



Livestock grazing, climatic variation, and breeding phenology jointly shape disease dynamics and survival in a wild amphibian

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ABSTRACT

Wildlife responses to infectious disease can be influenced by environmental stressors that alter host-pathogen dynamics. We investigated how livestock grazing, climatic variation, and breeding phenology influence disease prevalence and annual survival in boreal toad (*Anaxyrus boreas boreas*) populations challenged with *Batrachochytrium dendrobatidis* (*Bd*), a fungal pathogen implicated in global amphibian declines. We conducted a five-year (2015–2019) capture-recapture study of boreal toads ($n = 1301$) inhabiting pastures grazed by cattle in western Wyoming, USA. We employed structural equation models to determine whether the effects of climatic variation on *Bd* prevalence were direct or mediated through effects on breeding phenology and multi-state models to explore the interplay of grazing, weather, and *Bd* infection on adult survival. Higher winter snowpack was linked with shorter spring breeding seasons, which were associated with lower *Bd* prevalence. Boreal toads infected with *Bd* suffered increased mortality, but only at relatively cool temperatures. Although cattle grazing created warmer microclimates, likely by reducing vegetation cover, grazing-induced habitat changes did not scale up to influence adult survival. Our results suggest that boreal toads in cooler environments face increased risk of disease-induced mortality, possibly because infected individuals are not able to elevate body temperature to reduce or clear infection. More generally, we demonstrate that host-pathogen dynamics can be shaped jointly by independent and interactive effects of livestock grazing, breeding season length, and climatic variation. Future investigations of wildlife responses to disease therefore may benefit from considering anthropogenic land use and climatic regimes, including the effect of weather on host phenology.

1. Introduction

Emerging infectious diseases pose a major threat to global biodiversity (Daszak et al., 2000; Jones et al., 2008). Other potential stressors, such as habitat alteration and climate change, moreover, often increase the susceptibility of organisms to infection and exacerbate disease-induced declines (Patz et al., 2000; Goulson et al., 2015; Cohen et al., 2019). Environmental stressors also can interact counterintuitively, and even antagonistically, with disease, whereby the effect of a stressor attenuates the impact of disease on a population (Becker and Zamudio, 2011; Gahl et al., 2011). A better understanding of the complex interactions among putative stressors will therefore help guide management of populations challenged with infectious disease and preclude further declines.

The pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*), which causes the skin disease chytridiomycosis (Berger et al., 1998), is responsible for a catastrophic panzootic affecting hundreds of amphibian species worldwide (Scheele et al., 2019). *Bd* growth is highly sensitive to temperature and moisture: 15–25 °C is optimal, >28 °C is lethal (with variation in thermal sensitivity and optima among strains), and the fungus cannot tolerate desiccation (Piotrowski et al., 2004; Stevenson et al., 2013; Voyles et al., 2017). Infection rates in wild amphibian populations are therefore shaped largely by the thermal and hydric environment (Becker et al., 2012; Heard et al., 2014; Holmes et al., 2014). For instance, natural and anthropogenic disturbances that create warmer, drier conditions via changes to vegetation structure (e.g., deforestation, Becker and Zamudio, 2011; wildfire, Hossack et al., 2013; tropical cyclone, Roznik et al., 2015) resulted in lower *Bd* infection rates

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in host amphibians. However, many amphibians cannot physiologically tolerate warm, dry conditions, which increase evaporative water loss and/or decrease thermoregulatory ability, and most require extended hydroperiods for successful reproduction. Suboptimal habitat conditions for *Bd* may therefore not always improve survival or reproductive success despite lowering infection rates. Careful study of how habitat change interacts with *Bd* to influence host demography is needed, particularly if habitat manipulation is to be implemented as a conservation measure in populations challenged with disease (Heard et al., 2018).

Globally, amphibian distributions and critical habitat commonly overlap with land devoted to livestock grazing. Amphibian-livestock interactions have been relatively well-documented; albeit with mixed results (e.g., Jansen and Healey, 2003; Schmutzer et al., 2008; Burton et al., 2009; Roche et al., 2012). For instance, of the 46 articles included in a recent global review of amphibian responses to livestock use of wetlands, effects were positive in 15 studies, neutral or mixed in 21, and negative in 10 (Howell et al., 2019). Livestock activity can influence (positively, negatively, or neutrally) amphibians and their habitat via many potential pathways, including changes to water quality (Schmutzer et al., 2008), hydroperiod (Pyke and Marty, 2005), successional trajectory (Buckley et al., 2014), and direct trampling of egg masses, juveniles, and adults. Yet, the influence of livestock activity on *Bd* prevalence in host amphibian populations remains unexplored, despite demonstrated effects of livestock on vegetation structure (Fleischer, 1994; Jansen and Healey, 2003), and consequently the thermal and hydric environment.

Spatiotemporal variation in temperature and precipitation also shapes *Bd* infection rates (Rohr and Raffel, 2010; Holmes et al., 2014). The amount of snowpack and timing of snowmelt, for example, can influence *Bd* prevalence in montane amphibians (Clare et al., 2016), which generally have distinct breeding, foraging, and overwintering phenology. The interplay between climate, amphibian phenology, and *Bd* prevalence is largely unknown (but see Hossack et al., 2020), despite amphibians showing strong phenological responses to climate change (Todd et al., 2011; Ficetola and Maiorano, 2016). Winters with high snowpack delay amphibian emergence from hibernacula, for example, which influences the timing of spring breeding and can result in truncated breeding periods (Corn, 2003; Kissel et al., 2019). Shorter breeding seasons may decrease exposure to water-borne fungal zoospores and thus may decrease *Bd* infection rates (Carey et al., 2006). Disentangling the relationships between climatic regimes, season lengths, and disease dynamics is required to determine whether the effect of climatic variation on *Bd* prevalence is direct or mediated through its effect on breeding phenology.

Boreal toads (*Anaxyrus boreas boreas*) are a montane amphibian and one of the few species in North America with documented negative effects from *Bd* (Muths et al., 2003; Pilliod et al., 2010). Severe declines in boreal toad populations in the southern Rocky Mountains (southeast Wyoming, Colorado, northern New Mexico) have been attributed to chytridiomycosis (Muths et al., 2003; Scherer et al., 2005). In contrast, populations in western Wyoming persist despite high *Bd* prevalence (>65% in breeding adults; Murphy et al., 2009), though infected individuals can experience reduced survival (Pilliod et al., 2010). The majority of boreal toad populations in western Wyoming inhabit pastures leased for cattle (*Bos taurus*) grazing by the United States Forest Service (specifically the Bridger-Teton National Forest [BTNF]), which is in contrast with populations in the southern Rocky Mountains that experience little spatial overlap with cattle grazing (E. Muths, B. Wright, D. Cammack, personal communication). The influence of grazing, local climatic regimes, and breeding phenology in shaping *Bd* infection rates remains unclear. Further, boreal toads in the BTNF can clear *Bd* infection through actively seeking warm, open habitats that induce behavioral fever (Barrile et al., 2021). Whether this behavioral tactic translates to increased survival is unknown, as is the role of climatic variation and cattle grazing in creating habitat conditions conducive to this defensive

strategy.

