DISSERTATION

OF TOADS AND TOLERANCE: INTRASPECIFIC VARIATION IN HOST PERSISTENCE WHEN CHALLENGED BY DISEASE

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ABSTRACT

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Infectious diseases are increasingly known to drive population declines and extinctions and ultimately contribute to the loss of global biodiversity. This phenomenon is none more apparent than in the extinctions and extirpations of over 500 amphibian species worldwide due to a disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). This ongoing loss of amphibian diversity is concerning given the important roles amphibians serve for ecosystem function, ecosystem services, and pharmacology, among others. To mitigate these declines, scientists must understand the pathogen, environmental, and host factors that interact to affect disease outcomes.

Despite over 25 years of research on amphibian declines and Bd, there is still much we can learn that can crucially inform our conservation actions. For instance, while Bd is undoubtedly a lethal pathogen of severe threat to many amphibians around the world, researchers have observed substantial variation in host responses to Bd infection or presence in the wild and in the laboratory. While many host populations are extirpated by Bd, some persist at lower abundances or rebound completely. Understanding variation in the mechanisms of host population persistence may provide vital hints for how to better conserve vulnerable amphibian populations.

This leads me to the central question of my dissertation: “Why do some populations persist with Bd while others do not?”. I address this question in three chapters by studying

variation in boreal toad (*Anaxyrus boreas boreas*) responses to Bd at several locations across the toad's range. The boreal toad occurs from coastal southern Alaska down into Colorado. Boreal toads are susceptible to Bd and have experienced dramatic declines and extirpations at the southern edge of its range in New Mexico, Colorado, and southwestern Wyoming, which together are considered the southern Rocky Mountain population. Populations in the northern portion of its range, however, appear to be persisting despite the confirmed presence, and high prevalence, of Bd. Two major hypotheses for the difference in apparent persistence of boreal toad populations with Bd across regions include a) differences in demographic compensation and b) differences in host susceptibility. Therefore, I used observational and experimental studies to investigate these hypotheses to better understand the contexts in which boreal toads persist with Bd.

In my first chapter, I leveraged a long-term mark-recapture dataset from multiple populations of boreal toads across a high-elevation gradient in Colorado to test for the existence of compensatory recruitment. Compensatory recruitment is a hypothesized mechanism of host persistence when challenged by disease where hosts increase their baseline recruitment, which compensates for decreased survival attributed to disease, and ultimately stabilizes or slows population declines. Limitations from prior investigations of this phenomenon in other amphibian-Bd systems include the lack of pre-Bd monitoring data, the lack of population replication, and studies that primarily examine low elevation populations. I found a life history trade-off between survival and recruitment across elevations, where high-elevation toads have high survival but low recruitment and vice versa at lower elevations. Once Bd arrived, however, recruitment was reduced across all populations and survival was reduced to zero. Estimates of population abundance and population growth rates were also variable prior to Bd arrival, but

dramatically declined after. I did not find evidence for compensatory recruitment in these high elevation boreal toad populations. My findings highlight that demographic responses to disease may be environmentally context-dependent, and that high elevation amphibian populations are particularly vulnerable to the effects of Bd.

In Chapter 2, I used a laboratory exposure experiment to identify an appropriate isolate of Bd to use in a future experiment designed to investigate differential susceptibility of boreal toad populations to Bd (Chapter 3). While researchers have known about the potential for laboratory-maintained Bd cultures to lose pathogenicity over time (i.e., pathogen attenuation), most exposure studies use isolates that are available to them, regardless of how long they have been maintained in the laboratory, or are unaware of their chosen isolate's culture history. I exposed wild-caught, captive-reared boreal toads to three different isolates of Bd that varied in the amount of time maintained in the lab (old vs. new) and the geographic origin of the isolate compared to the host (local vs. novel) to determine the best isolate for use in Chapter 3. I found that boreal toads exposed to the older isolates had higher weekly survival probabilities than those exposed to the new isolate, indicating pathogen attenuation for older isolates. This effect was also mediated by individual body mass, where larger toads had higher survival. My findings indicate that newer, local isolates are likely better choices when exposing amphibian hosts to Bd and that isolate age and host weight can dramatically affect our inferences from exposure studies.

In my third chapter, I tested the hypothesis that boreal toads exhibit intraspecific variation in susceptibility to Bd. I expected that the host defense strategies of tolerance and resistance are stronger in boreal toads from Wyoming, and weaker in boreal toads from Colorado, and are primarily responsible for our observations of boreal toad population declines in Colorado, and relative population persistence in Wyoming. Previous studies investigating variation in

amphibian host tolerance and resistance to Bd are predominantly focused on species-level comparisons, with fewer focusing on intraspecific variation. Most studies also lack replication among strata of interest (e.g., geography, disease prevalence, host genotypes), and none use a robust methodological framework that can reveal host and pathogen dynamics throughout experimental exposures. Therefore, I conducted a laboratory experimental exposure of boreal toads to Bd, informed by the results of Chapter 2. I included toads from two populations in Colorado and two populations in Wyoming, representing replicates from our strata of interest (i.e., differences in decline severity). Using a multistate modeling approach, I modeled the effects of static covariates (e.g., host population origin, treatment dose of Bd, etc.) and dynamic, individual, time-varying covariates (e.g., weekly individual Bd load, weekly change in individual body mass, etc.) on boreal toad weekly survival and state transition probabilities. State transitions included the weekly probability an individual would clear their infection, or whether a cleared individual would re-gain infection, providing insight into typically hidden infection dynamics. I found that boreal toads from Colorado populations had lower weekly survival probabilities than those from Wyoming when comparing identical Bd loads. This is evidence of increased tolerance to Bd in Wyoming toad populations. As in Chapter 2, individual mass was also important at predicting the effects of Bd on weekly survival probabilities of boreal toads. Boreal toads from Colorado had similar peak Bd loads and cleared Bd at the same probabilities as Wyoming. Colorado boreal toads, however, were on average quicker to reach their peak Bd infection loads and had increased probabilities of re-gaining their infections. These results provide some support for increased resistance among boreal toads in Wyoming compared to those in Colorado. My findings highlight that differential susceptibility to Bd among boreal toads

from different regions may play a crucial role in generating the disparity in decline severity across the region.

In conclusion, my dissertation provides evidence that intraspecific variation in persistence, when challenged by disease, is an important driver of host-pathogen dynamics. My research has filled vital research gaps for an imperiled amphibian species with the goal of helping wildlife managers make tough conservation decisions about host translocations, reintroductions, and captive breeding. I hope my work aids in the persistence of an iconic Rocky Mountain amphibian for years to come.

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DEDICATION

This dissertation is dedicated to the memory of my mother, Patricia Kay Robinson Hardy, who left us unexpectedly in the middle of my degree. Love you mom.

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CHAPTER 1: COMPENSATORY RECRUITMENT UNLIKELY IN HIGH-ELEVATION AMPHIBIAN POPULATIONS CHALLENGED WITH DISEASE[1]

INTRODUCTION

Emerging infectious diseases are often important drivers of host population dynamics and significant threats to biodiversity as documented by recent outbreaks in human, domestic animal, and wildlife systems (Daszak et al., 2000; Plowright et al., 2021). While many diseases cause catastrophic harm to host populations, population level variation in response to disease exists, whereby some host populations are extirpated (e.g., Martin et al., 2018), some persist at lower densities or abundances (e.g., van Riper et al., 1986), and others rebound to pre-disease levels (e.g., Richards & Alford, 2005). However, assessing the effects of disease on wild host populations can be difficult due to the lack of long-term demographic data prior to, and after an outbreak (Plowright et al., 2008). In the cases where appropriate data are available, they can be used to increase understanding of host-pathogen dynamics and contribute to better management and conservation of hosts (Langwig et al., 2015).

When populations persist with disease, identification of the specific mechanisms underlying their resilience is crucial and may give managers the ability to translate successful mechanisms to other populations or species. Mechanisms of population persistence can be found on all sides of the epidemiological triangle, and include environmental, pathogen, or host-related factors, such as demographic, behavioral, or immunogenetic components of their biology (Russell et al,, 2020). The host-related demographic process of compensatory recruitment has

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gained support as an important mechanism of host population persistence across a variety of wildlife health cases. For example, studies on bovine tuberculosis (*Mycobacterium bovis*) in brushtail possums and European badgers (Arthur et al., 2004; Mcdonald et al., 2016), facial tumor disease in Tasmanian devils (Lachish et al., 2009), and toxicological contamination in common guillemots (Votier et al., 2008) all report evidence for compensatory recruitment maintaining population persistence.

Compensatory recruitment is defined as an increase in the recruitment of adults into a population in apparent response to decreased survival and can reverse declining population trajectories due to a stressor (e.g., disease) by stabilizing or increasing population growth rates. The processes acknowledged to lead to compensatory recruitment are increased reproductive effort (i.e., fecundity compensation; Minchella & Loverde, 1981), or increased juvenile survival. In turn, these changes could arise through several pathways, including negative density dependence (Rogowski et al., 2020), pathogen-induced adaptive plasticity (Agnew et al., 2000), or rapid evolutionary change. While researchers are just beginning to examine the exact pathways which lead to compensatory recruitment (Valenzuela-Sánchez et al., 2022), it is clear that if this mechanism is widespread, host populations may reach new equilibria post epidemic, thereby avoiding local extirpation (Langwig et al., 2015). However, the occurrence of demographic responses, such as compensatory recruitment, are expected to vary among populations and may be context-dependent (Valenzuela-Sánchez et al., 2022; West et al., 2020).

Environmental factors may play an important role in determining a population's ability to respond to disease via compensatory recruitment. For example, many host populations commonly occur across elevational gradients and may be subject to tradeoffs in survival and reproductive output. Life history theory predicts that high elevation populations have higher and

more stable adult survival rates but lower reproductive output, and vice-versa at lower elevations (see reviews: Hille & Cooper, 2015; Morrison & Hero, 2003). Such life history tradeoffs could present additional challenges for high elevation populations that are already vulnerable to stochastic processes (e.g., harsh winters), and reduced growing seasons, potentially limiting the capacity to increase recruitment and compensate for decreased adult survival when challenged with disease. Lower elevation populations are also vulnerable to stochastic processes (e.g., drought), but are likely buffered from environmental stressors due to their higher baseline reproductive output. Therefore, environmental variation, if ignored, could obscure the interpretation and generalization of demographic processes (e.g., compensatory recruitment) among populations across an environmental gradient.

There is increasing empirical support for compensatory recruitment as a mechanism of population persistence in amphibian populations affected by chytrid fungus (*Batrachochytrium dendrobatidis*; *Bd*; Brannelly et al., 2021), which is arguably responsible for the greatest loss of biodiversity ever attributed to a pathogen (Scheele et al., 2019). Examining recruitment rates, in addition to survival, is essential for uncovering the entire picture of how *Bd* impacts amphibian populations as recruitment may serve as the 'key' to population persistence (West et al., 2020). However, the scope of studies that examine compensatory recruitment have focused primarily on low elevation (<1,000 m) amphibian populations that were only sampled after disease arrival (Phillott et al., 2013; Spitzen-Van Der Sluijs et al, 2017; Valenzuela-Sánchez et al., 2022). To our knowledge, only one study has detected a potential signal of compensatory recruitment in a *Bd*-positive amphibian population at a higher elevation (Muths et al., 2011). These authors documented differences in apparent survival and recruitment between two spatially distant boreal toad (*Anaxyrus boreas boreas*) populations: one disease free in Colorado, USA (~3,300 m elev.)

and one *Bd*-positive in Wyoming, USA (~2,100 m elev.). Consistent with the compensatory recruitment hypothesis, the post-epidemic population showed lower apparent survival but higher recruitment relative to the disease-free population. However, like other studies, the demographic data prior to disease arrival were lacking, limiting the ability to differentiate between baseline differences in life histories (i.e., vital rates) across elevations and compensatory recruitment as explanations for the observed differences in survival and recruitment.

To better understand the demographic responses of high elevation amphibians to disease, we assessed population vital rates from five high elevation populations of boreal toads in Colorado. Boreal toads in the southern Rocky Mountains of Colorado have experienced dramatic declines that are primarily attributed to *Bd* (Mosher et al., 2018) and the species is listed as state endangered by Colorado Parks and Wildlife (https://cpw.state.co.us/learn/Pages/SOC-ThreatenedEndangeredList.aspx). The species' precipitous decline, and the existence of several ongoing long-term monitoring studies, have made this system ideal for investigating host-pathogen dynamics. Previous work on a subset of populations included in this study document their stability prior to *Bd* arrival and the influence of climate on population vital rates (Lambert et al., 2016). Here, we estimated survival and recruitment parameters before and after *Bd* arrival to determine if high elevation amphibian populations challenged with disease can respond with compensatory recruitment and how the environment (i.e., elevation) might impact population response. Our sampling of populations before and after *Bd* arrival provides a unique opportunity to gain insights on population dynamics that are rarely captured across multiple populations of marked individuals.

METHODS

Ethics Statement

All animal procedures were reviewed and approved by the Colorado State University Institutional Animal Care and Use Committee under IACUC protocol number 2018-070-CNHP. Field surveys and animal procedures were also permitted by Colorado Parks and Wildlife under Scientific Collecting Permit # HP0972.

Data collection and analysis

We surveyed five boreal toad populations in the southern Rocky Mountains of Chaffee County, Colorado, USA, and collected capture-mark-recapture data before and after Bd arrival (1998 – 2020, Table 1.1). Populations were located on U.S. Forest Service managed land in montane meadows surrounded by subalpine forests and varied in hydrology and size (Lambert et al., 2016). For a map of the study area and more details on the field sampling of toads and Bd see Supporting Information (Appendix S1).

We implemented the robust-design Pradel model (Pradel, 1996) using *Program MARK* (White & Burnham, 1999; *see Appendix S2*) to estimate annual apparent survival probabilities ϕ, per-capita recruitment rate f, and visit specific capture probabilities p for adult male boreal toads. Per-capita recruitment is the number of new adults in year t per adult at year t-1. Our dataset included a paucity of marked adult females, so they were excluded from the analysis. Females were captured less frequently because they spend less time at breeding sites than males and skip one or more years between breeding efforts (Muths et al., 2010). We developed hypotheses describing the effects of disease and the environment on annual male survival probability and per-capita recruitment in boreal toad populations (Table 1.2).

Disease

We hypothesized annual apparent survival probability would decrease after *Bd* arrival at each boreal toad population in our study area (Pilliod et al., 2010). However, we also expected

that boreal toad populations might compensate for this lower survival by increasing per-capita recruitment post-*Bd* arrival. This hypothesis (i.e., compensatory recruitment) provides a potential mechanism of persistence for populations challenged by emerging infectious diseases (Brannelly et al., 2021). We also hypothesized that an interactive effect of *Bd* and elevation is possible, whereby the negative impact of *Bd* on survival increases with elevation as suggested in other montane amphibian systems (Sapsford et al., 2013). We treated *Bd* presence as a binary covariate in our models, distinguishing years 'prior to' and 'after' *Bd* arrival at a given site via positive detection from at least two of three swab samples run in triplicate.

Environment

In accordance with life history theory, we expected our boreal toad populations to exhibit relatively high annual apparent survival (ϕ) and low fecundity (or per-capita recruitment as measured here (f)) in years unperturbed by other factors (i.e., disease) because all five populations occur at high elevation (>2,700 m). While we did not directly measure fecundity, we use per-capita recruitment (f) as an extension of the reproductive output linked to fecundity, where lower fecundity is connected to lower recruitment. Even though our populations occur at high elevation, the 600 m gradient between them allowed us to investigate if tradeoffs in vital rates existed at this spatial scale before Bd arrival.

We also incorporated two climate covariates, drought and winter onset date, to account for possible temporal variation in recruitment that is not associated with Bd. These covariates were found previously to be important influences on per-capita recruitment in boreal toad populations shared in the present study prior to Bd arrival (Muths et al., 2017). For example, Muths et al. (2017) found that increased drought and earlier winter onset in year t increased

recruitment three years later (year t+3), when metamorphs begin to recruit into the breeding population.

Drought conditions (DROUGHT) were defined as the number of days without precipitation during the active season (Table 1.2). Active season was defined as the period beginning after the first three consecutive days in spring (i.e., after March 20th) with minimum temperatures ≥ -4.4°C and ending after the first three consecutive days in fall (i.e., after September 22nd) with minimum temperatures ≤ -4.4°C. We chose this temperature threshold because it is sufficient to produce a killing frost, ending vegetative and invertebrate biomass growth, and would encourage toads to hibernate underground (Campbell, 1970). Winter onset date (WINSTART) was defined as the date of first sustained killing frost (minimum temperature ≤ -4.4°C; Table 1.2) where sustained is defined as ≥ three consecutive days. Daily minimum temperature and precipitation data for each population were retrieved from PRISM (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu).

Model Building

We employed a secondary candidate set model building approach (Bromaghin et al., 2013), where we fit models to test hypotheses about one focal parameter at a time (e.g., p) using a null model structure for other, non-focal parameters (e.g., ϕ, f). This approach is better than other model building strategies at recovering total Akaike weights, limiting inclusion of unimportant covariates, and identifying the top model when using the Pradel model (Morin et al., 2020). Specifically, we tested five capture probability structures (*see Appendix S3*). Similar studies of other boreal toad populations in the southern Rockies revealed little evidence of a behavioral effect on capture probabilities (Muths & Scherer, 2011). Accordingly, we set initial capture (p) and recapture (c) probabilities equal ($p=c$) and fit model structures where capture

probability was: constant (p(.)), varied spatially by population (i.e., site; p(s)), varied annually pre-*Bd* but was constant post-*Bd* ($p(t^{Bd})$), varied by population pre-*Bd* but was constant post-*Bd* ($p(s^{Bd})$), and varied by population and year pre-*Bd* but constant post-*Bd* ($p(s^{Bd} \times t^{Bd})$).

