MULTI-SCALE OCCUPANCY AND SPECIES INTERACTIONS OF A DESERT AMPHIBIAN

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of MATTHEW MICHAEL SMITH find it satisfactory and recommend that it be accepted.

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Abstract

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Species occupancy is influenced by abiotic and biotic factors across multiple spatial scales. To estimate relationships between occupancy and covariates, sensitive methods are necessary to detect species and account for imperfect detection. Environmental DNA (eDNA) is an emerging tool to survey aquatic species and is effective in detecting elusive species. We used field and eDNA techniques to identify Columbia spotted frog (*Rana luteiventris*) (CSF) occupancy in the Great Basin, U.S.A. At each site, we filtered 250ml of water through a 0.45 μm cellulose nitrate filter for three samples and one negative control per site. DNA was extracted using DNeasy Blood and Tissue Kit in a lab dedicated to low quality DNA. We analyzed samples using quantitative PCR to amplify species-specific DNA fragment. In addition to analyzing samples for eDNA of CSF, we created a species-specific assay for American beaver (*Castor canadensis*), which has a potentially positive relationship with CSF presence and tested samples for the pathogen *Batrachochytrium dendrobatidis (Bd)*. Environmental DNA increased the estimate of CSF at surveyed sites (naïve psi = 0.37) when compared to using traditional field methods alone (naïve psi = 0.20).

We employed a multi-scale occupancy model that simultaneously estimated the probability of occupancy at a watershed and site scale while accounting for availability within the watershed and imperfect detection. The top two models had a combined AIC_c weight of 0.82. The top supported model (AIC_c weight = 0.51) included precipitation and temperature as covariates of watershed occupancy.

We used two-species conditional occupancy model to determine if a species interaction existed between CSF and beavers and between CSF and *Bd*. For the two-species model between CSF and beavers, the top three models (combined AIC_c weight of 0.70) supported a species interaction. We found that CSF occupancy was higher at lower levels of annual precipitation when beaver was present then when absent. Additionally, *Bd* occupancy was strongly related to CSF being present (SIF = 2.63) and decreased as the distance to primary and secondary roads increased. Species interactions can have impacts on species occupancy that may influence species response to human impacts and climate change.

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Dedication

I would like to dedicate this to my parents, Mike and Mary. Even though I grew up in the big city, thank you for exposing me to the great outdoors through our many family vacations.

Multi-scale occupancy analysis from environmental DNA detections for landscape-scale monitoring of a desert amphibian

Introduction

Landscape-scale monitoring is important to understand species occupancy through time and possible impacts of climate change. Multiple challenges arise when developing an efficient and informative landscape-scale monitoring program. Riparian areas make up 1-3% of arid landscapes; aquatic species in these landscapes rely on these isolated areas of habitat (Patten 1998). These limited patches are further influenced by seasonal and between year variation that produce difficult conditions for survival and connectivity for aquatic species (Naiman & Decamps 1997). Consequently, changes in aquatic species occupancy can be difficult to detect and interpret, presenting challenges for species monitoring into the future.

Having accurate information on species distribution and habitat requirements across a landscape is required to improve ecological understanding and conservation and precise measures are necessary for detection of rare and difficult-to-detect species. Environmental DNA has emerged as a promising tool to survey and monitor aquatic species that are rare, elusive, and invasive (Goldberg *et al.* 2011; Jerde *et al.* 2011; Thomsen *et al.* 2012). Previous studies have shown that eDNA detection is influenced by degradation which is increased by higher water temperatures, likely through increased microbial activity within the water (Pilliod *et al.* 2014; Barnes *et al.* 2014; Strickler, Fremier & Goldberg 2015). Because eDNA is detected imperfectly it has been argued that it should be used in an occupancy modeling framework (Schmidt *et al.* 2013) and recent studies have applied this method to estimate detection and eDNA availability (Hunter *et al.* 2015; Schmelzle & Kinziger 2016; De Souza *et al.* 2016), but has not been applied

to estimate occupancy at multiple ecologically important scales or at this broad geographical area. The need for landscape level monitoring of amphibians has been an important point of emphasis in the literature (Hamer & Mahoney 2010) and has been implemented using amphibian occupancy (Gould *et al.* 2012; Adams *et al.* 2013) and the use of eDNA could allow for an efficient and effective approach to these assessments.

Due to the variability in suitable climate now and in the future, this species and other aquatic species in arid systems could benefit from efficient landscape level monitoring that considers occupancy at multiple spatial scales. The Great Basin clade of the Columbia spotted (*Rana luteiventris*) frog inhabits semi-arid portions of southeastern Oregon, southwestern Idaho, and Nevada in isolated populations and is a highly aquatic species that relies on aquatic habitat during all life stages (Reaser & Pilliod 2005). Under future climate scenarios, the Great Basin is predicted to become hotter and have increased variability in precipitation (Hurd *et al.* 1999; Cubashi *et al.* 2001) and the spotted frog is predicted to have a reduced area of suitable climate (Pilliod *et al.* 2015). Previous surveys in the northern Great Basin did not detect Columbia spotted frogs at a large portion of historical sites, but a relatively high proportion of proximal sites were occupied leading to difficulties in the interpretation of changes in occupancy and population declines (Wente, Adams & Pearl 2005).

In addition to the need for efficient landscape level monitoring, conservation and management decisions are complicated by differences in species occupancy and habitat selection at various spatial scales (George & Zack 2001). Occupancy modeling has improved estimation of species occurrence by accounting for false negatives when species are detected imperfectly (Mackenzie *et al.* 2002) and a multi-scale parameterization can allow for inferences of habitat relationships at multiple spatial scales (Nichols *et al.* 2008, Pavlacky *et al.* 2011). Habitat

relationships found through occupancy modeling at multiple spatial scales can aid conservation and management where species respond differently at different spatial scales (George & Zack 2001, Hagen *et al.* 2016). The unpredictability of the water regime in this system makes watershed occupancy a useful scale to monitor populations that rely on permanent aquatic environments. A loss of a single site could be negligible if connectivity to other occupied sites within the watershed remain, however, if watershed occupancy decreases substantially due to warmer and drier climate, connectivity and occupancy could be severely reduced.

One challenge with applying occupancy modeling to eDNA detections is assuming no false positives, but at the PCR replicate level, false positives are known to occur (Wilcox *et al.* 2013; Ficetola *et al.* 2015). These issues have been handled either by assuming false positives did not occur (Schmelzle & Kinziger 2016; De Souza *et al.* 2016) or by setting *ad hoc* thresholds (e.g., retesting ambiguous samples and considering only samples that test positive on the second run to be positive; Goldberg *et al.* 2013), but these methods can bias estimates (Ficetola *et al.* 2015, Lahoz-Monfort, Guillera-Arroita & Tingley 2016). To account for false positives in eDNA qPCR replicates, a site confirmation approach that relies on an alternate detection method can provide confirmation of site occupancy and be used to estimate the rate of false positives (Miller *et al.* 2011; Chambert, Miller & Nichols 2015). Multiple method false positive models have been used in simulation studies with eDNA data, but to date they have not been used with field data to improve occupancy model selection and inference (Lahoz-Monfort, Guillera-Arroita & Tingley 2016).

We conducted paired field and eDNA surveys across the Great Basin range of the Columbia spotted frog and applied a multi-scale occupancy modeling framework accounting for false positives to investigate factors influencing the occupancy of this amphibian in a highly

variable system. This is the first study to use eDNA in occupancy modeling at this extent and demonstrates the utility of this sensitive and accurate new tool for forwarding the field of applied ecology.

Methods

Site Selection

We selected 220 sites to survey throughout the range of the Great Basin clade of the Columbia spotted frog in southeastern Oregon, southwestern Idaho, and Nevada using a stratified random sampling approach. To gather information on potential locations of this rare amphibian, we used the historical dataset and predictive maps of Pilliod *et al.* (2015), the National Wetland Inventory (NWI; US Fish and Wildlife Service), and two meetings with biologists from the Bureau and Land Management, United States Fish and Wildlife Service, United States Forest Service, and Nevada Department of Wildlife. For site selection, first we randomly selected 21 known locations from historical records compiled by Pilliod *et al.* (2015), stratified by geographic area (Figure 1.1). To identify additional populations from these known populations, we then selected two 12th level hydrologic units (Watershed Boundary Dataset; US Geological Survey) adjacent or nearby the units containing historical sites. When multiple watersheds were available for selection, we selected watersheds in the following order of priority: those with sites suggested by resource managers, those with the highest proportion of predicted suitable climate (Pilliod *et al.* 2015), and those with the greatest wetland area (from NWI). Three individual sites within each watershed were then selected (in order of priority) from: on the ground knowledge of collaborators, random selection from semi-permanent wetlands (NWI), and those identified through Google Earth (Google Inc. 2016). When possible, 3 sites for each neighboring watershed

were sampled but because of water availability and limitations to public access, the number of sites varied and when the pre-selected sites where unavailable for sampling these were replaced when suitable habitat was observed in the area. The choice of watershed for neighboring population sampling was not influenced by the presence of additional historical locations. We took this neighboring watershed approach to increase the likelihood that we would have enough detections to model occupancy and to provide more detail on known locations at a fine scale. We selected additional survey sites based on model probability above 0.20 and below 0.20 from a climatic suitability model developed for Columbia spotted frogs in the Great Basin (Pilliod *et al.* 2015). In total, we surveyed 220 sites that consisted of: historical locations (N = 21), neighboring population sites (N = 126), model probability ≥ 0.20 (N = 49), and model probability < 0.20 (N = 24). All sites were selected to only be on public land. Sites were surveyed in May to August of 2015 and 2016 to target tadpole season, when breeding sites for the species can be most clearly identified.