We investigated the influence of cattle grazing, weather, and breeding phenology on the disease dynamics and annual survival of boreal toads (Fig. 1). Our objectives were to: (1) determine how live-stock grazing alters the vegetation structure and microclimate in habitats available to boreal toads; (2) assess the influence of grazing-induced habitat changes and climatic variables on *Bd* prevalence, including whether the effect of climate is mediated through breeding phenology; and (3) evaluate how grazing, climate, and disease influence rates of annual survival of adult toads. We expected cattle grazing to reduce vegetation cover and create warmer, drier microclimates (Jansen and Healey, 2003; Burton et al., 2009). We therefore predicted that higher levels of cattle grazing would be associated with lower *Bd* prevalence, as habitat changes resulting from grazing would increase opportunity for thermoregulation and the clearing of infection (Richards-Zawacki, 2010; Barrile et al., 2021). We further predicted that the effect of winter snowpack on *Bd* prevalence would be mediated through its effect on host breeding phenology. Specifically, higher winter snowpack delays spring emergence and results in shorter breeding seasons (Kissel et al., 2019), which we predicted would lower infection rates via decreased exposure time to fungal zoospores in breeding ponds (Carey et al., 2006). Finally, we hypothesized that grazing and climate would interact with disease to influence boreal toad survival. We expected *Bd* infection to increase mortality (Pilliod et al., 2010), with greater negative effects on survival during years with high precipitation (snowpack and rainfall), low average temperatures, and reduced cattle grazing. We reasoned that wet years were conducive to fungal growth and would result in severe *Bd* infections (Piotrowski et al., 2004; Holmes et al., 2014). We further reasoned that high temperatures and levels of cattle grazing would create conditions conducive to the elevation of body temperature, the subsequent clearing of infection, and therefore, higher survival.

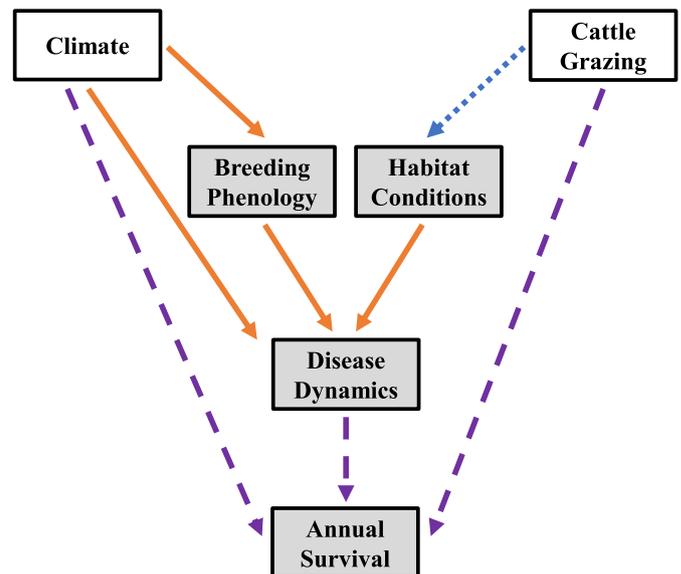


Fig. 1. Conceptual diagram illustrating our hypothesized relationships among climate, cattle grazing, local habitat conditions, breeding phenology, disease dynamics, and the annual survival rate of boreal toads. Grey-filled boxes indicate response metrics, and different colored arrows and line-types denote each of our objectives: the blue arrow (dotted line) corresponds to objective (1), to determine the effect of grazing on vegetation structure and microclimatic conditions; the orange arrows (solid lines) correspond to objective (2), to assess the influence of climate, breeding season length, and grazing-induced habitat changes on *Bd* prevalence; and the purple arrows (dashed lines) correspond to objective (3), to evaluate how grazing, climate, and infection with *Bd* influence boreal toad survival. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Methods

2.1. Study area

We studied boreal toads at four stream segments in the BTNF, two in the northern Wyoming Range (~1.4 km reach at Buck Creek and ~0.4 km reach at Chall Creek; 42°59' N, 110°24' W) and two in the northern Wind River Range (~0.2 km reach at Lower Gypsum Creek and ~0.3 km reach at Upper Gypsum Creek; 43°16' N, 109°57' W). Sites in the Wind River Range were at higher elevations (Lower Gypsum Creek = 2509 m; Upper Gypsum Creek = 2671 m) than Wyoming Range sites (Buck Creek = 2487 m; Chall Creek = 2441 m) and experienced longer winters and lower average temperatures. The landscape was similar between the two ranges; both contained mixed-conifer and aspen (*Populus tremuloides*) forests, sagebrush (*Artemisia tridentata*) and subalpine meadows, and riparian areas with willow (*Salix* spp.) complexes. Beaver ponds were common in montane stream channels and provided breeding habitat for boreal toads and other amphibian species. All of our study sites were located within grazing allotments managed by the BTNF. Cattle were turned onto pastures (which were nested within allotments) in early July, rotated to a second pasture in mid-August, and removed from the BTNF in early October. Several pastures were rested (no grazing) in a given year.

2.2. Grazing and habitat conditions

To test whether grazing altered habitat conditions, we deployed metal cages that excluded cattle from 1 m² plots. We installed nine cages at each of the four study sites, with three placed within the riparian zone, three in shrubland/grassland habitat, and three in the forest ($n = 72$ total cages during 2016–2017). We excluded cattle from 1 m² plots to reflect the scale at which we measured microhabitat use of boreal toads during a radio-telemetry study in 2016. We recorded ground temperature and relative humidity at plot center using a digital psychrometer (Extech Instruments®, Waltham, Massachusetts, USA), vegetation height using a meter stick, and vegetation cover using a spherical densiometer (held at ground height) in cages within three days before cattle entered and within three days after cattle exited a given pasture (vegetation height and cover were the average of five measurements taken at random locations within each plot). We repeated these methods at 1 m² plots exposed to grazing, which were 50 m in a random direction from each cage and in the same habitat type (e.g., riparian, forest). To validate that changes to habitat structure and conditions were caused by cattle and not wild ungulates (e.g., mule deer *Odocoileus hemionus*), we repeated our methodology at rested pastures (pastures with no cattle in a given year), which acted as a control.

We fit a two-way Multivariate Analysis of Variance (MANOVA) to determine whether cattle grazing altered vegetation structure and microclimatic conditions in amphibian habitat. We derived response variables by subtracting values recorded of our four habitat variables (vegetation height, vegetation cover, relative humidity, ground temperature) directly before the grazing season from values recorded directly after the grazing season in each plot. We then modeled the differences in habitat variables as the interactive function of two grouping variables: plot type (caged, grazed, control) and habitat type (riparian, shrubland, forest). We performed the two-way MANOVA using the *manova* function in Program R (R Core Team, 2020), specifying Pillai's trace as the test statistic. Prior to analysis, we tested for violations of MANOVA assumptions, including multivariate normality (using the *mshapiro.test* function in the 'mvnrmtest' package), homogeneity of variances (using the *levene.test* function in the 'rstatix' package), and linearity between response variables within each group via visual assessment of scatter plots.