Similarly, we tested seven structures for apparent survival probability (ϕ; *see Appendix S4*), including constant survival (ϕ(.)) and structures where apparent survival varied: spatially by elevation (ϕ(ELEV)), annually (ϕ(t)), before and after *Bd* arrival (ϕ(Bd)), or annually before *Bd* arrival, but was constant post-*Bd* ($\phi(t^{Bd})$). Because there were very few captures at any population post-*Bd* arrival (Table 1), we estimated a single post-*Bd* apparent survival probability using data from all populations combined. We also considered two additive structures that combined spatial and temporal variation in apparent survival probability ($\phi(ELEV + t^{Bd})$, ϕ(ELEV + Bd)) and an interactive relationship between elevation and *Bd* status (pre- vs. post-arrival; ϕ(ELEV × Bd)). Lastly, we tested 15 structures for per-capita recruitment (*f*; Appendix 5), including the seven structures listed above involving elevation and *Bd*, and univariate, additive, and interactive structures involving the two climate covariates, DROUGHT and WINSTART, that were found to be important influences on per-capita recruitment in healthy boreal toad populations (Muths et al., 2017; *see Appendix S5*).

Our final 'combined model set' included all combinations of supported structures (ΔAICc < 10) from each of the focal parameter model sets (i.e., ϕ, *f*, p; Appendices 3-5). Annual estimates of realized population growth rate (λ) and breeding population size (N) were derived parameters for all populations from the best supported model.

RESULTS

We captured 1,594 individual male boreal toads across all populations during the study period. Capture probability (p) varied across population and year ($\hat{p}$ range: 0.03 – 0.81). Focal

model sets yielded a single supported model ($\Delta AIC_c < 10$) for capture probability (*p*; *see Appendix S3*) and apparent annual survival (ϕ; *see Appendix S4*), and two supported models for recruitment (*f*; *see Appendix S5*). Our final combined model set therefore included only two models and yielded one clearly supported model (Table 1.3).

Disease

Bd had a strong negative effect on boreal toad annual apparent survival and per-capita recruitment. Adult apparent survival was reduced to 0 and recruitment reduced to 0.25 (SE=0.02) for all toad populations after *Bd* arrival (Fig. 1.1). Estimates of annual breeding population size varied among populations and among years prior to the arrival of *Bd*, but all populations declined in abundance after *Bd* arrival, with two populations (South Cottonwood and Morgan's Gulch) displaying especially severe declines (Fig. 1.2). Estimates of realized population growth (λ) for boreal toads were also highly variable across space and time prior to *Bd*, with annual values ranging between 0.65 (SE=0.08) and 1.46 (SE=0.24) (Fig. 1.3). After *Bd* arrival however, λ at all sites dramatically decreased to 0.25 (95% confidence interval (CI) 0.21 – 0.30) which indicates a severe decline in population growth rate where the populations are only sustained by recruitment, as annual apparent survival is essentially zero (Fig. 1.3). This is possible because adults may reproduce prior to *Bd* infection and succumb to death within a given year.

Environment

Prior to the arrival of *Bd*, we found strong support for higher survival ($\beta_{ELEV} = 0.28$ [SE=0.03], but lower per-capita recruitment ($\beta_{ELEV} = -0.12$ [SE=0.02]) with higher elevation (Fig. 1.1A). Mean annual survival ranged from 0.47 (SE=0.03) at the lowest elevation population (Delilah Pond; 2755m) to 0.83 (SE=0.01) at the highest elevation (Morgan's Gulch; 3366m). Additional annual variation in all parameters existed prior to the arrival of *Bd* (i.e., t^{Bd}), but this variation was not associated with the climate covariates we tested and was likely associated with

other unmeasured climate covariates. Specifically, recruitment was not strongly associated with drought severity or winter onset date (*see Appendix S5*).

DISCUSSION

Our results highlight several key findings: 1) We document severe negative impacts of *Bd* on host survival and per-capita recruitment across multiple populations of boreal toads; 2) We find no support for compensatory recruitment in these high elevation populations five to seven years post-*Bd* arrival; and 3) Our results reveal tradeoffs between survival and recruitment pre-*Bd* along a high elevation gradient with higher survival but lower recruitment at higher elevation sites. If demographic compensation for low survival was going to occur in our system, we would predict it to be most pronounced at lower elevations. While our lowest elevation population (Delilah Pond; 2755 m) prior to *Bd* arrival had >50% greater per-capita recruitment than our highest population, Delilah Pond also consistently had $\lambda<1$ and estimated abundances of <50 adult males. Therefore, mechanisms for demographic persistence may be context-dependent and influenced by host life histories, environmental factors, and baseline demographics. Taken together, high elevation amphibian populations are naturally vulnerable, and particularly so when presented with novel stressors such as introduced disease.

Bd impact on populations

To our knowledge, our unique dataset is one of relatively few from wildlife disease studies that include demographic data both pre-and-post pathogen arrival (n=18; *see Appendix S6*). Interestingly, we are one of only several studies to account for imperfect detection by having individually marked animals pre-and-post pathogen arrival (n=4; *see Appendix S6*). Most other studies include only raw or adjusted counts of captured or encountered individuals. While new methods are being developed to analyze count-based data of unmarked individuals to make inference on various aspects of host-pathogen dynamics while accounting for variable detection

probabilities (DiRenzo et al., 2019), capture-mark-recapture methods are still the most robust way to estimate host vital rates (e.g., apparent survival and per-capita recruitment).

Our findings highlight a strong negative effect of *Bd* on adult boreal toad apparent survival, where we report the lowest published estimate (0) from multiple *Bd*-positive populations. Previous studies on a variety of amphibian species have reported annual apparent survival of *Bd*-positive anuran populations from <0.1 (e.g., Murray et al., 2009) to >0.9 (e.g., Spitzen-Van Der Sluijs et al., 2017). Our findings emphasize the extreme susceptibility of boreal toad populations to *Bd* near their elevational range limits (~ 3,500 m, Mosher et al., 2018), challenging previous work positing that high elevations may serve as refugia from *Bd* (Mosher et al., 2018), thus changing managers' perception of vulnerability of high elevation populations that remain.

Our realized population growth rate estimate post-*Bd* reflects the sole contribution of recruitment to population growth: as survival is 0, therefore the entire growth rate is 0.25. Non-zero recruitment is possible when considering the following scenario. Once metamorphosis is completed, boreal toads leave the breeding site for 3-4 years. When not breeding, boreal toads are known to use drier, upland habitats (Muths, 2003) and do not congregate together, likely limiting their exposure to *Bd*. Once mature, males migrate to ponds where they encounter higher densities of breeding conspecifics, which facilitates exposure to, and transmission of *Bd*, in a highly aquatic environment. Although breeding adults are exposed to *Bd* and may contract chytridiomycosis at breeding sites, they are likely to mate and deposit eggs, with adult mortality occurring after breeding. This scenario supports the potential for some recruitment, but no adult survival.

Four of the five study populations are nearly extinct with 0-1 adults captured in 2020. South Cottonwood had only a single egg mass and two newly captured adult toads with no recaptures in 2020. Moreover, only 3 of 27 adult male toads captured since *Bd* arrival at South Cottonwood (6 years) have been recaptured. This capture to recapture ratio indicates a high turnover rate (Lampo et al., 2012) and provides support for the scenario we outline above. Although other boreal toad populations exist in the surrounding drainages from our five focal populations, most are now *Bd*-positive, and the potential for dispersal from these populations to facilitate demographic rescue declines dramatically at distances greater than 2-3km (Muths et al., 2018). Therefore, the combination of extremely low survival and recruitment makes the long-term persistence of robust, consistently breeding populations unlikely.

Lack of compensatory recruitment

We found no support for increased per-capita recruitment in boreal toad populations after *Bd* arrival which could compensate for reduced survival. Our findings contrast with estimates of compensatory recruitment documented at low elevations (< 1,000 m) in other amphibian species impacted by *Bd* around the world. Phillott et al., (Australia; 2013), Spitzen-van der Sluijs et al., (Europe; 2017), and Valenzuela-Sánchez et al., (South America; 2022) all report findings of higher-than-expected recruitment in *Bd*-positive amphibian populations, evidently compensating for decreased survival. In general, lower elevations provide an expectation of longer active seasons (i.e., time to grow), increased availability of consumptive resources (i.e., energy), and associated life history advantages (e.g., increased fecundity). These advantages, as well as the potential for greater connectivity among populations (i.e., gene flow) could facilitate compensatory recruitment. In contrast, high elevation populations experience very short growing seasons, reduced availability of consumptive resources, and life history constraints such as slow growth, delayed maturation, and lower fecundity. High elevation populations are often isolated

with limited gene flow, and likely limited genetic variation for selective pressures (e.g., disease) to act upon. Our results from high elevation populations experiencing these challenges suggest that there may be elevational limitations to the efficacy of compensatory recruitment as a demographic mechanism of population persistence. Recent work in Australia also supports this idea, providing evidence for response differences along an elevational gradient. West et al., (2020) used population projection models to look at population responses in *Litoria alpina* and suggest that lower elevation populations may compensate for reduced survival (due to *Bd*) via increased recruitment, which is mediated by life history advantages (e.g., earlier maturation, more eggs) that are not present in the higher elevation populations.

With this life history lens, we can re-examine the findings of Muths et al., (2011) that documented higher recruitment in a *Bd* endemic population of boreal toads in Wyoming compared to lower recruitment in a *Bd*-free population in Colorado at a higher elevation. This finding could be due to one of the following scenarios: 1) the lower elevation *Bd*-endemic population had a life history advantage whereby baseline recruitment of the population when healthy was already higher than its higher elevation counterparts, thus buffering it from some of the negative effects of *Bd* on its population trajectory; 2) compensatory recruitment occurred at the lower elevation site following *Bd* arrival which was facilitated by higher resource availability, habitat quality, genetic diversity, or other unknown pathways which promote population persistence with *Bd*; or 3) other unmeasured factors of the host (e.g., immunogenetic, behavioral), the environment (e.g., climate, habitat), or of the pathogen (e.g., strain virulence) are aiding population persistence that are yet unknown. None of these hypotheses are mutually exclusive, however, and could be working in concert to influence population persistence in Wyoming or other regions compared to our populations in Colorado.

Elevational tradeoffs in vital rates

Our results highlight a clear tradeoff between higher survival and lower recruitment with increased elevation among our boreal toad populations. Life history tradeoffs between survival and fecundity are well documented in avifauna across latitudinal gradients, and more recently documented across elevational gradients (Hille & Cooper, 2015). For example, avian clutch sizes (i.e., fecundity) generally decrease with elevation, whereas avian adult survival generally increases with elevation (Hille & Cooper, 2015). These studies typically compare species or populations at elevational differences of 1,000 m or more, and document large differences in survival across elevation (e.g., 0.38 increase in survival over 4,250 m; ~3% increase in survival per 100 m; Sandercock et al., 2005). Our findings of a 0.36 difference in survival (~13% increase per 100 m) and 0.28 difference in recruitment over ~600 m (or ~ 9% decrease per 100 m) highlight the strong effect of elevation on amphibian life histories over a much shorter elevational gradient. The differences we document here likely reflect the reduced dispersal ability of amphibians and create these differences in life histories over relatively small gradients (Smith & Green, 2005).

Though not as well-studied in amphibians, there is some support for a reduction in fecundity (i.e., clutch size) with increased elevation in temperate species (Morrison & Hero, 2003). Within our own region, trends of increased adult apparent survival with increased elevation have been documented in boreal toads (Barrile et al., 2021; Muths et al., 2018). But the tradeoff in fecundity or recruitment has rarely been investigated until now (but see Pilliod et al., 2022). Our results support the notion of a common trend of increased adult survival at high elevations in the Rocky Mountains. We suggest that increased survival comes at the cost of lower reproductive effort. In the case of our five populations, this tradeoff ultimately led to our observations of lower recruitment of adults into the breeding population (i.e., per-capita

recruitment). This life history tradeoff (higher survival but reduced recruitment) in conjunction with reduced dispersal and gene flow typically observed among high elevation amphibian populations (Funk et al., 2005), has clear potential to contribute to the increased vulnerability of high elevation amphibian populations to local extinction (Morrison & Hero, 2003). Recent genomic studies found low levels of genomic diversity, small effective population sizes, and surprisingly low historical genetic differentiation among known, extant boreal toad populations throughout the Southern Rocky Mountains (D. Trumbo, Virginia Polytechnic University, oral comm., July 1, 2022). Collectively, our demographic findings coupled with new genomic information suggests that, without further conservation action, remaining toad populations in the region may have limited ability to recover following *Bd* arrival.

Conclusions

To aid the recovery of host populations challenged by disease, conservation managers benefit from understanding host-pathogen dynamics in both persisting, and near-extirpated, populations. The benefits of studying persisting populations are obvious – to clarify the mechanisms of host-pathogen coexistence to inform disease mitigation in susceptible populations. The value of studying non-persisting or near-extirpated populations may not be as clear but is equally as informative. Investigating these scenarios, as we have done here, leads to a better understanding of when some persistence mechanisms may not be applicable, and clarifies the bounds or thresholds of such mechanisms.

Our study highlights the value of long-term demographic monitoring for the ability to assign cause when stressors arrive (e.g., disease) and detecting change at the scale of populations versus individuals (Grogan et al., 2018; Preece et al., 2017). The importance of such ecological monitoring will only continue to grow. For example, several amphibian-*Bd* systems have

documented dramatic recoveries of host populations near extinction, but only after decades of *Bd* endemicity (Knapp et al., 2016; Voyles et al., 2018). These studies provide some hope for our system, which is only 4-6 years since *Bd* arrival, and emphasizes the need for continued monitoring of these populations.

Our results document substantial demographic differences between *Bd*-positive boreal toad populations in Colorado (this study) and Wyoming (Barrile et al., 2021; Muths et al., 2011). We also illustrate how a mechanism of population persistence (compensatory recruitment) may be elevationally context-dependent. Without the potential for these populations to increase per-capita recruitment on their own, conservation actions focused on increasing recruitment and survival in-situ, such as probiotic host treatments in the wild or translocations, may be warranted. For example, at high elevations, compensatory recruitment may not occur or be less effective in achieving host-pathogen coexistence, thus limiting the ability of these populations to persist with *Bd*. Therefore, we caution that the generalization of potential persistence mechanisms across the entire geographic or environmental range of a given host is unwise.

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TABLES AND FIGURES

Table 1.1. Captures of Boreal toad (*Anaxyrus boreas boreas*) at study sites across elevations and spanning pre-and-post- arrival of Batrachochytrium dendrobatidis (Bd) in the southern Rocky Mountains of Colorado, USA.

Site	Time Period	Captures	Time Period	Captures	**Elevation (m)**
Delilah Pond	2008-2014	232	2015-2020	42	2755
Collegiate Camp	1998-2014	1014	2015-2020	10	2951
South Cottonwood	1998-2013	2073	2014-2020	253	3183
Denny Creek	1998-2015	934	2016-2020	21	3238
Morgan's Gulch	1998-2013	370	2014-2020	37	3366

Table 1.2. Hypothesized relationships between disease (*Batrachochytrium dendrobatidis, Bd*), elevation, and climate covariates, and apparent survival (ϕ) and per-capita recruitment (*f*) in five populations of boreal toads in the southern Rocky Mountains of Colorado, USA. N/A indicates that the variable was not applied to a given parameter.

			Expected Variable Effect (+/-) on vital rate parameters	
Variable	Explanation	Model Notation	Survival Φ	Per-capita Recruitment *f*
Disease	Categorical covariate: before (0) and after (1) Bd arrival at a given population	Bd	-	+
Environment				
Elevation	Elevation/100	ELEV	+	-
Drought	Number of days without precipitation during the active season in year t-3	DROUGHT	N/A	+
Winter Onset	Date of first sustained (≥ 3 consecutive days) killing frost (≤ -4.4°C) in year t-3	WINSTART	N/A	+

Table 1.3. Model selection results from robust-design Pradel models fit to capture-mark-recapture data collected from boreal toads (*Anaxyrus boreas boreas*) at five populations in Chaffee, Co., Colorado, USA (1998-2020). Elevation and *Batrachochytrium dendrobatidis (Bd)* were important in the top model. ^{Bd} superscript notation indicates that time (*t*) or population (i.e., site; *p*(s)) variation was included up until the arrival of *Bd.*

Model	AIC_c	ΔAIC_c	W_t	*K*	-2Log(L)
$p(s^{Bd} \times t^{Bd})\ \phi(\text{ELEV}+ t^{Bd})\ f(\text{ELEV}+ t^{Bd})$	26649.10	0.00	1.00	115	10503.97
$p(s^{Bd} \times t^{Bd})\ \phi(\text{ELEV}+ t^{Bd})\ f(t^{Bd})$	26705.91	56.81	0.00	114	10562.87

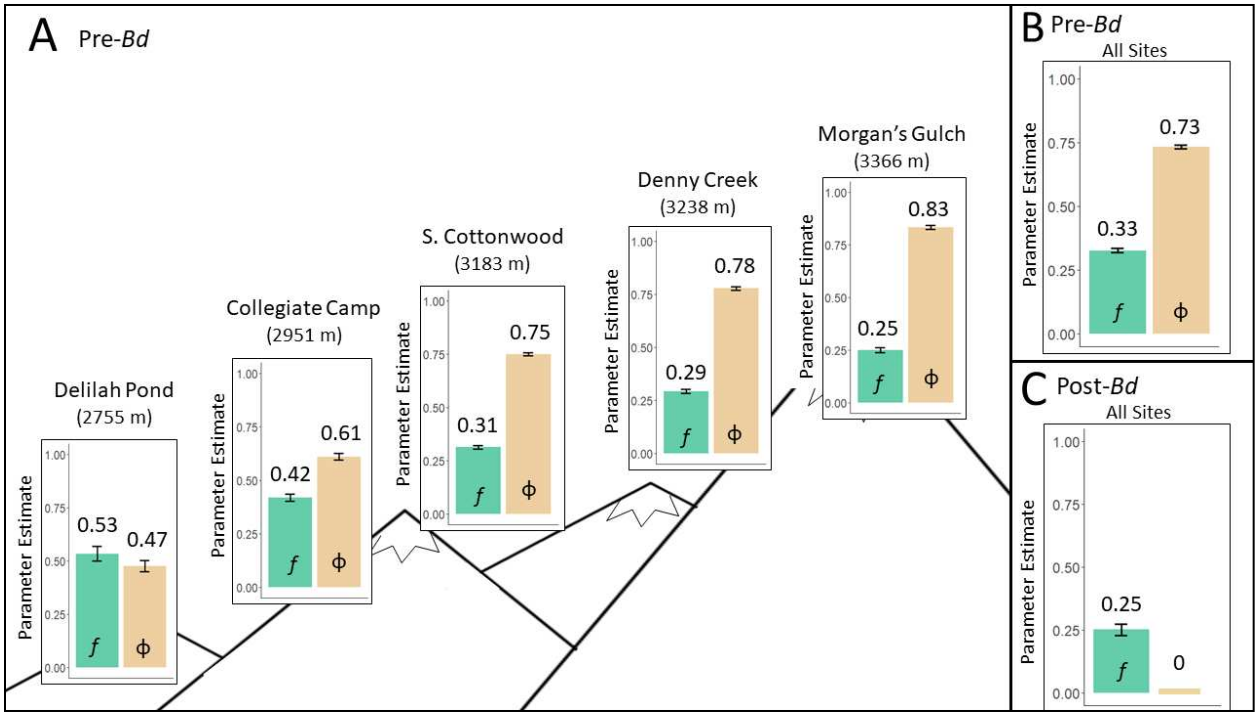

Figure 1.1. Opposing elevational trends in annual survival (ϕ) and per-capita recruitment (f) estimates for five boreal toad (*Anaxyrus boreas boreas*) populations in Colorado, USA. (A) Survival and recruitment estimates among five populations pre- *Batrachochytrium dendrobatidis (Bd)*. (B) Survival and recruitment estimates of all populations combined pre-*Bd*. (C) Survival and recruitment estimates of all populations combined post-*Bd*. Error bars indicate ± 1 standard error. Parameter estimates for (A) and (B) are generated from models not included in our formal analysis, $p(s^{Bd} \times t^{Bd})$ ϕ(ELEV + Bd) f(ELEV + Bd) and $p(s^{Bd} \times t^{Bd})$ ϕ(Bd) f(Bd), respectfully, to display average estimates across elevations. Parameter estimates for (C) come from our top model in our final candidate set: $p(s^{Bd} \times t^{Bd})$ ϕ(ELEV+ t^{Bd}) f(ELEV+ t^{Bd}).

