip*	4	196.62	16.14	0.00	188.31
method	*mtnRange + acidHuc*	6	197.00	16.52	NA	184.35

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	*mtnRange + pH*	6	197.37	16.89	NA	184.73
method	*precip + pH*	5	197.92	17.44	NA	187.46
method	*eDnaBd + acidWatershed*	5	198.69	18.21	0.00	188.23
method	*areaHa + acidWatershed*	5	199.31	18.83	0.00	188.85
method	*eDnaBd + potassium*	5	199.65	19.17	0.00	189.19
method	*frostDays + acidHuc*	5	199.67	19.19	0.00	189.21
method	*eDnaBd + depth*	5	200.19	19.71	0.00	189.73
method	*areaHa + frostDays*	5	200.26	19.78	0.00	189.80
method	*areaHa + potassium*	5	200.27	19.79	0.00	189.82
method	*frostDays + acidWatershed*	5	200.36	19.87	0.00	189.90
method	*areaHa + pH*	5	200.41	19.93	0.00	189.95
method	*acidWatershed + depth*	5	200.50	20.02	0.00	190.04
method	*eDnaBd + pH*	5	200.50	20.02	0.00	190.04
method	*areaHa + acidHuc*	5	200.56	20.08	0.00	190.11
method	*eDnaBd + phosphate*	5	200.89	20.41	0.00	190.43
method	*areaHa + phosphate*	5	201.12	20.64	0.00	190.66
method	*areaHa*	4	201.23	20.75	0.00	192.93
method	*eDnaBd + chloride*	5	201.25	20.77	0.00	190.80
method	*areaHa + forestPct*	5	201.29	20.80	NA	190.83
method	*acidWatershed + forestPct*	5	201.36	20.88	0.00	190.90
method	*areaHa + chloride*	5	201.73	21.25	NA	191.27
method	*eDnaBd + forestPct*	5	201.74	21.26	0.00	191.29
method	*frostDays + phosphate*	5	201.88	21.39	0.00	191.42
method	*eDnaBd*	4	202.11	21.62	0.00	193.80
method	*eDnaBd + geologyClass*	7	202.47	21.99	NA	187.60
method	*frostDays + potassium*	5	202.65	22.16	0.00	192.19
method	*acidWatershed + potassium*	5	202.70	22.22	0.00	192.25
method	*eDnaBd + acidHuc*	5	202.71	22.22	NA	192.25
method	*phosphate + pH*	5	202.72	22.24	0.00	192.26
method	*acidWatershed + phosphate*	5	202.81	22.32	0.00	192.35
method	*depth + pH*	5	202.82	22.34	0.00	192.36
method	*frostDays + forestPct*	5	202.87	22.38	0.00	192.41
method	*frostDays*	4	202.90	22.42	0.00	194.60
method	*acidWatershed*	4	202.96	22.48	0.00	194.66
method	*frostDays + depth*	5	203.03	22.54	NA	192.57
method	*depth + phosphate*	5	203.34	22.86	0.00	192.88
method	*potassium + forestPct*	5	203.47	22.99	0.00	193.02
method	*frostDays + pH*	5	203.51	23.03	NA	193.05
method	*depth + geologyClass*	7	203.57	23.09	0.00	188.70
method	*acidWatershed + chloride*	5	203.78	23.30	NA	193.33
method	*depth + potassium*	5	203.79	23.31	0.00	193.33
method	*forestPct + acidHuc*	5	204.06	23.58	0.00	193.61
method	*acidWatershed + pH*	5	204.12	23.63	NA	193.66
method	*frostDays + chloride*	5	204.19	23.71	NA	193.74
method	*depth + forestPct*	5	204.24	23.75	0.00	193.78
method	*chloride + forestPct*	5	204.27	23.79	0.00	193.81
method	*potassium + phosphate*	5	204.30	23.82	0.00	193.84
method	*depth + chloride*	5	204.36	23.88	0.00	193.90
method	*depth*	4	204.40	23.91	0.00	196.09
method	*depth + acidHuc*	5	204.44	23.96	NA	193.98
method	*potassium*	4	204.46	23.98	0.00	196.16
method	*geologyClass*	6	204.53	24.05	0.00	191.88
method	*phosphate + acidHuc*	5	204.54	24.06	0.00	194.08
method	*phosphate + chloride*	5	204.62	24.14	0.00	194.17