Environmental DNA Sample Collection and Visual/Dip Net Surveys

We performed visual/dip net surveys and collected eDNA samples at each site. Upon initial approach of a site, we conducted a visual survey where two biologists surveyed different portions of the site until the entire site was observed. After the visual survey, we collected eDNA samples before any other equipment or clothing touched the water to prevent cross-contamination between sites. Following the collection of water and eDNA filtering, we performed dip netting around the observed site moving along the shoreline approximately every 1 meter accessible locations.

We visually assessed sites to identify areas where frogs or tadpoles would most likely occur to collect water samples. Areas without canopy cover, with emergent vegetation, and south facing shorelines were given preference when collecting water samples. We collected three filter samples by filtering 250 mL of water for each filter at a site; per sample detection rate in a pilot study with Columbia spotted frogs was found to be 0.66, indicating that >0.95 detection can be achieved with three samples (Goldberg *et al.* unpublished data). Most sites had 1 sampling location; however, we collected samples from 2-6 additional locations within the site and combined equal portions from each location into a single sample for 14 sites, when sites were approximated to be >1200 m^2. We used single-use whirl-paks (Nasco, Inc.) to collect the individual water samples. We attempted to filter 250 mLs for each sample; when the filter clogged before reaching this amount we recorded the amount successfully filtered. We filtered eDNA samples through a 0.45 μm cellulose nitrate filter within single use sterile analytical test filter funnel (Nalgene, Inc). After filtration was complete, we folded the filter in half inwards and placed into an individual coin envelope marked with unique identification, location and date. The coin envelope was then either placed into a larger bag containing silica desiccant until completely dried (2015) or placed into an individual bag with silica desiccant (2016); this approach was adapted from Carim *et al.* (2014). There was no evidence of contamination from either method, but to further safeguard against possible contamination we took the more rigorous approach in 2016. Any contact with the filter was either with a new clean pair of latex gloves or with forceps sterilized in 50% bleach and rinsed with distilled water. Samples were stored out of direct light. We also collected one negative control at each site by filtering distilled water on site using the same materials and methods as the field samples. We decontaminated boots with 10%

commercial bleach solution between each site and filtration materials downstream of filter once per week or sooner if moving to new geographical area.

eDNA Lab Protocol and Analysis

We extracted and prepared all eDNA samples for quantitative PCR (qPCR) in a lab dedicated to low quality DNA extraction where high quality DNA and PCR product are restricted. Access is limited and researchers must shower and change clothes before entering after being exposed to high quality DNA or PCR product. We extracted DNA from samples using the Qiagen DNeasy Blood and Tissue Kit with the addition of the Qiagen QIAshredder as described in Goldberg *et al.* (2011). Each extraction batch included a negative control to test for contamination.

Great Basin Columbia spotted frog presence was assessed using targeted sequence detection through quantitative PCR (qPCR). We used a previously developed qPCR assay for Great Basin Columbia spotted frogs from mitochondrial DNA sequences (Table 1.1). We analyzed each sample in triplicate. Each qPCR included 3 μL of DNA extract in a total volume of 15 μL. Reactions were run using 1X QuantiTect Multiplex PCR Mix (Qiagen Inc.), 0.2 μM of each primer, and 0.2 μM of probe on a Bio-rad CFX96 Touch Real-Time PCR Detection System. To test for inhibition of the qPCR, each well included an exogenous internal positive control (IPC; Applied Biosystems). Reactions activated for 15 minutes at 95℃ then ran for 50 cycles of 94℃ for 60s followed by 60℃ for 60s. All qPCR plates included negative controls to test for contamination. Positive standards from a serial dilution of 10^{-3} through 10^{-6} were included in duplicate on each qPCR plate from extracted DNA of Great Basin Columbia spotted frog quantified on a NanoDrop (Thermo Fisher Scientific Inc.; 28.10 ng/uL).

Multi-scale Occupancy

The multi-scale occupancy detection histories were made up of 3 independent eDNA filter samples that were determined to be a detection (1) or non-detection (0) based on the amplification of Columbia spotted frog mtDNA through qPCR. The model parameters consisted of a broad-scale occupancy probability (Psi) which represented the probability of occupancy within a watershed, a fine-scale occupancy probability (theta) of a site being occupied conditional on the watershed being occupied, and the probability of detection (p) of an eDNA filter sample conditional on occupancy of the site and watershed. An example detection history of 101 000 would indicate that Columbia spotted frogs were detected in sample 1 and sample 3 at site 1 and were not detected at site 2 with both sites located within the same watershed.

We hypothesized that watershed occupancy (Psi) was influenced by up to 4 different watershed covariates: average precipitation, average temperature, total length of perennial streams, and average slope of the stream network (Table 1.2a). We hypothesized that average precipitation and total length of perennial streams would have a positive relationship and average temperature and average slope would have a negative relationship on watershed occupancy. We considered average slope as a potential influence on occupancy because Columbia spotted frogs occur mostly in stream habitats in the study area, but in slow or still side channels or margins of streams. We hypothesized that more gradual slopes within the stream network would be associated with these aquatic site characteristics. Average precipitation included rainfall and snow melt. We hypothesized site occupancy (theta) was influenced by up to 6 covariates: emergent vegetation, water type, livestock impact, average precipitation, average temperature, and elevation (Table 1.2b). Finally, we hypothesized that eDNA detection at a site was

influenced by up to two covariates: temperature (°C) in a negative relationship and pH in a positive relationship (Table 1.2c). At each site, we measured these parameters just under the surface of the water after eDNA samples were collected with a multiparameter meter (OAKTON Instruments, Vernon Hills, IL). We modeled detection probability (p) for eDNA filter samples as a constant, covariate of pH, or covariate of water temperature (Table 1.3). We calculated multicollinearity of covariates within each level of the multi-scale occupancy model (psi, theta, and p) by calculating variance inflation factors.

We ran all combinations of hypotheses at each level for a total of 75 models (Table 1.3). Multi-scale occupancy models were fitted using Program Mark 8.1 (White & Burnham 1999). Models were ranked according to Akaike's information criterion corrected for small sample size (AICc) and assessed for support based on AICc weights and delta AICc.

We applied the estimated probability of a false positive to each positive qPCR replicate from samples in our multi-scale occupancy dataset. The resampled qPCR replicates for each sample were then converted to a sample level detection (1) if any of the three replicates were positive or no detection (0) if none. We resampled the dataset 30 times and applied the new encounter histories to our multi-scale occupancy models to assess differences in model selection and parameter inference.

False positive estimation

We estimated the probability of false positives at the qPCR replicate level using the Miller *et al.* (2011) false positive occupancy model. We considered visual/dip net surveys as unambiguous detections and qPCR replicates of eDNA samples as ambiguous detections. Encounter histories were individual eDNA filter samples with three qPCR replicates (1 =

exponential amplification of target DNA or 0 = no amplification) and detection (1) or non-detection (0) using visual/dip net survey. We modeled a constant parameterization (psi(.)fp(.)p(.)) of the false positive occupancy modeled in the Unmarked R package (Fiske & Chandler 2011).

Results

We tested a total of 660 eDNA samples from 220 sites. Two sites had evidence of on-site contamination of target DNA (field negatives testing positive) and were excluded from occupancy analyses. Target DNA was not detected in any extraction negatives or qPCR negatives. We detected Columbia spotted frogs at a greater proportion of sites using eDNA then traditional surveys, with a naïve occupancy estimate of 0.37 with eDNA and 0.20 with visual/dipnet surveys (Table 1.4). Of the sites where eDNA detections occurred, qPCR replicates were highly consistent, with only 17% of positive sites having only 1 positive qPCR replicate (Table 1.5).

False positive estimation

The false positive occupancy model sampled each individual sample as a unique encounter history. The full dataset had 504 qPCRs that amplified for our target sequence. False positive rate per qPCR was estimated at 0.023 (SE = 0.004; 95% CI: 0.016 – 0.033) for the constant parameter model.

Multi-scale occupancy

Out of the full dataset, sixty-one watersheds had >1 (2-8) spatially replicated sites per watershed for a total of 168 sites available for use in the multi-scale analysis. The top two candidate models were nested and together had 0.82 of the evidence weight (Table 1.6). The most supported model from the candidate set included climatic covariates of precipitation and temperature at the watershed occupancy scale, emergent vegetation and water type as covariates of site occupancy, and water temperature as a covariate of detection (Table 1.6). The next model included all covariates of the top model with the addition of stream length as a covariate of watershed occupancy (Table 1.6). Variance inflation factors did not exceed 4.39.

The parameter estimate for precipitation had a positive relationship with watershed occupancy (Figure 1.2a) while temperature had a negative impact on watershed occupancy (Figure 1.2b). Both climatic relationships supported our hypotheses that precipitation had a positive influence and temperature had a negative influence on watershed occupancy and neither parameter estimate for average precipitation and average temperature overlapped zero (Table 1.6). Aquatic covariates of emergent vegetation, stream habitat, and beaver pond water type had a positive influence on site occupancy (Table 1.7). Water temperature had a positive effect on eDNA detection (Table 1.7, Figure 1.3), opposite to our hypothesis. At the mean water temperature (18.4℃), eDNA sampling had a high probability of detection per sample, $p = 0.78$ (SE = 0.03; 95% CI: 0.71 – 0.84).

The resampled datasets that accounted for false positives had an average of 379.4 positive qPCRs (N = 30) out of the original 389 positive qPCRs. Twenty-seven resampled datasets that accounted for false positives did not lead to any change in model selection with a combined model weight of the top two models ranging from 0.66 – 0.88 (mean = 0.80). Two resampled datasets showed slight changes in model selection compared to when false positives were not

accounted for. Model selection for 2 of the 30 resampled datasets showed support for an additional third model that no longer included water temperature as a covariate of detection. An additional resampled dataset also saw the top two models switch in most supported with the top model including streams as a covariate of psi with an AICc weight of 0.37. Both resampled datasets described above had a loss of occupancy at the site level that resulted in a loss of occupancy at the watershed.