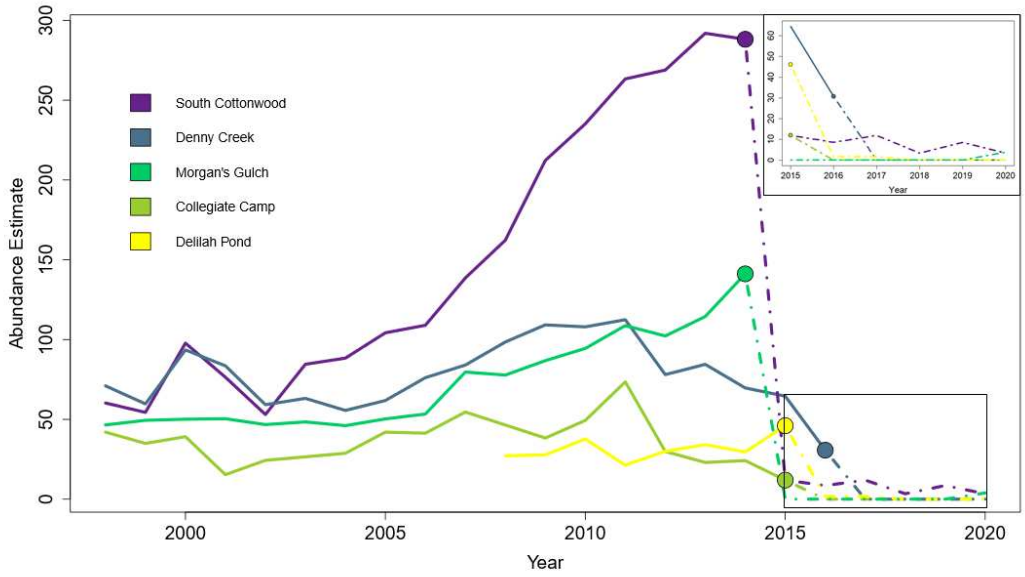

Figure 1.2. Estimates of abundance and severe decline of breeding males for five populations of boreal toads (*Anaxyrus boreas boreas*) in Chaffee County, Colorado, USA. Filled circles denote *Batrachochytrium dendrobatidis* (*Bd)* arrival and dashed lines track the estimated abundance of breeding males post-*Bd* arrival. The insert represents the abundance estimates for the final 6 years of our study (2015-2020).

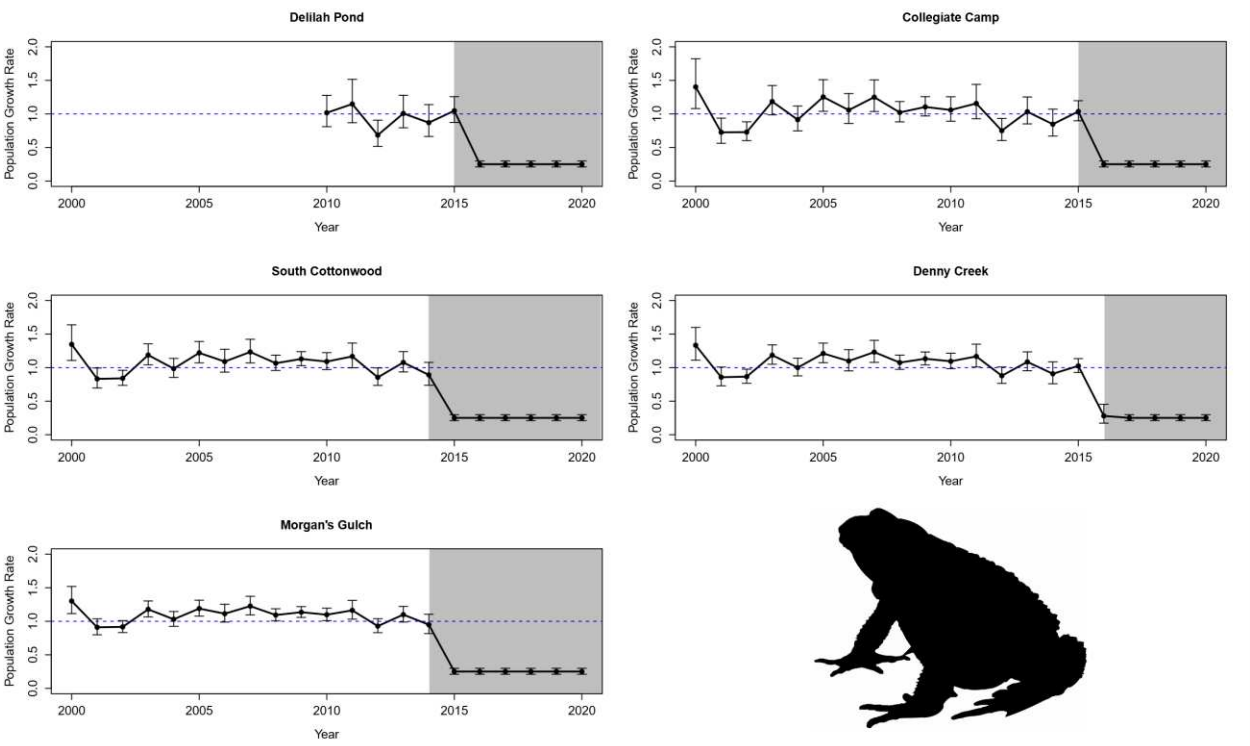

Figure 1.3. Realized annual population growth rates (λ, solid black lines) with 95% confidence intervals for five Boreal toad (*Anaxyrus boreas boreas*) populations in Chaffee County, Colorado USA derived from the top model in our candidate model-set. Values >1 (above the horizontal dashed line) indicate population growth and values <1 indicate population decline. Gray shading indicates the presence of *Batrachochytrium dendrobatidis (Bd)* in the population. Toad silhouette sourced from phylopic.com (with permission via creative commons license).

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CHAPTER 2: ISOLATE-SPECIFIC MORTALITY IN A THREATENED AMPHIBIAN

INTRODUCTION

Laboratory experiments that expose hosts to pathogens or parasites are commonly used in disease ecology to gain new insights into host-pathogen dynamics. In these experiments, researchers manipulate focal factors (e.g., pathogen genotype) while controlling for others (e.g., environmental conditions or host genotype) to determine the relative effect of focal factors in the disease process. Several factors are often manipulated or controlled for to recreate or replicate natural conditions, but experiments are ultimately simplified models of host-pathogen dynamics owing to the complexity of disease processes. If careful thought and biological reasoning are not incorporated into the experimental design, we risk limiting our inference from these 'simplified models' by unintentionally introducing biases. For example, a recent meta-analysis of inconsistences across experimental studies that exposed amphibian hosts to the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), the pathogenic agent responsible for global amphibian declines (Skerratt et al. 2007; Scheele et al. 2019), found likely unintentional disproportionate effects of temperature on host mortality in experimental studies (Sauer et al. 2020). Cool-adapted hosts subjected to warmer experimental conditions and warm-adapted hosts subjected to cooler experimental conditions had higher mortality when exposed to Bd relative to hosts subjected to their adapted optimum temperatures. These results highlight the potentially inadvertent consequences of experimental design choices that can ultimately bias inference when not considering ecologically relevant experimental conditions.

Other common inconsistencies across Bd exposure studies include Bd isolate origin (e.g., local or novel) and variation in pathogenicity due to in-vitro attenuation. Previous studies suggest that some isolates are more pathogenic than others (Berger et al. 2005; Retallick and Miera 2007;

Fisher et al. 2009), but maintenance of these isolates in culture over multiple generations can select for decreased pathogenicity (attenuation; Brem et al. 2013; Langhammer et al. 2013). This inadvertent, artificial selection can severely bias experimental inference if unaccounted for (Kumar et al. 2020) and could lead to misguided conservation or management decisions for hosts based on flawed information from isolates not experienced by wild hosts. For example, exposing hosts to an attenuated pathogen could mask variation in host susceptibility, resulting in falsely deeming populations or species as 'resistant' or 'tolerant' to infection. In addition, choosing isolates that are not distributed within the host's range may limit the ecological relevance of an experiment if hosts are exposed to isolates that occur outside of their range, as is common in Bd exposure experiments (Sauer et al. 2020). Naïve hosts may be particularly susceptible to novel isolates due to the lack of co-evolution between host and pathogen (Doddington et al. 2013). Using a novel isolate could result in widespread reduction in host fitness, thus falsely overgeneralizing the high susceptibility of populations or species when it is not warranted. Therefore, careful consideration of the potential effects of both pathogen attenuation and novelty are warranted when selecting isolates prior to initiation of pathogen exposure experiments.

Here, we test the relative importance of in-vitro pathogen attenuation (isolate age: older vs. newer) and isolate geographic origin (novel vs. local relative to the host geographic origin) on the weekly survival of boreal toads (*Anaxyrus boreas boreas*), a species known to be susceptible to Bd (Carey et al. 2006; Murphy et al. 2009). We tested a new, local Bd isolate from Colorado, an older, local Bd isolate collected in Colorado, and an older, novel Bd isolate from Panama. Because there is evidence for regional variation in population responses to Bd within the Eastern Clade of boreal toads (Goebel et al. 2009; Pilliod et al. 2010; Muths et al. 2011; Oyler-McCance et al. 2017; Hardy et al. 2023) we sourced toads from a location within the

Eastern Clade where toads are thought to be highly susceptible to Bd, and from a second location where they may be less susceptible. These differences allow us to determine if any effects of pathogen attenuation are consistent in the face of different host genotypes. Our study represents a necessary first step to determine the appropriate isolate for use in future studies that investigate variation in host population susceptibility.

We developed several hypotheses based on each isolate's history and possible evolutionary context. If the novelty of an isolate strongly influences host outcomes (e.g., fitness), boreal toads exposed to local Colorado isolates will have higher weekly survival compared to those exposed to a novel Panamanian isolate. Under this hypothesis, we expect hosts to be more susceptible to a novel isolate that lacks a shared coevolutionary history (Doddington et al. 2013). Alternatively, if pathogen attenuation is more important, boreal toads exposed to older isolates should have higher weekly survival compared to those exposed to the fresh isolate, regardless of the geographic source of the isolate. Finally, if both novelty and pathogen attenuation influence host outcomes antagonistically, we expect boreal toad weekly survival to be highest when exposed to the older, local Colorado isolate, lowest when exposed to the fresh, local isolate, and in between the two when exposed to the older novel Panamanian isolate.

METHODS

Site selection, egg collection, and animal husbandry

We selected two boreal toad populations to collect eggs from for use in our exposure experiment. Both populations have a history of Bd presence and tested positive for Bd in 2021. We selected one population located on US Forest Service land in western Wyoming (Chall Creek) and one population in Colorado also located on US Forest Service land (Panhandle Creek). Toads from Wyoming are thought to be less susceptible to Bd (Murphy et al.

2009,2011), while those from Colorado represent highly susceptible populations (Carey et al. 2006). This allows us to determine if any effects of pathogen attenuation are consistent across host genotypes. In collaboration with state and federal biologists, we collected up to 250 eggs from a given eggmass (i.e., < 5% of available eggs) at each population. Eggs were collected and stored in a large Ziploc bag filled with water inside cylindrical Coleman coolers for transport to the Colorado Parks and Wildlife (CPW) Fish Research Hatchery in Bellvue, CO within 24h for rearing.

Upon arrival at the hatchery, eggs were counted and inspected for viability. All eggs from each population were placed in 50-gallon aquaria and reared together until Gosner stage 30 (Gosner 1960). At this stage, larvae (i.e., tadpoles) were transferred to the Colorado State University Aquatic Research Laboratory and were haphazardly assigned to 10-gallon tanks with ~100 individuals per tank. Tadpoles were fed algae wafers and mixtures of puréed squash, zucchini, and collard greens daily. Aquaria were subject to a 50% water change daily and a 12hr light-dark cycle. We used natural well water that ranged from 15-18 °C. Once tadpoles began metamorphosis and developed limbs, we transferred individuals to transition containers consisting of a small plastic container of shallow water with stones to assist larval transition from aquatic to terrestrial habitat through metamorphosis. Fully terrestrial toadlets were then moved to 10-gallon terraria consisting of Eco Earth® (Zoo Med Laboratories Inc., San Luis Obispo, California, USA) substrate and water dishes. Toads were fed flightless *Drosophila melanogaster* dusted in vitamins *ad libidum*. Terraria housed up to 50 toadlets from the same collection site. When toads from a population averaged 2-3 weeks post-metamorphosis, we transferred them to a temperature-controlled experimental room for individual Bd treatments. During the experiment toads were individually housed in 946ml plastic container 'habitats' with Eco Earth® substrate, a

small dish of water, and a mesh lid for air circulation. Toads were fed *D. melanogaster* every other day and water was re-filled every day.

Experimental design and isolate selection

Toads from each population were randomly assigned to one of three Bd treatments where they were exposed to: a formerly cryopreserved isolate from Campana, Panama, isolated from *Hyalinobatrachium vireovittatum* in 2013 with a minimum of 15 passes in culture and maintained at the University of Colorado at Boulder (novel, old; *NovOld*); a formerly cryopreserved isolate *JEL-275* from Clear Creek County, Colorado, USA, isolated from *Anaxyrus boreas boreas* in 1999 with a minimum of 40 passes in culture and maintained at the Collection of Zoosporic Enfungi at the University of Michigan (local, old; *LocOld*); and a fresh isolate from Boulder County, Colorado, USA, isolated from an American bullfrog (*Lithobates catesbeianus*) in 2021 with one passage in culture and maintained at the University of Colorado at Boulder (local, new; *LocNew*). It was infeasible to procure a new, novel isolate due to logistical challenges associated with animal developmental timing for the experiment and delivering a pathogen across country borders during the COVID-19 pandemic. We therefore used what isolates were reasonably available to us, as the majority of Bd exposure studies have historically done. We provide estimates of the minimum number of passages in culture for older isolates based on conversations with the researchers who maintained the isolates prior to our acquisition of them. Researchers provided either: the number of passages that have occurred while the isolate was in their possession, with unknown passages prior (*NovOld*), or an estimated number of passages per year prior to cryopreservation that aligns with the lab's standard practices (*LocOld*). We then added the known number of passages for each isolate since arriving in our possession to generate our reported minimum passage value.

Toad exposure and monitoring

Prior to exposure, toads were weighed to the nearest 0.1 gram. Twenty-nine to 30 toads from each population were randomly assigned to each exposure treatment and we insured that the average mass between treatment groups of a given host population was similar at the beginning of the experiment. Toads exposed to Bd were 2-3 weeks post-metamorphosis at the beginning of the experiment.

To prepare Bd inocula, we grew Bd in sterile 1% Tryptone broth without antibiotics at room temperature for 5-7 days prior to plating on sterile Tryptone-agar media. Plates incubated at room temperature for another 5-7 days prior to zoospore harvest. We washed plates with 1% Tryptone to gather zoospores while leaving zoosporangia on the plate. This process ensured accurate counts of zoospores with a hemacytometer without biasing counts with zoosporangia. We used a 3x2 factorial design to expose boreal toads from each of two populations to three isolates of Bd at a single dose (10^6 zoospores). Hemacytometer counts of zoospores informed the dilutions necessary to achieve desired dose. Toads were individually exposed to Bd with a 24hr bath in 15ml solution of Bd in a 150mm diameter petri-dish in the experimental room. After 24hrs, toads were removed from the bath and placed in their individual habitat. Toad habitats were arranged on metal shelving racks in a blocked design within the experiment room by treatment/population combination to avoid any environmental inconsistencies (e.g., temperature, air-flow) within the room vertically or horizontally. We checked on toads once daily to feed, water, and detect mortalities. Toads were reweighed once every seven days post-exposure until day 28. Toads alive on day 28 were euthanized humanely via overdose of MS-222.