2.3. Breeding phenology

Boreal toads in western Wyoming congregate at breeding ponds shortly after snowmelt in the spring (~mid-May) and continue spawning through mid to late June. We conducted standardized visual encounter surveys to hand-capture toads at breeding sites within our four stream segments at night during the breeding season in 2015–2019. We defined the breeding season at each site from when we observed the first clutch of eggs to when only a few or no males were present during nighttime surveys. Captured individuals were sexed by the presence of darkened nuptial pads on the thumbs of males and marked using passive integrated transponder tags (8 mm × 1.2 mm FDX tag; Oregon RFID, Portland, Oregon, USA) for individual identification. We implanted tags subcutaneously on the dorsal side of each individual and positioned the tag to fit securely beneath the cranial crest. Assuming no tag loss, individuals retain their tag throughout the entirety of their lifetime. Total handling time for each toad remained under 10 min. We tagged adult males only, assuming individuals ≤55 mm were juveniles (Carey et al., 2005). We excluded female toads because they do not remain at breeding sites post-amplexus and often skip breeding years (Muths et al., 2010), both of which could bias demographic estimates. We sampled each stream segment in its entirety during each nighttime survey, and conducted multiple recapture surveys within each breeding season at all streams (mean = 5 surveys per site, per year; range = 3–9).

2.4. Bd prevalence

We collected disease samples from every individual encountered in a given breeding season using standardized protocols to swab ventral skin surfaces of host amphibians for *Bd* (Brem et al., 2007). For individuals captured more than once in a given breeding season, we randomly selected one sample within the breeding season for analysis ($n = 1757$ total samples during 2015–2019). Disease samples were analyzed by Pisces Molecular® (Boulder, Colorado, USA) via a quantitative polymerase chain reaction (qPCR) to detect *Bd*. The qPCR approach has the advantage of high sensitivity and low false negative rate (Skerratt et al., 2011). The assay developed by Pisces Molecular® used a TaqMan qPCR and targeted the ITS-1 region at a specific rDNA locus, similar to the assay developed by Annis et al. (2004). Samples were screened initially in singlicate with the following criteria: failure to show log-linear amplification crossing a fluorescent threshold before or by cycle 45 (samples scored as positive met this criteria). Any weak or questionable samples were repeated in triplicate in a second qPCR run, during which samples that scored two of the three replicates as positive were scored as positive (J. Wood, personal communication). Although we standardized our swabbing protocol (e.g., each toad was swabbed the same number of times in the same areas of the body), we ultimately did not view the collection method as a quantitative method. Therefore, we did not use the quantitative results from the qPCR (i.e., swabbing an animal is not a quantitative technique, so we were not comfortable using a quantitative result). However, given the sensitivity of the assay used by Pisces Molecular®, we were confident in the positive/negative result for the presence or absence of *Bd*.

We developed a suite of covariates related to grazing, weather, and breeding phenology to test our hypothesized relationships with disease prevalence (Tables 1, 2; Fig. S1). Covariates were measured every year at each site and uniquely derived for each site-year combination. We determined *Bd* prevalence at a site during a given breeding season by dividing the number of *Bd* positive individuals by the total number of individuals tested (*PREV*). We calculated a metric for grazing pressure by dividing the number of cattle by the area of the pasture and multiplying that value by the number of days those cattle spent on the pasture (i.e., density of cattle × duration of grazing; *GRAZE*). To determine whether our grazing metric accurately characterized the amount of cattle activity in amphibian habitat, we calculated a Pearson's correlation coefficient between our metric and the number of cattle that visited

Table 1

Name, abbreviation, and mean values (with standard errors in parentheses) of grazing, disease, and climate variables at Chall Creek (CC) and Buck Creek (BC) in the Wyoming Range and Lower and Upper Gypsum Creek (LG and UG, respectively) in the Wind River Range, averaged across 2015–2019.

Variable	Abbreviation	CC	BC	LG	UG
Length of breeding season (days)	<i>BL</i>	41 (2)	32 (1)	26 (3)	20 (2)
Disease prevalence	<i>PREV</i>	74 (7)	76 (6)	65 (8)	71 (8)
Grazing pressure	<i>GRAZE</i>	917 (325)	534 (181)	1633 (101)	814 (46)
Snow water equivalent (mm)	<i>SWE</i>	354 (37)	354 (37)	363 (48)	363 (48)
Precipitation in breeding season (mm)	<i>PPT.B</i>	103 (12)	101 (12)	59 (7)	64 (8)
Minimum temperature (°C)	<i>TMIN.B</i>	0.1 (0.4)	0.7 (0.4)	1.5 (0.5)	2.4 (0.6)
Precipitation in active season (mm)	<i>PPT.A</i>	253 (27)	243 (26)	216 (22)	253 (23)
Maximum temperature (°C)	<i>TMAX.H</i>	22.9 (0.4)	23.2 (0.4)	22.5 (0.4)	20.4 (0.4)

Table 2

Name and definitions of grazing, disease, and climate variables included in the analysis of boreal toad infection rates and annual survival in the Bridger-Teton National Forest, USA, during 2015–2019. Asterisks (*) denote variables included in the analysis of infection rates only, crosses (+) denote variables included in survival analyses only, and B superscripts (B) denote variables included in both analyses.

Variable	Definition
Length of breeding season (days)*	Number of days from the first occurrence of an egg clutch until only a few/zero males were present at a breeding site
Disease prevalence*	Percent <i>Bd</i> positive out of the total number of individuals tested in a breeding season
Grazing pressure ^B	Cattle density (number of cattle per square km) × the number of days cattle spent on a pasture
Snow water equivalent (mm) ^B	Maximum snow water equivalent (amount of water contained within the snowpack) in the winter directly preceding the breeding season
Precipitation in breeding season (mm)*	Total amount of precipitation during the breeding season
Minimum temperature (°C)*	Mean daily minimum air temperature during the breeding season
Precipitation in active season (mm) ⁺	Total amount of precipitation during the active season
Maximum temperature (°C) ⁺	Mean daily maximum air temperature in the warmest months (July and August)

Notes: Active season was defined as the number of days from the first occurrence of SWE = 0 in spring until the last occurrence of SWE = 0 in fall (SWE, snow water equivalent).

boreal toad breeding ponds. During 2018–2019, we deployed game cameras (Stealth Cam, Model: STC-RX24, Grand Prairie, Texas, USA) at two breeding ponds at each of our four study sites. Cameras were set to capture an image every 10 min. Using the same time frame used to calculate our metric for grazing pressure, we found that the correlation coefficient between the total number of cattle photographed in and around (i.e., within ~20 m) breeding ponds and our metric for grazing pressure was 0.82. We therefore used our grazing metric (*GRAZE*) as a covariate in subsequent analyses.