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	*potassium + pH*	5	204.78	24.30	NA	194.32
method	*potassium + acidHuc*	5	204.91	24.43	NA	194.46
method	*phosphate*	4	205.16	24.68	0.00	196.86
method	*pH + forestPct*	5	205.32	24.83	0.00	194.86
method	*pH*	4	205.33	24.85	0.00	197.02
method	*pH + chloride*	5	205.51	25.02	NA	195.05
method	*chloride*	4	205.56	25.08	0.00	197.26
method	*forestPct*	4	205.59	25.11	0.00	197.29
method	*phosphate + forestPct*	5	205.61	25.13	NA	195.15
method	*acidHuc*	4	205.75	25.26	0.00	197.44
method	*pH + acidHuc*	5	205.81	25.33	NA	195.35
method	*chloride + acidHuc*	5	205.83	25.35	NA	195.38
method	1	3	205.99	25.51	0.00	199.81

Tiger salamander

Table A12. Stage 1 model selection results for tiger salamander. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	sub-global	15	212.26	0.00	0.91	178.30
method + mtnRange	sub-global	17	213.28	1.02	NA	174.14
method + julian	sub-global	16	213.80	1.53	NA	177.26
method + areaHa	sub-global	16	214.02	1.76	NA	177.49
method + year	sub-global	16	214.60	2.34	NA	178.07
1	sub-global	14	216.87	4.61	0.09	185.43
mtnRange	sub-global	16	217.44	5.17	NA	180.90
julian	sub-global	15	217.86	5.60	NA	183.89
areaHa	sub-global	15	218.68	6.42	NA	184.72
year	sub-global	15	219.28	7.02	NA	185.31

Table A13. Stage 2 model selection results for tiger salamander. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	*waterTemp*	4	225.43	0.00	0.96	217.13
method	*tpi*	4	234.32	8.89	0.01	226.02
method	*fish*	4	234.86	9.43	0.01	131.82
method	*stream*	4	236.21	10.77	0.00	133.17
method	*chloride*	4	236.81	11.38	0.00	228.51
method	*potassium*	4	237.11	11.68	0.00	228.81
method	*depth*	4	237.36	11.93	0.00	134.32
method	*spc*	4	237.78	12.35	0.00	229.48
method	*magnesium*	4	239.26	13.83	0.00	230.96
method	*calHuc*	4	239.45	14.01	0.00	231.15
method	*elev*	4	239.54	14.11	0.00	231.24
method	*anc*	4	240.21	14.78	0.00	231.91
method	*geologyClass*	6	240.39	14.95	0.00	133.00
method	*wetlandEdge*	4	240.87	15.44	0.00	232.57
method	*mtnRange*	5	240.89	15.46	0.00	135.70
method	*limeHuc*	4	241.88	16.44	0.00	233.57
method	*calWatershed*	4	242.12	16.69	0.00	233.82
method	*calcium*	4	242.21	16.77	0.00	233.90
method	*acidWatershed*	4	242.70	17.27	0.00	234.40
method	*pH*	4	243.02	17.58	0.00	234.71
method	*nitrate*	4	243.22	17.78	0.00	234.91
method	1	3	243.52	18.09	0.00	142.60
method	*sulfate*	4	244.20	18.76	NA	235.89
method	*frostDays*	4	244.52	19.08	NA	236.21
method	*areaHa*	4	244.69	19.26	NA	236.39
method	*year*	4	244.74	19.31	NA	141.70
method	*eDnaBd*	4	244.86	19.42	NA	141.82
method	*limeWatershed*	4	244.87	19.44	NA	236.57
method	*nDeposition*	4	245.01	19.58	NA	236.71
method	*aspect*	4	245.34	19.91	NA	237.04

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	ψ					
method	*precip*	4	245.38	19.95	NA	237.08
method	*acidHuc*	4	245.42	19.98	NA	237.11
method	*forest*	4	245.49	20.06	NA	237.19
method	*julian*	4	245.53	20.10	NA	237.23
method	*ephemeral*	4	245.64	20.20	NA	142.60
method	*phosphate*	4	245.64	20.21	NA	237.34

Table A14. Stage 3 model selection results for tiger salamander. Variable names are defined in table 1.