Data analysis

We used a Cormack-Jolly-Seber (CJS) model to estimate weekly survival probabilities for toads exposed to different Bd isolates and to test our *a priori* hypotheses regarding Bd isolate

age or geographic origin using regional host populations that may vary in susceptibility. We fit models where weekly survival was equal or different for toads from different populations (*POP*). We also explored survival differences among toads exposed to: local isolates collected within the region vs. a novel isolate (*LOCvNOV*), isolates with longer passage and cryopreservation histories vs. the fresh isolate (*OLDvNEW*), or a combination of geographic origin and isolate age where host survival differs among all isolates (*Isolate*). We also explored the effect of each individual toad's body mass at the beginning of each week (*MASS*) in additive or interactive combinations with the survival structures described for isolate age and geographic origin. Lastly, we fit a null model (*NULL*) that estimated constant weekly survival for all individuals across all isolates. Our candidate model set consisted of 23 models (Table 1). We fit all models using the CJS model type in Program MARK (White and Burnham 1999) fixing capture probability to 1, and used a model selection approach using AICc (Burnham and Anderson 2004) to evaluate relative support for each hypothesis or model.

RESULTS

We found strong evidence that boreal toad weekly survival probability varied based on the age and geographic origin of the Bd isolate, in addition to the individual toad body mass at the beginning of each week (Table 2.1, Figure 2.1). Toads exposed to *LocNew* had much lower weekly survival probabilities ($\hat{\beta}_{\text{LocNew}}$ = -5.93; 95% CI [-7.37,-4.49]), compared to *LocOld* ($\hat{\beta}_{\text{LocOld}}$ = -3.23; 95% CI [-4.25,-2.21]) and the *NovOld* isolate ($\hat{\beta}_{\text{NovOld}}$ = -2.59; 95% CI [-3.54,-1.65]). These results support the attenuation hypothesis, whereby inadvertent artificial selection in laboratory cultures creates less pathogenic isolates, regardless of isolate novelty in relation to the exposed host. We found weak support for weekly survival differences by host populations (cumulative model weight of 0.15; Table 2.1). Average body mass of toads decreased the first

week after exposure for all isolates (Figure 2.2), but those exposed to *LocNew* continued to decrease until all toads in this treatment died by 21 days post-exposure.

DISCUSSION

Our results suggest that fungal passage history, geographic origin, and host body mass strongly influence host weekly survival probabilities of boreal toad hosts in experimental exposure settings. The *LocNew* Bd isolate was highly pathogenic to toads across all body weights, resulting in the lowest weekly survival probabilities of all isolates tested. The two older isolates were pathogenic for smaller toads, but less so for heavier toads. We believe that the difference in pathogenicity between isolates is largely a result of pathogen attenuation whereby fungal pathogens become less pathogenic when maintained over long periods in culture (Butt et al. 2006; Safavi 2012; Langhammer et al. 2013; Brem et al. 2013; Kumar et al. 2020). Our results highlight that newer isolates are likely the best choice for experiments that investigate host susceptibility to Bd and that older isolates may be pathogenic for smaller hosts only. Here we discuss our findings in detail and their implications for future pathogen exposure studies.

Boreal toads were highly susceptible to our fresh, local isolate of Bd (*LocNew*) compared to older isolates. None of the toads exposed to the *LocNew* isolate survived longer than 20 days post-exposure. Our findings support previous work that demonstrates that newer Bd isolates are more pathogenic than older isolates with longer passage histories (Langhammer et al. 2013, Brem et al. 2013, Kumar et al. 2020). Both of our older isolates may exhibit less pathogenicity via lower zoospore production and growth (Langhammer et al. 2013), or smaller sporangium size (Fisher et al. 2009) as shown in other attenuated isolates. Alternatively, our older isolates may have become less efficient at zoospore production while growing on amphibian skin compared to artificial media, as has been shown for other pathogenic fungal species (Butt et al. 2006). Our

results highlight the considerable variation in the number of passages in culture that should be considered for pathogen attenuation and that this number may vary among isolates. In other words, 20 passes for one isolate may not exhibit the same degree of attenuation as 20 passes for another isolate. Future research could address the presently unknown differences in attenuation rates among Bd isolates or strains by comparing Bd phenotypic traits *in-vitro* (e.g., zoospore production, sporangium size, etc) or host mortality *in-vivo* across a series of passages (e.g., 1, 3, 5, 10, 15, 20, 50). A study like this would not only describe the relationship between passage number and attenuation, but provide evidence for whether the relationship was generalizable across isolates or strains and aid in the design of future experimental exposures.

While both of our older isolates affected boreal toad weekly survival similarly, we found evidence to support isolate-specific differences among all three isolates, including small differences in both older ones. In fact, toads exposed to the *LocOld* isolate had lower weekly survival probabilities than those exposed to *NovOld*, opposite of what we initially predicted. While unexpected, there are several hypotheses that could explain our findings. First, research from other Bd isolates shows that temperature plays an important role in the variation in growth and zoospore production among different isolates (Sheets et al. 2021). Because the *NovOld* isolate is originally from Central America, it is possible that this isolate has retained its unique preferential growth conditions which may be warmer than what it was subjected to in our experiment (~15 °C). Thus, the *LocOld* isolate originally taken from a high elevation, temperate region, may exhibit a growth advantage over the *NovOld* isolate if the *LocOld* isolate is adapted to the cooler temperatures. In addition, because *LocOld* was originally isolated from the same species that we exposed it to, something rarely done in Bd exposure experiments (Sauer et al. 2020), it may also exhibit a growth advantage from selection to a particular host species (Byrne

et al. 2022). Byrne et al. (2022) found that specific Bd genotypes may be associated with specific host species, providing evidence for the potential for coevolutionary relationships between host and pathogen that may benefit the pathogen. While our study was not designed to test these hypotheses, they provide further opportunities for future research into the effects of pathogen evolutionary history and maintenance of these traits in culture that may help explain the differences we observed.

Lastly, we found little evidence for differences in weekly survival between our two host populations when exposed to a given Bd isolate. We originally included toads from two populations that came from regions experiencing different degrees of population declines due to Bd (Pilliod et al. 2010, Muths et al. 2011, Hardy et al. 2023) to test whether Bd isolate pathogenicity was consistently reproducible across multiple host genotypes. While we found weak support for population differences, these results should be interpreted with caution with respect to host susceptibility for several reasons. First, to properly investigate differences in host tolerance or resistance, one would want to expose hosts from replicate populations from each of the focal areas of comparison (e.g., regions with different rates of population declines). We only included one population from each of the hypothesized regions of differing susceptibility. Second, experiments should expose hosts to a variety of pathogen doses to generate a distribution of pathogen infection burdens. We used a single, high dose (10^6) that is known to be lethal to our host species (Carey et al. 2006). Third, researchers would need to measure individual pathogen burdens (e.g., via qPCR) to assess differences in susceptibility across the distribution of pathogen burdens generated (Råberg et al. 2009). Therefore, our study here represents the first step in designing an investigation of differential host susceptibility by

selecting an appropriate pathogen isolate for host exposure with the goal of improving the realism and inference gained from future experimental studies.

In conclusion, we show that selecting pathogen isolates with even relatively short passage histories (e.g., 15 passages) can severely bias inference from host exposure experiments due to in-vitro pathogen attenuation. Our results highlight the reduced pathogenicity in older isolates and their effects on host survival regardless of the isolate's novelty to the host. If we unknowingly chose either of the older isolates for future experiments, we would gain inaccurate inference on the responses of exposed hosts, potentially leading to erroneous management actions (or inactions) based on apparent low susceptibility. We therefore recommend that isolate passage history be prioritized when selecting isolates for exposure experiments that focus on host responses and to collect and use fresh (new) isolates when possible. We also strongly encourage detailed record-keeping of in-vitro passages to improve transparency in isolate selection and reporting.

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TABLES AND FIGURES

Table 2.1. Model selection results from Cormack Jolly-Seber survival models of boreal toads (*Anaxyrus boreas boreas*) exposed to three *Batrachochytrium dendrobatidis* (Bd) isolates in a laboratory experiment.

Model	*AICc*	Delta AICc	w_i	*k*	-2log(L)
Isolate * MASS	389.25	0	0.48	6	377.02
Isolate + MASS	391.32	2.07	0.17	4	383.21
OLDvNEW * MASS	391.32	2.07	0.17	4	383.21
Isolate + POP + MASS	391.66	2.40	0.14	5	381.14
OLDvNEW + MASS	394.87	5.62	0.03	3	388.80
OLDvNEW + POP + MASS	397.30	8.04	0.01	5	387.13
LOCvNOV* MASS	436.85	47.60	0	4	428.74
LOCvNOV + MASS	440.79	51.53	0	3	434.72
LOCvNOV +POP + MASS	443.06	53.81	0	5	432.89
Isolate + POP	455.36	66.10	0	4	447.25
POP + OLDvNEW	456.30	67.05	0	3	450.24
Isolate	457.72	68.47	0	3	451.66
OLDvNEW * POP	458.28	69.03	0	4	450.17
POP * Isolate	458.42	69.17	0	6	446.19
OLDvNEW	458.92	69.66	0	2	454.88
POP + MASS	471.06	81.80	0	3	464.99
MASS	471.26	82.01	0	2	467.22
POP * MASS	473.10	83.85	0	4	464.99
POP + LOCvNOV	489.40	100.15	0	3	483.33
LOCvNOV * POP	490.20	100.95	0	4	482.09
LOCvNOV	491.56	102.31	0	2	487.53
POP	509.83	120.58	0	2	505.80
Null	512.63	123.38	0	1	510.62

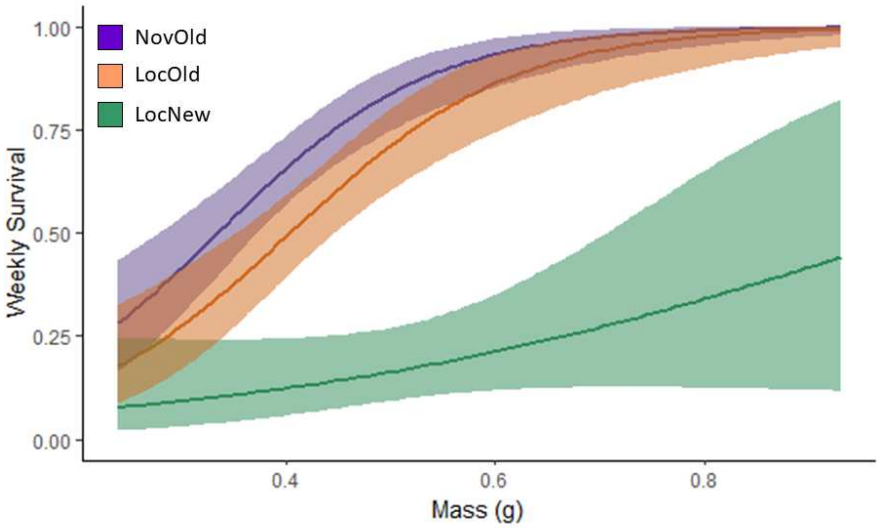

Figure 2.1. Estimated boreal toad (*Anaxyrus boreas boreas*) weekly survival probabilities as a function of weekly body mass (g) after exposure to three isolates of *Batrachochytrium dendrobatidis* (Bd). Lines (estimates) and shading (95% confidence intervals) are displayed for toads exposed to the novel, old (NovOld; purple), local, old (LocOld; orange), and local, new (LocNew; green). Estimates are from our top model (Isolate * Mass; $w = 0.48$) and are graphed between the minimum and maximum values of body mass observed.

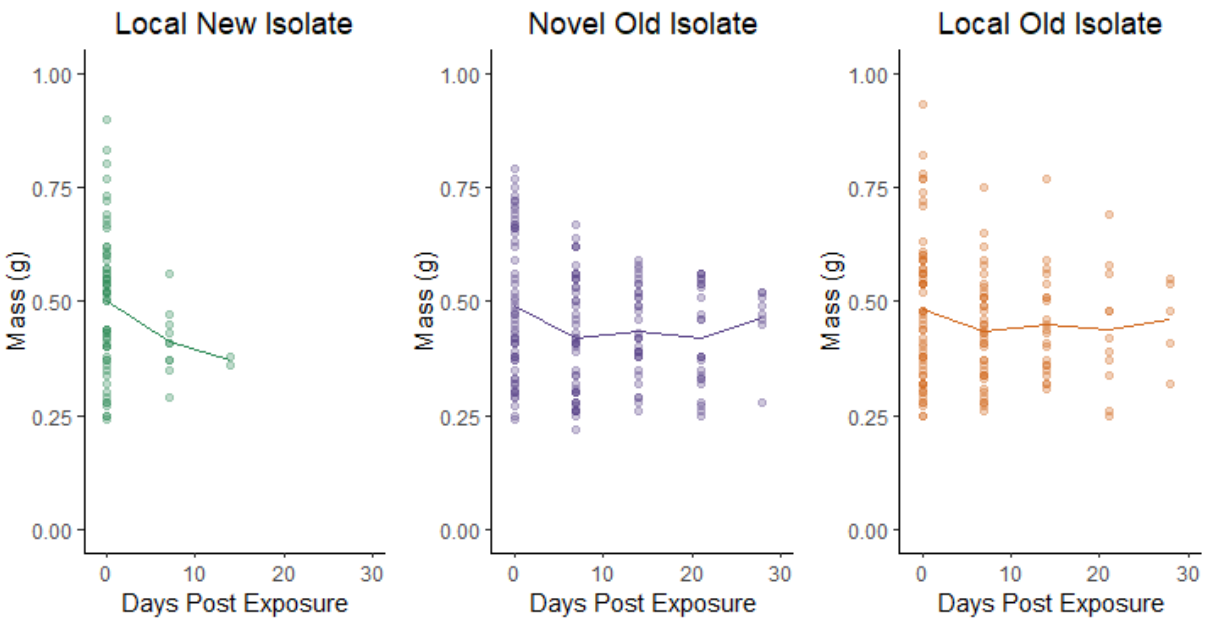

Figure 2.2. Boreal toad (*Anaxyrus boreas boreas*) weekly body mass (g) after exposure to one of three isolates of *Batrachochytrium dendrobatidis* (Bd) during a 28-day laboratory experiment. Lines represent average body mass and circles represent body mass measurements for individual toads exposed to the local, new isolate (LocNew; n = 60; green), the novel, old isolate (NovOld; n = 59; purple), or local, old isolate (LocOld; n =60; orange).

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CHAPTER 3: DISEASE TOLERANCE EXPLAINS INTRASPECIFIC VARIATION IN HOST-PATHOGEN DYNAMICS

INTRODUCTION

Disease strongly affects all scales of biological organization; from influencing individual behavior (e.g., Buck et al. 2018) and population dynamics (Hudson et al. 1998), to structuring communities (Wood et al. 2007), affecting whole ecosystems (Monk et al. 2022), and ultimately shaping the evolution of both host and pathogen (May and Anderson 1983). Due to the ubiquity of disease in natural systems, hosts have evolved strategies to defend themselves from infection, or further harm once infected. These defenses fall into two general categories: 1) mechanisms of disease resistance, and 2) mechanisms of disease tolerance. Host resistance mechanisms are defined as those that directly affect the pathogen and limit its growth (Råberg et al. 2009). For example, skin or gut microbiota that naturally occur in many organisms are responsible for limiting infections by controlling pathogen growth and are considered a mechanism of disease resistance (Van Den Elsen et al. 2017). Host tolerance mechanisms are defined as those that limit the damage or negative fitness consequences caused by the pathogen and do not directly inhibit pathogen growth (Råberg et al. 2009). Host cellular and tissue repair responses are examples of tolerance mechanisms that aid in reducing damage caused by infection (Medzhitov et al. 2012). For instance, mice with a specific enzyme were able to tolerate bacterial infections because the enzyme was necessary for preventing tissue damage due to circulating free heme (Larsen et al. 2010). As ecologists are increasingly aware of the growing contribution of emerging infectious diseases to the loss of biodiversity (Daszak et al. 2000; Smith et al. 2006,2009), investigating whether host resistance or tolerance exists in wildlife species of concern could be particularly important for predicting host-pathogen dynamics and informing conservation actions.

Distinguishing between resistance or tolerance mechanisms in host populations can inform our expectations for evolutionary dynamics of both host and pathogen and guide conservation strategies. Because host resistance mechanisms impact pathogens negatively by limiting their growth, host resistance may select for pathogens that can overcome resistance mechanisms due to shorter pathogen generation times, and lead to antagonistic coevolution (Roy and Kirchner 2000). The presence of resistance traits in a host population is predicted to fluctuate via a negative feedback loop between the proportion of resistant individuals in a population and how beneficial the trait is (Roy and Kirchner 2000). Because maintaining resistance is assumed to have an energetic cost, selection will act against hosts when pathogen prevalence is nearly absent, such that the cost of maintaining resistance traits is relatively higher when their advantage is minimal (Roy and Kirchner 2000). Thus, resistance strategies may be optimal for hosts where infection risk is transient or unpredictable (e.g., pathogen seasonality or vector driven), and in large, diverse host communities that exist along a continuum of susceptibility, where several resistant hosts will benefit those that are less resistant. Therefore, if resistance is identified in a given host-pathogen system, this may signal that conservation actions aimed at assisting pathogen clearance or eradication will be beneficial (e.g., increasing environmental features that aid in host clearance).

In contrast, tolerance mechanisms do not impact the pathogen negatively and therefore pathogen prevalence is expected to increase as tolerant hosts increase. While a tolerance approach may seem like the superior host strategy, the increased emergence of tolerant reservoir hosts may amplify and promote pathogen persistence and could aid in increased transmission to more vulnerable, intolerant hosts. Tolerance mechanisms therefore may be optimal host strategies where risk of infection is more constant through space or time (e.g., environmental

persistence or environmental transmission of the pathogen could lead to more consistent pathogen presence), and where susceptible host communities are less diverse or exist at lower densities to minimize the effect of tolerant reservoir hosts that may act as superspreaders to others (Råberg et al. 2009). If host tolerance is identified, this might signal that pathogen prevalence or infection risk is relatively high, and that measures aimed at eradicating the pathogen may be ineffective. Though resistance and tolerance are presented as a dichotomy, it is important to note that these host defense strategies are not mutually exclusive, and both can coexist within a single host population (Pagán and García-Arenal 2018). These differences in host-pathogen dynamics ultimately illustrate the importance of identifying variation in host resistance and tolerance in natural systems as each strategy begets a different outcome for host and pathogen.