Discussion

We found that multi-scale modeling of occupancy informed by eDNA sampling lead to improved landscape-scale monitoring power that increased detections and had consistent model results when false positives were considered. Using eDNA increased our total detections in this system and proved to be more sensitive than our visual/dip net surveys allowing for an efficient sampling strategy across the range of the Great Basin clade of Columbia spotted frogs. The greater number of detections helped estimate factors that influence spotted frog occupancy across multiple spatial scales.

The results from our eDNA surveys were consistent with previous studies that described emergent vegetation and climatic variables as important influences on Columbia spotted frog presence in the Great Basin (Arkle & Pilliod 2015; Pilliod *et al.* 2015). The results in this study highlight the importance of climatic factors influencing Columbia spotted frog occupancy at a broader scale. While site presence was influenced by finer scale aquatic characteristics. Arid and semi-arid landscapes like the Great Basin; have a high degree of natural variability in water availability; an increase in precipitation variability and hotter temperatures are predicted under future climate scenarios (Hurd *et al.* 1999; Cubashi, Meehl & Boer 2001). By using this multi-

scale approach, habitat relationships at multiple scales can be incorporated into landscape level monitoring while accounting for future variability across watersheds and sites.

Contrary to expectations, we found that eDNA detection probabilities increased with increasing temperatures, indicating that degradation was not the primary influence on detection. Increased eDNA detection of amphibians in warmer water could be caused by several factors, including increased activity in warmer water, increased metabolism, and greater frequency of time spent in the water during hot conditions. Warmer water temperatures can increase amphibian activity (Brattstrom 1963), leading to the possibility of greater eDNA shedding and higher detection. However, previous studies observed no effect of water temperature on eDNA production for fish in microcosm experiments (Takahara *et al.* 2012; Klymus *et al.* 2015). It is likely that the increase in detection probability at warmer temperatures is being influenced by behavioral choices of spotted frogs in this system.

Estimating false positive rates with eDNA has been recognized as an important step forward to improve methodology and inference (Ficetola *et al.* 2015; Lahoz-Monfort *et al.* 2015). Quantitative PCR replication can help improve parameter estimates when a degree of uncertainty is assigned to the number of positive replications and to a greater degree using an additional detection method (Ficetola *et al.* 2015; Lahoz-Monfort *et al.* 2015). This study used empirical data to estimate the rate of false positives for eDNA qPCRs using multiple detection methods and found that it had a minimal influence on model results in this dataset. When available, an estimation of the probability of false positives from eDNA samples is relatively straightforward and can improve or support the inferences being made of occupancy and detection (Lahoz-Monfort *et al.* 2015). While minimal differences in model selection or parameter inference were observed with our resampling, not all false positives during qPCR have

the same impact on inference. A false positive that occurs for where it is the only positive replicate for the site or watershed would have a larger influence on inference than a change in one qPCR replicate for a site with multiple positive replicates or multiple occupied sites within the watershed.

This study demonstrates the utility of eDNA sampling across a large geographical area to assess ecological questions of species occurrence at multiple scales while accounting for false positives. Other studies have used eDNA within an occupancy modeling framework but have focused on detection (Schmidt *et al.* 2013, Hunter *et al.* 2016, Schmelzle and Kinziger 2016). We implemented a robust eDNA sampling scheme that had greater detection sensitivity over traditional detection methods, as has been observed in other studies (Dejean *et al.* 2012, Pilliod *et al.* 2013, Schmelzle and Kinziger 2016). The multi-scale approach we applied allowed us to examine factors at the individual habitat patch but also the watershed scale that is more relevant to a landscape with highly variable habitat. We demonstrated that eDNA can be a highly sensitive and highly detectable method to assess amphibian occupancy across a broad geographic area. The greater sensitivity and efficiency afforded by eDNA detection can improve landscape scale monitoring and create greater confidence in determining baseline data needed to continue to monitoring species trends into the future.

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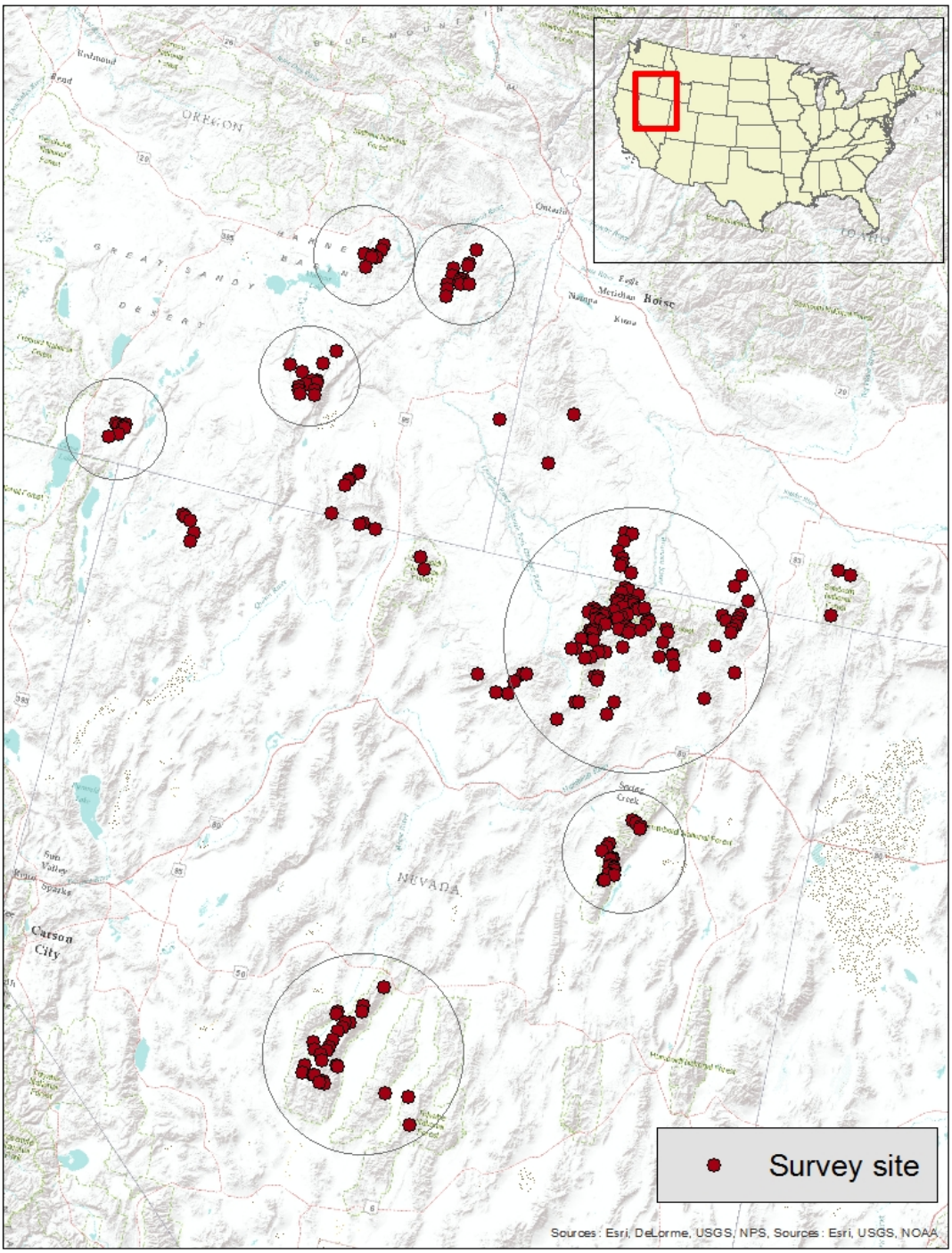

Figure 1.1: Columbia spotted frog (*Rana luteiventris)* surveys in the Great Basin. 220 survey sites in seven geographically distinct areas (black circles). Historical sites were selected from geographically distinct areas.

Table 1.1: Quantitative PCR assay design for Columbia spotted frog in the Great Basin (*Rana luteiventris)*. Assay was validated with 5 of each species listed.

Forward Primer	TTCAATGAATCTGAGGAGGCTTTT
Reverse Primer	GAAAGGAAGGATGAAGTGGAATGT
Probe	6FAM-AGTAGACAATGCTACCCTC-MGB

Publication: Goldberg *et al.* unpublished

Validated species:

Northern leopard frog (*Lithobates pipiens*)

American bullfrog (*Lithobates catesbeianus*)

Woodhouse's toad (*Anaxyrus woodhousii*)

Pacific treefrog (*Pseudacris sierrae*)

Western toad (*Anaxyrus boreas*)

Great Basin spadefoot (*Spea intermontana*)

Table 1.2a: A list of covariates used to model occupancy at the watershed level (psi). Includes predicted influence on occupancy, mean and range of covariate as well as description and source information.

Covariate	Mean (range)	Description	Source
Precipitation	415.8 (13.8 – 1060.8 mm)	Mean annual precipitation within watershed	PRISM climate group; 30 year normals (1981-2010); 800 m
Temperature	3.39 (0.68 – 7.09 ℃)	Mean annual temperature within watershed	PRISM climate group; 30 year normals (1981-2010); 800 m
Slope	0.048 (0.015 – 0.159)	Average slope along stream flowlines within watershed	NHDPlus Dataset
Streams	74.4 (23.3 – 151.6 km)	Sum of stream lengths within watershed	NHDPlus Dataset

Table 1.2b: A list of covariates used to model occupancy at the site level (theta). Includes predicted influence on occupancy, mean and range of covariate as well as description and source information.