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	ψ					
method	*waterTemp + spc*	5	218.68	0.00	0.16	208.22
method	*waterTemp + depth*	5	218.72	0.04	0.16	208.26
method	*waterTemp + wetlandEdge*	5	218.85	0.17	0.15	208.39
method	*waterTemp + chloride*	5	219.58	0.90	0.10	209.12
method	*waterTemp + anc*	5	220.73	2.05	0.06	210.27
method	*waterTemp + magnesium*	5	220.91	2.22	0.05	210.45
method	*waterTemp + fish*	5	221.03	2.35	0.05	210.58
method	*waterTemp + potassium*	5	221.21	2.52	0.05	210.75
method	*waterTemp + tpi*	5	221.44	2.76	0.04	210.99
method	*waterTemp + mtnRange*	6	222.02	3.34	0.03	209.37
method	*tpi + magnesium*	5	222.37	3.68	0.03	211.91
method	*waterTemp + calHuc*	5	222.60	3.91	0.02	212.14
method	*waterTemp + acidWatershed*	5	223.28	4.60	0.02	212.82
method	*waterTemp + elev*	5	223.54	4.86	0.01	213.08
method	*tpi + spc*	5	223.64	4.96	0.01	213.18
method	*waterTemp + calcium*	5	223.77	5.09	0.01	213.32
method	*waterTemp + stream*	5	224.68	6.00	0.01	214.22
method	*fish + depth*	5	225.02	6.34	0.01	214.57
method	*waterTemp*	4	225.43	6.75	0.01	217.13
method	*waterTemp + pH*	5	225.94	7.26	NA	215.48
method	*waterTemp + limeHuc*	5	226.08	7.40	NA	215.62
method	*tpi + anc*	5	226.27	7.59	0.00	215.81
method	*waterTemp + nitrate*	5	226.81	8.12	NA	216.35
method	*tpi + stream*	5	227.32	8.64	0.00	216.87
method	*fish + calHuc*	5	227.53	8.85	0.00	217.07
method	*fish + spc*	5	228.85	10.17	0.00	218.39
method	*tpi + fish*	5	228.96	10.28	0.00	218.50
method	*tpi + chloride*	5	228.99	10.31	0.00	218.54
method	*tpi + potassium*	5	229.01	10.33	0.00	218.56
method	*tpi + elev*	5	229.24	10.56	0.00	218.78
method	*fish + stream*	5	229.30	10.62	0.00	218.84
method	*stream + magnesium*	5	229.48	10.80	0.00	219.02
method	*tpi + depth*	5	229.48	10.80	0.00	219.02
method	*fish + chloride*	5	229.58	10.90	0.00	219.13
method	*tpi + calHuc*	5	229.88	11.20	0.00	219.42
method	*tpi + acidWatershed*	5	229.88	11.20	0.00	219.42
method	*stream + spc*	5	229.91	11.23	0.00	219.45
method	*fish + elev*	5	230.00	11.32	0.00	219.54
method	*stream + chloride*	5	230.46	11.78	0.00	220.00
method	*tpi + pH*	5	230.83	12.15	0.00	220.37

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	*tpi + mtnRange*	6	230.93	12.25	0.00	218.28
method	*stream + anc*	5	231.04	12.36	0.00	220.58
method	*tpi + calcium*	5	231.07	12.39	0.00	220.61
method	*fish + magnesium*	5	231.22	12.54	0.00	220.76
method	*tpi + wetlandEdge*	5	231.32	12.64	0.00	220.86
method	*fish + potassium*	5	231.36	12.68	0.00	220.90
method	*depth + anc*	5	231.61	12.93	0.00	221.15
method	*fish + anc*	5	231.62	12.94	0.00	221.16
method	*chloride + depth*	5	231.73	13.05	0.00	221.27
method	*stream + potassium*	5	231.84	13.16	0.00	221.38
method	*stream + elev*	5	232.07	13.38	0.00	221.61
method	*stream + wetlandEdge*	5	232.11	13.43	0.00	221.65
method	*fish + geologyClass*	7	232.28	13.60	0.00	217.42
method	*depth + magnesium*	5	232.29	13.61	0.00	221.83
method	*depth + calHuc*	5	232.37	13.69	0.00	221.92
method	*stream + depth*	5	232.62	13.94	0.00	222.16
method	*fish + mtnRange*	6	232.65	13.97	0.00	220.00
method	*depth + spc*	5	232.67	13.99	0.00	222.22
method	*stream + calHuc*	5	232.91	14.23	0.00	222.46
method	*fish + wetlandEdge*	5	232.98	14.30	0.00	222.53
method	*stream + geologyClass*	7	233.04	14.36	0.00	218.17
method	*fish + calcium*	5	233.39	14.71	0.00	222.94
method	*potassium + depth*	5	233.53	14.85	0.00	223.07
method	*fish + pH*	5	233.62	14.94	0.00	223.16
method	*fish + acidWatershed*	5	233.70	15.01	0.00	223.24
method	*chloride + limeHuc*	5	233.76	15.08	0.00	223.31
method	*spc + limeHuc*	5	234.04	15.36	0.00	223.58
method	*tpi*	4	234.32	15.64	0.00	226.02
method	*fish + limeHuc*	5	234.38	15.70	0.00	223.92
method	*depth + wetlandEdge*	5	234.43	15.75	0.00	223.97
method	*stream + pH*	5	234.47	15.79	0.00	224.01
method	*anc + limeHuc*	5	234.66	15.98	0.00	224.21
method	*fish*	4	234.86	16.18	0.00	226.56
method	*chloride + calHuc*	5	234.93	16.25	0.00	224.47
method	*stream + calcium*	5	235.19	16.51	0.00	224.74
method	*stream + limeHuc*	5	235.27	16.59	0.00	224.82
method	*depth + mtnRange*	6	235.27	16.59	0.00	222.63
method	*fish + nitrate*	5	235.33	16.65	NA	224.87
method	*stream + acidWatershed*	5	235.36	16.68	0.00	224.90
method	*depth + pH*	5	235.40	16.72	0.00	224.95
method	*magnesium + limeHuc*	5	235.47	16.79	0.00	225.01
method	*chloride + spc*	5	235.50	16.81	0.00	225.04
method	*depth + acidWatershed*	5	235.54	16.86	0.00	225.08
method	*tpi + nitrate*	5	235.62	16.94	NA	225.16
method	*stream + mtnRange*	6	235.84	17.16	0.00	223.20
method	*potassium + spc*	5	236.02	17.34	0.00	225.56
method	*depth + calcium*	5	236.03	17.35	0.00	225.57
method	*chloride + magnesium*	5	236.04	17.36	0.00	225.59
method	*depth + elev*	5	236.05	17.37	0.00	225.59
method	*tpi + limeHuc*	5	236.14	17.46	NA	225.68
method	*chloride + nitrate*	5	236.15	17.47	0.00	225.69
method	*stream*	4	236.21	17.53	0.00	227.91
method	*potassium + calHuc*	5	236.31	17.63	0.00	225.85
method	*calHuc + elev*	5	236.31	17.63	0.00	225.85