Host tolerance or resistance is a major component in determining whether hosts persist or perish in the face of disease (Brannelly et al. 2021; Russell et al. 2020). Since Råberg et al. (2007) initiated research on resistance and tolerance in model animal systems, many studies have described various lines of evidence for these defense strategies in host-pathogen systems of wildlife, for example: various amphibian species to *Batrachochytrium dendrobatidis (Bd)* (Savage and Zamudio 2011; Searle et al. 2014; Murone et al. 2016; Waddle et al. 2019; Woodhams et al. 2007), Hawaiian Amakihi to *Plasmodium relictum* (Atkinson et al. 2013), Little brown bats to *Pseudogynmnoascus destructans* (Langwig et al. 2017), Tree Swallows to *Protocalliphora sialia* (DeSimone et al. 2018; Grab et al. 2019), Darwin's finches and Galapagos mockingbirds to *Philornis downsi* (Knutie et al. 2016), Cuban treefrogs to *Aplectana* sp. (Knutie et al. 2017), House finches to *Mycoplasma gallisepticum* (Bonneaud et al. 2019; Adelman et al. 2013; Bonneaud et al. 2011), and Black-tailed prairie dogs to *Yersinia pestis* (Rocke et al. 2012;

Russell et al. 2019b). Yet inference from these studies should not be weighed equally. For instance, most experimental exposure studies only sample individuals from one population of a given species (e.g., Woodhams et al. 2007, Sears et al. 2011, Murone et al. 2017, DeSimone et al. 2018, Grab et al. 2019, Knutie et al. 2016, Knutie et al. 2017). Those that do include multiple populations often chose a single representative population across different strata (e.g., strong and weak demographic responses or high and low disease prevalence) such that there is no replication within strata (e.g., Waddle et al. 2019, Atkinson et al. 2013, Bonneaud et al. 2011, Adelman et al. 2013, Bonneaud et al. 2019). Evidence of host defense strategies using multiple population replicates across strata is extremely rare (but see: Savage and Zamudio 2011, Rocke et al. 2012, Russell et al. 2019a), but vitally necessary to confidently assess variation in resistance or tolerance.

Without population replication across strata of interest, we risk over-generalizing host defense strategies to describe an entire species as 'tolerant' or 'resistant', without accounting for the underlying population variation that is likely common. Over-generalization of a given species' susceptibility to disease, or ability to persist with disease, may lead to missed management opportunities, or even un-mitigated population declines. Regardless of which defense strategy is present, describing variation in host tolerance or resistance across the landscape is important for understanding the relative vulnerability of populations to disease. For example, information on population variation in tolerance or resistance could be used to identify potential source populations for population supplementation (moving individuals from relatively tolerant or resistant populations to those that are less tolerant or resistant), reintroduction (moving tolerant or resistant individuals to locations that were historically occupied but not currently), and for captive breeding and assurance programs. The consequences of ignoring

intraspecific variation across biology have recently gained attention in the study of species traits in evolutionary and community ecology (Bolnick et al. 2011; Des Roches et al. 2018), and impacts of climate change on organismal physiology (Bennett et al. 2019; Cicchino et al. 2023). Thus, continuing the coarse-scale investigation of species' resistance, tolerance, and susceptibility to pathogens and parasites without assessing intraspecific host variation may also hinder our understanding of host-pathogen dynamics and species conservation.

Furthermore, many experimental exposure studies are limited in inference to the specific study and duration of that study regardless of ecological relevance. Host-pathogen dynamics are just that, dynamic. Analyses of experimental exposures and biomedical studies are limited historically by representing only static, group-level effects on fitness outcomes (e.g., odds of survival from time-to-event analyses [e.g., Cox proportional hazard models, Kaplan-Meir estimator] Le-Rademacher et al. 2022). These classical methodologies not only miss the dynamic processes of disease through the duration of the study (e.g., changes in host condition or changes in pathogen growth) but are limited by not accounting for a host's transition between various states of interest (e.g., infected, cleared, symptomatic). Multistate models provide a flexible approach to estimate parameters of interest in epidemiological studies that include multiple states, events, and measures of relevant covariates throughout the experiment (Le-Rademacher et al. 2022). Multistate models are commonly employed in various capture-mark-recapture (CMR) analyses involving wild populations and used in epidemiological field studies of marked hosts (e.g., Cooch et al. 2012; Conn and Cooch 2009; Muths et al. 2020), though their advantages have yet to be implemented broadly in studies of experimental exposures in the context of wildlife disease dynamics.

We use an experimental exposure of boreal toads (*Anaxyrus boreas boreas*) to Bd to illustrate the utility of the multistate framework in revealing intraspecific differences in host tolerance and resistance to pathogen infection. Bd is the fungal pathogen contributing to global amphibian declines and extirpations (Skerratt et al. 2007; Wake and Vredenburg 2008) and is considered responsible for the largest loss of biodiversity due to a single pathogen (Scheele et al. 2019). While many studies have used experimental exposures to describe variation in susceptibility to Bd among amphibian species (see Sauer et al. 2020 for meta-analysis of >50 studies), only a handful have investigated intraspecific population variation in susceptibility (Tobler and Schmidt 2010; Savage and Zamudio 2011; Bataille et al. 2015; Waddle et al. 2019). No studies, to our knowledge, have examined the relative roles of tolerance vs. resistance using a robust quantitative framework that directly links host fitness and pathogen burden.

The boreal toad system is ideal for investigating intraspecific variation in host responses to disease and highlights the potential danger in overgeneralizing a species' response range-wide. Boreal toad populations in the United States have experienced severe declines and extirpations due to Bd in the southern Rocky Mountains region of their range (Hardy et al. 2023; Mosher et al. 2018; Muths et al. 2003). Conversely, populations in the middle Rocky Mountains region have persisted despite high Bd prevalence within and among populations (Hossack et al. 2020; Pilliod et al. 2010; Muths et al. 2011; Russell et al. 2019a). These two regions also correspond with apparent population genetic structuring (Goebel et al. 2009, Oyler-McCance et al. 2017). This apparent dichotomy in demographic responses provides an ideal laboratory to investigate whether intraspecific variation in resistance or tolerance to Bd exists within boreal toads. We hypothesize that the differences in the relative severity of declines in populations that have been exposed to Bd is due to intraspecific variation in tolerance, resistance, or both. To test this

hypothesis, we use a common garden exposure experiment to investigate differences in host fitness when challenged with Bd.

METHODS

Site selection, egg collection, and animal husbandry

We selected four boreal toad populations to collect eggs from for use in our exposure experiment. All populations have a history of Bd presence and tested positive for Bd in 2021. We selected two populations on US Forest Service lands in western Wyoming (Chall Creek and Blackrock) that represent the Eastern Clade (EC) in genetic analyses (Oyler-McCance et al. 2017) and two populations in Colorado (Lost Lake, located in Rocky Mountain National Park, and Panhandle Creek on US Forest Service land) that represent the southern Rocky Mountain genetic subpopulation (SRM) of the Eastern clade (Oyler McCance et al. 2017). In collaboration with state and federal biologists, we collected up to 250 eggs from a given eggmass (i.e., < 5% of available eggs) at each population. Eggs were collected and held in a large Ziploc® (SC Johnson, Racine, Wisconsin, USA) bag filled with water and placed inside cylindrical insulated coolers for transport to a facility for rearing. Eggs were housed at the Colorado Parks and Wildlife (CPW) Fish Research Hatchery in Bellvue, CO within 24h of collection.

Upon arrival at the hatchery, eggs were counted and inspected for viability. Eggs from each population were placed in 50-gallon aquaria and reared together until Gosner stage 30 (Gosner 1960). At this stage, larvae (i.e., tadpoles) were transferred to the Colorado State University Aquatic Research Laboratory and were haphazardly assigned to 10-gallon tanks with ~ 100 individuals per tank. Fifty percent of the water in aquaria was changed daily with natural well water that ranged from 15-18 °C. Tadpoles were fed algae wafers and mixtures of puréed squash, zucchini, and collard greens daily and experienced a 12hr light-dark cycle. Once tadpoles began metamorphosis and developed limbs, we transferred individuals to transition containers

consisting of a small plastic container of shallow water with stones to assist larval transition from aquatic to terrestrial habitat through metamorphosis. Fully terrestrial toadlets were then moved to 10-gallon terraria consisting of Eco Earth® (Zoo Med Laboratories Inc., San Luis Obispo, California, USA) substrate and water dishes. Toads were fed flightless *Drosophila melanogaster* dusted in vitamins *ad libidum*. Terraria housed up to 50 toads from the same collection site. When toads from a population averaged 6-7 weeks post-metamorphosis, we transferred them to a temperature-controlled experimental room for individual Bd treatments. During the experiment toads were individually housed in 946ml plastic containers with Eco Earth® substrate, a small dish of water, and a mesh lid for air circulation. Toads were fed *D. melanogaster* every other day and water was re-filled every day.

Experimental design

Toads from each collection site were randomly assigned to one of three Bd exposure treatments: low ($1x10^2$ zoospores), medium ($1x10^3$), or high ($1x10^5$). Doses were selected to ensure infections were distributed across a range of zoospore burdens to assess potential differences in tolerance or resistance. Prior to exposure, toads were weighed to the nearest 0.10 gram and swabbed with sterile cotton swabs (Fisher Scientific) following Livo et al. (2004) to verify that toads were Bd-free. Toads were randomly assigned to each exposure treatment group and we conducted an ANOVA to confirm that average body mass was not different among treatment groups. Toad habitats were arranged on metal shelving racks in a randomized block design to avoid environmental inconsistencies (e.g., temperature, airflow) within the room.

Bd isolation, toad exposure, and monitoring

We isolated Bd from American bullfrog (*Lithobates catesbeianus*) tadpoles collected in Boulder County, CO following Fisher et al. (2018). This isolate had three passages prior to host exposure, limiting potential for pathogen attenuation as documented in other studies (Brem et al.

2013; Langhammer et al. 2013). Recent data also suggest that isolates in Colorado and Wyoming are phylogenetically similar and unlikely to display differences in pathogenicity (Rothstein et al. unpubl. data). To prepare Bd inocula, we grew Bd in sterile 1% Tryptone broth without antibiotics at room temperature for 5-7 days prior to plating on sterile Tryptone-agar media. Plates were incubated at room temperature for another 5-7 days prior to zoospore harvest. We washed plates with 1% Tryptone to gather zoospores while leaving zoosporangia on the plate. This process ensured accurate counts of zoospores with a hemacytometer without biasing counts with zoosporangia. We used a 3x4 factorial design to expose boreal toads from each of four collection sites to the three doses of Bd (Table 3.1). Hemacytometer counts of zoospores informed the dilutions and makeup of the three doses (i.e., 10^2, 10^3, 10^5). In the experimental room, toads were individually exposed to Bd with a 24hr bath in a 15ml solution of Bd in a 150mm x 25mm petri-dish per their treatment dose (Table 3.1). After 24hrs, toads were placed back into their individual habitat. We checked toads once daily to feed, water, and detect mortalities. Beginning seven days post-exposure, and continuing once every seven days until day 56, toads were weighed and swabbed for Bd. Swabs were stored in 70% ETOH at -20°C prior to DNA extraction and qPCR. Toads alive on day 56 were humanely euthanized via overdose of MS-222.

Swab DNA extraction and qPCR

We used Qiagen Blood and Tissue DNEasy® spin-column kits and followed the manufacturer's protocol (Qiagen, Venlo, Netherlands) for animal tissues to extract fungal DNA from swab samples with alterations to the tissue digestion methods which included keeping the swabs in buffer in Step 1 and transferring swabs to spin columns for the primary centrifuge cycle (Step 5). We performed fast-qPCR of extracted DNA in triplicate in the lab of Dr. Jacob Kerby

at the University of South Dakota following established protocols (Kerby et al. 2013). We developed an index of pathogen burden by taking the natural log of the mean copy number + 1 for each sample to use as a quantitative covariate in our analyses and refer to this metric as 'Bd load' throughout.

Data analysis approach

We used a multistate mark-recapture approach (Schwarz et al. 2008; Nichols et al. 1992) to investigate differences in resistance or tolerance to Bd infection among boreal toad populations. This modeling framework allowed us to estimate host weekly survival and state transition probabilities that allow inference on processes of resistance and tolerance with two distinct advantages. First, we were able to model weekly survival probabilities as a function of static and dynamic covariates. Static covariates include treatment or group level factors like geographic origin, exposure dose, or host mass prior to exposure and are typically used in epidemiological models. Dynamic covariates on the other hand are those that are allowed to change throughout the experiment, such as individual pathogen burden or the change in host weight. Therefore, we modeled weekly survival not only as a function of static treatments (e.g., exposure dose), but also as a function of individual host (e.g., mass change) and pathogen conditions (e.g., Bd load). This allowed us to capture individual variation and ask questions about which static or dynamic factors are most important in determining host fitness (i.e., survival). Importantly, this approach allowed us to assess differences in host tolerance by comparing estimates of weekly survival probabilities for different populations across a gradient of pathogen burdens. Second, because some toads lost and re-gained Bd infections multiple times throughout the study, a common occurrence in host-pathogen dynamics that is rarely modeled, we were able to investigate the effect of several static or dynamic covariates on the probability an individual transitions from infected to cleared (Ψ^{IC}) and cleared to re-infected

(Ψ^{CI}). These state transition probabilities also serve as lines of evidence for disease resistance. Pathogen clearance (Ψ^{IC}) implies a direct host mechanism of resisting pathogen growth and presence to zero, and pathogen reinfection implies a lack of resistance to infection (Ψ^{CI}). Together, these state transition parameters provide valuable information on host-pathogen dynamics and host resistance relative to simply reporting the raw counts of each transition or ignoring the clearance phenomenon completely.

Capture Histories and Covariates

We constructed 'capture' histories for each individual on a weekly scale to estimate survival and state transition probabilities. These histories denote whether each individual is alive and infected (I) or cleared (C), or dead (0) at each weekly sampling period. Using these states, we modeled weekly survival and state transitions as a function of several static or dynamic covariates. We included static covariates that described an individual's geographic population of origin (Blackrock WY, Chall Creek WY, Panhandle Creek CO, Lost Lake CO; *POP*), geographic region (WY or CO; *REG*), and exposure treatment (low, mid, high dose; *TRT*).We also used several dynamic covariates of interest that were measured each week. Weekly measurements of an individual's mass (*MASS*) and a quantitative index of weekly pathogen burden from qPCR analysis of swabs (*BD*) were used as individual, time-varying covariates. We developed an additional metric that represented the relative change in body mass from prior to exposure (t_0) until the weekly sample t by subtracting the mass at t_0 from the mass at t and dividing the difference by t_0. This metric gave us a measure of the change in body mass ($\Delta MASS$) for each individual, each week they were alive, relative to their starting mass prior to exposure. These individual, time-varying covariates (*MASS*, *BD*, $\Delta MASS$) allowed us to investigate their effects on weekly host survival probabilities, overcoming limitations of classical approaches that only allow static, group-level comparisons that describe conditions at the onset

of the study (e.g., initial mass or exposure treatment). This is especially important in studies where host outcomes depend on the pathogen or parasite burden, such as amphibian hosts exposed to Bd, where a static exposure treatment factor may not capture the variation in burdens experienced and their progression.

Hypotheses

We tested for boreal toad tolerance to Bd by fitting models that reflected differences in weekly survival probability (*S*) attributed to geography (*POP* or *REG*), disease status (*BD* or *TRT*), and body mass (*MASS* or Δ*MASS*) and additive or interactive combinations of these covariates. We hypothesized that Wyoming toads would have higher weekly survival per a given Bd load than Colorado toads, either without variation among populations within a region (*REG*) or with variation among populations within and across regions (*POP*). We also expected that toads with lower Bd would have higher weekly survival, and that this relationship would be better modeled by the individual, time-varying measure of Bd load (*BD*) than by the static factor of initial exposure dose treatment (*TRT*). Finally, we hypothesized that larger toads (*MASS*) or those that had the smallest relative change in body mass (Δ*MASS*) would have higher weekly survival. We also fit several null weekly survival models that reflected the effect of infection alone based on disease state (cleared or infected) or constant survival probabilities for all individuals regardless of disease state, geography, disease status, or body mass.

We tested for boreal toad resistance to Bd in several ways. We fit models to estimate and model Ψ^{IC}, the weekly state transition probability from infected to cleared, and Ψ^{CI}, the weekly state transition probability from cleared to reinfected. Using the multistate framework, we hypothesized that if Wyoming toads are more resistant, they would have higher probabilities of clearing infections (Ψ^{IC}) and lower probabilities of regaining infections (Ψ^{CI}). Therefore, we fit

models where these transition probabilities are associated with an individual's region (*REG*). We did not have enough transitions between states to fit models with population effects (*POP*). We also hypothesized that an individual's pathogen burden may influence their ability to clear infections between sampling occasions, thus we fit models where this transition probability (Ψ^{IC}) was a function of the individual's Bd load (BD) at time *t*. We also included models where the state transition probabilities (Ψ^{IC} or Ψ^{CI}) remained constant from week to week (.).

Additionally, we assessed boreal toad resistance to Bd by testing for differences in the average peak Bd loads and average time to reach peak Bd loads between Colorado and Wyoming toads. We used t-tests to assess these differences and evaluate the strength of effects by reporting the mean differences and associated measures of precision (95% confidence intervals). If boreal toad populations in Wyoming are more resistant to Bd than those in Colorado, we predicted that Wyoming populations would experience lower peak Bd loads on average or take longer to reach peak loads.