Covariate	Mean (range) or count	Description	Source
Precipitation	481.5 (202.3 – 1102.5 mm)	Mean annual precipitation of site	PRISM climate group; 30 year normals (1981-2010); 800 m
Temperature	6.30 (3.62 – 9.15 ℃)	Mean annual temperature of site	PRISM climate group; 30 year normals (1981-2010); 800 m
Elevation	1917 (1089 – 2721 m)	Elevation of site	National Elevation Dataset; 10m

Emergent Vegetation	0% - 27; <25% - 32; 25-50% - 10; >50% - 100	Percent shoreline covered with emergent vegetation	On site survey
Water type	Lake/pond - 44; stream - 68; beaver pond - 56	Aquatic habitat description at survey site	On site survey

Table 1.2c: A list of covariates used to model detection at the eDNA sample level (p). Includes predicted influence on detection, mean and range of covariate as well as description and source information.

Covariate	Mean (range)	Description	Source
Water temperature	18.2 (6.3 – 29.1 ℃)	Measured water temperature from sample location	On site survey
pH	8.5 (6.8 – 10.6)	Measured pH from sample location	On site survey

Table 1.3: List of hypotheses at each scale for watershed occupancy (psi), site occupancy (theta), and detection (p). All combinations were modeled for a total of 75 candidate models.

psi(.)	theta(.)	p(.)
psi(streams)	theta(elevation)	p(pH)
psi(slope)	theta(livestock impact)	p(water temperature)
psi(precipitation + temperature)	theta(precipitation + temperature)	
psi(precipitation + temperature + streams)	theta(emergent vegetation + water type)	

Table 1.4: Proportion of Great Basin Columbia spotted frog detections (+) and non-detections (-) by method. A site was considered positive for eDNA if ≥ 1 sample had ≥ 1 positive qPCRs.

eDNA detection	Visual/dip net detection	Proportion of sites (Count)
-	+	0.01 (2)
+	-	0.17 (38)
+	+	0.19 (43)
-	-	0.62 (137)

Table 1.5: Possible encounter histories by site and percentage of sites with encounter history

Possible encounter histories	% of sites
000	63.2%
100, 010, 001	8.2%
110, 101, 011	4.1%
111	24.5%

Proportion of positive samples that had either 1, 2, or 3 positive qPCR replicates.

Number of positive qPCR replicates in triplicate set	Proportion of samples
1	0.17
2	0.07
3	0.76

Table 1.6: The top 6 models from the candidate set. K = number of parameters. Δ AICc is the difference in AICc from the top model and the AICc weight is the relative strength of the model given the candidate set. Veg - % emergent vegetation; (.) – intercept only

Model	K	AICc	Δ AICc	AICc Weight
Psi(precip + temp)theta(veg + water type)p(water temp)	10	369.73	0	0.51
Psi(precip + temp + streams)theta(veg + water type) p(water temp)	11	370.69	0.96	0.31
Psi(precip + temp)theta(veg + water type)p(.)	9	373.87	4.14	0.06
Psi(precip + temp + streams)theta(veg + water type) p(.)	10	374.67	4.94	0.04
Psi(.)theta(veg + water type)p(water temp)	8	375.71	5.98	0.03
Psi(precip + temp)theta(veg + water type)p(pH)	10	376.78	7.05	0.01

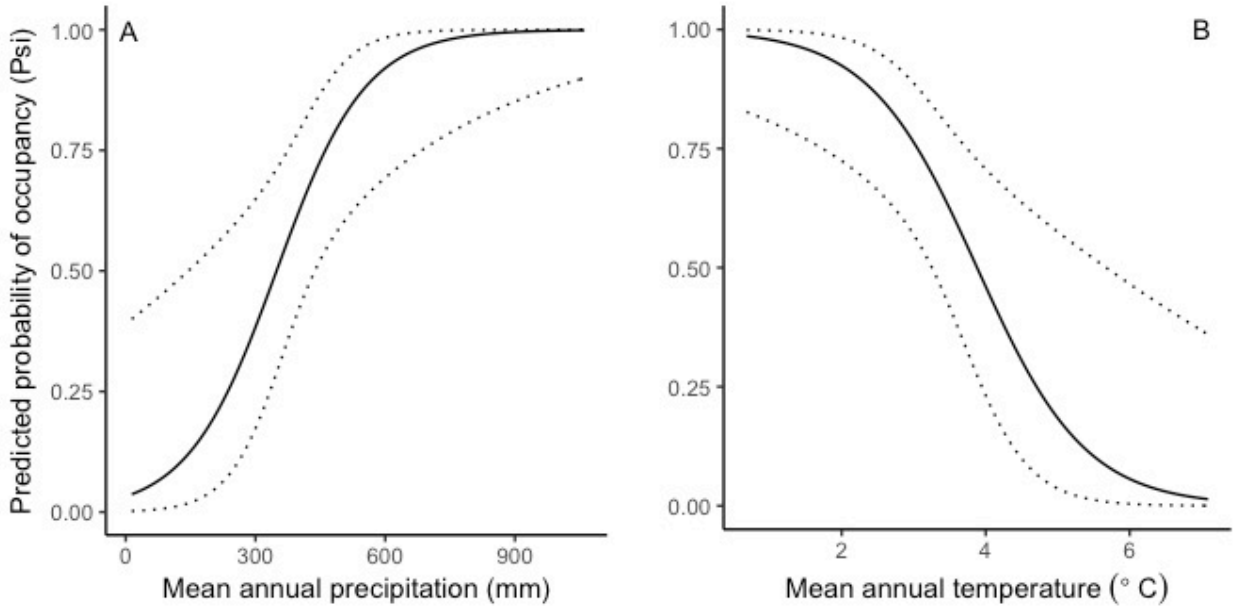

Figure 1.2: Predicted probability of watershed occupancy from a multi-scale occupancy model for Columbia spotted frogs (*Rana luteiventris)* in the Great Basin by (**A**) mean annual precipitation and (**B**) mean annual temperature for 30-year normals from strongest supported model based on AICc (AICc weight = 0.51) with other covariate values held at the mean. Shaded gray area is equal to 95% confidence interval.

Table 1.7: Coefficient estimates from the strongest supported candidate model for multi-scale occupancy of the Columbia spotted frog (*Rana luteiventris)* in the Great Basin, psi(precipitation + temperature)theta(emergent vegetation + water type)p(water temperature). Lower and upper confidence intervals at 95%.

	Estimate	Standard Error	Lower C.I.	Upper C.I.
Psi				
Precipitation	0.91	0.36	0.21	1.61
Temperature	-1.31	0.50	-2.28	-0.34
Theta				
Emergent vegetation	1.10	0.25	0.60	1.59
Water type – Lake/pond	-0.19	0.37	-0.92	0.55
Water type – Stream	0.63	0.38	-0.13	1.38
Water type – Beaver pond	0.57	0.35	-0.11	1.27
P				
Water temperature	0.11	0.04	0.03	0.20

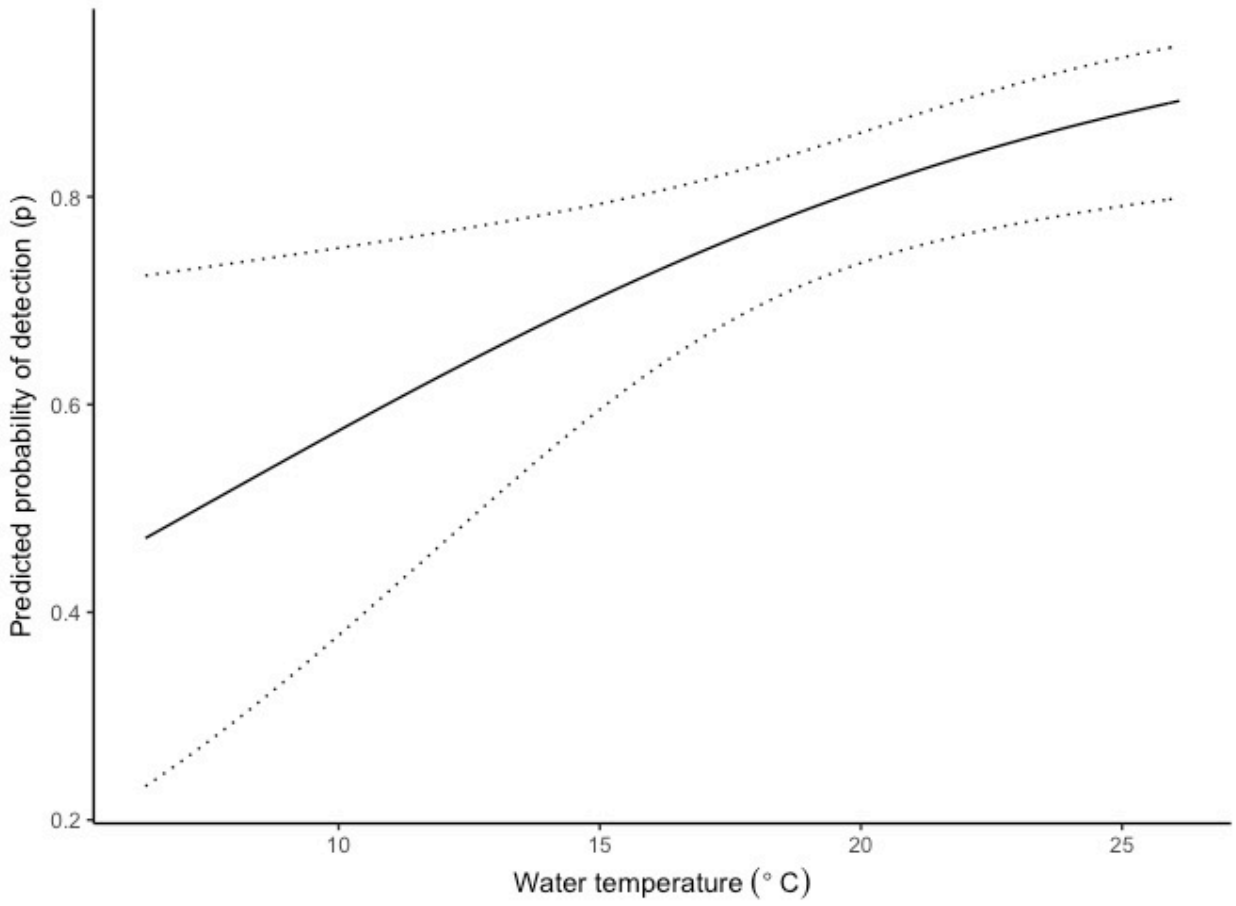

Figure 1.3: Multi-scale occupancy analysis of the Columbia spotted frog (*Rana luteiventris*) in the Great Basin and predicted probability of detection per eDNA sample as a function of water temperature for the strongest supported model according to AICc (AICc weight = 0.51) with all other covariates held at mean values. Shaded gray area represents 95% confidence interval.

