



Host–pathogen metapopulation dynamics suggest high elevation refugia for boreal toads

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Abstract. Emerging infectious diseases are an increasingly common threat to wildlife. Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), is an emerging infectious disease that has been linked to amphibian declines around the world. Few studies exist that explore amphibian–*Bd* dynamics at the landscape scale, limiting our ability to identify which factors are associated with variation in population susceptibility and to develop effective in situ disease management. Declines of boreal toads (*Anaxyrus boreas boreas*) in the southern Rocky Mountains are largely attributed to chytridiomycosis but variation exists in local extinction of boreal toads across this metapopulation. Using a large-scale historic data set, we explored several potential factors influencing disease dynamics in the boreal toad–*Bd* system: geographic isolation of populations, amphibian community richness, elevational differences, and habitat permanence. We found evidence that boreal toad extinction risk was lowest at high elevations where temperatures may be suboptimal for *Bd* growth and where small boreal toad populations may be below the threshold needed for efficient pathogen transmission. In addition, boreal toads were more likely to recolonize high elevation sites after local extinction, again suggesting that high elevations may provide refuge from disease for boreal toads. We illustrate a modeling framework that will be useful to natural resource managers striving to make decisions in amphibian–*Bd* systems. Our data suggest that in the southern Rocky Mountains high elevation sites should be prioritized for conservation initiatives like reintroductions.

Key words: amphibian decline; *Anaxyrus boreas boreas*; *Batrachochytrium dendrobatidis*; elevation; host–pathogen dynamics; multispecies occupancy.

INTRODUCTION

Emerging infectious diseases (EIDs) can increase species extinction rates, shape community dynamics, and fundamentally alter how ecosystems function (Whiles et al. 2012, Langwig et al. 2016, Rothermel et al. 2016). Mechanisms underlying disease emergence are often poorly understood because populations may decline before sampling can occur, pathogens may be newly described, and static patterns of disease metrics (e.g., occurrence or prevalence) are not informative (Hastings 2001, Yackulic et al. 2015). Despite these difficulties, understanding the ecology of host–pathogen relationships and the factors that influence disease-related extinction events are central goals of ecologists and resource managers (Langwig et al. 2015). Identifying factors that moderate or intensify interactions between hosts and their pathogens is essential for predicting disease risk and for planning management interventions.

Although most pathogens are not predicted to drive their hosts to extinction (Anderson and May 1991, De Castro and Bolker 2005), evidence of this phenomenon exists in many natural settings (Thorne and Williams 1988, Schloegel et al. 2006, Smith et al. 2006, Ryan et al. 2008). Moreover, population-level responses to pathogens can vary within a single species over small spatial and temporal scales (Hosseini et al. 2004, Murphy et al. 2009, Savage and Zamudio

2011). Variation in extinction risk may be linked to elements of host susceptibility, pathogen infectivity and virulence, environmental features, or interactions of these elements. Understanding the mechanisms that give rise to variation in disease dynamics underlies sound ecological insights and is the basis for conservation and management activities (Garner et al. 2016).

Chytridiomycosis has emerged as an infectious disease of amphibians and is caused by the aquatic fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al. 1999). Despite almost 20 years of research, questions remain about which factors shape local host–*Bd* dynamics (Venesky et al. 2014b). The global impacts of chytridiomycosis (Skerratt et al. 2007) coupled with local extirpations and differential susceptibility of amphibian populations (Schloegel et al. 2006, Murphy et al. 2009), make identifying the factors that lead to extinction a conservation priority (Wilber et al. 2017). Geographic isolation of populations, host species community richness, variation in climate, and habitat differences across sites have all been suggested to influence amphibian–*Bd* dynamics (Searle et al. 2011, Heard et al. 2013, Addis et al. 2015, Clare et al. 2016). Assessing the relative contributions of these factors to host–pathogen dynamics will improve our ability to successfully manage landscapes, species, and populations challenged by chytridiomycosis (Venesky et al. 2014b, Garner et al. 2016).

Populations that are geographically isolated from one another may differ in their responses to pathogens. Some amphibian species may be especially prone to population isolation due to limited dispersal abilities (Blaustein et al. 1994), high breeding site fidelity (Smith and Green 2005), and

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vulnerability to barriers like roads (Fahrig et al. 1995, Marsh et al. 2005) and inhospitable terrain (Funk et al. 2005, Murphy et al. 2010a, Watts et al. 2015). In laboratory trials, individual amphibians of the same species from geographically isolated populations have demonstrated differential susceptibility to chytridiomycosis (Murphy et al. 2009, Savage and Zamudio 2011, Bradley et al. 2015). The cause of the variation in susceptibility to chytridiomycosis can be related to a variety of factors including differences in habitat (Lips et al. 2006), skin microbiome (Kueneman et al. 2015), infection history (Murphy et al. 2011), or genetics (Addis et al. 2015).

The presence of an environmental or alternate host species “reservoir” could increase the potential of a pathogen to drive a host species to extinction (Rosà et al. 2003, Dobson 2004). While evidence for a long-lived or resting stage of *Bd* is minimal (Johnson and Speare 2003, but see Di Rosa et al. 2007), multiple amphibian species often occupy the same breeding habitat and could serve as reservoir species that may alter extinction risk for susceptible species (Reeder et al. 2012). Field and laboratory studies in amphibian–*Bd* systems have found that host species community richness can either dilute or amplify disease risk depending on the composition and traits of co-occurring hosts (Searle et al. 2011, Venesky et al. 2014a, Han et al. 2015, Scheele et al. 2017).

Elevational differences in climate can also influence patterns of disease (Selig et al. 2006, Lafferty 2009). Changes in moisture or temperature across elevations can interact with the physiological tolerances of free-living pathogens or vectors to shape pathogen distributions (Minakawa et al. 2002) and can influence host stress and associated immune responses (Raffel et al. 2006). *Bd* growth and survival are sensitive to cold and hot temperatures and, in culture, optimal growth of *Bd* occurs between 15° and 25°C (Piotrowski et al. 2004). In the tropics, chytridiomycosis-related amphibian declines are most pronounced in colder months and at high elevations where temperatures are near the thermal optimum for *Bd* growth (Pounds et al. 2006, Gründler et al. 2012, Sapsford et al. 2013, Whitfield et al. 2016). Nevertheless, studies of whether cold climates at northern latitudes limit the effects of *Bd* on amphibians are equivocal, for example, finding positive (Muths et al. 2008), neutral (Knapp et al. 2011), or negative (Savage et al. 2011) relationships between temperature and *Bd* occurrence, infection intensity, and prevalence, respectively.