Model building and fitting

We fit all models using the multistate model type in *Program MARK* (White and Burnham 1999). We employed a secondary candidate set model building approach (Bromaghin et al. 2013), where we fit models to test hypotheses about one focal parameter at a time (i.e., *S*) using a null model structure for the other, non-focal parameters (i.e., Ψ^{IC} or Ψ^{CI}). This approach is better than other model building strategies at recovering total Akaike weights, limiting inclusion of unimportant covariates, and identifying the top model when using model types with multiple parameters (Morin et al. 2020). Our final 'combined model set' included all combinations of supported structures (with AICc < 10) from each of the focal parameter model sets (i.e., weekly survival and state transition probabilities).

RESULTS

To address hypotheses regarding boreal toad tolerance and factors influencing host weekly survival (*S*), our focal model set contained 49 survival models (Appendix 1), two of which were well-supported ($\Delta AIC_C < 10$) and retained in our final combined model set. To test hypotheses regarding boreal toad resistance and factors influencing host weekly transition probabilities (Ψ^{IC} and Ψ^{CI}) we fit six transition probability models (Appendix 2) and all were supported ($\Delta AIC_C < 10$) and retained in our final candidate model set. Thus, our final candidate model set consisted of 12 models that included all combinations of the six state transition probability structures and two survival structures (Table 3.2).

Tolerance

Overall, we found strong support for intraspecific variation in Bd tolerance among boreal toad populations. Boreal toads from populations in Wyoming consistently displayed higher weekly survival probabilities at a given Bd load when compared to those in Colorado, particularly as Bd loads increased (Figure 3.2). Toads from populations in Colorado were more variable in tolerance. Panhandle Creek toads exhibited moderate weekly survival compared to those from Wyoming, and Lost Lake toads exhibited the lowest weekly survival especially at higher Bd loads. This effect of Bd load was mediated by an interaction between population origin and weekly body mass. For example, if comparing weekly survival of toads that weighed 0.4 grams across populations at an identical Bd load of natural log 10, those in Colorado had lower weekly survival probabilities (Lost Lake = 0.43, 95% CI [0.31-0.56]; Panhandle Creek = 0.50, 95% CI [0.36-0.65]; Figure 3.3) compared to Wyoming toads (Blackrock = 0.71, 95% CI [0.58-0.82]; Chall Creek = 0.81, 95% CI [0.68-0.90]; Figure 3.3).

Resistance

We found mixed support for increased resistance of Wyoming boreal toads to Bd compared to those in Colorado. The probability an infected toad cleared Bd was low ($\widehat{\Psi}^{IC}$ =0.05) and similar between regions (Figure 3.4), with some support (w = 0.29) for a weak negative effect of increased Bd load on the probability of clearance ($\beta_{Bd} = 4.706242 \times 10^{-8}$, ± 1 SE [0.63]; Model 2, Table 3.2). However, Wyoming boreal toads that did clear Bd infections had a much lower probability of being reinfected ($\widehat{\Psi}^{CI}$ = 0.16 [95% CI 0.07-0.32]) compared to those from Colorado ($\widehat{\Psi}^{CI}$ = 0.50 [95% CI 0.22-0.78]; Figure 4). We also observed differences in weekly survival probabilities among toads that completely cleared their infections (see y-intercept of Figure 3.2).

We found no evidence that average peak Bd loads differed for toads from Colorado and Wyoming (t=-1.3, p-value=0.19, mean difference = -0.62 [95% CI = -1.57-0.32]; Figure 3.5). However, boreal toads from Wyoming reached peak Bd loads later than Colorado toads (t=-2.1, p=0.04, mean difference = -4.24 days [95% CI = -8.26 - -0.22]; Figure 3.6).

DISCUSSION

To test for intraspecific variation in host tolerance or resistance to a fungal pathogen, we conducted an experimental exposure of boreal toad hosts sourced from multiple populations in Wyoming and Colorado, where population decline severity differs among regions. We found strong evidence of high tolerance to Bd in toads from Wyoming, where these toads had higher weekly survival probabilities at a given Bd burden compared to toads in Colorado (Figure 2). Boreal toads from Wyoming may also be more resistant to Bd. While we found no differences in the average peak Bd load or probability of Bd clearance across regions, we found some evidence for longer times to reach peak burdens, and strong support for lower probabilities of reinfection in Wyoming toads who had cleared their infections. Our results demonstrate that intraspecific

variation in boreal toad tolerance and resistance to Bd infection likely plays an important role in shaping regional differences in host-pathogen dynamics in wild populations and has important implications for species conservation.

Boreal toads from Wyoming may have several advantages that could contribute to the regional differences in tolerance and resistance that we observed. First, boreal toads in our study area are nested in two genetic groups: an Eastern Clade that includes the Wyoming populations sourced here, and a Southern Rocky Mountain subpopulation embedded in the Eastern Clade that includes our Colorado source populations. The ranges of these two genetic groups are currently disjunct and may have experienced restricted gene flow for a long time, owing to the unsuitable habitat and low elevations between them (e.g., the Red Desert of Wyoming; Hammerson et al. 1999) and the likely colonization of Colorado after glacial retreat during the last glacial maximum (~12kya; Brugger et al. 2019; Guido et al. 2007). The potential for historical isolation between genetic groups may have contributed to the low genomic diversity found in boreal toad populations in Colorado, where populations exist at their elevational limits and are now fragmented across the landscape due to Bd-related local extirpations (Mosher et al. 2018; Trumbo et al. *In Revision*). Thus, the low diversity found in Colorado may contribute to the lack of adaptive response to a novel stressor (i.e., disease) over the last 50 years. In contrast, boreal toad populations in the Eastern Clade, including northwestern Wyoming, have higher genomic variation than those in the southern Rocky Mountains subpopulation found in Colorado (Oyler-McCance et al. 2017, Trumbo et al. *In Revision*). Overall genomic diversity is the source for genetic adaptation and is predictive of both inbreeding depression and adaptive potential (Kardos et al. 2021). Therefore, we might expect that boreal toad populations in Wyoming, and the Eastern Clade as a whole, possess greater variation in beneficial Major Histocompatibility

Complex (MHC) genotypes for natural selection to act on than those in Colorado. Previous research on amphibian susceptibility to Bd has found that some species have MHC profiles and subsequent immune responses that improve Bd resistance relative to others (Savage and Zamudio 2011; Bataille et al. 2015; Savage et al. 2018; Trujillo et al. 2021). In fact, research outside of the amphibian-Bd system has shown that specific MHC genotypes are correlated with unique microbial communities in mice (Kubinak et al. 2015), highlighting the potential for MHC diversity to influence anti-fungal microbial diversity on amphibian skin, a known resistance mechanism (Harris et al. 2006; Rebollar et al. 2020). Further research on the MHC diversity of boreal toads across regions is warranted to investigate whether certain MHC genotypes exist in specific populations, and if those genotypes contribute to tolerance or resistance to Bd.

While we documented strikingly clear differences in host tolerance and mixed support for differences in host resistance, we are still unsure of the exact functional mechanisms behind these pathogen defense strategies. Boreal toads from Wyoming were more tolerant to Bd infection and displayed higher weekly survival probabilities when compared across identical pathogen burdens with toads from Colorado. How are Wyoming boreal toads able to have such high weekly survival? Tolerance mechanisms are, in general, much more understudied than resistance mechanisms (Råberg et al. 2009). Wyoming boreal toads are somehow limiting the damage caused by the pathogen and limiting the fitness consequences of Bd infection. Recent research investigating the differential expression of genes in Bd-infected boreal toads may shed some light on the mechanisms of tolerance (Corey-Rivas et al. *pers. com.*). Corey-Rivas et al. found that Bd-infected boreal toads from Utah had upregulated immune responses, and Colorado boreal toads had downregulated molecular functioning, suggesting a disruption of electrolyte balance. Importantly, sampled Utah populations are also part of the Eastern Clade and more

closely related to the boreal toad populations in Wyoming sampled in our study. Therefore, this promising research may begin to link which gene regions are associated with boreal toad tolerance to Bd and guide genomic surveillance or captive breeding strategies to enhance the prevalence of certain genotypes.

We also observed some evidence for Wyoming boreal toad resistance to Bd with lower probabilities of becoming reinfected and the slightly longer time needed to reach peak pathogen burdens. There are several mechanisms of resistance that could be responsible for the patterns we observed, including functional immune responses (Grogan et al. 2018; Rollins-Smith 2001), anti-microbial peptides (Woodhams et al. 2006; Rollins-Smith and Conlon 2005; Rollins-Smith 2009), anti-fungal skin flora (Rebollar et al. 2020), or other behavioral or physiological traits (e.g., skin sloughing rates Ohmer et al. 2017). For example, researchers studying the effects of *Batrachochytrium salamandrivorans* (Bsal) on newts (*Ichthyosaura alpestris*) found that infected individuals routinely switched habitats from aquatic to terrestrial to aid in desiccating and clearing their infections (Daversa et al. 2018). We did not observe individual toads moving between their aquatic or terrestrial habitat within fairly small, individual containers, but future studies could include video recording of several individuals in each treatment to determine if behavioral differences exist that could explain and aid in Wyoming boreal toads' resistance to Bd. In fact, a field study of radio-telemetered boreal toads at a low elevation population in Wyoming found that infected individuals preferentially select open habitats that are warmer and have higher probabilities of lowering their Bd burden through behavioral thermoregulation (Barrile et al. 2021).

In addition to differences in tolerance and resistance observed between Wyoming and Colorado boreal toads, weekly survival estimates also revealed differences among regions in the

absence of Bd (y-intercept Figure 2). Colorado toads that cleared their infections and had zero pathogen burden had weekly survival probabilities that were lower (~12% difference) than boreal toads from Wyoming who had also cleared their infections. In other words, uninfected Colorado boreal toads have lower weekly survival probabilities than uninfected Wyoming toads. This reduced fitness in Colorado toads that cleared previous infections could indicate a lag effect from their prior infection and the cost of clearing their infection ultimately resulted in lower survival probabilities. Alternatively, this difference could imply that Colorado toads have inherently lower vigor, attributed to the lower genomic diversity observed in Colorado populations (Trumbo et al. *In Revision*) that could potentially contribute to increased incidence of inbreeding depression (Keller and Waller 2002).

Our findings have significant implications for management of boreal toads in the SRM subpopulation. Reintroduction and translocation efforts to conserve and recover boreal toads in Colorado currently follow a nearest-neighbor approach where source populations are chosen based on distance to the selected reintroduction site (H Crockett, Colorado Parks and Wildlife, pers. com.). With the knowledge that Colorado boreal toad genomic diversity is low SRM-wide (Trumbo et al. *In Review*) and with new evidence that tolerance and resistance to Bd is also low (this study), success of reintroduction efforts may be improved by incorporating genetic material from other sources in the Eastern Clade. Indeed, high-profile success stories from the Florida panther (*Puma concolor*) illustrate the benefits of reintroducing and translocating individuals from different genetic lineages to mask deleterious alleles and improve vigor and resiliency (Johnson et al. 2010). In addition to reintroduction and translocation implications, our findings have strong potential to influence captive rearing and breeding efforts. Managers might consider introducing Eastern Clade genetic material to improve the potential for heritable tolerance traits

to be carried in crosses between lineages. Further research is needed to evaluate the heritability of tolerance in Eastern Clade boreal toads.

In conclusion, we provide evidence that intraspecific variation in host tolerance and resistance are responsible for shaping regional differences in host-pathogen dynamics in response to a deadly fungal pathogen. We also leverage the power of multistate modeling to reveal traditionally hidden disease dynamics that contribute to our understanding of the disease ecology of our system and we encourage its use in future experimental exposures of pathogens or parasites to hosts. Finally, by documenting differences in host defense strategies across a large portion of a host's range, we demonstrate that characterizing an entire species as 'tolerant' or 'resistant' can be unwise and potentially detrimental to host conservation as a whole without accounting for intraspecific variation.

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TABLES AND FIGURES

Table 3.1. Number of boreal toads (*Anaxyrus boreas boreas*) from each population exposed to each *Batrachochytrium dendrobatidis* (Bd) treatment dose.

	Bd Treatment Dose		
Population	10^2 (low)	10^3 (mid)	10^5 (high)
Blackrock, WY	11	12	11
Chall Creek, WY	13	14	14
Lost Lake, CO	18	21	20
Panhandle Creek, CO	13	13	13

Table 3.2. Model selection results from multistate mark-recapture models of boreal toads (*Anaxyrus boreas boreas*) exposed to *Batrachochytrium dendrobatidis* (Bd) in a laboratory experiment. Parameters are Ψ^{IC}, transition probability from infected to cleared; Ψ^{CI}, transition probability from cleared to infected; *S*, weekly survival probability; covariates include REG, regional variation in host source (Colorado or Wyoming); POP, population variation in host source; BD, weekly Bd load; MASS, weekly measurement of mass in grams; (.), no variation.

Model	AICc	Δ AICc	*w*	*K*	-2log(L)
Ψ^{IC}(.), Ψ^{CI}(REG), *S*(BD + MASS * POP)	848.71	0.00	0.37	14	820.11
Ψ^{IC} (BD), Ψ^{CI}(REG), *S*(BD + MASS * POP)	849.16	0.45	0.29	15	818.47
Ψ^{IC} (REG), Ψ^{CI}(REG), *S*(BD + MASS * POP)	850.58	1.87	0.14	15	819.88
Ψ^{IC} (.), Ψ^{CI}(REG), *S*(BD + MASS * REG)	852.94	4.23	0.04	8	836.74
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + MASS * POP)	853.22	4.51	0.04	14	824.61
Ψ^{IC} (BD), Ψ^{CI}(REG), *S*(BD + MASS * REG)	853.35	4.64	0.04	9	835.09
Ψ^{IC} (BD), Ψ^{CI}(.), *S*(BD + MASS * POP)	853.67	4.95	0.03	15	822.97
Ψ^{IC} (BD), Ψ^{CI}(REG), *S*(BD + MASS * REG)	854.77	6.06	0.02	9	836.51
Ψ^{IC} (REG), Ψ^{CI}(.), *S*(BD + MASS * POP)	855.09	6.37	0.02	15	824.39
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + MASS * REG)	857.45	8.73	0.00	8	841.24
Ψ^{IC} (BD), Ψ^{CI}(.), *S*(BD + MASS * REG)	857.86	9.14	0.00	9	839.60
Ψ^{IC} (REG), Ψ^{CI}(.), *S*(BD + MASS * REG)	859.28	10.56	0.00	9	841.02

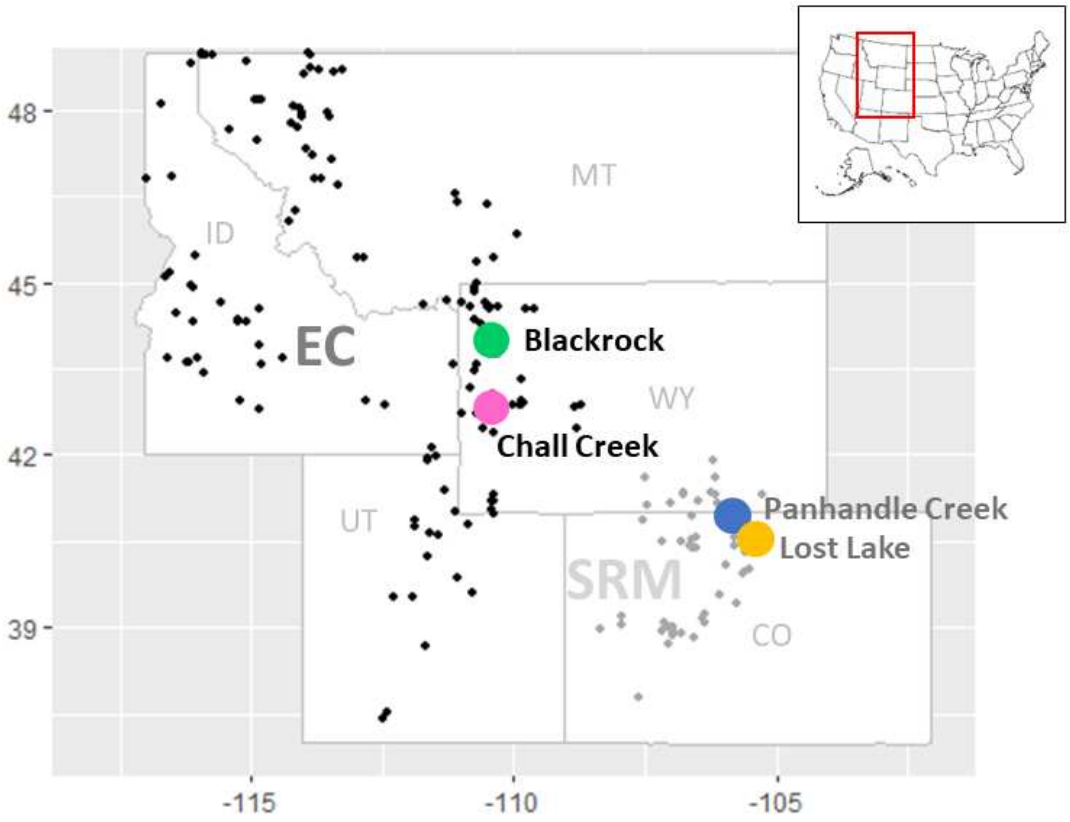

Figure 3.1. Map of boreal toad (*Anaxyrus boreas boreas*) historical range throughout the Intermountain West. Black points represent historical and contemporary boreal toad populations belonging to the Eastern Clade (EC) genetic population and grey points represent boreal toad populations in the southern Rocky Mountain (SRM) genetic subpopulation. Colored points indicate boreal toad egg mass sources for the experimental exposure study.