Columbia spotted frog and *Batrachochytrium dendrobatidis* occupancy are influenced by biotic interactions across environmental gradients in the Great Basin, USA

Introduction

Biotic interactions affect species distributions through predation, competition, host-pathogen interactions, and facilitation (Wisz *et al.* 2013). Species distribution is influenced by both abiotic conditions and biotic interactions; understanding how these limit occupancy at a local and range-wide scale is important for predicting the potential effects of climate change (Davis *et al.* 1998; Martin 2001). While most predictive models for species occurrence focus on abiotic limitations, biotic interactions have been well documented at local scales and there is an increasing acknowledgment of the importance of considering biotic interactions at broader spatial scales (Wiens 2011; Wisz *et al.* 2013).

Inferences about biotic relationships can be incorrect when the uncertainty of absence data is not considered. To address this issue, occupancy modeling that incorporates detection probability of two species and includes species-specific occupancy covariates can be applied (Mackenzie, Bailey & Nichols 2004; Richmond, Hines & Beissinger 2010). This modeling approach minimizes bias from imperfect detection and can account for differences in habitat requirements. The two-species occupancy framework was originally developed with competition and predator-prey relationships in mind (Mackenzie, Bailey & Nichols 2004) and most applications have examined competitive interactions (Yackulic *et al.* 2014; Robinson, Bustos & Roemer 2014; Steen *et al.* 2014). However, there is a growing area of research that highlights the importance of facilitation in species relationships that can influence distribution and potentially mitigate abiotic stress (Bruno, Stachowicz & Bertness 2003; Afkhami, McIntyre & Strauss

2014). There are limited examples of the use of two-species occupancy models to understand facilitative species interactions (e.g., Peoples & Frimpong 2016).

Many amphibians benefit from a facilitative relationship with American beavers (*Castor canadensis*). The habitat created by this ecosystem engineer can lead to increased amphibian population sizes and species richness (Cunningham, Calhoun & Glanz 2007; Karraker & Gibbs 2009). A study looking at long-term trends in amphibian occupancy in the western United States found that beaver-created habitat was important for multiple species and played a role in increasing colonization over a ten-year period (Hossack *et al.* 2015). The colonization of beaver-created habitat could be an important pathway for amphibians to maintain or increase occupancy when landscape or climate change occurs. Furthermore, the creation of new habitat in areas that have high variability of surface water could benefit amphibian occupancy by providing greater stability.

The Great Basin system has high variability in precipitation within and between years (Hurd *et al.* 1999; Seager *et al.* 2007). Under future climate scenarios, this area is predicted to be become hotter and have even greater variability in precipitation (Hurd *et al.* 1999; Cubashi, Meehl & Boer 2001). The Columbia spotted frog (*Rana luteiventris*) is a highly aquatic amphibian that occurs in isolated patches within northern and central portions of the Great Basin (Reaser & Pilliod 2005). Understanding the biotic interaction between Columbia spotted frog and beavers in this system could identify factors important to Columbia spotted frog occupancy in the future. Beaver-created ponds were associated with higher occupancy then other water types for Columbia spotted frog in the Great Basin in an analysis of known locations (Arkle & Pilliod 2015); this study builds on that work by adding an estimation of detection probability for

both species, accounting for species-specific habitat covariates, and using active beaver detections across the whole range.

An additional species interaction that potentially exists for Columbia spotted frogs is with the pathogen *Batrachochytrium dendrobatidis* (Bd)**.** Bd causes the infectious disease chytridiomycosis and has been implicated in amphibian declines worldwide (Rohr *et al.* 2008; Vredenburg *et al.* 2010). Bd has been found in the Great Basin (Pearl *et al.* 2007; Arkle & Pilliod 2015) and in a closely related species (*Rana pretiosa*), where a slight decrease in juvenile body condition of infected individuals was found, indicating the potential for sub-lethal effects and the need for further investigation (Pearl *et al.* 2009). Bd is not known to survive desiccation (Johnson *et al.* 2003) and has been found to be strongly associated with stream dwelling amphibians and permanent water bodies (Kriger & Hero 2007). The Great Basin has few highly aquatic amphibians; Columbia spotted frogs are likely the primary host for this pathogen in this system as all life stages are aquatic (Reaser & Pilliod 2005). However, Bd is currently not believed to be directly involved in declines of Columbia spotted frog populations and experimentally isolated peptides from the Columbia spotted frog inhibited Bd growth (Rollins-Smith *et al.* 2002, Rollins-Smith *et al.* 2005). The potential for sub-lethal effects highlight the importance of understanding how Bd and Columbia spotted frog co-occur across the landscape.

The spread of Bd globally has been linked to the human aided transport and introduction of non-native amphibians (Weldon *et al.* 2004; Fisher & Gardner 2007). Bd presence and Bd with similar genotypes have been associated with areas of greater human impact and access (Morgan *et al.* 2007; Pauza, Driessen & Skerratt 2010). Additionally, the distance from roads and other human influences have been implicated in ranavirus (another emerging amphibian pathogen) presence (Miller, Gray & Storfer 2011). However, Bd is also present in remote

amphibian populations (Obendorf & Dalton 2006; Puschendorf, Bolanos & Chaves 2006; Seimon *et al.* 2007). Bd has been detected in water samples (Kirshtein *et al.* 2007, Hyman and Collins 2011, Chestnut *et al.* 2014) and on other species [e.g., birds (Johnson and Speare 2005, Garmyn *et al.* 2011); crayfish (McMahon *et al.* 2013, Betancourt-Roman *et al.* 2016)]. Understanding local factors that contribute to Bd occupancy is important to consider as amphibian declines continue to occur across the world.

In this study, we used two-species occupancy models to investigate species interactions between Columbia spotted frogs, beaver, and Bd and their relationship to habitat covariates. For data collection, we utilized environmental DNA (eDNA) samples to detect multiple species from a single sample. By incorporating biotic interactions, we can better understand species occupancy and interactions between biotic and abiotic conditions.

Methods

Site selection (same as manuscript 1)

We selected 220 sites to survey throughout the range of the Great Basin clade of the Columbia spotted frog in southeastern Oregon, southwestern Idaho, and Nevada using a stratified random sampling approach. To gather information on potential locations of this rare amphibian, we used the historical dataset and predictive maps of Pilliod *et al.* (2015), the National Wetland Inventory (NWI; US Fish and Wildlife Service), and two meetings with biologists from the Bureau and Land Management, United States Fish and Wildlife Service, United States Forest Service, and Nevada Department of Wildlife. For site selection, first we randomly selected 21 known locations from historical records compiled by Pilliod *et al.* (2015), stratified by geographic area. To potentially identify new populations, we then selected two 12^{th} level

hydrologic units (Watershed Boundary Dataset) adjacent or nearby the units containing historical sites. When multiple watersheds were available for selection, we selected watersheds in the following order of priority: those with sites suggested by resource managers, those with the highest proportion of predicted suitable climate (Pilliod *et al.* 2015), and those with the greatest wetland area (from NWI). Three individual sites within each watershed were then selected (in order of priority) from: on-the-ground knowledge of collaborators, random selection from semi-permanent wetlands (National Wetland Inventory), and those identified through Google Earth (Google Inc. 2016). When possible, 3 sites for each neighboring watershed were sampled but because of water availability and limitations to public access, the number of sites varied and when the pre-selected sites where unavailable for sampling these were replaced when suitable habitat was observed in the area. The choice of watershed for neighboring population sampling was not influenced by the presence of additional historical locations. We took this neighboring watershed approach to increase the likelihood that we would have enough detections to model occupancy and to provide more detail on known locations at a fine scale. We selected additional survey sites based on model probability above 0.20 and below 0.20 from a climatic suitability model developed for Columbia spotted frogs in the Great Basin (Pilliod *et al.* 2015). In total, we surveyed 220 sites that consisted of: historical locations (N = 21), neighboring population sites (N = 126), high model probability (N = 49), and low model probability (N = 24). All sites were selected to only be on public land. Sites were surveyed in May to August of 2015 and 2016 to target tadpole season, when breeding sites for the species can be most clearly identified.