We obtained estimates of maximum snow water equivalent (*SWE*, an estimate of the amount of water contained in snowpack) and the total amount of precipitation during the breeding season (*PPT.B*). Further, cold temperatures during the breeding season can stress reproductive individuals via reduced immune function and lowered growth and energy ingestion (Lillywhite et al., 1973), which may increase susceptibility to infection. However, colder temperatures may also create less favorable conditions for *Bd* growth (Piotrowski et al., 2004; Pilliod et al.,

2010). We therefore calculated the average daily minimum air temperature during the breeding season (*TMIN.B*). Temperature and precipitation variables were derived from the PRISM data set (PRISM climate group, accessed June 2020) and snow water equivalent from the nearest SNOTEL station with similar elevation (East Rim Divide [2417 m], approximately 21 km from Buck and Chall Creeks; and Gros Ventre Summit [2667 m], approximately 18 km from Lower and Upper Gypsum Creek).

To assess how grazing and climate influenced *Bd* infection rates, and whether the effect of climate was direct or mediated through breeding phenology, we inserted grazing, climate, breeding phenology, and disease prevalence variables (Tables 1, 2) into a path analysis. Path analysis took the form of a structural equation model (SEM) parameterized in the ‘piecewiseSEM’ package (*psem* function) in Program R (R Core Team, 2020). The SEM consisted of two models, a linear mixed-effects model and a generalized linear mixed-effects model, both specifying stream as a random intercept (fit using *lmer* and *glmer* functions in the *lme4* package, respectively). Breeding season length (*BL*) was modeled as a function of climate variables (*SWE*, *PPT.B*, *TMIN.B*) in the linear mixed-effects model and disease prevalence (*PREV*) was modeled as a function of the same climate variables, our grazing metric (*GRAZE*), and breeding season length (*BL*) in the generalized linear mixed-effects model, which specified a binomial error distribution (logit link function).

Model coefficients were standardized (placed in units of standard deviations of the mean via scaling by the ratio of the standard deviation of x over the standard deviation of y) such that the relative strength of predictors could be compared across multiple responses. Because *PREV* was summarized during the breeding season (May–June), covariate values for *GRAZE* and *SWE* corresponded to the period directly preceding a given breeding season (e.g., *PREV* during the breeding season in 2018 was modeled as a function of snow water equivalent (*SWE*) during the winter of 2017–2018, and *GRAZE* during the summer/fall of 2017). Values of *BL*, *PPT.B*, and *TMIN.B* corresponded to the breeding season itself (e.g., *PREV* during the breeding season in 2018 was modeled as a function of precipitation during the breeding season (*PPT.B*) in 2018).

2.5. Annual survival

Our sampling method produced capture-recapture data at two distinct temporal scales (within and among breeding seasons) thereby comprising a robust design. Robust designs consider multi-season studies of open populations as series of short-term studies of closed populations, allowing for demographic estimates that are robust to sources of variation in capture probabilities (Kendall et al., 1997). We used multi-state closed robust design (hereafter, multi-state) models to estimate boreal toad annual survival from our mark-recapture dataset. Multi-state models estimated apparent survival (ϕ ; ‘apparent’ because mortality cannot be separated from permanent emigration) and state-transition probabilities (ψ) between primary periods (i.e., breeding seasons) and capture probability (p) and f_0 (the number of individuals never captured/tagged) within primary periods. ‘States’ in our models referred to individual infection status (*Bd* positive/*Bd* negative) and the transition parameter represented the probability of switching infection status between seasons (i.e., transitioning from an infected to an uninfected state, or vice-versa). Multi-state models permit the estimation of state-specific survival probabilities (Chabanne et al., 2017), which allowed us to test hypotheses about the effects of disease on annual survival.

We retained snow water equivalent (*SWE*) as a potential covariate on survival, as adequate snowpack provides insulation to prevent hibernating individuals from freezing (Corn, 2003) and higher *SWE* has been positively correlated with boreal toad survival (Scherer et al., 2008; Muths et al., 2020). We also retained our grazing metric (*GRAZE*) as a covariate on survival, as we predicted that higher grazing pressure would increase survival through creating basking habitats (Jansen and Healey, 2003) for infected toads to clear infection (Barrile et al., 2021).

We derived two additional covariates from the PRISM and SNOTEL databases for use in survival analyses. First, we determined the total amount of precipitation ($PPT.A$) during the boreal toad active season, which we defined as the number of days from the first occurrence of $SWE = 0$ in spring until the last occurrence of $SWE = 0$ in fall. Drought conditions during the active season are linked to lower amphibian survival (Walls et al., 2013), though we predicted that years with high precipitation would facilitate fungal growth and result in severe *Bd* infections, ultimately decreasing survival (Holmes et al., 2014). Second, we calculated the average daily maximum temperature during the hottest months (July and August; $TMAX.H$) as warm temperatures during the active season present opportunity for infected individuals to elevate body temperature (Pilliod et al., 2010) and boreal toads perform optimally at relatively warm temperatures (e.g., maximal growth and energy ingestion at $\sim 27^\circ\text{C}$; Lillywhite et al., 1973).

Rather than fitting an extremely large model set incorporating all plausible combinations for model parameters, we adopted a step-down approach (Lebreton et al., 1992) to identify supported models representing competing hypotheses about the interplay among grazing, disease, and climate on boreal toad survival. We fixed survival, transition, and the $f0$ parameter at a high dimensionality (φ and $f0$ varied over time and among streams; ψ varied between infection states) while identifying the most parsimonious structure for capture probability (Lebreton et al., 1992; Doherty et al., 2012). We ran models in which capture probability remained constant or varied by year (i.e., breeding season), capture occasion, stream, and state, including additive and interactive combinations of these variables (32 structures; Table S1). We next identified the most parsimonious structure for $f0$. Our model set for $f0$ included time-constant, time-dependent, and stream-dependent models, including additive and interactive combinations (five structures; Table S2). We then modeled time-, stream-, and state-dependent structures for transition probability (14 structures; Table S3). Finally, retaining the most parsimonious structures for $f0$, capture probability, and transition probability (highest AIC_c weight; Burnham et al., 2011), we modeled apparent survival as a function of our grazing, disease, and climate-related covariates (see Tables 1, 2). The interval for mortality ranged from the end of the breeding season (late June) in year t to the beginning of the breeding season (early May) in year $t + 1$. Covariate values therefore corresponded to the period directly preceding the breeding season in year $t + 1$ (e.g., survival between 2015 and 2016 was modeled as a function of the grazing pressure ($GRAZE$) during the summer/fall of 2015). At each step of the modeling procedure, we dropped models that did not converge or models with singular parameters.