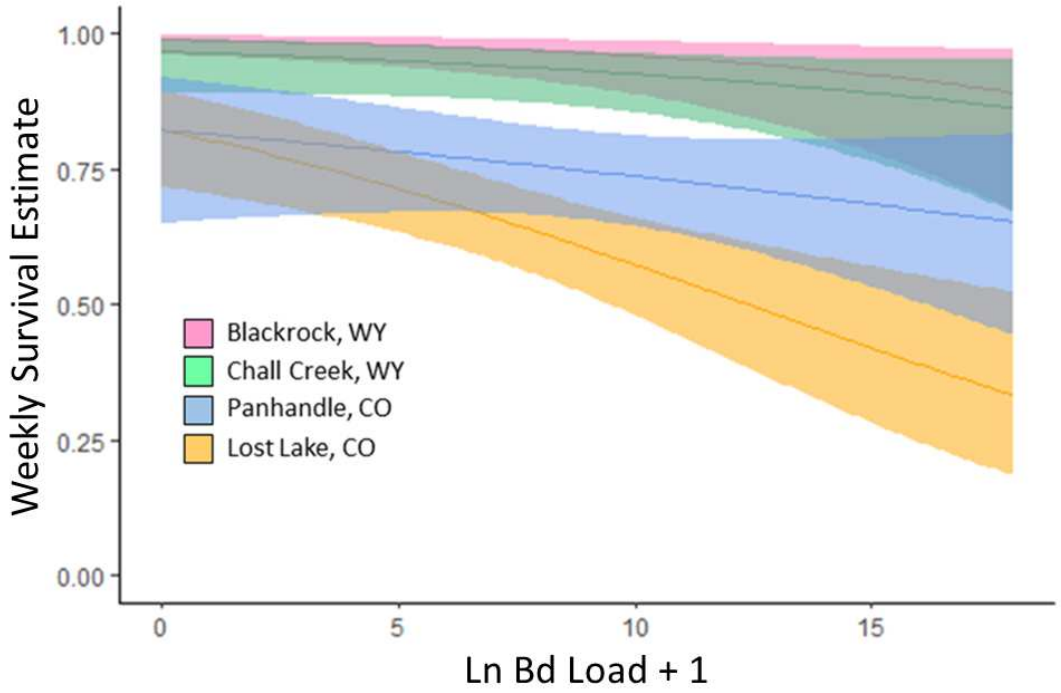

Figure 3.2. Estimated weekly survival probabilities of boreal toads (*Anaxyrus boreas boreas*) from four populations exposed to *Batrachochytrium dendrobatidis* (Bd). Survival estimates (solid lines) are displayed as a function of natural log Bd load and are given for our top model in our final combined model set where toad mass was set at 0.5g. Bands represent 95% confidence intervals around survival estimates.

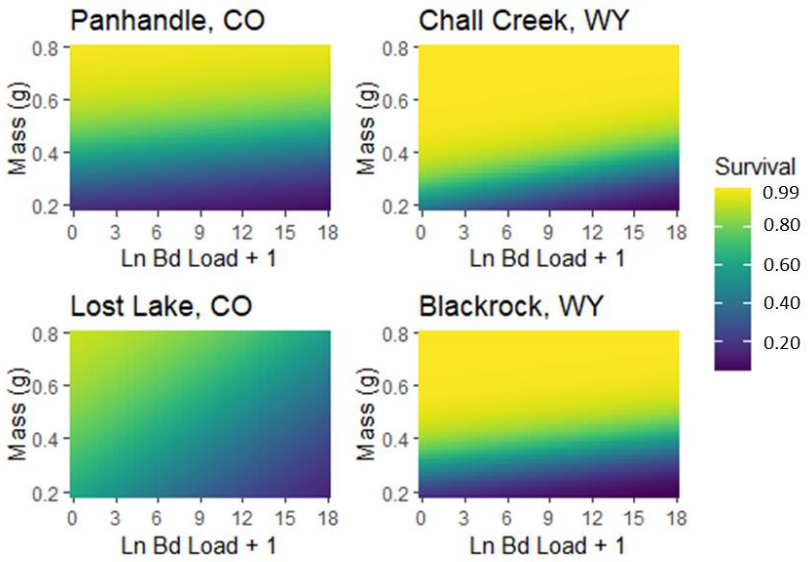

Figure 3.3. Heatmaps displaying the interaction between mass (g) and *Batrachochytrium dendrobatidis* (Bd) load on weekly survival probability for four populations of boreal toads (*Anaxyrus boreas boreas*). Yellow and green colors indicate high weekly survival estimates where darker blues and purples represent lower weekly survival estimates. The range in mass (g) is condensed to 0.2-0.8g to better show the gradient of survival estimates, but individuals ranged from 0.2-1.12g during the experiment, with only six individuals from Wyoming populations above 0.8g.

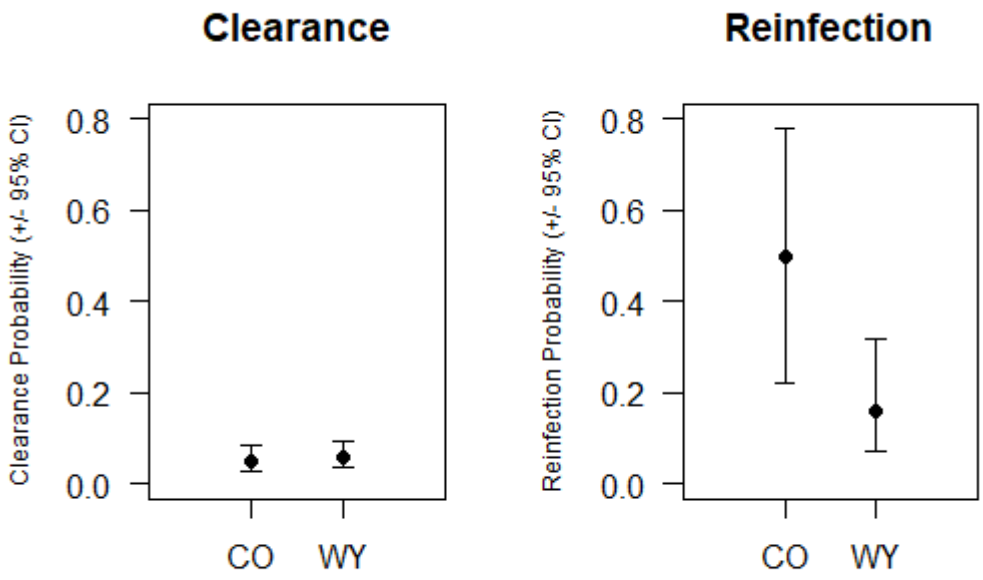

Figure 3.4. State transition probabilities (ψ) of boreal toads (*Anaxyrus boreas boreas*) from Colorado (CO) and Wyoming (WY) exposed to *Batrachochytrium dendrobatidis* (Bd). (a) The probability that an infected toad clears their infection (ψ^{IC}). (b) The probability that a cleared toad becomes infected (ψ^{CI}).

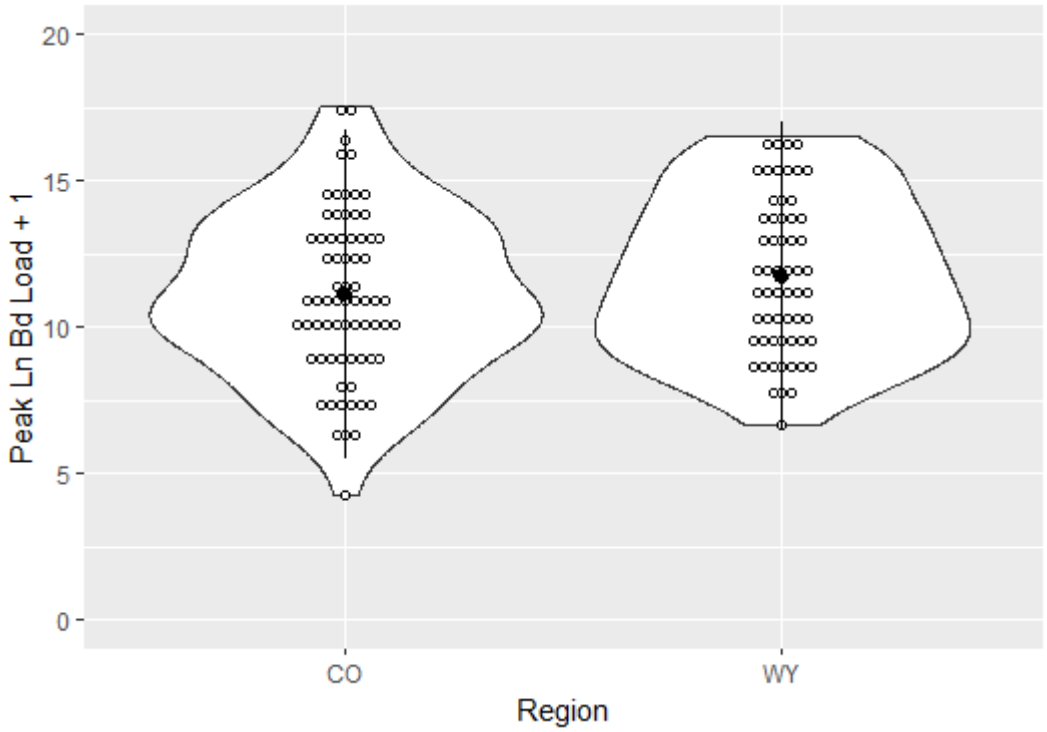

Figure 3.5. Log peak pathogen (*Batrachochytrium dendrobatidis* (Bd)) loads experienced by exposed boreal toads (*Anaxyrus boreas boreas*) from Colorado (CO) and Wyoming (WY) are similar. Black filled circles denote the region-specific mean, open circles represent individual toads. Vertical lines indicate ± 1 standard deviation from the mean.

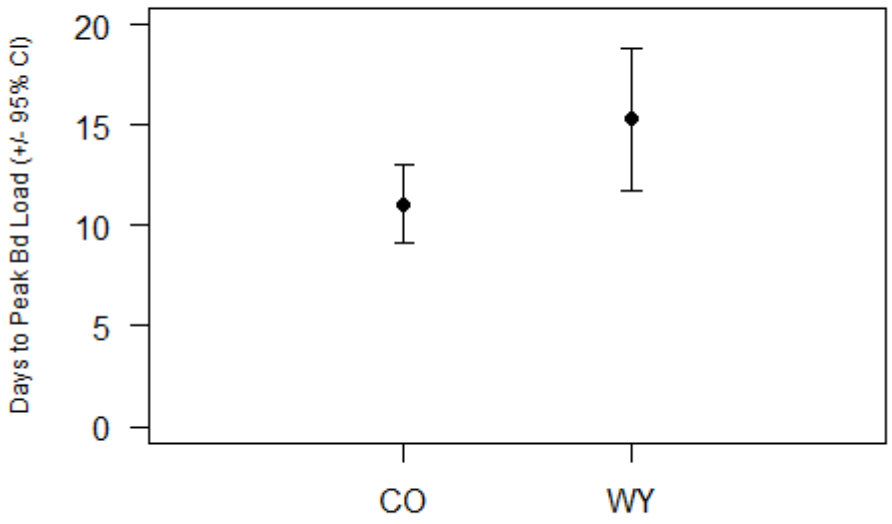

Figure 3.6. The average number of days for boreal toads (*Anaxyrus boreas boreas*) from Colorado (CO) and Wyoming (WY) to reach peak *Batrachochytrium dendrobatidis* (Bd) loads. Boreal toads from Wyoming take longer to reach peak Bd loads than those from Colorado.

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APPENDICES

CHAPTER 1 APPENDIX

Appendix S1: Sampling methods and map

Briefly, we visited populations three times each year during the breeding season (May – June) except for Morgan's Gulch that was visited 1-2 times each year. Night surveys were conducted by at least two observers looking for boreal toads with headlamps, except for Morgan's Gulch which was surveyed during the day. Toads were captured with gloved hands and unmarked toads received a passive integrated transponder (PIT) tag with a unique number that was inserted subcutaneously on the dorsal side. Starting in 2004, up to 20 individuals per population, per year, were swabbed non-invasively to determine the presence of *Batrachochytrium dendrobatidis (Bd)* following Livo et al. (2004). Swabs were assessed for the presence of *Bd* at Pisces Molecular Laboratory (Boulder, CO) using PCR, and later qPCR techniques. This level of sampling ensures detection of *Bd* arrival at a site, given prevalence is ≥ 0.05 (i.e., the probability of detecting *Bd* at least once with n=20 swabs is ≥95%). After arrival, *Bd* prevalence at these and other populations in the southern Rocky Mountains greatly exceeded 0.05 (Colorado Parks and Wildlife, unpublished data, 1998-2020). Therefore, we are confident that we document the actual year of *Bd* arrival at each site.

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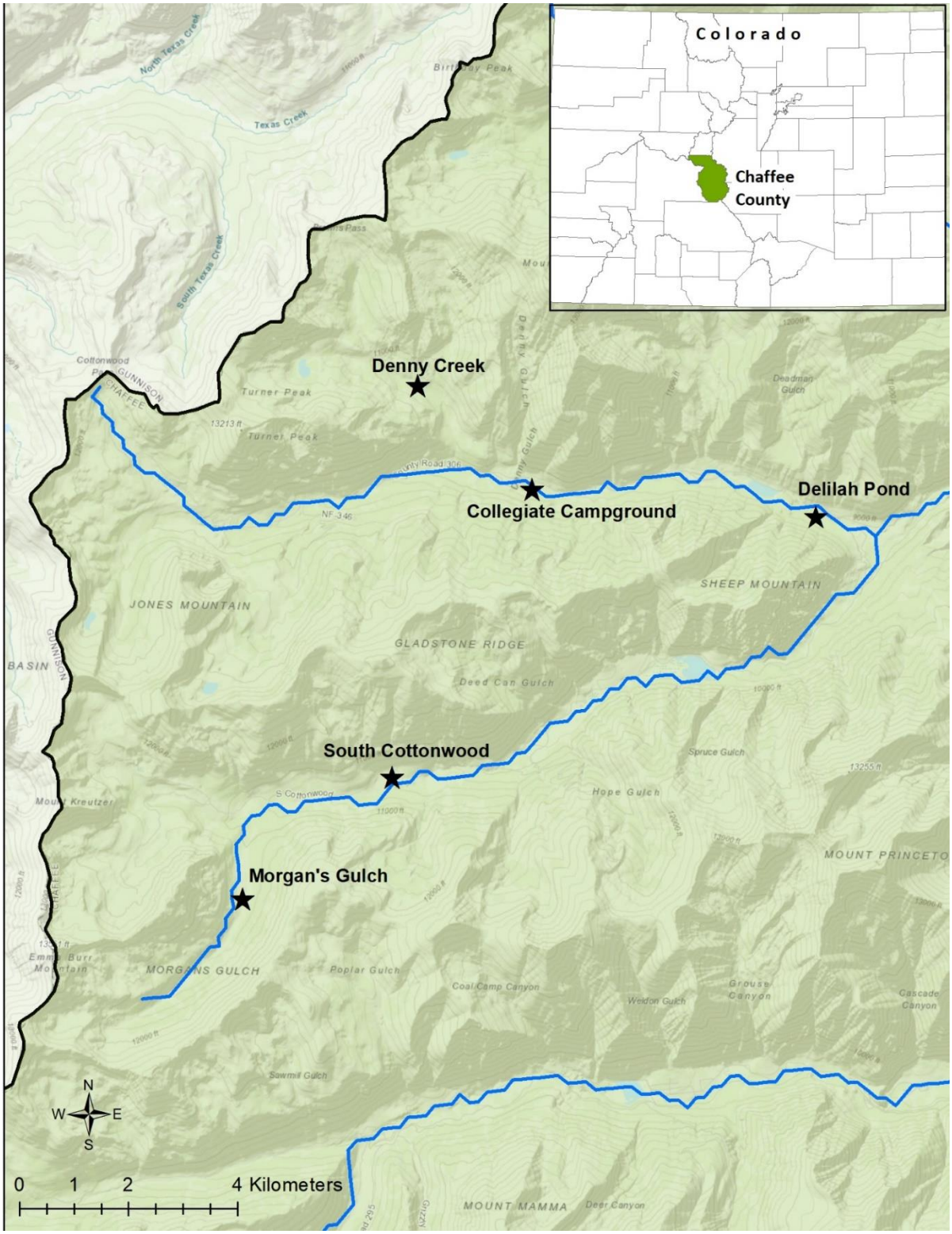

Figure S1: Map of boreal toad (*Anaxyrus boreas boreas*) study area and populations within Chaffee, County, Colorado, USA. Surface Layer Credits: Sources: Esri, HERE, Garmin,

Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAn, GeoBase, IGN, Kadaster NNL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo ©.

Appendix S2: Pradel Model

Model description

The Pradel model is described in detail in the seminal paper (Pradel 1996) and in Williams et al. 2002 (Section 18.4). The Pradel model(s) has also been referred to as 'temporal symmetry' or 'reverse-time' model(s) in the literature. An excellent tutorial on the Pradel model(s) and how to fit these models in program *MARK* is available at: http://www.phidot.org/software/mark/docs/book/pdf/chap13.pdf. It is difficult to improve on the explanation of the model provided in the referenced pdf, so we present excerpts from that document, so readers understand model parameters and their link to the capture history data. In the explanation below, we define the following parameters:

N_i= the population size at time *i*.

λ_i= the realized or observed population growth rate between time *i* and *i* +1: $\lambda_i = N_{i+1}/N_i$

ϕ_i= apparent survival probability = probability than an individual in the population at time *i* survives and remains in the population at time *i* +1

B_i= the number of new individuals entering the populations between time *i* and *i* +1

f_i= the per-capita recruitment rate between time *i* and *i* +1

γ_i= 'seniority' probability = the probability that an individual in the population at time *i* was alive and in the population at time *i* -1

p_{ij} = the probability of capturing an individual in survey *j* during primary time period *i*, given the individual was alive and in the population.

p_i^* = cumulative capture probability = the probability of capturing an individual in at least one survey during primary time period *i*, given the individual was alive and in the population.