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	*mtnRange + limeHuc*	6	236.38	17.70	0.00	223.74
method	*potassium + limeHuc*	5	236.39	17.71	0.00	225.93
method	*potassium + magnesium*	5	236.47	17.79	0.00	226.02
method	*elev + limeHuc*	5	236.63	17.95	0.00	226.17
method	*potassium + wetlandEdge*	5	236.64	17.96	0.00	226.19
method	*potassium + nitrate*	5	236.66	17.98	0.00	226.20
method	*potassium + anc*	5	236.67	17.99	0.00	226.21
method	*chloride + wetlandEdge*	5	236.74	18.06	0.00	226.28
method	*chloride*	4	236.81	18.13	0.00	228.51
method	*depth + limeHuc*	5	236.86	18.18	0.00	226.40
method	*potassium + elev*	5	236.88	18.20	0.00	226.42
method	*stream + nitrate*	5	236.99	18.31	NA	226.53
method	*potassium*	4	237.11	18.43	0.00	228.81
method	*spc + nitrate*	5	237.16	18.48	0.00	226.70
method	*chloride + calcium*	5	237.20	18.52	NA	226.74
method	*chloride + elev*	5	237.27	18.59	NA	226.81
method	*depth + geologyClass*	7	237.30	18.62	0.00	222.43
method	*chloride + mtnRange*	6	237.30	18.62	NA	224.66
method	*chloride + pH*	5	237.31	18.63	NA	226.85
method	*depth*	4	237.36	18.68	0.00	229.06
method	*potassium + pH*	5	237.36	18.68	NA	226.91
method	*spc + wetlandEdge*	5	237.46	18.77	0.00	227.00
method	*calHuc + wetlandEdge*	5	237.50	18.81	0.00	227.04
method	*potassium + mtnRange*	6	237.60	18.92	NA	224.95
method	*spc + elev*	5	237.61	18.93	0.00	227.15
method	*depth + nitrate*	5	237.77	19.09	NA	227.31
method	*spc*	4	237.78	19.10	0.00	229.48
method	*chloride + acidWatershed*	5	237.87	19.19	NA	227.41
method	*spc + calHuc*	5	237.90	19.22	NA	227.44
method	*magnesium + nitrate*	5	238.05	19.37	0.00	227.59
method	*potassium + acidWatershed*	5	238.17	19.49	NA	227.71
method	*magnesium + wetlandEdge*	5	238.25	19.57	0.00	227.79
method	*chloride + anc*	5	238.30	19.62	NA	227.84
method	*magnesium + calHuc*	5	238.34	19.66	0.00	227.88
method	*limeHuc + pH*	5	238.59	19.91	0.00	228.13
method	*elev + wetlandEdge*	5	238.63	19.95	0.00	228.17
method	*magnesium + elev*	5	238.77	20.09	0.00	228.32
method	*anc + nitrate*	5	238.80	20.12	0.00	228.34
method	*potassium + calcium*	5	238.80	20.12	NA	228.34
method	*limeHuc + acidWatershed*	5	238.84	20.15	0.00	228.38
method	*calHuc + anc*	5	238.86	20.18	0.00	228.40
method	*calHuc + limeHuc*	5	238.95	20.27	0.00	228.49
method	*spc + mtnRange*	6	239.00	20.32	NA	226.35
method	*elev + nitrate*	5	239.06	20.38	0.00	228.60
method	*magnesium*	4	239.26	20.58	0.00	230.96
method	*calHuc*	4	239.45	20.77	0.00	231.15
method	*elev + anc*	5	239.46	20.77	0.00	229.00
method	*spc + pH*	5	239.53	20.85	NA	229.08
method	*Elev*	4	239.54	20.86	0.00	231.24
method	*anc + wetlandEdge*	5	239.57	20.88	0.00	229.11
method	*spc + acidWatershed*	5	239.60	20.92	NA	229.14
method	*elev + mtnRange*	6	239.64	20.95	NA	226.99
method	*calHuc + nitrate*	5	239.66	20.98	NA	229.20
method	*wetlandEdge + mtnRange*	6	239.72	21.04	0.00	227.07