Differences in habitat quality or structure may also alter host–pathogen dynamics (Penczykowski et al. 2014) or the effects of disease on individuals (Sauter et al. 2006). Some evidence suggests that warmer water and drier microhabitats may inhibit *Bd* growth (Puschendorf et al. 2011, Heard et al. 2013), indicating that semi-permanent wetlands may serve as an environmental refuge from high pathogen loads and/or high infection probabilities. However, these semi-permanent sites require tadpoles to metamorphose quickly and may lead to frequent reproductive failure even in the absence of disease (Karraker and Gibbs 2009, Zipkin et al. 2012).

We evaluated the competing roles of geographic isolation, host species community richness, elevation, and habitat permanence in shaping amphibian disease dynamics using a long-term data set on boreal toads (*Anaxyrus boreas boreas*) and *Bd* in the Southern Rocky Mountains (SRM). We used a dynamic two-species occupancy model to account for the

imperfect detection of both toads and *Bd* and to obtain unbiased estimates of host and pathogen dynamics (Richmond et al. 2010, Mosher et al. 2018). Our objectives were to (1) identify the major factors driving local variation in amphibian–*Bd* occurrence dynamics in a temperate amphibian–*Bd* system and (2) quantify this variation, providing empirical estimates of boreal toad–*Bd* dynamics that will be useful for understanding amphibian extinction risk in the SRM and other temperate and high elevation settings.

METHODS

Study species

Boreal toads are a species native to western North America that has experienced *Bd*-related declines throughout the SRM (Muths et al. 2003, Scherer et al. 2005), though some populations with *Bd* persist within this region (Carey et al. 2005). Boreal toad breeding sites in the SRM vary in their elevation, habitat type, and whether other amphibian species occur in sympatry with boreal toads. In addition, some sites have been isolated from others by rugged terrain or major interstate highways for many years leading to measurable genetic differences in boreal toad populations (Goebel 2000, Colorado Parks and Wildlife, *unpublished data*). These sources of variation, coupled with long-term monitoring of both boreal toads and *Bd*, make this an ideal system to investigate factors that may shape disease-related amphibian extinctions.

Data collection

Boreal toad surveys were conducted annually from 2001 to 2010 at 82 historic breeding wetlands (hereafter, sites) in mountainous regions of Colorado and Wyoming (Fig. 1). Boreal toads breed in a variety of habitat types including beaver ponds, wet meadows, oxbows, and lake or pond margins and sites range in elevation from 2,470 to 3,680 m. Visual encounter surveys (range 1–10, mean = 3) were conducted at sites each year during the boreal toad breeding and developmental seasons (May–October). Boreal toad skin swabs were collected opportunistically during surveys from adult boreal toads of both sexes from 2003 to 2010. An average of 3.5 swabs per survey were collected over the course of the study, but swabs were not necessarily collected on each visit and could not be collected unless boreal toads were present and detected.

To develop pathogen detection/non-detection histories, we first extracted *Bd* DNA from swabs using a Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, California, USA; Bletz et al. 2015). Extracted DNA was analyzed in triplicate using either standard polymerase chain reaction (PCR; years 2003–2005) or quantitative real-time PCR (qPCR; years 2006–2010) techniques, and samples were only considered positive if DNA was successfully amplified in two of three replicates. For a replicate to be classified as positive, evidence of an exponential, log-linear increase in fluorescence across at least three cycles must have been observed (J. Wood, *personal communication*).

In addition, we determined whether each breeding site was semi-permanent (prone to seasonal drying) or permanent, and whether the sites were species rich (providing breeding

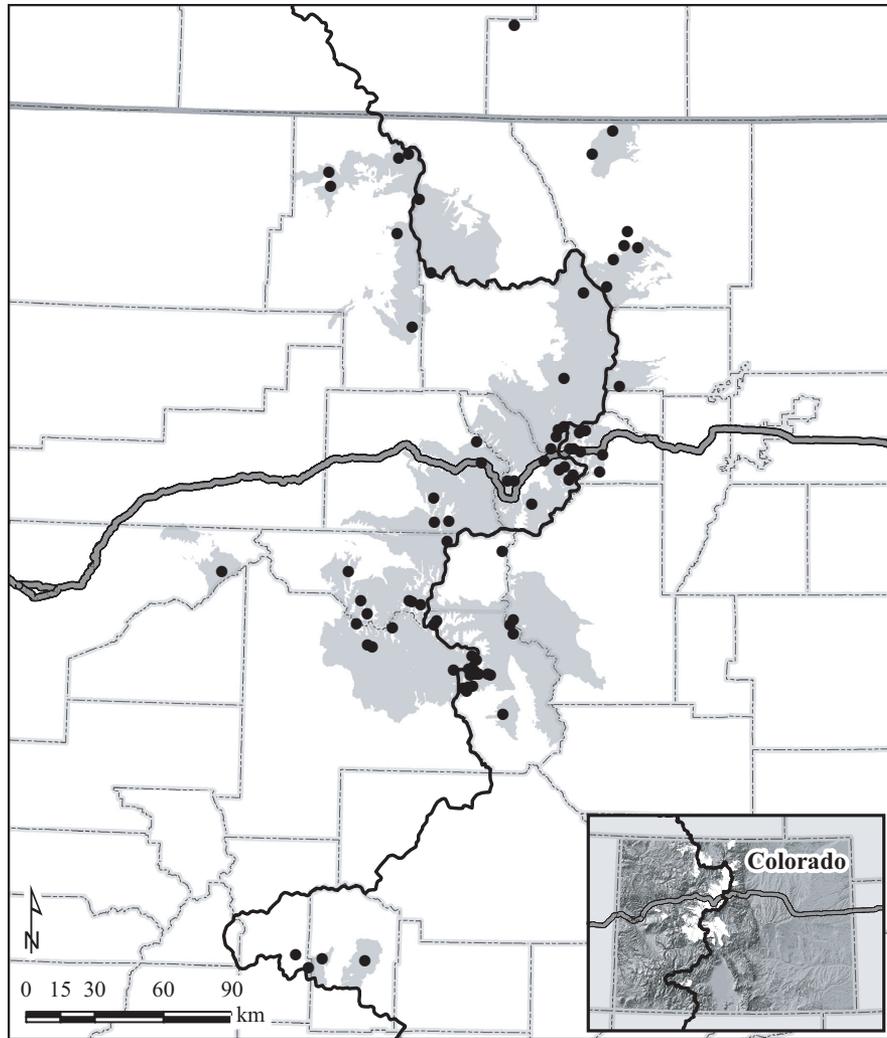


FIG. 1. Map of 82 historic boreal toad breeding sites (black points) in Colorado and southern Wyoming with two potential geographic barriers (the Continental Divide [black line] and Interstate 70 [gray line]). The shaded region represents the known historic range of boreal toads in Colorado (Colorado Parks and Wildlife, CPW Boreal Toad Shapefile Download—Overall Range, Species Activity Mapping Project. <http://www.arcgis.com/home/item.html?id=3723aadaf0eb41acaed0b95289e1b5f6>).

habitat for boreal toads and at least one other amphibian species) or species poor (providing breeding habitat for boreal toads only). Two other native amphibian species overlap in distribution, breeding habitat, and breeding phenology with boreal toads in the southern Rocky Mountains: tiger salamanders (*Ambystoma tigrinum*) and boreal chorus frogs (*Pseudacris maculata*) (Hammerson 1999). Habitat permanence and species richness data were elicited from agency biologists who sampled these sites for the duration of the study, and were treated as binary, static covariates (Table 2).