We considered univariate models with each covariate and tested our hypotheses that grazing and climate would interact with disease to influence boreal toad survival (nine total model structures). Given the presence of transient individuals in other amphibian systems (Frétey et al., 2004; Schmidt et al., 2007), including some boreal toad populations (Muths et al., 2018), we expected some degree of transience in our system. We defined transient individuals as newly captured toads that were marked, released, and then permanently emigrated from our study area, such that those toads were no longer available for encounter during subsequent surveys (Pradel et al., 1997). Resident (adult males that remained in our study area) survival probability can be negatively biased if transience is not modeled appropriately. We therefore fit each model structure for survival both with and without transients (18 total models; Table 3). We incorporated transience into survival models following the methods employed by Muths et al., 2018 (see Appendix S2 in Muths et al., 2018 for details).

We used AIC_c (Akaike information criterion corrected for sample size) model selection to compare and rank models and to calculate model weights for all analyses described below (Burnham et al., 2011). Multi-state analyses were conducted using Program MARK (White and Burnham, 1999), with models constructed via the 'RMark' package (Laake, 2013) in Program R. To our knowledge, a goodness-of-fit test is

Table 3

Model selection results including model name, AIC_c , ΔAIC_c , model weights, number of parameters (K), and $-2\log(L)$ for multi-state capture-recapture models used to estimate the survival probability (φ) of adult boreal toads ($n = 1301$) at four stream segments in the Bridger-Teton National Forest, USA, during 2015–2019.

Model	AIC_c	ΔAIC_c	Model Wt	K	$-2\log(L)$
$\varphi(\text{state} \times TMAX.H + 2a)$	3833.05	0.00	0.76	59	3712.71
$\varphi(TMAX.H + 2a)$	3835.33	2.29	0.24	57	3719.15
$\varphi(GRAZE + 2a)$	3855.21	22.15	0.00	57	3739.01
$\varphi(\text{state} + 2a)$	3855.70	22.66	0.00	57	3739.52
$\varphi(SWE + 2a)$	3856.95	23.90	0.00	57	3740.76
$\varphi(\text{state} \times TMAX.H)$	3857.54	24.49	0.00	58	3739.28
$\varphi(PPT.A + 2a)$	3857.59	24.54	0.00	57	3741.40
$\varphi(\text{state} \times SWE + 2a)$	3857.71	24.67	0.00	59	3737.37
$\varphi(\text{state} \times GRAZE + 2a)$	3857.85	24.81	0.00	59	3737.51
$\varphi(\text{state} \times PPT.A + 2a)$	3858.81	25.77	0.00	59	3738.47
$\varphi(TMAX.H)$	3859.30	26.25	0.00	56	3745.19
$\varphi(GRAZE)$	3871.37	38.32	0.00	56	3757.26
$\varphi(\text{state})$	3874.13	41.08	0.00	56	3760.02
$\varphi(\text{state} \times GRAZE)$	3874.33	41.29	0.00	58	3756.07
$\varphi(SWE)$	3875.12	42.08	0.00	56	3761.02
$\varphi(PPT.A)$	3875.77	42.72	0.00	56	3761.66
$\varphi(\text{state} \times SWE)$	3876.11	43.06	0.00	58	3757.85
$\varphi(\text{state} \times PPT.A)$	3877.32	44.27	0.00	58	3759.06

Notes: We modeled apparent survival probability (φ) as a function of grazing, disease, and climate metrics (see Tables 1, 2 and Fig. S1). We considered univariate models with each covariate and tested our hypotheses that grazing and climate would interact with disease (*state*) to influence survival. We fit each model structure both without and with transients (denoted *2a*). All models included the most supported structures for capture probability, $p(\text{site} \times \text{survey} + \text{year})$ [Table S1], transition probability, $\psi(\text{state} \times \text{tostate})$ [Table S3], and the number of individuals never captured, $f0(\text{site} \times \text{year})$ [Table S2]. Additive and interactive effects in models are denoted by (+) and (x), respectively.

not available for these model types when including site-specific and time-varying covariates, so we used our global structures and the median \hat{c} approach to adjust for possible overdispersion (White and Burnham, 1999).