Parameter		K	AIC_c	ΔAIC_c	w_i	-2*LL
p	**ψ**					
method	*elev + calcium*	5	239.74	21.06	NA	229.29
method	*wetlandEdge + nitrate*	5	239.80	21.12	0.00	229.34
method	*magnesium + mtnRange*	6	239.82	21.14	NA	227.17
method	*geologyClass + mtnRange*	8	239.89	21.21	0.00	222.77
method	*limeHuc + calcium*	5	240.12	21.44	0.00	229.66
method	*Anc*	4	240.21	21.53	0.00	231.91
method	*elev + acidWatershed*	5	240.32	21.64	NA	229.86
method	*calHuc + mtnRange*	6	240.34	21.66	NA	227.69
method	*geologyClass*	6	240.39	21.71	0.00	227.74
method	*calHuc + pH*	5	240.52	21.83	NA	230.06
method	*mtnRange + nitrate*	6	240.55	21.86	0.00	227.90
method	*elev + pH*	5	240.57	21.89	NA	230.11
method	*calHuc + calcium*	5	240.58	21.90	NA	230.12
method	*wetlandEdge + calcium*	5	240.81	22.13	0.00	230.35
method	*wetlandEdge*	4	240.87	22.19	0.00	232.57
method	*mtnRange*	5	240.89	22.21	0.00	230.44
method	*anc + mtnRange*	6	240.94	22.26	NA	228.29
method	*wetlandEdge + acidWatershed*	5	241.10	22.41	NA	230.64
method	*magnesium + acidWatershed*	5	241.12	22.44	NA	230.66
method	*calHuc + acidWatershed*	5	241.16	22.48	NA	230.71
method	*magnesium + pH*	5	241.19	22.51	NA	230.73
method	*mtnRange + calcium*	6	241.42	22.74	NA	228.77
method	*calcium + nitrate*	5	241.43	22.75	0.00	230.97
method	*anc + calcium*	5	241.51	22.83	NA	231.05
method	*limeHuc*	4	241.88	23.19	0.00	233.57
method	*anc + acidWatershed*	5	242.00	23.32	NA	231.54
method	*anc + pH*	5	242.02	23.34	NA	231.57
method	*wetlandEdge + pH*	5	242.04	23.36	NA	231.58
method	*limeHuc + nitrate*	5	242.08	23.40	NA	231.62
method	*acidWatershed + nitrate*	5	242.20	23.52	0.00	231.74
method	*Calcium*	4	242.21	23.53	0.00	233.90
method	*pH + nitrate*	5	242.21	23.53	0.00	231.75
method	*mtnRange + acidWatershed*	6	242.31	23.63	NA	229.67
method	*mtnRange + pH*	6	242.59	23.91	NA	229.95
method	*acidWatershed*	4	242.70	24.02	0.00	234.40
method	*pH*	4	243.02	24.34	0.00	234.71
method	*calcium + pH*	5	243.15	24.47	NA	232.69
method	*calcium + acidWatershed*	5	243.19	24.51	NA	232.73
method	*Nitrate*	4	243.22	24.54	0.00	234.91
method	1	3	243.52	24.84	0.00	237.34
method	*acidWatershed + pH*	5	243.59	24.91	NA	233.13

Appendix B

Variable plots

Figure B1. Variables from competitive models for amphibian occupancy and *Bd* occurrence summarized by mountain range. All variables are zero-centered and scaled for comparison. Violin plots show density of data as colored shapes, with the interquartile range as boxes, medians as bold horizontal lines, 1.5 times the interquartile range as error bars, and outliers as dots. Unique combinations of letters indicate groups that were significantly different in comparisons by ANOVA with Tukey's honest significance test ($\alpha = 0.05$). Variable names are defined in Table 1.