Modeling framework

We used a dynamic two-species occupancy model (Richmond et al. 2010, Miller et al. 2012a, Mosher et al. 2018) to estimate boreal toad (species A) and *Bd* (species B) occurrence dynamics simultaneously (Table 1). The model assumes that sites (i.e., wetlands) are in one of four mutually exclusive states in any year and allows for estimation of these state probabilities: sites can be occupied by boreal toads only (state A:

$\psi^A(1 - \psi^{BA})$), occupied by *Bd* only (state B: $(1 - \psi^A)\psi^{BA}$), occupied by boreal toads and *Bd* (state AB: $\psi^A\psi^{BA}$), or unoccupied by either species (state U: $(1 - \psi^A)(1 - \psi^{BA})$). This model assumes that *Bd* can exist in isolation of boreal toads (state B), either in the environment or in an alternate host species. Transitions among states can occur between seasons (Fig. 2), but sites are closed to changes in states within a season. Colonization (γ) and extinction (ϵ) parameters characterize these transitions and are estimated for each species conditional on whether the other species is present in season t (Table 1). For instance, the probability of a site transitioning from state A (boreal toad only) in season t to state AB (boreal toad and *Bd*) in season $t + 1$ is the product of the probabilities of boreal toad persistence in the absence of *Bd* in season t , and *Bd* colonization in the presence of boreal toads in season t (i.e., $(1 - \epsilon^A)(\gamma^{BA})$; Fig. 2, transition 4).

Detection parameters (Table 1) describe the probability of detecting each species when they occur alone (p^A and p^B) or when they occur together (r^A and r^{BA}). Finally, the model allows for a different detection probability for *Bd* when

TABLE 1. List of parameters estimated in the dynamic two-species occupancy model of toads and *Bd* including initial state parameters (ψ), vital rate parameters (colonization, γ and local extinction, ε), and detection probabilities (p and r).

Parameter	Description
$\psi_{t=1}^A$	initial probability of occupancy for species A
$\psi_{t=1}^{BA}$	initial probability of occupancy for species B, given species A is present
$\psi_{t=1}^{Ba}$	initial probability of occupancy for species B, given species A is absent
γ_t^A	probability of colonization by species A, given species B is absent
γ_t^{AB}	probability of colonization by species A, given species B is present
γ_t^B	probability of colonization by species B, given species A is absent
γ_t^{BA}	probability of colonization by species B, given species A is present
ε_t^A	probability of extinction of species A, given species B is absent
ε_t^{AB}	probability of extinction of species A, given species B is present
ε_t^B	probability of extinction of species B, given species A is absent
ε_t^{BA}	probability of extinction of species B, given species A is present
p_t^A	detection probability of species A, given species B is absent
p_t^B	detection probability of species B, given species A is absent
r_t^A	detection probability of species A, given species B is present
r_t^{BA}	detection probability of species B, given species A is present and detected
r_t^{Ba}	detection probability of species B, given species A is present and undetected

Notes: We fixed the detection of *Batrachochytrium dendrobatidis* (*Bd*; species B) to be 0 when boreal toads (species A) were absent (p^B) or present but undetected (r^{Ba}) to reflect that *Bd* is only detected on boreal toad skin swabs in this system, and therefore estimate only 14 of the 16 parameters listed. While these parameters can vary through time (noted with subscript t), the presence of an unobservable state in our study restricted us to assume that colonization and extinction parameters were constant through time.

toads are present but not detected (r^{Ba}). In our study, *Bd* was detected from skin swabs and cannot be detected when boreal toads are absent or undetected. Therefore, we fixed the detection of *Bd* (species B) to zero when boreal toads (species A) were absent or present but undetected ($p^B = r^{Ba} = 0$). This sampling limitation creates an unobservable state that has implications for parameter estimation. Mosher et al. (2018) examined the ramifications of this limitation and found some parameters were unidentifiable when models contained combinations of time-specific vital rates. In time-constant models, colonization and extinction probabilities for *Bd* in the absence of the host (γ^B and ε^B) were estimated imprecisely. Accordingly, we explored only models with time-constant vital rates with the understanding that some estimates may be extremely imprecise.

Biological hypotheses

Because we lacked genetic data at the broad spatial scale encompassed by the study, we focused on populations

isolated by barriers to movement where local adaptation and thus putative genetic or other differences may be present. A major interstate highway established in 1956 runs east-west (Interstate 70) and intersects with the Continental Divide (which often exceeds 4,000 m in elevation): these features separate boreal toad populations into four regions (northeast, northwest, southwest, and southeast; Fig. 1) whose connectivity may be limited by impervious surfaces and topography (Murphy et al. 2010b). We note that this coarse measure of isolation may not accurately depict real genetic differences in toads but could reflect differences due to the isolation of populations from dispersal of infected animals, different strains of *Bd*, or habitat differences among regions.

To explore potential impacts of amphibian host richness on disease dynamics, we categorized sites as sustaining either species-poor (boreal toads only) or species-rich (boreal toads and at least one additional species) amphibian communities. Categorization was based on whether boreal chorus frogs and/or tiger salamanders had ever been detected at the wetland during the study. Due to the small number of sites with tiger salamanders ($n = 6$) we were unable to separate species-specific effects (chorus frogs vs. tiger salamanders). We used elevation as a proxy for climatic differences, where high elevation sites are generally colder with higher snowpack than low elevation sites. Finally, to investigate the impacts of seasonal drying, we classified sites as semi-permanent (and subject to periodic drying) or permanent (no drying). We made a priori predictions about which vital rate parameters these factors would be most likely to influence and why (Table 2A).

We anticipated heterogeneity in the detection probability of both boreal toads and *Bd* across space and/or time. Accordingly, we generated a priori hypotheses and predictions for covariates influencing detection parameters as well (Table 2B).

Modeling approach

We initially fit a simple model where all parameters were time constant (14 total parameters); this model indicated that extinction probabilities for toads and *Bd*, in the absence of the other species, were zero ($\varepsilon^A = \varepsilon^B = 0.00$; 95% CIs: [0.00, 0.02] and [0.00, 0.35], respectively). Therefore, we modeled these extinction parameters as time constant, rather than as a function of covariates, in subsequent models. We employed a step-down modeling strategy (Lebreton et al. 1992) whereby we first explored factors resulting in variation in detection probability using the most general (highly parameterized) vital rate structures and constant, state-specific initial occupancy probabilities. Specifically, we fit additive models to explore whether boreal toad detection probabilities in the presence (r^A) and absence (p^A) of *Bd* were constant or varied either linearly or quadratically with day of year (DOY or DOY²). In addition, we investigated whether *Bd* detection probability (r^{BA}) was constant or varied either linearly or quadratically with elevation (elev or elev²). We then used the best-supported detection structures to explore hypotheses about factors influencing host–pathogen dynamics.