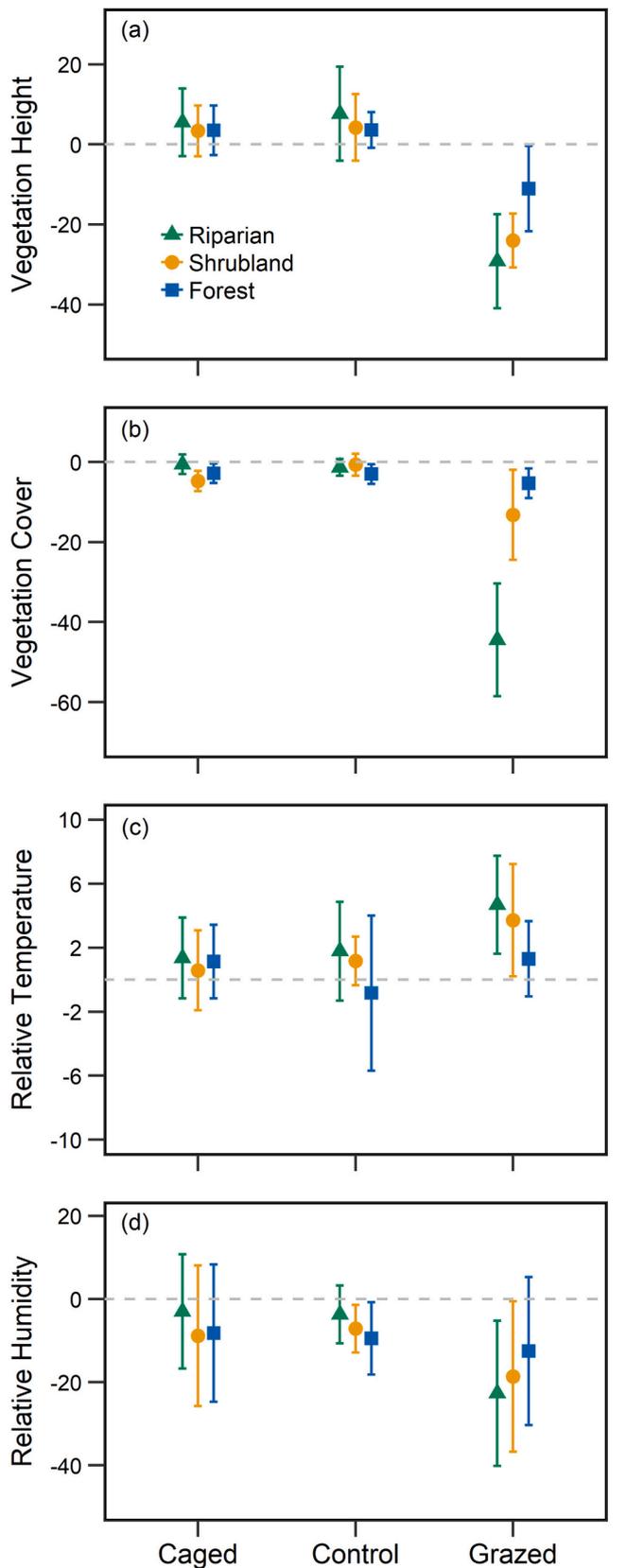
3. Results

3.1. Grazing and habitat conditions

We did not discover any violations of MANOVA assumptions in the habitat data collected during our cattle enclosure deployment (e.g., Shapiro-Wilk test for multivariate normality, $p > 0.05$; Levene's test for homogeneity of variances, $p > 0.05$). The effect of cattle grazing (plot type; caged, control, grazed) on the change in vegetation structure and microclimatic conditions before versus after the grazing season was dependent on habitat type (riparian, shrubland, forest; $F(4, 266) = 4.046$, $p < 0.001$, Pillai's Trace = 0.745). Cattle grazing decreased vegetation height, vegetation cover and relative humidity, and increased relative ground temperature (ground - ambient temperature), with the most pronounced effects in riparian habitat and least pronounced in forested habitat (Fig. 2). In caged and control (uncaged but no cattle present) plots, by contrast, vegetation height increased, whereas percent vegetation cover, relative ground temperature, and relative humidity remained similar over the grazing season, with similar effects across all habitat types (Fig. 2).

3.2. *Bd* prevalence

We captured and tagged 1301 unique individuals during 2015–2019: 317 adult male boreal toads during 27 capture surveys at Buck Creek, 782 toads during 32 surveys at Chall Creek, 123 toads during 21 surveys at Lower Gypsum Creek, and 79 toads during 19 surveys at Upper Gypsum Creek. *Bd* prevalence during the breeding period was $\sim 70\%$ across all years and study streams (1234 positives out of 1757 total



(caption on next column)

Fig. 2. Plots exposed to cattle grazing (denoted by 'Grazed' on the x-axis; $n = 54$) experienced decreases in (a) vegetation height, (b) vegetation cover and (d) relative humidity, and an increase in (c) relative ground temperature (ground - ambient temperature), with the most pronounced effects in riparian habitat and least in forested habitat. In caged (cattle excluded from grazing; $n = 72$) and control (uncaged but no cattle present; $n = 18$) plots, by contrast, (a) vegetation height increased, whereas (b) percent vegetation cover, (c) relative ground temperature, and (d) relative humidity remained similar over the grazing season, with similar effects across all habitat types. Values on the y-axis represent the change in each microhabitat variable before versus after the grazing season (i.e., value recorded after grazing season - value recorded before grazing season, in each plot), with mean values (triangles, circles, squares) and standard deviations (error bars) plotted for each habitat (riparian, shrubland, forest) and plot type (caged, control, grazed). The horizontal dotted line at $y = 0$ in each plot denotes no change in the respective microhabitat variable before versus after the grazing season.

samples; Fig. S1). Breeding season length was the strongest predictor of *Bd* prevalence, with longer breeding periods resulting in higher infection rates (Fig. 3). Snow water equivalent indirectly affected *Bd* prevalence through its effect on breeding phenology. Higher winter snowpack resulted in shorter breeding periods, which were associated with lower infection rates (Fig. 3). Spring precipitation did not affect the length of the breeding period but had a direct and positive association with *Bd* prevalence (Fig. 3). Average minimum temperature did not affect the length of the breeding season nor *Bd* prevalence, and cattle grazing was positively associated with *Bd* prevalence (Fig. 3).

3.3. Annual survival

We found little evidence of overdispersion ($\hat{c} = 1.43$) in the multi-state model containing the most general structures for all parameters, thus we used AIC_c to compare models and calculate model weights. The best-supported structure for capture probability (model weight, $w = 1.0$) indicated variation among streams, breeding seasons, and secondary capture occasions (i.e., surveys within the breeding season; Table S1). Capture probability ranged from 0.06 to 0.55 (mean = 0.29) and was generally higher at Wind River sites, during surveys earlier in the breeding season, and gradually increased over 2015–2019. The most parsimonious structure for f_0 (model weight, $w = 1.0$) varied by stream and breeding season (Table S2) and the best structure for transition probability (model weight, $w = 1.0$) included transitioning between infection states (Table S3). The probability of a toad becoming infected was significantly higher than the probability of clearing infection; the mean probability of transitioning from *Bd* negative to *Bd* positive was 0.65 (95% CI = 0.58, 0.71) and the mean probability of transitioning from *Bd* positive to *Bd* negative was 0.29 (95% CI = 0.25, 0.34).

The best-supported model for the annual survival of boreal toads (model weight, $w = 0.76$) included the interactive effect of infection status and the average maximum temperature during the hottest months of the year (Table 3). Toads infected with *Bd* were less likely to survive, but only when summer temperatures were relatively cool (Fig. 4a). Average maximum temperature in July and August varied across our study sites, with higher temperatures at lower elevations in the Wyoming Range compared to the higher elevations in the Wind River Range (Table 1; see Table 2 for variable description). Accordingly, the negative effect of *Bd* infection on host survival was more pronounced at sites in the Wind River Range, particularly at the coolest, highest elevation site at Upper Gypsum Creek (Fig. 4b). Boreal toads experienced higher survival at Upper Gypsum Creek ($\beta = 0.50$; 95% CI = 0.09, 0.92), whereas survival probabilities at all other sites were not statistically different from one another. Further, the negative effect of *Bd* on survival was statistically significant at Upper Gypsum Creek ($\beta = -0.99$; 95% CI = -1.81, -0.17), whereas survival probabilities between *Bd* positive and *Bd* negative individuals were not statistically different at all other sites.

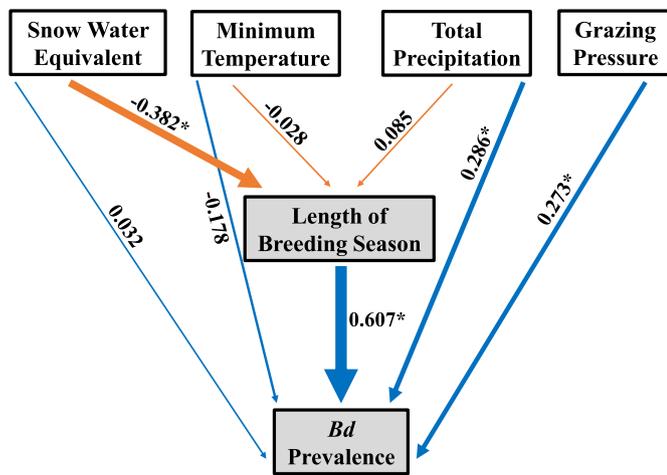


Fig. 3. Structural equation model indicating relationships among cattle grazing, climate variables, breeding phenology, and *Bd* infection rates (see Table 2 for variable definitions) in adult male boreal toads in the Bridger-Teton National Forest, USA, during 2015–2019. Grey-filled boxes indicate response metrics, with orange arrows corresponding to the linear mixed-effects model (response = length of breeding season) and blue arrows denoting the generalized linear mixed-effects model (response = *Bd* prevalence). Numbers above arrows are standardized path coefficients with the relative strength of each indicated by line width. Asterisks denote significance (i.e., $p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The best-supported model included the transience parameter, as did nine of the top ten survival models (denoted by 2a in Table 3), suggesting that accounting for transient adults was important for modeling the survival of resident individuals. Transience varied across sites: the proportion of transients was 0.15 at Lower Gypsum Creek, 0.16 at Upper Gypsum Creek, 0.20 at Buck Creek, and 0.24 at Chall Creek. Reported survival estimates (e.g., Fig. 4) reflect those of resident individuals only.