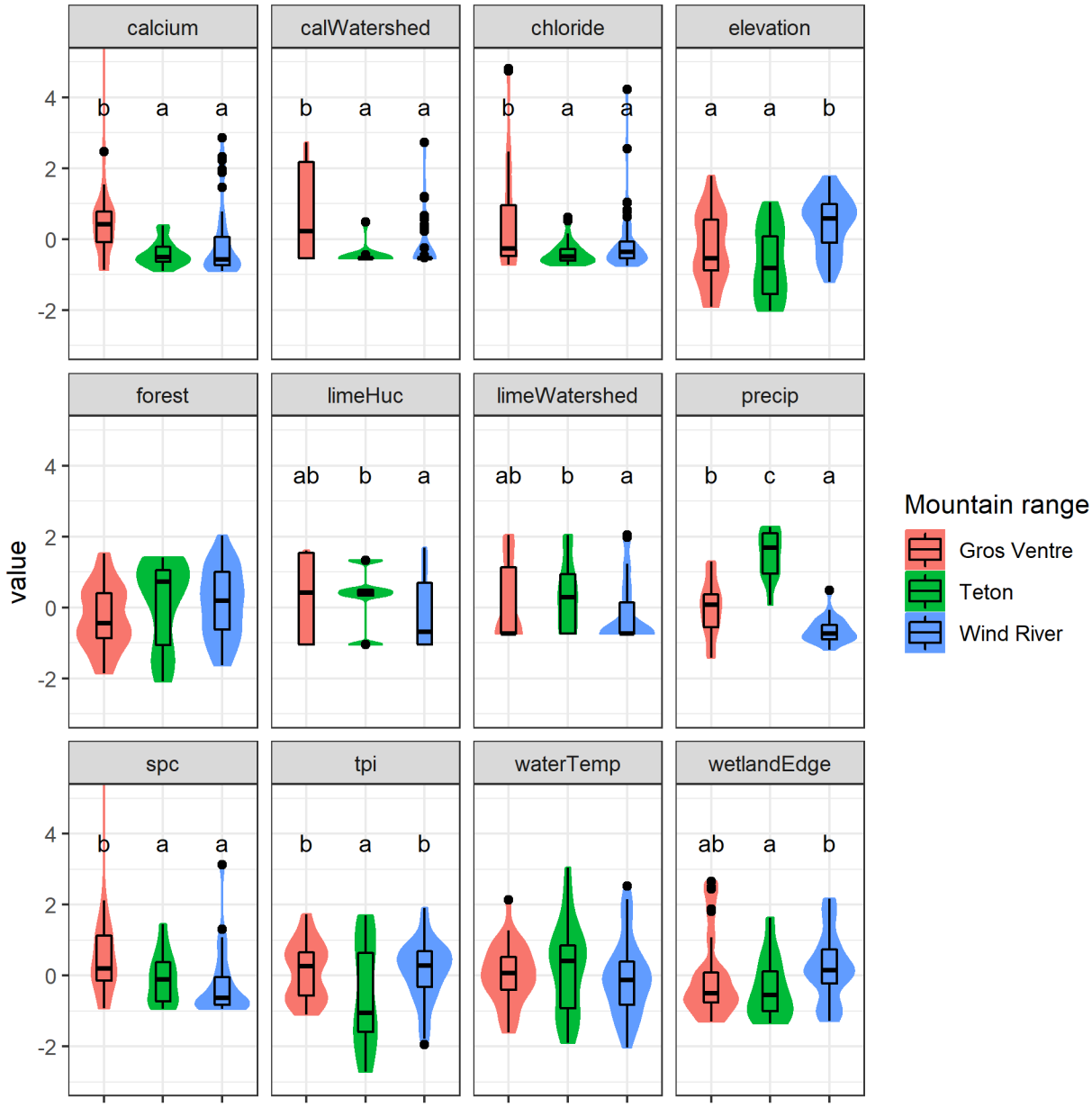