Several factors hypothesized to influence host–pathogen dynamics were correlated: elevation and host species richness were negatively associated (Spearman’s rank correlation

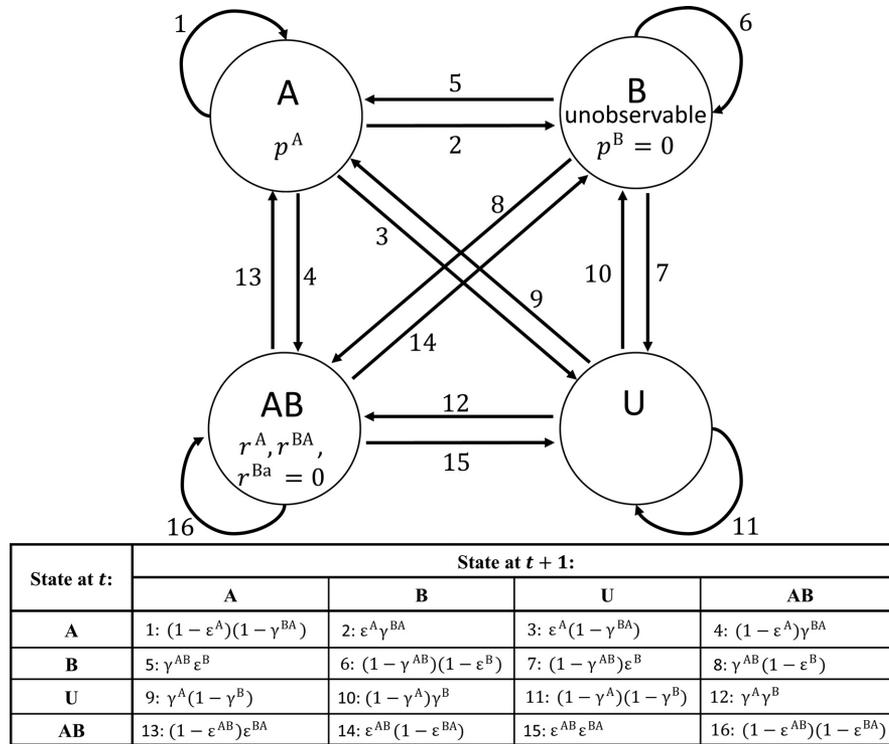


FIG. 2. State transition diagram and detection probabilities for a two-species dynamic occupancy model. Boreal toads (species A) and *Batrachochytrium dendrobatidis* (*Bd*; species B) can occur separately (states A or B), together (state AB), or not at all (unoccupied, state U). Arrows represent the annual transitions among states, with transition probabilities consisting of products of state-specific colonization (γ) and extinction (ϵ) probabilities. Species detection probabilities (p and r) for occupied states are listed within each circle. Parameters are defined in detail in Table 1. Unobservable states and events are signified by detection probabilities being fixed at zero; these occur when *Bd* exists in the absence of toads (state B, $p^B = 0$) and when toads and *Bd* occur together (state AB), but toads are not detected ($r^{BA} = 0$).

TABLE 2. Factors hypothesized to influence (A) disease dynamics and (B) detection probability in a boreal toad–*Bd* system.

Factor (proxy)/Driver	Covariate type	Hypothesized Effects†	Rationale
(A) Colonization and extinction probabilities for boreal toads and <i>Bd</i>			
Geographic differences (isolated regions)	categorical, static	differences expected, but direction unknown $\gamma^{AB}, \epsilon^{AB}$	Geographically isolated boreal toad populations may adapt differently to disease.
Species richness (species poor vs. species rich)	binary, static (1 = species-rich)	$\gamma^{AB}(-), \epsilon^{AB}(+) \gamma^B(+), \gamma^{BA}(+), \epsilon^{BA}(-)$	Additional amphibian species may amplify or dilute impacts of disease on boreal toads, and amplification was hypothesized in this system.
Climate (elevation)	continuous, static	negative (linear) or quadratic $\gamma^{AB}, \epsilon^{AB}, \gamma^B, \gamma^{BA}, \epsilon^{BA}$	Very cold or very warm temperatures may limit <i>Bd</i> growth.
Habitat type (semi-permanent vs. permanent)	binary, static (1 = permanent)	$\gamma^{AB}(-), \epsilon^{AB}(+) \gamma^B(+), \gamma^{BA}(+), \epsilon^{BA}(-)$	Semi-permanent wetlands are prone to desiccation and could lower <i>Bd</i> loads.
(B) Detection probabilities for boreal toads and <i>Bd</i>			
Elevation	continuous	linear (-) or quadratic r^{BA}	Very warm or very cold temperatures may limit <i>Bd</i> growth.
Day of year	continuous	linear (+) or quadratic p^A, r^A	Boreal toad life stages present throughout the breeding season vary in their conspicuousness.

Note: For each factor of interest, we hypothesized which parameters were affected, the expected effect (positive [+] or negative [-]), and the rationale for these predictions.

coefficient, $r_s = -0.43$), elevation and region were positively associated ($r_s = 0.35$), and permanence and the presence of other amphibians were positively associated ($r_s = 0.39$). To avoid overparameterization, we included just one factor per

model. We expected the geographic isolation of populations, or coarse region, to be associated with boreal toad vital rates in the presence of *Bd* only, while other factors were hypothesized to be linked to *Bd* abundance and therefore could be

associated with vital rate parameters for either *Bd* or boreal toad dynamics (Table 2A). In these cases, we constructed models where the factor of interest could be associated with boreal toad dynamics, *Bd* dynamics, or both, resulting in a candidate model set of 33 models. State-specific initial occupancy probabilities were modeled as constant across sites. Models were fit using the dynamic conditional-binomial two-species occupancy model in Program MARK (White and Burnham 1999) and were evaluated using Akaike's information criterion corrected for sample size (AIC_c ; Burnham and Anderson 2002).

RESULTS

Bd was detected throughout the region over the course of the study, though pathogen sampling was at times difficult or impossible due to a paucity of post-metamorphic individuals to swab (Appendix S1: Fig. S1). We found evidence of within-season variation in boreal toad detection probability, which was best modeled as a quadratic relationship with DOY (Fig. 3A; Appendix S2: Table S1). The detection peak in mid-July corresponds with the appearance of large, conspicuous, boreal toad tadpoles. Detection of boreal toads (across life stages) at sites with *Bd* (r^A) was considerably lower than when *Bd* was absent (p^A), potentially due to disease-induced differences in toad abundance. Detection of boreal toads was highest in the middle of the sampling season and declined toward the end of the season. The detection probability of *Bd* declined with elevation; linear and quadratic relationships received similar support (Fig. 3B, Appendix S2: Table S1). We retained the simpler linear *Bd* detection structure for the subsequent vital rate analysis because it received more support and the estimated 95% confidence intervals largely overlapped those from the quadratic structure (Fig. 3B, Appendix S2: Table S1).