Environmental DNA Sample Collection

We visually assessed sites to identify areas where frogs or tadpoles would most likely occur to collect water samples. Areas without canopy cover, with emergent vegetation, and south facing shorelines were given preference when collecting water samples. We collected three filter samples by filtering 250 mL of water for each filter at a site; per sample detection rate in a pilot study with Columbia spotted frogs was found to be 0.66, indicating that >0.95 detection can be achieved with three samples (Goldberg *et al.* unpublished data). Most sites had 1 sampling location; however, we collected samples from 2-6 additional locations within the site and combined equal portions from each location into a single sample for 14 sites, when sites were approximated to be >1200 m^2. We used single-use whirl-paks (Nasco, Inc.) to collect the individual water samples. We attempted to filter 250 mLs for each sample; when the filter clogged before reaching this amount we recorded the amount successfully filtered. We filtered eDNA samples through a 0.45 μm cellulose nitrate filter within single use sterile analytical test filter funnel (Nalgene, Inc). After filtration was complete, we folded the filter in half inwards and placed into an individual coin envelope marked with unique identification, location and date. The coin envelope was then either placed into a larger bag containing silica desiccant until completely dried (2015) or placed into an individual bag with silica desiccant (2016); this approach was adapted from Carim *et al.* (2014). There was no evidence of contamination from either method, but to further safeguard against possible contamination we took the more rigorous approach in 2016. Any contact with the filter was either with a new clean pair of latex gloves or with forceps sterilized in 50% bleach and rinsed with distilled water. We also collected one negative control at each site by filtering distilled water on site using the same materials and methods as the field samples. We decontaminated boots with 10% commercial bleach solution

between each site and filtration materials downstream of filter once per week or sooner if moving to new geographical area.

eDNA Lab Protocol and Analysis

We extracted and prepared all eDNA samples for quantitative PCR (qPCR) in a lab dedicated to low quality DNA extraction where high quality DNA or PCR product are restricted. Access is limited and researchers must shower and change clothes before entering after being exposed to high quality DNA or PCR product. We extracted DNA from samples using the Qiagen DNeasy Blood and Tissue Kit with the addition of the Qiagen QIAshredder as described in Goldberg *et al.* (2011). Each extraction batch included a negative control to test for contamination.

Great Basin Columbia spotted frog, American beaver, and Bd presence were assessed using targeted sequence detection through quantitative PCR (qPCR). We used previously developed qPCR assays for Great Basin Columbia spotted frogs from mitochondrial DNA sequences (Goldberg *et al.* unpublished) and Bd (Boyle *et al.* 2004). We designed a qPCR assay for American beavers from previously published control region sequences obtained through Genbank (Serrano 2011). We used ClustalX2 (Larkin *et al.* 2007) to align sequences and compiled the consensus sequence using Sequencher (Gene Codes Corporation). We used Primer Express (verison 3.0; Applied Biosystems) to identify potential assays and tested them for specificity using the Primer-BLAST algorithm (Ye *et al.* 2012) requiring greater than or equal to 2 base pair changes with at least 1 located within 4 base pairs from the 3' end of each primer and at least 1 base pair change towards the middle of the probe sequence. The resulting assay was tested against a panel of 10 beaver tissue samples (Table 2.1).

We analyzed each sample in triplicate qPCRs for the detection of the species of interest. Each qPCR included 3 μL of DNA extract in a total volume of 15 μL. Reactions were run using 1X QuantiTect Multiplex PCR Mix (Qiagen Inc.), 0.2 μM of each primer, and 0.2 μM of probe on a Bio-rad CFX96 Touch Real-Time PCR Detection System. To test for inhibition of the qPCR, each well included an exogenous internal positive control (IPC; Applied Biosystems). Reactions activated for 15 minutes at 95℃ then ran for 50 cycles of 94℃ for 60s to 60℃ for 60s. All qPCR plates included negative controls to test for contamination. Columbia spotted frog and beaver assays along with IPC were tested to confirm that interference did not occur when multiplexed (Goldberg *et al.* 2016). Similarly, we multiplexed Bd with an additional species (western toad (*Anaxyrus boreas)*, data not included) and did not observe any interference. Positive standards from a serial dilution of 10^{-3} through 10^{-6} were included in duplicate on each qPCR plate from extracted DNA from Columbia spotted frog and beaver or a synthesized gene (gBlocks; Integrated DNA Technologies; 10000 – 10 copies) for Bd. Columbia spotted frog and beaver samples were quantified on a Qubit 3.0 Fluorometer (Life Technologies; 67.4 ng/uL and 40.8 ng/uL, respectively). For each species, qPCRs were assigned a detection (1) if exponential amplification occurred or non-detection (0) if no amplification occurred.

Two-species occupancy model

We used the conditional parameterization of the two-species occupancy model (Richmond, Hines & Beissinger 2010) to evaluate the co-occurrence of beavers and Bd with Columbia spotted frog in the Great Basin. The encounter histories were made up of three independent eDNA filter samples and a sample was considered positive if any of the qPCRs had a detection. This parameterization requires an *a priori* designation of a dominant and subordinate

species, where conditional occupancy parameters are estimated for the subordinate species (psiBA and psiBa) and an unconditional occupancy parameter is estimated for the dominant species (psiA). For modeling the beaver/Columbia spotted frog interaction, we assigned the beavers as the dominant species because the presence or absence of Columbia spotted frog was not thought to impact beaver presence. For the interaction between Columbia spotted frog and Bd, we assigned Columbia spotted frog as the dominant species as Bd requires a host for reproduction and survival. This relationship could also be modeled in the opposite direction, but Bd has not been implicated in any Columbia spotted frog declines to date and Columbia spotted frogs are often the only amphibian in these systems, making them the primary host. Additionally, this parameterization estimate a derived species interaction factor (SIF) that assesses whether the two species occur together more often than random (SIF > 1), independently (SIF = 1), or less often than random (SIF < 1) (Richmond, Hines & Beissinger 2010). Two-species occupancy model sets become very large by estimating 4-8 parameters depending on whether parameters are conditional and unconditional and then each parameter can be modeled with various environmental covariates in different combinations; because of this multiplicative effect with the addition of covariates we limited our model set (Richmond, Hines & Beissinger 2010).

We focused on two main questions for each model set: (1) is there support for a biotic interaction? and (2) if there is an interaction how might this change across an environmental gradient? We also had no *a priori* reason to believe that the detection of beavers or Columbia spotted frog would be impacted by the presence of the other and for this reason we set conditional probabilities of detection equal to their unconditional probabilities of detection (pA = rA; pB = rBA = rBa). Similarly, we set conditional probabilities of detection equal to their unconditional probabilities of detection for Columbia spotted frog and Bd to limit the total model

set, but incorporated Columbia spotted frog presence as a covariate of detection for Bd (pB). To address Question 1, we compared two model structures. The first structure modeled the probability of Columbia spotted frog or Bd occupancy given the presence of beavers/Columbia spotted frog (psiBA) and the probability of Columbia spotted frog or Bd occupancy given the absence of beavers/Columbia spotted frog (psiBa) as two separate parameters. The alternative structure set psiBA = psiBa and support for this model structure would suggest that occupancy was independent of the dominant species (Yackulic *et al.* 2014; Haynes *et al.* 2014; Steen *et al.* 2014).

Degradation has been shown to be an important process in eDNA detection; likely through microbial activity influenced by abiotic conditions (Barnes *et al.* 2014; Strickler, Fremier & Goldberg 2015). To account for this process, we hypothesized that Columbia spotted frog detection would be influenced by either water temperature, pH, or intercept only (p(.)) (Table 2.2). At each site, we measured water temperature and pH just under the surface of the water after eDNA samples were collected with a multiparameter meter (OAKTON Instruments, Vernon Hills, IL).

For Question 2, we asked if the co-occurrence of Columbia spotted frog and beavers were influenced by an effect of climatic variables of annual precipitation and temperature or by elevation (Table 2.2). We hypothesized that Columbia spotted frog and beavers will have higher co-occurrence at sites with less annual precipitation and with warmer temperatures; we had no *a priori* expectation of the influence of elevation. Annual precipitation and temperature data was extracted from 30 year normals dataset (PRISM climate group; 800 m cells) and elevation was acquired from a digital elevation model at 1/3 arc second (US Geological Survey 2014). We hypothesized that beaver occupancy (psiA) would be influenced by both slope and area of tree-

dominated vegetation classification within 200 meters of the site or climatic covariates of annual precipitation and temperature (Table 2.2). The sum of tree-dominated vegetation classification was extracted from LANDFIRE Existing Vegetation Type (2014, 30 m) for a 200 m buffer around the site location; beavers have been observed foraging up to this distance (Müller-Schwarze & Schulte 1999). We hypothesized that beaver detection (pA) would be influenced by eDNA degradation by including covariates of water temperature and pH (Table 2.2).

Similarly, we asked if the occurrence of Bd was influenced by the distance from a primary or secondary road or if the water temperature given the presence or absence of Columbia spotted frog (Table 2.2). Road distance was calculated from 2016 TIGER/Line primary and secondary roads (U.S. Census Bureau 2016). In laboratory settings, Bd growth and reproduction occurred between temperatures of 4-25℃ and had higher growth and reproduction rates between 17-23℃ (Piotrowsk, Annis & Longcore 2004); because of this we hypothesized that water temperature would positively influence Bd presence. We also included three potential covariates of detection for Bd (pB) that hypothesized Bd detection would be influenced by the presence of Columbia spotted frog or covariates that could influence degradation (water temperature and pH) (Table 2.2).

We ran all combinations of hypotheses for psiA, psiBA, psiBa (or psiB), pA, and pB for a total of 72 models (Table 2.2). Two additional null models were included in the model set, psiA(.)psiBA(.)psiBa(.)pA(.)pB(.) and psiA(.)psiB(.)pA(.)pB(.). Two-species occupancy models were fitted using Program Mark 8.1 (White & Burnham 1999). Models were ranked according to Akaike's information criterion corrected for small sample size (AICc) and assessed for support based on AICc weights and delta AICc.