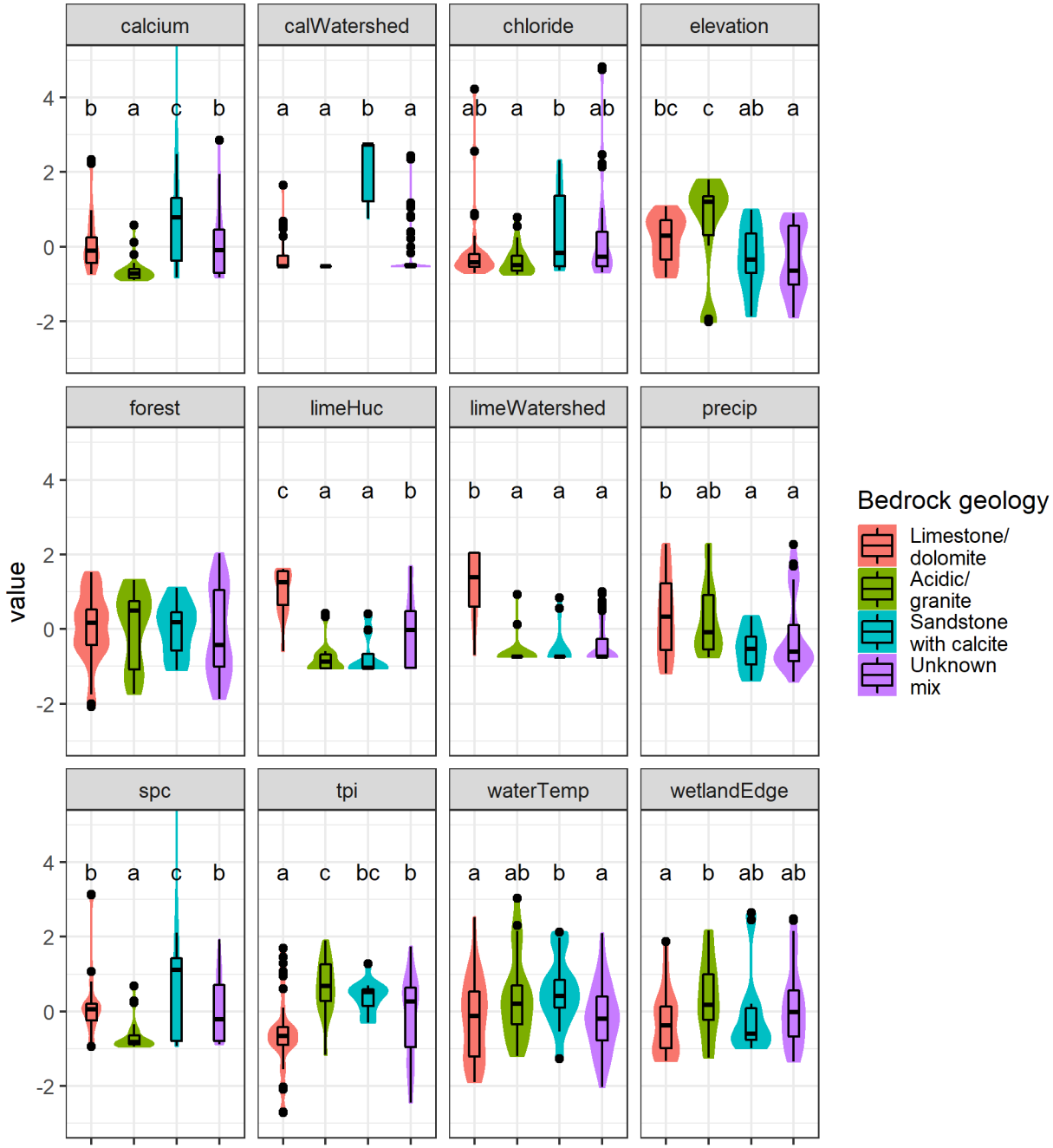