A null model, indicating that vital rate parameters were not state dependent (i.e., $\gamma^A = \gamma^{AB}$, $\gamma^B = \gamma^{BA}$, etc.), was the least supported of the 33 models investigated (model weight = 0.00, Table 3), suggesting that *Bd* alters the occurrence dynamics of boreal toads and that the presence of

boreal toads influences the colonization and persistence of *Bd*. A model that assumed the state-specific vital rates were identical among all sites was also not supported, providing evidence that toad-*Bd* dynamics vary across the landscape (model weight = 0.01, Table 3). Models that included our hypothesized factors were all better supported than these null hypotheses. Elevation was the best-supported hypothesis: models with either linear or quadratic relationships between elevation and host and/or pathogen vital rates were included in the top six models (Table 3). Together, these six models received >60% of the cumulative model weight. We present graphical and numerical results for the best-supported model, which differed from the next-best model only in the form of the elevation effect (quadratic vs. linear). Because the best model was almost twice as likely as the second model (weight = 0.28 vs. weight = 0.15), we felt comfortable making inferences from just one model.

Models suggesting that habitat permanence or the presence of other amphibian species influenced boreal toad-*Bd* dynamics had limited support (Table 3). The presence of other amphibian species was somewhat associated with a higher probability that boreal toads would recolonize a site occupied by *Bd*, but the mechanism for this relationship is unclear given the data available (Fig. 4A). Colonization and extinction estimates from the best model containing the effect of habitat permanence (model weight = 0.04, Table 3) showed poor precision, though *Bd* colonization probability was potentially higher at semi-permanent sites, contrary to our predictions (Fig. 4B). Alternatively, *Bd* extinction probability in the presence of toads was also higher at semi-permanent sites, consistent with our a priori predictions (Fig. 4C). Models suggesting that boreal toad-*Bd* dynamics differed among geographically isolated regions were not supported.

Consistent with our expectations, extinction probabilities for both boreal toads and *Bd* were strongly influenced by the presence of the other species (host or pathogen) and were highest at intermediate elevations within our study system (Fig. 5A, B). In the absence of *Bd*, boreal toads always persisted (extinction probability = 0.00), but toad populations with *Bd* were vulnerable to extirpation at intermediate

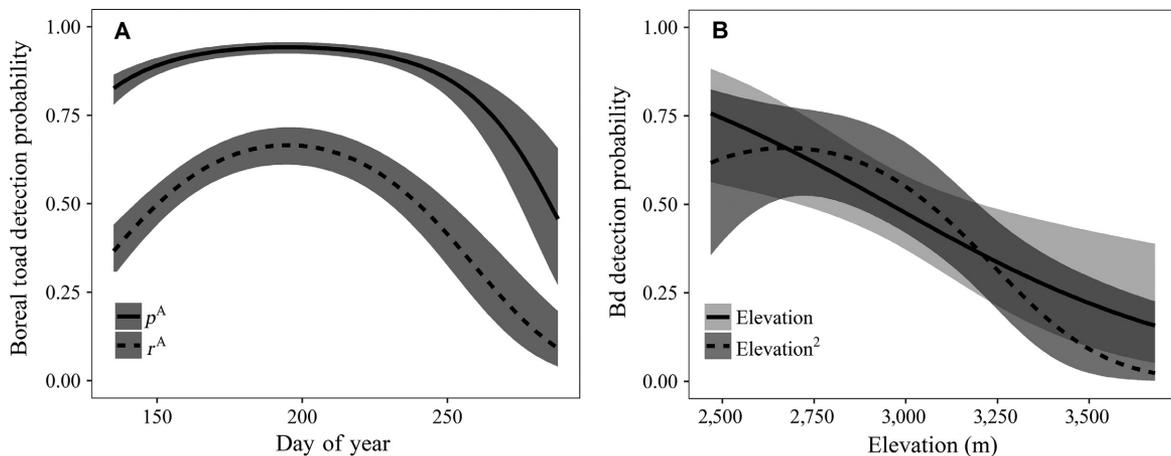


FIG. 3. Best-supported detection probability relationships from the step-down modeling procedure for boreal toads and *Bd*. (A) Detection probability for boreal toads was related to day of year and was lower when *Bd* was present (dashed line; r^A) compared to absent (solid line; p^A). (B) *Bd* detection probability varied with elevation in either a linear (solid line) or quadratic (dashed line) fashion. Estimates and 95% confidence intervals (shaded areas) are given for the best-supported detection structures given general structures for other parameters.

TABLE 3. Model selection table for boreal toad and *Bd* disease dynamics showing the 10 best-supported models.

Model name	AIC _c	ΔAIC _c	Model weight	K	Deviance
$\gamma^A(.) \gamma^{AB}(elev^2) \gamma^B(elev^2) \gamma^{BA}(elev^2) \varepsilon^A(.) \varepsilon^{AB}(elev^2) \varepsilon^B(.) \varepsilon^{BA}(elev^2)$	2,228.07	0.00	0.28	25	2,175.3
$\gamma^A(.) \gamma^{AB}(elev) \gamma^B(elev) \gamma^{BA}(elev) \varepsilon^A(.) \varepsilon^{AB}(elev) \varepsilon^B(.) \varepsilon^{BA}(elev)$	2,229.27	1.20	0.15	21	2,185.31
$\gamma^A(.) \gamma^{AB}(.) \gamma^B(elev^2) \gamma^{BA}(elev^2) \varepsilon^A(.) \varepsilon^{AB}(.) \varepsilon^B(.) \varepsilon^{BA}(elev^2)$	2,231.05	2.97	0.06	21	2,187.09
$\gamma^A(.) \gamma^{AB}(.) \gamma^B(elev) \gamma^{BA}(elev) \varepsilon^A(.) \varepsilon^{AB}(.) \varepsilon^B(.) \varepsilon^{BA}(elev)$	2,231.42	3.35	0.05	19	2,191.82
$\gamma^A(.) \gamma^{AB}(.) \gamma^B(elev) \gamma^{BA}(elev) \varepsilon^A(.) \varepsilon^{AB}(.) \varepsilon^B(.) \varepsilon^{BA}(.)$	2,231.61	3.53	0.05	18	2,194.17
$\gamma^A(.) \gamma^{AB}(.) \gamma^B(.) \gamma^{BA}(.) \varepsilon^A(.) \varepsilon^{AB}(.) \varepsilon^B(.) \varepsilon^{BA}(elev)$	2,232.02	3.95	0.04	18	2,194.58
$\gamma^A(.) \gamma^{AB}(.) \gamma^B(perm) \gamma^{BA}(perm) \varepsilon^A(.) \varepsilon^{AB}(.) \varepsilon^B(.) \varepsilon^{BA}(perm)$	2,232.20	4.13	0.04	19	2,192.6
$\gamma^A(.) \gamma^{AB}(.) \gamma^B(.) \gamma^{BA}(.) \varepsilon^A(.) \varepsilon^{AB}(.) \varepsilon^B(.) \varepsilon^{BA}(perm)$	2,232.55	4.48	0.03	18	2,195.11
$\gamma^A(.) \gamma^{AB}(amph) \gamma^B(.) \gamma^{BA}(.) \varepsilon^A(.) \varepsilon^{AB}(.) \varepsilon^B(.) \varepsilon^{BA}(.)$	2,232.65	4.58	0.03	18	2,195.21
$\gamma^A(.) \gamma^{AB}(.) \gamma^B(.) \gamma^{BA}(.) \varepsilon^A(.) \varepsilon^{AB}(.) \varepsilon^B(.) \varepsilon^{BA}(elev^2)$	2,233.00	4.77	0.03	19	2,193.40
$\gamma^A(.) \neq \gamma^{AB}(.), \gamma^B(.) \neq \gamma^{BA}(.), \varepsilon^A(.) \neq \varepsilon^{AB}(.), \varepsilon^B(.) \neq \varepsilon^{BA}(.)$	2,235.26	7.03	0.01	17	2,199.98
Null model: $\gamma^A(.) = \gamma^{AB}(.), \gamma^B(.) = \gamma^{BA}(.), \varepsilon^A(.) = \varepsilon^{AB}(.), \varepsilon^B(.) = \varepsilon^{BA}(.)$	2,261.03	32.79	0.00	14	2,232.15