4. Discussion

Wildlife populations face many, often concomitant, challenges (e.g., infectious disease, exotic introductions, habitat loss). Understanding how potential stressors interact with critical biological processes

therefore is imperative for effective conservation. We investigated the interplay of climatic variation and cattle grazing with the breeding phenology, disease prevalence, and survival rates of adult boreal toads. Cattle grazing reduced vegetation cover and created warmer microclimates in terrestrial toad habitat, which we predicted would decrease *Bd* prevalence and increase boreal toad survival. Contrary to our predictions, grazing was associated with higher *Bd* infection rates and grazing-induced habitat changes did not scale up to influence adult survival. Winter snowpack and spring precipitation also were associated with disease prevalence, although the effect of snowpack was mediated through its effect on breeding phenology. Higher winter snowpack was linked to shorter spring breeding seasons, which were associated with lower *Bd* prevalence. Boreal toads infected with *Bd* suffered increased mortality, but only when temperatures during the hottest months of the year were relatively cool. Overall, our results underscore the importance of environmental context when evaluating wildlife responses to infectious disease.

Habitat changes as the result of grazing should have increased the availability of microclimates in which infected toads could behaviorally thermoregulate and possibly clear infection (Barrile et al., 2021), assuming that warm, open patches were to some degree limited in our system. We reasoned, for instance, that clearing infection during the summer/fall (prior to entering hibernacula) would result in more uninfected toads emerging from hibernacula during the following spring, thereby decreasing *Bd* prevalence in the spring breeding season. However, individuals could have become reinfected at breeding ponds, possibly prior to being sampled for disease. Further, the mismatch in the temporal scale at which we measured grazing (summer/fall) and *Bd* prevalence (spring) likely limited our ability to detect an effect and may have resulted in a potentially spurious positive relationship between grazing and *Bd*.

Grazing in the summer/fall could have decreased habitat quality such that toads entered hibernacula in poorer condition and thus were more susceptible to infection during the following spring. For example, high stocking rates can compact soil (Pyke and Marty, 2005), which collapses small mammal burrows, and reduce willow cover, both of which serve as important refuge sites for boreal toads to meet biophysical demands (e.g., thermoregulation, osmoregulation) and escape predation (Long and Prepas, 2012). However, levels of grazing intensity currently permitted in the Bridger-Teton National Forest may not reach those required to meaningfully alter boreal toad habitat, either positively or negatively (e.g., pastures in our study typically were stocked

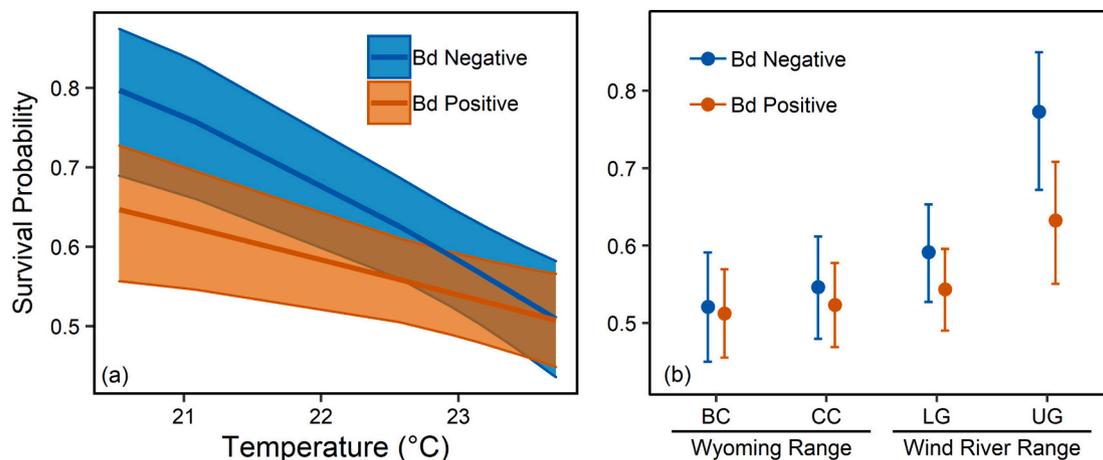


Fig. 4. Adult boreal toads ($n = 1301$) experienced (a) greater annual survival when mean maximum temperatures during the hottest months (July and August) were relatively low at four sites in the Bridger-Teton National Forest, USA, during 2015–2019. The effect of temperature on survival, moreover, was (a) dependent on individual infection status. The negative effect of *Bd* infection on host survival was (b) more pronounced at cooler sites in the Wind River Range (LG and UG = Lower and Upper Gypsum Creek, respectively) compared to warmer sites in the Wyoming Range (BC and CC = Buck and Chall Creeks, respectively), particularly at the coolest site, UG. Mean predicted survival estimates (solid lines in (a); dots in (b)) and 95% confidence intervals (shaded ribbons in (a); error bars in (b)) of resident individuals were derived from the best-supported multi-state model (model weight, $w = 0.76$; Table 3).

with approximately 1000 cattle, or 23.74 cattle per km²). Cattle grazing was not associated with boreal toad survival in our study, suggesting that grazing-induced habitat changes may have manifested at too small of a scale to affect demographic rates, as opposed to larger scale disturbance events such as wildfire and tropical cyclones (Hossack et al., 2013; Roznik et al., 2015). Large-scale experimental manipulations involving grazing treatments (e.g., altering the timing, duration, and density of cattle) are needed to better test whether amphibians use habitat patches created by livestock to reduce or clear infection, and ultimately if those individuals experience increased survival. However, a cautious approach is warranted, as increases in the level of grazing intensity could result in negative impacts to amphibian habitat and disease dynamics (Pfennig et al., 1991).