Figure B2. Variables form competitive models for amphibian occupancy and *Bd* occurrence summarized by bedrock geology class. All variables are scaled and zero-centered for comparison. Violin plots show density of data as colored shapes, with the interquartile range as boxes, medians as bold horizontal lines, 1.5 times the interquartile range as error bars, and outliers as dots. Unique combinations of letters indicate groups that were significantly different in comparisons by ANOVA with Tukey's honest significance test ($\alpha = 0.05$). Variable names are defined in Table 1.



Appendix C

Field Data Sheets

Amphibians & Geology

Site and Survey Data

Sub-site			–			–				Surveyors		
Date			/			/	2	0	1	8	Time	

eDNA

Volume filtered (mL)	

eDNA notes (issues with filtering, notes on habitats where samples were collected, …)**:**

Water quality (Recorded with a YSI Pro Plus Multiprobe)

Last calibrated		DO			
Water temperature (°C)			Specific conductivity (SPC, µS/cm)		
Barometric pressure (mmHg)			Conductivity (µS/cm)		
DO (% saturation)			pH		
DO (mg/L)			ORP (mV)		

Water samples taken? (check box) NH_4^+ ☐ ANC ☐ Ions ☐

Site characteristics

Waterbody type (circle all that apply)	Permanent Lake/Pond	Temporary Lake/Pond	Active Beaver Pond	Inactive Beaver Pond		
	Wet Meadow	Marsh/Bog	Spring/Seep	Backwater/Oxbow	Stockpond	Stream
Primary substrate	Silt/mud	Sand	Gravel	Cobble	Boulder/rock	
Maximum water depth	<1 m	>1 m	Water color	Clear	Stained	Turbidity: Clear Cloudy
Fish present?	Yes	No	Fish species			
% of shoreline with emergent vegetation			Snails present?	Yes	No	Snail sample #
Evidence of cattle grazing (current season)	None	Light	Heavy veg	Heavy shore	Heavy veg and shore	
Evidence of cattle grazing (past)	None	Light	Heavy veg	Heavy shore	Heavy veg and shore	
Site photo #		Description				
Geology photo #		Description				
Geol. sample description			HCl test result	None	1	2 3

Site notes (describe wetland size, type, vegetation; geology, additional photos, …)**:**

Survey conditions

Air temperature (°F)		Wind	Calm	Light	Moderate	Strong	Cloud cover (%)	
Precipitation	None	Rain	Snow	Hail	Survey difficulty (explain below)		Low	Medium High

Survey conditions notes (difficulty of survey, changes in weather conditions, …)**:**

Amphibians & Geology

Visual Encounter Survey — Observer 1

Sub-site			–			–			

Date			/			/	2	0	1	8

Observer Name	

Please record all times, including break and resume

Start time		Break		Resume		Stop		Total (min)	

Track name		GPS number		Downloaded?	

Record tracks of survey routes and name with site name-observer #, e.g., "WR0101A1"

Species Detected (record additional species and samples on extra data sheets)

1. Species		Detection Method:	Call Visual Dipnet	# Adults		# Metamorphs	
# Juveniles		# Egg Masses		# Tadpoles	1-25 25-50 50-100 100-500 >500		

Sample #	Type	Sample #	Type
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip

2. Species		Detection Method:	Call Visual Dipnet	# Adults		# Metamorphs	
# Juveniles		# Egg Masses		# Tadpoles	1-25 25-50 50-100 100-500 >500		

Sample #	Type	Sample #	Type
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip

3. Species		Detection Method:	Call Visual Dipnet	# Adults		# Metamorphs	
# Juveniles		# Egg Masses		# Tadpoles	1-25 25-50 50-100 100-500 >500		

Sample #	Type	Sample #	Type
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip

4. Species		Detection Method:	Call Visual Dipnet	# Adults		# Metamorphs	
# Juveniles		# Egg Masses		# Tadpoles	1-25 25-50 50-100 100-500 >500		

Sample #	Type	Sample #	Type
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip

Species codes: **BCF** = Boreal Chorus Frog **BT** = Boreal Toad **TS** = Tiger Salamander **CSF** = Columbia Spotted Frog **NLF** = Northern Leopard Frog **UNID** = Unidentified

Notes:

Continued on back? Yes No

Amphibians & Geology

Visual Encounter Survey — Observer 2

Sub-site			–			–			

Date			/			/	2	0	1	8

Observer Name	

Please record all times, including break and resume

Start time					**Break**					**Resume**					**Stop**					**Total** (min)				

Track name		**GPS number**		**Downloaded?**	

Record tracks of survey routes and name with site name-observer #, e.g., "WR0101A1"

Species Detected (record additional species and samples on extra data sheets)

1. Species		**Detection Method:**	Call Visual Dipnet	**# Adults**		**# Metamorphs**	
# Juveniles		**# Egg Masses**		**# Tadpoles**	1-25 25-50 50-100 100-500 >500		

Sample #	**Type**	**Sample #**	**Type**
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip

2. Species		**Detection Method:**	Call Visual Dipnet	**# Adults**		**# Metamorphs**	
# Juveniles		**# Egg Masses**		**# Tadpoles**	1-25 25-50 50-100 100-500 >500		

Sample #	**Type**	**Sample #**	**Type**
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip

3. Species		**Detection Method:**	Call Visual Dipnet	**# Adults**		**# Metamorphs**	
# Juveniles		**# Egg Masses**		**# Tadpoles**	1-25 25-50 50-100 100-500 >500		

Sample #	**Type**	**Sample #**	**Type**
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip

4. Species		**Detection Method:**	Call Visual Dipnet	**# Adults**		**# Metamorphs**	
# Juveniles		**# Egg Masses**		**# Tadpoles**	1-25 25-50 50-100 100-500 >500		

Sample #	**Type**	**Sample #**	**Type**
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip
	Swab Tadpole Clip		Swab Tadpole Clip

Species codes: **BCF** = Boreal Chorus Frog **BT** = Boreal Toad **TS** = Tiger Salamander **CSF** = Columbia Spotted Frog **NLF** = Northern Leopard Frog **UNID** = Unidentified

Notes:

Continued on back? Yes No