Notes: Vital rate parameters were either constant (.) or varied with covariates, including elevation (elev), host amphibian species richness (amph), habitat permanence (perm), and the geographic isolation of populations (geo). Model names reflect the model structure for each vital rate parameter. Model selection information including the Akaike information criterion corrected for sample size (AIC_c), the change in AIC_c attributable to each model (ΔAIC_c), model weights, number of parameters (K), and deviance are shown for each model. We explored only additive relationships between parameter pairs (γ^A and γ^{AB} , γ^B and γ^{BA} , etc.). Geographic isolation was explored but did not appear in the top 10 models. Each model in this analysis had the same detection structure (p^A/r^A (DOY²) r^{BA} (elev) where DOY² represents a quadratic effect of day of year while elev represents a linear effect of site elevation), which was identified as the most parsimonious in the first part of the step-down procedure (Appendix S2: Table S1).

elevations where annual extinction probabilities were ~0.30 (Fig. 5A). When boreal toads were present, the annual extinction probability of *Bd* was also highest at intermediate elevations (~0.30) and *Bd* was unlikely to go extinct at elevational extremes (Fig. 5B). In the absence of boreal toads, *Bd* was likely to persist, though this parameter was difficult to estimate precisely (extinction probability = 0.02, 95% profile likelihood confidence interval from 0.00 to 0.15) because *Bd* could not be detected in the absence of boreal toads. Because we used only boreal toad skin swabs in this study, we have very little information about *Bd* dynamics in the absence of toads such that the estimate of ε^B should be interpreted with caution (Mosher et al. 2018).

The colonization probabilities of boreal toads and of *Bd* were also associated with elevation. When host and pathogen co-occurred, boreal toads were more likely to recolonize high elevation sites should they become locally extinct

(Fig. 5C) and *Bd* was more likely to colonize low and mid-elevation sites (Fig. 5D). Boreal toads were very unlikely to recolonize low and mid-elevation sites after going locally extinct (Fig. 5C). When *Bd* was absent, boreal toads were very likely to colonize unoccupied historic breeding sites at any elevation (colonization probability = 1.00, 95% profile likelihood confidence interval from 0.21 to 1.00), but this parameter was estimated imprecisely because boreal toads rarely go extinct in the absence of *Bd* (recall = 0). In the absence of toads, *Bd* colonization was difficult to estimate well and should be interpreted with caution, again due to a lack of information about *Bd* in the absence of boreal toads (γ^B ; Fig. 5D).

To summarize, *Bd* readily colonized low elevation sites and was unlikely to go extinct in those locations, while boreal toad sites at low elevations that went extinct were very unlikely to be recolonized by toads. At intermediate elevations,

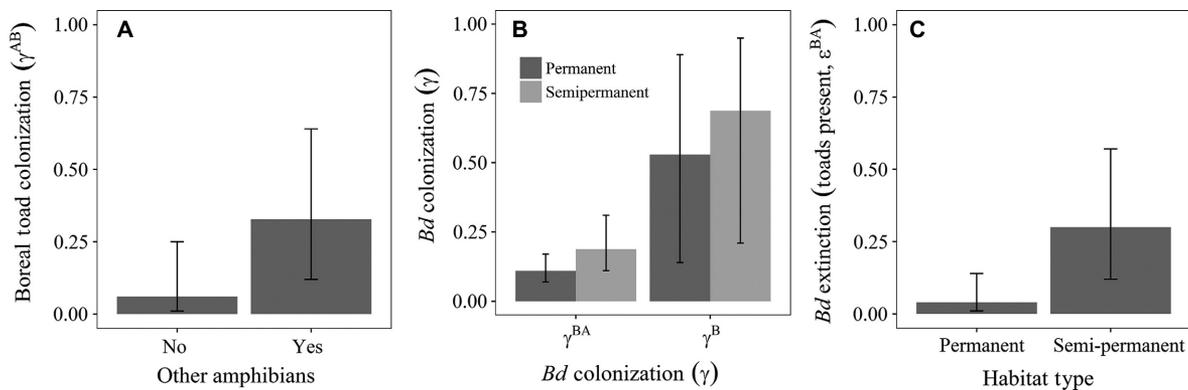


FIG. 4. Estimates of boreal toad colonization and *Bd* extinction with 95% confidence intervals for Southern Rocky Mountain populations of toads studied from 2001 to 2010. We present (A) estimates of colonization for boreal toads when they co-occur with *Bd* and when other amphibian species are, or are not, present. We also present (B) estimates of *Bd* colonization when boreal toads are and are not present for permanent (dark gray) and semi-permanent sites (light gray). (C) *Bd* extinction probability when boreal toads are also present differed slightly between permanent and semi-permanent habitat types. Each set of estimates comes from the best-supported model containing these factors, though these covariates were not well supported.