Results

Two-species occupancy model: Columbia spotted frog and beavers

The most supported model for Columbia spotted frog with beavers included the covariates of slope and tree-dominated area as influences on beaver occupancy (psiA), climatic covariates of precipitation and temperature as influences on Columbia spotted frog occupancy given the presence or absence of beaver (psiBA, psiBa), and water temperature as a covariate of detection for Columbia spotted frog (pB) (Table 2.3). The top three models (total model weight = 0.70) included parameters of psiBA and psiBa, indicating support for a biotic interaction between Columbia spotted frogs and beavers (Table 2.3). Consistent with our hypothesis, we found that Columbia spotted frog occupancy at sites with less annual precipitation is higher when beavers are present and lower when beavers are absent (Figure 2.1). Unexpectedly, we found that Columbia spotted frog occupancy also decreased with higher annual temperature when beavers are present (β = -1.41, SE = 0.407, 95% CI: -2.22 - -0.62), which would be expected for Columbia spotted frog occupancy without the hypothesized role of beaver-created areas. In our comparison of covariates that influence American beaver occupancy, physical site characteristics were supported over climate covariates and slope had a negative impact on beaver occupancy (β = -0.069, SE = 0.028, 95% CI: -0.13 - -0.01). Tree-dominated habitat had a positive relationship with beaver occupancy but the 95% confidence interval overlapped zero. At the eDNA sample level, Columbia spotted frog detection was positively influenced by water temperature (β = 0.11, SE = 0.04, 95% CI: 0.04 - 0.19).

Two-species occupancy model: Columbia spotted frog and Bd

The most supported two-species occupancy model (AICc weight = 0.78) included water temperature as a covariate of Columbia spotted frog occupancy (psiA), distance from a road as influence on Bd occupancy given the presence or absence of Columbia spotted frog (psiBA, psiBa), water temperature as a covariate of detection for Columbia spotted frog (pA), and the presence of Columbia spotted frog as a covariate of Bd detection (pB) (Table 2.4). This top model included parameters of psiBA, psiBa, and environmental covariates that indicated support for a species interaction between Bd and Columbia spotted frog. The occupancy probability of Bd given Columbia spotted frog was higher closer to roads (Figure 2.2). Consistent with our hypothesis, the SIF of Bd and Columbia spotted frog varied with site distance from primary or secondary roads and when a site was next to a road, Bd and Columbia spotted frog were more likely to occur together (SIF = 2.63, SE = 0.25, 95% CI: 2.13 – 3.12. At mean road distance (15.5 km) and water temperature (18.4℃), unconditional Bd occupancy was estimated at 0.18 (SE = 0.04, 95% CI: 0.13 – 0.26) while Bd occupancy given the presence or absence of Columbia spotted frog was 0.41 (SE = 0.07, 95% CI: 0.29 – 0.54) and 0.05 (SE = 0.03, 95% CI: 0.02 – 0.18), respectively. Road distance was not supported as a factor of occupancy for Columbia spotted frog. However, Columbia spotted frog occupancy (psiA) had a positive relationship with water temperature (β = 0.07, SE = 0.03, 95% CI: 0.01 - 0.13). When Columbia spotted frog was detected, the detection probability of Bd was higher (pB = 0.68) than without Columbia spotted frog (pB = 0.28) (β = 1.68, SE = 0.58, 95% CI: 0.53 - 2.82) and water temperature increased our detection of Columbia spotted frog (β = 0.11, SE = 0.04, 95% CI: 0.03 – 0.18).

Discussion

Using multi-species detection of eDNA in water samples, we found that biotic interactions and abiotic factors influence Columbia spotted frog occupancy in the Great Basin. This work quantifies the importance of beaver to an amphibian in a water-limited system across a precipitation gradient, a relationship that could gain greater importance with increased precipitation variability in this area predicted under future climate scenarios (Hurd *et al.* 1999). Additionally, we found that Bd occupancy is impacted by Columbia spotted frog occupancy and that proximity to a primary or secondary road increases Bd occupancy when Columbia spotted frogs are also present.

While the importance of beaver-modified habitat for amphibians has been documented in other studies (Popescu & Gibbs 2009, Hossack *et al.* 2015, Karraker and Gibbs 2009, Cunningham *et al.* 2007; Arkle & Pilliod 2015), this study shows how active beaver presence within areas that receive less precipitation directly impacts amphibian occupancy. Beaver occupancy in the driest portions of the range could benefit the Columbia spotted frog in the Great Basin by increasing availability of habitat. Future climate scenarios predict a decrease in spring snow pack and precipitation in the Great Basin (Cayan *et al.* 2013) that could negatively impact Columbia spotted frog breeding; this could potentially be mitigated by beaver-created dams that raise water levels during spring snow melt (DeVries *et al.* 2012; Zero & Murphy 2016).

There are growing efforts to understand how beaver influence the restoration and management of riparian areas in arid and semi-arid systems for both wildlife conservation and human use (Albert & Trimble 2000; Gibson & Olden 2014). Through dam building, beavers raise water tables, broaden floodplains, expand wetlands, improve water quality, increase habitat complexity, and promote greater species richness and diversity. In arid and semi-arid systems, there is great potential for beaver-created riparian areas to increase water storage and maintain

perennial flows during drought conditions that would benefit wildlife and livestock (Demmer & Beschta 2008; Wild 2011). In arid and semi-arid systems like the Great Basin, riparian vegetation can limit beaver establishment (Baker *et al. 2005;* White & Rahel 2008; Gibson & Olden 2014), but the addition of dam support structures has successfully promoted dam building in beavers and increased net aggradation that can promote riparian vegetation, increase groundwater storage and possibly increase summer flows (Pollock *et al.* 2013). Alternatively, the placement of dam structures that mimic beaver dams has improved floodplain connectivity and could be used in areas where riparian resources cannot support or limit beaver (DeVries *et al.* 2012).

The probability of occupancy of Bd in this system was influenced by the proximity to primary and secondary roads, with the closeness to road increasing the probability that Bd would occur at the same site with Columbia spotted frogs. This is similar to other findings that suggested gravel roads in Tasmania were strongly associated with Bd presence, likely through the movement of contaminated water (Pauza *et al.* 2010). The association of higher co-occurrence and proximity to roads in this study could relate to the potential of spread through the bait trade. Bd-positive water has been reported in bait shops selling larval tiger salamanders (*Ambystoma tigrinum*) (Picco and Collins 2008). Most of the Bd positive sites where located in northern Nevada where outdoor recreation and large reservoirs on main roads are used for fishing that could lead to greater opportunity for the introduction Bd into the system through fishing bait. However, if Bd is being introduced at these fishing locations, a possible mechanism for the spread along roads needs further research. Populations of Columbia spotted frogs located near roads may be more likely to have Bd; this pattern could be used to help identify populations or sites that could be at higher risk for pathogen spread and sublethal effects.

Species interactions are important in determining occupancy through predation, competition, host-pathogen interactions, and facilitation. This study quantified the co-occurrence of Columbia spotted frog and beavers in a facilitative relationship and a host-pathogen interaction between Bd and Columbia spotted frogs in a semi-arid landscape. Using multiple species detections from eDNA samples, we accounted for detection probabilities for all species and modeled covariates of detection and occupancy. As landscapes are altered by human factors and climate change, species interactions have the potential to mitigate or aggravate these effects and should be considered in current and future occupancy and conservation goals.

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Table 2.1: Quantitative PCR assay design for American beaver (*Castor Canadensis)*. Quasar 670 and CAL Fluor Red 610 Fluorophore dye (Biosearch Technologies).

Forward Primer	TCCCGAGCGGGTTGCT
Reverse Primer	CATAAACAATCCACYTCAAAATGGA
Probe	TTTCACGGAGGATGGTAGATTAAGA-BHQplus

Table 2.2: List of hypotheses for each parameter of the two-species occupancy model. All combinations were modeled for a total of 72 candidate models. Two null models were also included for each species combinations (psiA(.)psiBA(.)psiBa(.)pA(.)pB(.) and psiA(.)psiB(.)pA(.)pB(.)). Csf – presence of Great Basin Columbia spotted frog, tree – the sum of tree classified cells within 200 meters of site, (.) – intercept only

American beaver (*Castor canadensis)* and Great Basin Columbia spotted frog (*Rana luteiventris)*				
psiA(slope + tree)	psiBA(precip + temp)	psiBa(precip + temp)	pA(.)	pB(.)
psiA(precip + temp)	psiBA(elevation)	psiBa(elevation)	pA(pH)	pB(pH)
			pA(water temp)	pB(water temp)

Great Basin Columbia spotted frog (*Rana luteiventris) and Batrachochytrium dendrobatidis*				
psiA(road)	psiBA(road)	psiBa(road)	pA(.)	pB(csf)
psiA(water temp)	psiBA(water temp)	psiBa(water temp)	pA(pH)	pB(pH)
			pA(water temp)	pB(water temp)

Table 2.3: Top two-species occupancy modeling results from model set (N = 74) examining the co-occurrence of Columbia spotted frogs and American beaver in the Great Basin. Δ AICc is the difference in AICc from the top model and the AICc weight is the relative strength of the model given the candidate set. K = number of parameters. Phy – physical covariates of slope and the number of cells in a 200 m area around point with tree-dominated vegetation classification from LANDFIRE existing vegetation type (30m). Climate – climate covariates of annual precipitation and temperature of 30 year normal dataset from PRISM at 800 m cell size. Wt – water temperature recorded on site during survey. Ph – ph recorded on site during survey. (.) – intercept only.