Greater precipitation during the spring breeding season resulted in increased *Bd* prevalence during the same breeding period, likely because the fungus requires moist conditions (Piotrowski et al., 2004), thrives in wet environments (Van Sluys and Hero, 2009), and infection rates in host amphibians increase with rainfall (Holmes et al., 2014). Winter precipitation (i.e., the amount of snowpack) and the timing of snowmelt also can influence amphibian-*Bd* dynamics (Clare et al., 2016). Snowpack was associated with *Bd* prevalence at our sites, albeit through its effect on the length of the breeding period. Greater snowpack has been linked to higher survival rates of boreal toads (Scherer et al., 2008; Muths et al., 2020) and other amphibians that hibernate terrestrially (e.g., O'Connor and Rittenhouse, 2016) as snowpack insulates hibernating individuals from harsh winter conditions (Corn, 2003). Although snowpack was weakly, positively associated with overwinter survival in our study ($\beta = 0.06$, [SE = 0.07]), models that included snow water equivalent did not perform well in comparison to other multi-state analyses. Nonetheless, positive associations between snowpack and amphibian survival are troubling in the context of climate change predictions, as snowpack is expected to dramatically decline in the western US (Mote et al., 2018), with more precipitation falling as rain than snow (Safeeq et al., 2016).

Our results suggest that decreased snowpack under projected future conditions will result in longer breeding periods, at least in permanent and semipermanent ponds such as the beaver impoundments in our system. Amphibians indeed are among the taxa that show the strongest phenological responses to climate change (Todd et al., 2011; Ficetola and Maiorano, 2016). We report that amphibian breeding phenology can influence *Bd* infection rates as variation in breeding season length was the strongest predictor of disease prevalence in our system. Longer breeding periods likely prolonged exposure to fungal zoospores. Male boreal toads do not vocalize to attract mates, rather males move around scrambling for females and attempt to dislodge other males in amplexus. Male-male competition can be intense, and we often observed large knots of males (~2–20 individuals in a ball) during the breeding period. Increased male-male contact in these circumstances likely increased disease spread (Rowley and Alford, 2007) at a higher rate than contact with moist terrestrial substrates and/or post-breeding habitat use of aquatic habitats (i.e., infection by free-swimming fungal zoospores).

Breeding season length and climate metrics varied across our study sites, including average daily maximum temperature during the hottest months (July and August). Boreal toads can clear *Bd* infection via seeking out warm, open habitat patches (Barrile et al., 2021); however, Barrile et al. (2021) was conducted at lower elevation sites in the Wyoming Range, which offered warmer summer temperatures than sites at higher elevations in the Wind River Range. Indeed, the negative effect of *Bd* on survival was only statistically significant at the coolest, highest elevation site at Upper Gypsum Creek. Colder summer temperatures likely limit the opportunity for individuals to behaviorally thermoregulate and combat infection, which may help explain disease-induced declines in boreal toads occupying high elevation sites in Colorado (Muths et al., 2003). For example, basking increased the body temperature of boreal toads to 23 °C at a high-elevation site in Colorado (2810 m; Muths and Corn, 1997), which is insufficient to kill *Bd* and/or achieve

optimal body temperature for boreal toad growth and immune function (Lillywhite et al., 1973).

The creation of microhabitats with elevated temperatures (e.g., via heat lamps or habitat modification) has been proposed as the best in situ countermeasure to *Bd* in terms of practicality and minimization of collateral damage to ecosystems (Hettyey et al., 2019). Small-scale microhabitat manipulation to create warm patches therefore comprises a potentially effective mitigation action against *Bd* and possibly other amphibian diseases. For instance, although grazing-induced habitat changes were not associated with decreased *Bd* prevalence in our study, managers could alter the timing, duration, and density of cattle to achieve desired habitat outcomes. Further, populations that experience cooler temperatures could benefit more from localized heating, provided the target species can tolerate warm temperatures. Alternatively, populations experiencing thermal regimes nearby, but not quite, warm enough to suppress *Bd* growth and/or achieve optimal body temperature could benefit most, as relatively modest increases in temperature could result in a favorable shift in thermal regime. In colder environments, comparable interventions may not shift thermal regimes enough to result in favorable outcomes with respect to *Bd* suppression and host immune response.

Given that boreal toads experience optimal growth and immune function at relatively warmer temperatures, the negative effect of temperature (i.e., average daily maximum temperature during the hottest months) on adult survival likely was not causative. Rather, we reason that this relationship was probably a site effect. Populations inhabiting cooler environments often display demographic characteristics of a slow life-history strategist (Muths et al., 2011; Liao et al., 2014), including high and stable adult survival and low but variable rates of recruitment (Gaillard et al., 1998; Cayuela et al., 2015). Survival rates in our study were consistent with this pattern; annual survival was greater at cooler sites in the Wind River Range compared to warmer sites in the Wyoming Range, with significantly higher survival at the coolest site at Upper Gypsum Creek. Greater disease-induced mortality at Upper Gypsum Creek is particularly concerning because lower recruitment is expected at cool, high-elevation sites (Muths et al., 2011). Further, compensatory recruitment is key to amphibian persistence with *Bd* (Scheele et al., 2015; Spitzen-van der Sluijs et al., 2017), suggesting that boreal toad populations at sites such as Upper Gypsum Creek are at a higher risk of disease-related extirpation. Therefore, as a countermeasure to *Bd*-induced mortality in adults, managers could focus on bolstering recruitment rates via the protection and creation of breeding habitat to improve reproductive success (West et al., 2020). Boreal toads at our study sites bred almost exclusively in beaver ponds, the availability of which has been declining over the past few decades (Hossack et al., 2015). Protecting resident beavers from trapping, relocating problem beavers from other areas, and maintaining abandoned ponds may help increase the quality and quantity of breeding habitat and improve recruitment rates (Lambert et al., 2016).

Bd is thought to have been involved with the extinction of over a hundred amphibian species and remains a major threat to global biodiversity (Scheele et al., 2019). Increasing temperatures and temperature variability associated with climate change are expected to increase the susceptibility of many amphibians to chytridiomycosis and exacerbate *Bd*-related declines (Rohr and Raffel, 2010; Cohen et al., 2019). We demonstrate that the opposite relationship also can occur in some amphibians, whereby *Bd*-infected individuals suffer decreased survival only during cooler temperatures, likely via decreased opportunity for infected individuals to induce behavioral fever. In certain contexts, therefore, amphibians in cooler environments are likely to face increased risk of disease-induced mortality compared to conspecifics at warmer locations (Puschendorf et al., 2009).

Emerging infectious diseases continue to threaten wildlife populations, but likely rarely act independently (Goulson et al., 2015). We demonstrate that infection rates can be influenced by livestock grazing and climatic variation. Although the observed relationships among

disease, grazing, weather, and host demography are correlative, our results provide direction for future examination into the underlying mechanisms. Our results also suggest that the effects of climatic variation on host-pathogen dynamics can manifest via altered season lengths. Future investigations of wildlife responses to disease therefore may benefit from considering anthropogenic land use and climatic regimes, including the effect of weather on host phenology.

CRedit authorship contribution statement

Gabriel Barrile: Conceptualization, Methodology, Software, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration. **Anna Chalfoun:** Resources, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition. **Annika Walters:** Resources, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition.

Data availability statement

Data will be made available in the Dryad Digital Repository upon publication acceptance.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocon.2021.109247>.

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