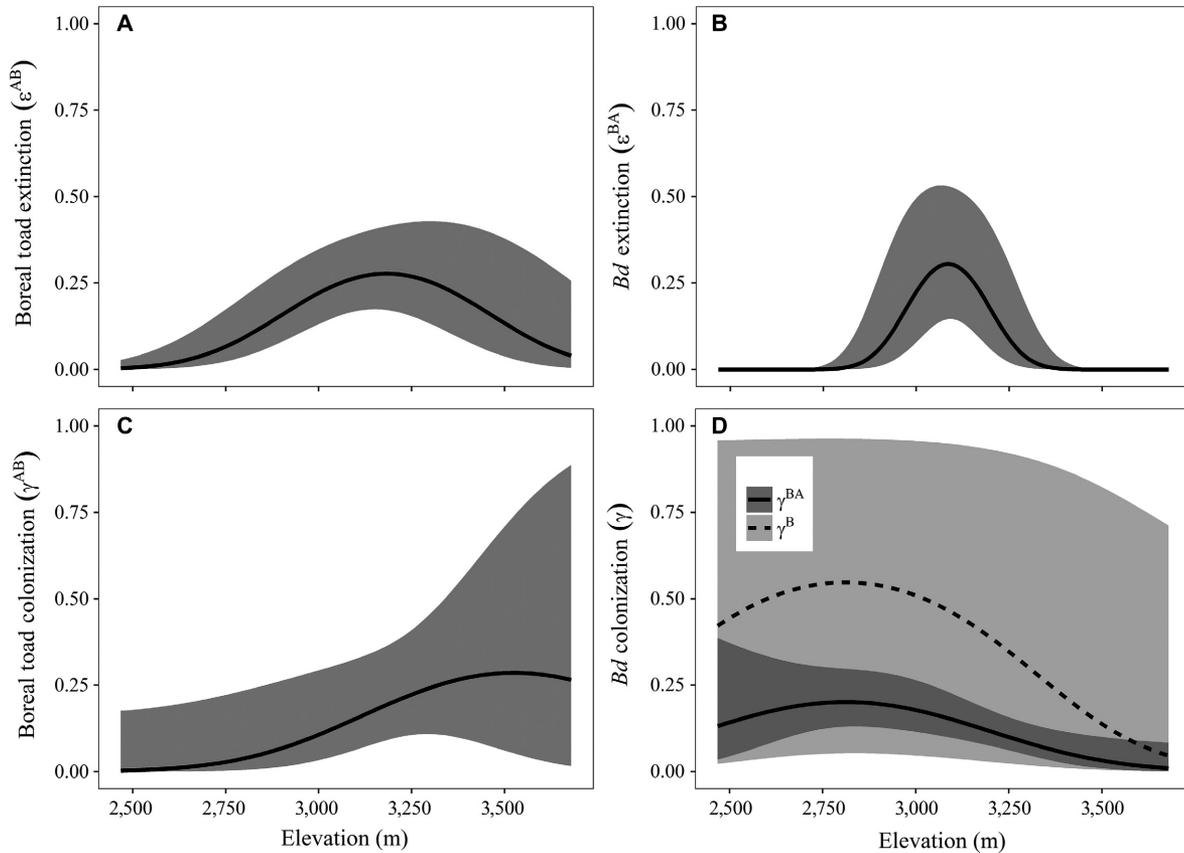


FIG. 5. Effects of elevation on boreal toad and *Bd* dynamics for Southern Rocky Mountain populations studied from 2001 to 2010. Estimates and 95% confidence intervals are from the best-supported model of boreal toad–*Bd* dynamics. A quadratic effect of elevation was associated with (A) boreal toad and (B) *Bd* extinction when host and pathogen co-occurred. (C) Boreal toad colonization in the presence of *Bd* also showed a quadratic association with elevation in the presence of *Bd*, (D) as did *Bd* colonization independent of boreal toad presence.

Bd colonization and local extinction both occurred to some degree. Boreal toads were also likely to go extinct at intermediate elevations, but had some chance of recolonizing sites after extinction events. Finally, *Bd* was unlikely to successfully colonize high elevation sites, but if the pathogen did become established it was unlikely to go extinct at these sites. At high elevation sites, boreal toads were unlikely to go locally extinct but, if they did, the site could often be recolonized.

Based on these spatially varying dynamic patterns from the best-supported model, the proportion of sites where only boreal toads occur has declined steadily over time, and these declines were most pronounced at low elevations (Fig. 6A). Simultaneously, the proportion of sites with *Bd* increased over the course of the study and was also highest at low elevations (Fig. 6B). To confirm that the elevation relationships generated by the best model were not merely a function of assuming constant initial occupancy states, we ran a post-hoc model where the initial conditional distribution of *Bd* (ψ^{BA} and ψ^{Ba}) varied as an additive function of elevation. This model was not as well-supported as the model with constant *Bd* occupancy probabilities ($\Delta\text{AIC}_c = 1.83$), and produced nearly identical trajectories as those from the constant model (Fig. 6).

It is important to note that we could not model time variation in vital rate parameters (γ and ϵ), as the presence of an unobservable state yields biased estimates of most time-

varying parameters (Mosher et al. 2018). Without time variation in vital rate parameters, the state distributions (i.e., the proportion of sites in each of the four mutually exclusive states) will reach an equilibrium (Caswell 2001, Martin et al. 2009), which may or may not reflect the biology of this system. Even with the simplifying assumption of time-constancy, we had difficulty identifying covariate relationships for ϵ^A , ϵ^B , γ^A , and γ^B due to data sparseness for various states. The only way to relax this assumption and to explore time-variation in host and pathogen vital rate parameters is to sample *Bd* when boreal toads are not detected, thus removing the unobservable state (Mosher et al. 2018).

DISCUSSION

Capturing the dynamics of both hosts and pathogens is important for understanding coevolution, the potential for local adaptation, and the factors that influence disease dynamics across landscapes and systems. We present the first landscape-scale analysis of an amphibian–*Bd* system where both host and pathogen dynamics are modeled explicitly and imperfect and variable detection of both species is considered. We present a framework for evaluating competing factors associated with local variation in host–pathogen dynamics and provide estimates of parameters in a boreal toad–*Bd* system. This framework will be applicable to other host–