Model	AICc	Δ AICc	AICc Weight	K
psiA(phy) psiBA(climate) psiBa(climate) pA(.) pB(wt)	987.06	0	0.42	12
psiA(phy) psiBA(climate) psiBa(climate) pA(wt) pB(wt)	989.22	2.17	0.14	13
psiA(phy) psiBA(climate) psiBa(climate) pA(ph) pB(wt)	989.25	2.19	0.14	13
psiA(phy) psiB(climate) pA(.) pB(wt)	990.40	3.35	0.08	9
psiA(climate) psiBA(climate) psiBa(climate) pA(.) pB(wt)	990.82	3.76	0.06	12
psiA(phy) psiB(climate) pA(wt) pB(wt)	992.51	5.45	0.03	10
psiA(phy) psiB(climate) pA(ph) pB(wt)	992.53	5.47	0.03	10
psiA(climate) psiBA(climate) psiBa(climate) pA(wt) pB(wt)	992.98	5.92	0.02	13
psiA(climate) psiBA(climate) psiBa(climate) pA(ph) pB(wt)	993.01	5.95	0.02	13
psiA(phy) psiBA(climate) psiBa(climate) pA(.) pB(.)	993.43	6.37	0.02	11
psiA(climate) psiB(climate) pA(.) pB(wt)	994.16	7.10	0.01	9
psiA(phy) psiBA(climate) psiBa(climate) pA(wt) pB(.)	995.57	8.52	0.01	12
psiA(phy) psiBA(climate) psiBa(climate) pA(ph) pB(.)	995.60	8.54	0.01	12
psiA(climate) psiB(climate) pA(wt) pB(wt)	996.26	9.20	0.00	10
psiA(climate) psiB(climate) pA(ph) pB(wt)	996.29	9.23	0.00	10
psiA(phy) psiB(climate) pA(.) pB(.)	996.99	9.93	0.00	8
psiA(climate) psiBA(climate) psiBa(climate) pA(.) pB(.)	997.19	10.13	0.00	11

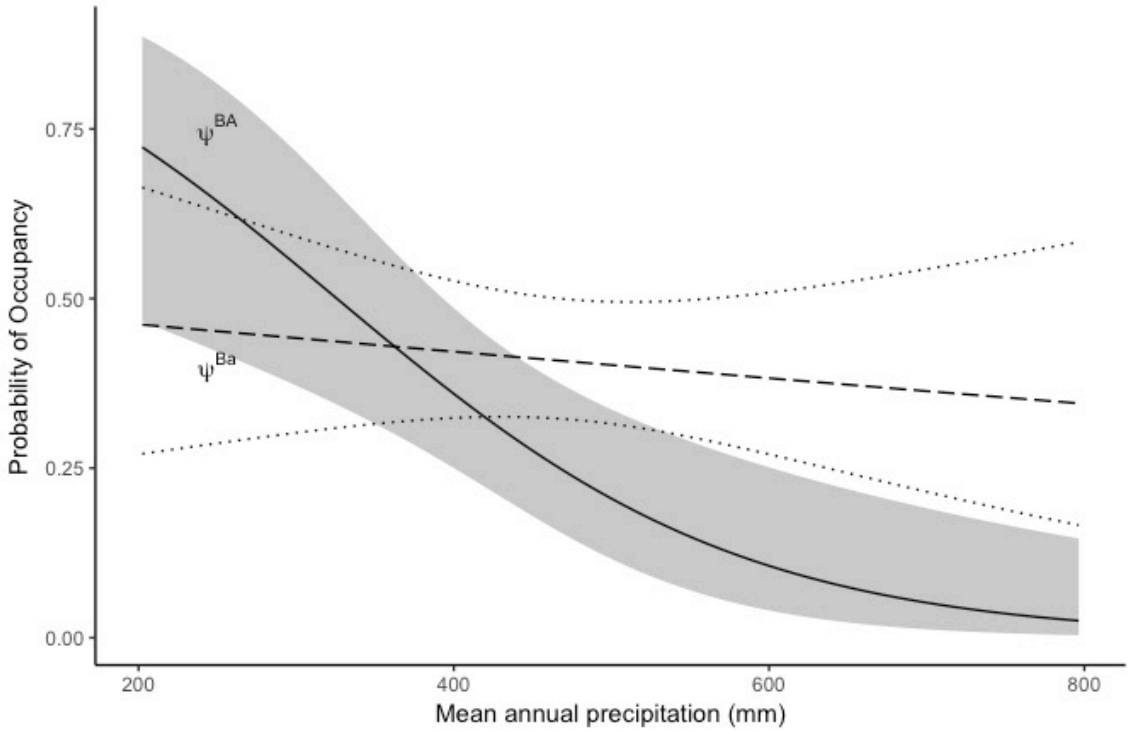

Figure 2.1: The probability of Columbia spotted frog occupancy given American beaver is present (ψ^{BA}) or absent (ψ^{Ba}) from two-species occupancy model of the most supported model (AICc weight = 0.42) by the mean annual precipitation. The 95% confidence interval is shaded in gray for ψ^{BA} and dotted lines for ψ^{Ba}.

Table 2.3: Top two-species occupancy modeling results from model set (N = 74) examining *Batrachochytrium dendrobatidis* occurrence with Columbia spotted frogs and in the Great Basin. Δ AICc is the difference in AICc from the top model and the AICc weight is the relative strength of the model given the candidate set. K = number of parameters. wt – water temperature recorded on site during survey. Road – distance of site from primary or secondary road. Csf – presence of Columbia spotted frog. (.) – intercept only.

Model	AICc	Δ AICc	AICc Weight	K
psiA(wt) psiBA(road) psiBa(road) pA(wt) pB(csf)	820.92	0	0.78	10
psiA(road) psiBA(road) psiBa(road) pA(wt) pB(csf)	825.54	4.62	0.08	10
psiA(wt) psiBA(road) psiBa(road) pA(.) pB(csf)	826.04	5.12	0.06	9
psiA(wt) psiBA(road) psiBa(road) pA(ph) pB(csf)	827.73	6.82	0.03	10
psiA(wt) psiBA(road) psiBa(road) pA(wt) pB(wt)	828.31	7.39	0.02	10
psiA(wt) psiBA(road) psiBa(road) pA(wt) pB(ph)	829.38	8.46	0.01	10
psiA(wt) psiBA(wt) psiBa(wt) pA(wt) pB(csf)	830.07	9.15	0.00	10
psiA(road) psiBA(road) psiBa(road) pA(.) pB(csf)	831.44	10.52	0.00	9

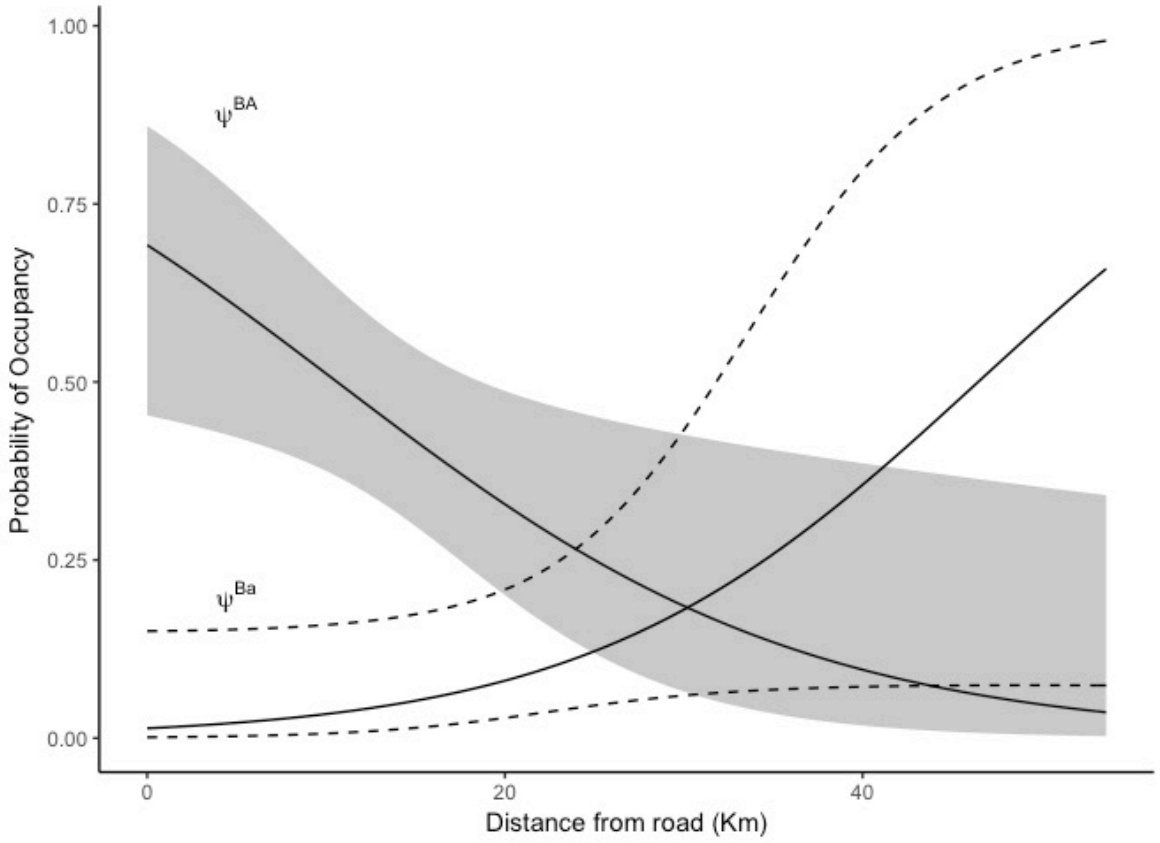

Figure 2.2: The probability of *Batrachochytrium dendrobatidis* occupancy given Great Basin Columbia spotted frog (*Rana luteiventris*) is present (ψ^{BA}) or absent (ψ^{Ba}) by the distance from a primary or secondary road from the most supported model (AICc weight = 0.78) of a two-species occupancy model set. The 95% confidence interval is shaded in gray for ψ^{BA} and dashed lines for ψ^{Ba}.

APPENDIX

Western toads (*Anaxyrus boreas*) occur across the Great Basin and declines from occupied historical sites have been documented (Wente et al. 2005). We used a previously developed quantitative PCR assay for western toads from mitochondrial DNA sequences to test for the presence of western toads in environmental DNA samples. We tested 220 sites for the presence of western toad. We detected target DNA at 2 survey sites and opportunistically observed western toads at 2 additional locations. We hypothesized that limited water would require western toads and Columbia spotted frogs to co-occur at sites across the Great Basin, but a lack of western toad detections suggests that they are utilizing different habitat during our sampling period of May-August.