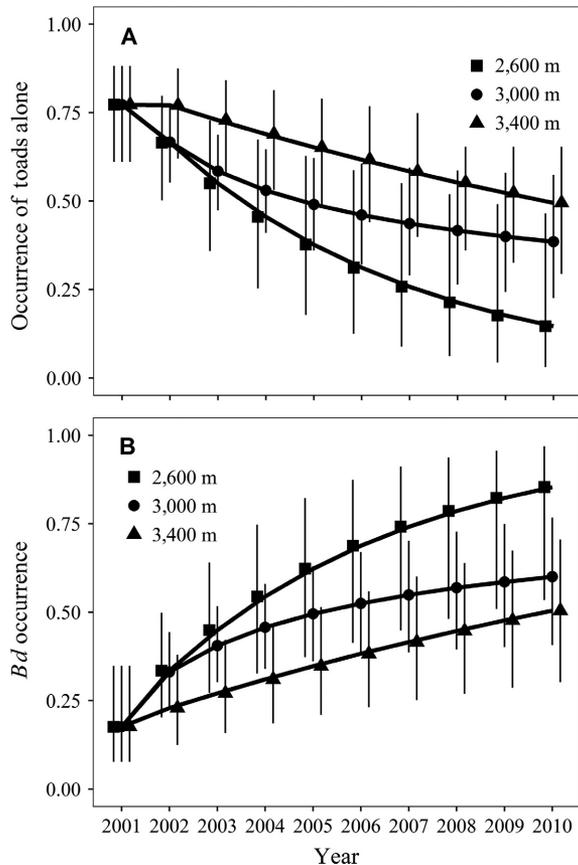


FIG. 6. Derived annual estimates of boreal toad and *Bd* occurrence for Southern Rocky Mountain populations studied from 2001 to 2010. (A) Annual estimates of toad occurrence in the absence of *Bd* (i.e., the proportion of occupied toad sites without *Bd*) and (B) estimated annual proportion of sites with *Bd*. Estimates are given for three elevations of interest, representing low, mid, and high elevation historic toad sites. Estimates and 95% confidence intervals are based on the best-supported model.

pathogen systems and will be immediately useful for guiding amphibian-pathogen monitoring and conservation efforts.

We found differences in boreal toad and *Bd* dynamics across elevations that were associated with less pronounced boreal toad declines at high elevations where *Bd* was also less likely to occur. High elevation sites in the SRM experience lower temperatures, shorter growing seasons, and higher daily temperature fluctuations than sites at lower elevations (Carey et al. 2005). Thus, the variation in dynamics that we observed across an elevational gradient may relate to (1) temperature-dependent pathogen growth where *Bd* grows better at lower temperatures and elevations, (2) elevational influences on transmission dynamics where small toad populations at high elevations may limit efficient pathogen transmission, or (3) an interaction between these factors.

Temperature-dependent pathogen growth has been shown for *Bd*, and experimental studies have identified upper and lower temperature thresholds between which *Bd* growth is optimal (Johnson et al. 2003, Piotrowski et al. 2004). Climate has also been linked to chytridiomycosis outbreaks in natural settings (Pounds et al. 2006, Bosch et al. 2007, Kriger et al. 2007). The colder temperatures at high elevations in temperate systems likely limit the pathogen's growth, resulting in

lower loads and *Bd* detection probabilities (Muths et al. 2008, Chestnut et al. 2014), reduced infection prevalence (Murphy et al. 2009), and decreased infection intensities (Kriger et al. 2007). Our work corroborates the hypothesis that temperature is related to *Bd* growth, as we found lower *Bd* occupancy and detection at high elevations. Differences in *Bd* detection probability likely reflect heterogeneity in *Bd* load (Miller et al. 2012b; Mosher et al., 2017), and we found lower *Bd* detection probabilities at high elevations.

An alternative, but not mutually exclusive, explanation for the elevational effects that we identified relates to amphibian life history characteristics. Like most amphibian species, high elevation boreal toad populations tend to have more frequent reproductive failure (Carey et al. 2005) and lower recruitment than low elevation populations. In addition, female boreal toads do not breed every year (Muths et al. 2010) and breeding frequency may be reduced at higher elevation sites (Muths et al. 2013). These processes can lead to lower population densities at high elevations. While it has been suggested that the high growth rate and reproductive potential of *Bd* sometimes drive hosts to extinction before low densities cause the pathogen to die out (Vredenburg et al. 2010, Fisher et al. 2012), the naturally low density of boreal toads at high elevations may prevent *Bd* from being transmitted efficiently upon arrival. Suboptimal growth conditions for *Bd* may interact with low boreal toad densities at high elevations, lowering infection loads and/or decreasing transmission rates, providing a potential mechanism(s) for host population persistence in the face of disease (Briggs et al. 2010, Wilber et al. 2017). We lack empirical measures of annual density at sites in our study, but stress that this important state variable could be incorporated into future monitoring efforts and could be a valuable predictor of disease dynamics.

While we assume that the elevational relationships we identified were the result of temperature differences, additional variables such as canopy cover, precipitation, and occurrence of aquatic predators may also be correlated with elevation and may be partially responsible for the differences we found (Becker et al. 2012, Searle et al. 2013). In our system, elevation was negatively correlated with the presence of other amphibian species ($r_s = -0.43$); the highest elevation sites lacked other amphibians. Experimental studies of multi-species amphibian host communities have found both positive and negative relationships between host richness and the risk of chytridiomycosis depending on species traits, life stages studied, and the strength of competitive interactions (e.g., Searle et al. 2011, Venesky et al. 2014a, Han et al. 2015). Future work on this topic requires sampling efforts that target the occurrence and infection status of multiple host species.

Monitoring implications

Our ability to estimate some parameters related to *Bd* persistence, colonization, and occurrence in the absence of boreal toads was limited because skin swabs were only collected when toads were both present and detected. Empirical estimates of these parameters would be valuable to management agencies as they consider mitigation strategies that target reducing the colonization and persistence of *Bd* (e.g., Converse et al. 2017, Gerber et al. 2017). Knowing how long *Bd* persists in the environment, or in reservoir hosts, after the

extirpation of target amphibian hosts can help guide amphibian reintroduction or translocation efforts and other management actions. We stress that long-term monitoring initiatives in this and other host-pathogen systems with free-living hosts would benefit from employing methods to survey for pathogens outside of their target hosts and in the environment.

In *Bd* systems, collecting skin swabs from co-occurring, but non-target, amphibian species and surveying for *Bd* zoospores using water filtration can provide additional information on the presence of the pathogen when the target host is not detected (Kirshtein et al. 2007, Schmidt et al. 2013, Chestnut et al. 2014; Mosher et al., 2017). Expanding survey methods to include standardized surveys for non-target amphibians may also provide better information about host community structure and stability and disease prevalence in these other species. Samples collected using water filtration (i.e., environmental DNA) could document the presence of multiple amphibian and pathogen species simultaneously with a single water sample (Bloom et al. 2013).

Chytridiomycosis has been linked to the decline or extinction of up to 200 amphibian species (Skerratt et al. 2007), but there are strikingly few examples of in situ management actions and their impacts on amphibian-*Bd* dynamics (Scheele et al. 2014, Garner et al. 2016). This is partly due to a lack of empirical measures of host-pathogen dynamics and subsequent modelling efforts to examine the effect of potential management activities. We provide empirical estimates of host and pathogen dynamics, and highlight next directions. For example, our work suggests that the physiological tolerances of *Bd* may interact with boreal toad density resulting in high elevation refugia. Results from our work can be used to develop long-term monitoring plans for amphibians and their pathogens, and to inform immediate management decisions.

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SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/eap.1699/full>

DATA AVAILABILITY

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.1c990>