The relationship between the realized population growth rate, the per-capita recruitment rate, and apparent survival starts with developing an expression for the population size at time *i* +1, namely:

$$N_{i+1} = N_i\phi_i + B_i \quad \text{(Eq 1)}$$

The population size at *i* +1 is simply the individuals present at time *i* that survived and remained in the population plus the new individuals in the population. Simply, dividing each side of the equation by N_i yields:

$$\lambda_i = \phi_i + \frac{B_i}{N_i} = \phi_i + f_i \quad \text{(Eq 2)}$$

To understand how the model parameters connect to the observed capture histories, its beneficial to consider the apparent survival probability, ϕ_i, and the seniority parameter, γ_i. Apparent

survival probability is estimated by considering the capture history forward in time, while the seniority parameter is estimated by consider the capture history going backward in time, hence the terms 'temporal symmetry' or 'reverse-time' for this model. For example, consider the capture history '100110' where the individual is captured, marked, and released for the first time in period 1, recaptured at least once in period 4 and 5, then not seen in period 6. Note, the robust design model incorporated in MARK includes multiple surveys per period, but here we use the cumulative capture probability, p_i^*, to highlight the between-period dynamics. The probability expression associated with the capture history moving forward would be:

$$\phi_1(1-p_2^*)\phi_2(1-p_3^*)\phi_3 p_4^*\phi_4 p_5^*(1-\phi_5 p_6^*)$$

Then moving backwards or reversing the capture history ('100110' → '011001') and conditioning on the individual being alive and in the population during period 5, the probability expression is:

$$\gamma_5 p_4^*\gamma_4(1-p_3^*)\gamma_3(1-p_2^*)\gamma_2 p_1^*$$

The likelihood of this model is described in detail in Pradel 1996 and Williams et al. 2002 (Section 18.4) and consists of the product of the conditional probabilities associated with all the observed capture histories. While the seniority parameter is useful in understanding the temporal symmetry of the Pradel model, Pradel (1996) described three parameterizations. Because we were interested in modeling apparent survival and per-capita recruitment directly, we chose the parameterization where the seniority parameter is replaced by substituting the following expression for γ_i in the likelihood:

$\gamma_i = \frac{\phi_{i-1}}{\phi_{i-1}+f_{i-1}}$ (Eq 3; Pradel 1996, Williams et al. 2002)

Model parameter structures

We chose to use the 'Robust Design Pradel Recruitment' parameterization that directly estimates apparent survival probability, ϕ_i, per-capita recruitment, f_i, and survey- or visit-specific capture probability. We modeled each of these parameters as a function temporal or spatial covariates or constraints using a logit link function for apparent survival probability, ϕ_i and visit-specific capture probability, p_{ij}. We used a log link function for per-capita recruitment as this parameter is not bounded by 1. Realized or observed population growth rate λ_i and abundance of male breeding toads, N_i, are derived quantities (see Eq. 2; Pradel 1996, Williams et al. 2002 for details on derived quantities).

For example, a capture probability structure where capture probability varied by population prior to *Bd* by was constant post-*Bd* ($p(s^{Bd})$) would be expressed as:

logit $(p_{s,i,j}) = \beta_0 + \beta_1(Pop1) + \beta_2(Pop2) + \beta_3(Pop3) + \beta_4(Pop4)$ for visits conducted prior to *Bd* detection at site (or population) *s,* and

logit $(p_{s,i,j}) = \beta_5$ for visits conducted after *Bd* was detected at site (or population) *s*.

Similarly, an apparent survival structure that included additive relationships between elevation and *Bd*-status (ϕ(ELEV + Bd)) would be expressed as:

$logit\ (\phi_{s,i}) = \beta_0 + \beta_1(Elevation\_s) + \beta_2(Bd\_s)$ where Bd_s is an indicator variable that is equal to 0 for years prior to Bd detection at site (or population) *s*, and Bd_s = 1 for years including and after *Bd* detection at site (or population) *s.*

Finally, a per-capita recruitment structure that included spatial (e.g., elevation) and annual temporal variation before *Bd* arrival but constant afterwards (*f* (ELEV + t^{Bd})) would be expressed as:

$log\ (f_{s,i}) = \beta_0 + \beta_1(Elevation\_s) + \beta_2(1998) + \beta_3(1999) + \cdots + \beta_{17}(2014)$ for years prior to *Bd* detection at site (or population) *s* and

$log\ (f_{s,i}) = \beta_{18}$ for years after *Bd* was detected at site (or population) *s*.

Low population size (Fig 2) and few captures post-*Bd* arrival (Table 1) limited modeling spatial and temporal variation post-*Bd*.

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Appendix S3: Capture probability model set

Model results for the five capture probability structures fit to boreal toad capture-recapture data at five breeding sites (1998-2020) using a robust-design Pradel model. Model names, Akaike Information Criterion for small sample sizes (AICc), Δ AICc, model weights (wt), number of parameters (K), and -2*log(L)* are given for each model. We assumed initial and recapture probabilities were equal and refer to these collectively as capture probability (*p*). We investigated structures where capture probability was constant over time and sites (.), varied spatially between populations or sites (*s*), varied annually before *Bd* arrival and was constant thereafter (t^{Bd}), varied spatially among populations before *Bd* arrived and was constant thereafter (s^{Bd}), or varied by site and year before *Bd* arrived and was constant thereafter ($s^{Bd} \times t^{Bd}$). All models included the null structures associated with apparent survival (ϕ) and per-capita recruitment (*f*).

Model	AICc	Δ AICc	Wt	K	-2log(L)
$p(s^{Bd} \times t^{Bd})$	27825.75	0	1	77	27669.30
$p(s^{Bd})$	28692.40	866.66	0	8	28676.37
$p(t^{Bd})$	28711.71	885.96	0	21	28669.52
$p(s)$	28919.71	1093.96	0	7	28905.69
$p(.)$	29340.28	1514.53	0	3	29334.27

Appendix S4: Apparent survival probability model set

Model results for the seven apparent survival probability structures fit to boreal toad capture-recapture data at five sites (1998-2020) using a robust-design Pradel model. Model names, AICc, Δ AICc, model weights (wt), number of parameters (K), and -2*log(L)* are shown for each model. We investigated structures where apparent survival probability (ϕ) was constant over time and sites (.) or varied: spatially by elevation (ELEV), before and after *Bd* arrival (Bd), or annually before *Bd* arrival, but was constant post-*Bd* (t^{Bd}). We also considered additive structures between elevation and annual or constant structures pre-*Bd* ((ELEV + t^{Bd}), (ELEV + Bd)) and an interactive relationship between elevation and *Bd* status (pre- vs. post-arrival; (ELEV × Bd)). Note: Elevation (m) values for each sites were re-scaled by dividing by 100. All models included the null structures associated with capture probability (*p*) and per-capita recruitment (*f*).

Model	**AICc**	**Δ AICc**	**Wt**	**K**	**-2log(L)**
ϕ(ELEV+ t^{Bd})	28090.61	0	1	22	28046.40
ϕ(t^{Bd})	28128.04	37.43	0	21	28085.86
ϕ(ELEV + Bd)	28235.03	144.42	0	5	28225.02
ϕ(ELEV x Bd)	28237.04	146.43	0	6	28225.02
ϕ(Bd)	28278.37	187.77	0	4	28270.37
ϕ(ELEV)	29302.14	1211.53	0	4	29294.13
ϕ(.)	29340.28	1249.67	0	3	29334.27

Appendix S5: Per capita recruitment model set

Model results for the 15 per-capita recruitment (*f*) structures fit to boreal toad capture-recapture data at five sites (1998-2020) using a robust-design Pradel model. Model names, AICc, Δ AICc, model weights (wt), number of parameters (K), and -2*log(L)* are shown for each model. Structures included constant per-capita recruitment (.) and univariate structures where per-capita recruitment varied spatially by elevation (ELEV), or temporally according to: *Bd* arrival (pre- vs. post-arrival, *Bd*), annual variation before *Bd* arrival but constant post-*Bd* (t^{Bd}), drought conditions during the active season three years prior (DROUGHT), or winter onset date three years prior (WINSTART). We considered additive and interactive structures between *Bd* status (pre- vs. post-arrival) and each of the following: drought conditions, winter onset date, and elevation, and additive relationships between elevation and the two climate covariates. Because there were very few captures at any site post-*Bd* arrival, we estimated a single post-*Bd* per-capita recruitment parameter using data from all sites combined. Lastly, we considered an additive structure with spatial variation associated with elevation and annual variation before *Bd* arrival (but constant post-*Bd* (ELEV + t^{Bd}).

Model	AICc	Δ AICc	Wt	K	-2log(L)
$f(t^{Bd})$	28612.27	0	0.72	21	28570.08
f(ELEV + t^{Bd})	28614.20	1.93	0.28	22	28570.00
f(DROUGHT + Bd)	28760.74	148.47	0	5	28750.73
f(DROUGHT x Bd)	28762.73	150.47	0	6	28750.72
f(Bd)	28764.21	151.94	0	4	28756.20
f(ELEV + Bd)	28765.89	153.63	0	5	28755.88
f(WINSTART + Bd)	28766.21	153.94	0	5	28756.20
f(ELEV x Bd)	28766.38	154.11	0	6	28754.36
f(WINSTART x Bd)	28768.2069	155.9408	0	6	28756.19
f(WINSTART + ELEV)	29276.912	664.6459	0	5	29266.9
f(WINSTART)	29286.11	673.8439	0	4	29278.102
f(DROUGHT + ELEV)	29333.911	721.6449	0	5	29323.899
f(DROUGHT)	29337.83	725.5639	0	4	29329.822
f(ELEV)	29338.679	726.4129	0	4	29330.671
f(.)	29340.2758	728.0097	0	3	29334.271

Appendix S6: Wildlife disease studies

Wildlife disease studies that contain host demographic data before and after pathogen arrival.

Study	Host Taxon	Disease Agent	Data Type	Comparison
(Edwards et al., 2002)	Rabbits	*Rabbit Hemorrhagic Disease Virus* (RHDV)	Transect counts	Relative abundance
(Scherer et al., 2005)	Toads	*Batrachochytrium dendrobatidis*	Individually marked animals	Survival estimates
(Dhondt et al., 2005)	Birds	*Mycoplasma gallisepticum*	Citizen Science counts	Group size
(Lips et al., 2006)	Frogs	*Batrachochytrium dendrobatidis*	Transect counts	Captures/person and proportion dead
(Lachish et al., 2007)	Tasmanian Devils	*Devil Facial Tumor Disease* (DFTD)	Individually marked animals	Abundance and survival estimates
(LaDeau et al., 2007)	Birds	*West Nile Virus*	Citizen Science counts	Counts/route
(Frick et al., 2010)	Bats	*Pseudogymnoascus destructans*	Winter counts	Population growth rate and counts
(Hill et al., 2010)	Birds	*West Nile Virus*	Individually marked animals	Fecundity and repro. success
(Robinson et al., 2010)	Birds	*Trichomonas gallinae*	Citizen Science counts	Counts
(Vredenburg et al., 2010)	Frogs	*Batrachochytrium dendrobatidis*	Visual encounter survey counts	Counts
(Foppa et al., 2011)	Birds	*West Nile Virus*	Citizen Science Counts	Counts
(Pellegrini et al., 2011)	Birds	*West Nile Virus*	Individually marked animals	Survival estimates

(Langwig et al., 2012)	Bats	*Pseudogymnoascus destructans*	Winter count	Population growth rate
(Lawson et al., 2012)	Birds	*Trichomonas gallinae*	Citizen Science counts	Abundance
(Frick et al., 2015)	Bats	*Pseudogymnoascus destructans*	Colony count	Colony size
(Frick et al., 2017)	Bats	*Pseudogymnoascus destructans*	Colony count	Colony size
(Kilpatrick & Wheeler, 2019)	Birds	*West Nile Virus*	Citizen Science Counts	Counts/route
(Kilpatrick et al., 2020)	Bats	*Pseudogymnoascus destructans*	Colony count	Population growth rate

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CHAPTER 3 APPENDIX

Appendix 1: Transition probability model set

Model results for the six state transition probability (ψ) structures fit to boreal toad survival and disease data after exposure to *Batrochochytrium dendrobatidis* (Bd) in a laboratory experiment using a Cormack Jolly-Seber model. Model names, Akaike Information Criterion for small sample sizes (AICc), ΔAICc, model weights (*w*), number of parameters (*K*), and *-2log(L)* are given for each model. We modeled the weekly probability an individual transitions from Bd infected, to cleared (ψ^{IC}), as a function of Bd load (BD), geographic region of origin of the host (REG), or as a constant (.). We modeled the weekly probability an individual transitions from cleared, back to infected (ψ^{CI}), as a function of the geographic region of origin of the host (REG), or as a constant (.). Because the focal parameters of interest were ψ, we only included basic structures associated with weekly survival (*S*), where weekly survival was different for infected (I) versus cleared (C) individuals.

Model	AICc	Δ AICc	*w*	*K*	-2log(L)
Ψ^{IC}(.), Ψ^{CI}(REG), *S*(I/C)	998.60	0.00	0.35	5	988.52
Ψ^{IC} (BD), Ψ^{CI}(REG), *S*(I/C)	999.0	0.39	0.29	6	986.88
Ψ^{IC} (REG), Ψ^{CI}(REG), *S*(I/C)	1000.41	1.81	0.14	6	988.29
Ψ^{IC} (.), Ψ^{CI}(.), *S*(I/C)	1001.1	2.48	0.10	4	993.02
Ψ^{IC} (BD), Ψ^{CI}(.), *S*(I/C)	1001.47	2.86	0.08	5	991.38
Ψ^{IC} (REG), Ψ^{CI}(.), *S*(I/C)	1002.88	4.28	0.04	5	992.80

Appendix 2: Weekly survival probability model set

Model results for the 49 weekly survival probability (*S*) structures fit to boreal toad survival and disease data after exposure to *Batrochochytrium dendrobatidis* (Bd) in a laboratory experiment using a Cormack Jolly-Seber model. Model names, Akaike Information Criterion for small sample sizes (AICc), ΔAICc, model weights (*w*), number of parameters (*K*), and *-2log(L)* are given for each model. We modeled weekly survival probability for individual toads as a function of static and/or dynamic covariates of interest and additive or interactive combinations where appropriate. Static covariates include: exposure treatment dose of Bd at the start of the experiment (i.e., low, medium, or high; TRT), host population of origin (POP), or host geographic region of origin (REG). Dynamic covariates include: the weekly measure of Bd load (BD), the weekly measure of body mass (MASS), or the weekly change in body mass from time *t* to time t_0 (ΔMASS). Two null models were also fit, one with weekly survival as a constant (.), and one with weekly survival differing between infected (I) and cleared (C) individuals. Because the focal parameter of interest was *S*, we only included constant null structures (.) associated with weekly state transition parameters (ψ).

Model	AICc	Δ AICc	*w*	*K*	-2log(L)
Ψ^{IC}(.), Ψ^{CI}(.), *S*(BD + MASS * POP)	853.22	0.00	0.89	14	824.61
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + MASS * REG)	857.45	4.23	0.11	8	841.24
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + MASS + POP)	865.23	12.01	0.00	8	849.03
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + MASS + REG)	866.71	13.49	0.00	6	854.59
Ψ^{IC} (.), Ψ^{CI}(.), *S*(MASS * POP)	886.65	33.43	0.00	12	862.20
Ψ^{IC} (.), Ψ^{CI}(.), *S*(MASS * REG)	890.02	36.80	0.00	8	873.82
Ψ^{IC} (.), Ψ^{CI}(.), *S*($MASS^{IC}$ + REG)	890.06	36.84	0.00	8	873.85
Ψ^{IC} (.), Ψ^{CI}(.), *S*(TRT + MASS * REG)	891.50	38.28	0.00	12	867.05
Ψ^{IC} (.), Ψ^{CI}(.), *S*(TRT + MASS * POP)	893.76	40.54	0.00	20	852.53
Ψ^{IC} (.), Ψ^{CI}(.), *S*($MASS^{IC}$ + POP)	894.82	41.60	0.00	12	870.37
Ψ^{IC} (.), Ψ^{CI}(.), *S*($MASS^{I}$ + REG)	895.12	41.90	0.00	7	880.96
Ψ^{IC} (.), Ψ^{CI}(.), *S*($MASS^{I}$ + POP)	895.85	42.63	0.00	9	877.60
Ψ^{IC} (.), Ψ^{CI}(.), *S*(TRT + MASS + REG)	896.08	42.86	0.00	9	877.82
Ψ^{IC} (.), Ψ^{CI}(.), *S*(TRT + MASS + POP)	897.32	44.10	0.00	11	874.94
Ψ^{IC} (.), Ψ^{CI}(.), *S*(MASS + BD)	911.67	58.45	0.00	5	901.58
Ψ^{IC} (.), Ψ^{CI}(.), *S*(MASS + ΔMASS)	924.01	70.79	0.00	6	911.89
Ψ^{IC} (.), Ψ^{CI}(.), *S*(TRT+MASS)	933.94	80.72	0.00	8	917.74
Ψ^{IC} (.), Ψ^{CI}(.), *S*(MASS)	936.80	83.58	0.00	5	926.71
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + ΔMASS + POP)	940.31	87.09	0.00	8	924.10
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + ΔMASS * POP)	945.87	92.65	0.00	14	917.26
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + POP)	951.68	98.46	0.00	7	937.52
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + ΔMASS + REG)	954.46	101.24	0.00	6	942.34
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD * POP)	955.69	102.47	0.00	10	935.37
Ψ^{IC} (.), Ψ^{CI}(.), *S*(BD + ΔMASS * REG)	956.33	103.11	0.00	8	940.13
Ψ^{IC} (.), Ψ^{CI}(.), *S*(TRT + ΔMASS + POP)	956.38	103.16	0.00	11	934.00
Ψ^{IC} (.), Ψ^{CI}(.), *S*($\Delta MASS^{I}$ + POP)	959.56	106.34	0.00	9	941.30

Ψ^{IC} (.), Ψ^{CI}(.), S(ΔMASS * POP)	959.96	106.74	0.00	12	935.51
Ψ^{IC} (.), Ψ^{CI}(.), S(BD + REG)	961.61	108.39	0.00	5	951.52
Ψ^{IC} (.), Ψ^{CI}(.), S(TRT + ΔMASS + REG)	962.29	109.07	0.00	9	944.03
Ψ^{IC} (.), Ψ^{CI}(.), S(ΔMASS^{IC} + POP)	963.22	110.00	0.00	12	938.77
Ψ^{IC} (.), Ψ^{CI}(.), S(BD * REG)	963.49	110.27	0.00	6	951.37
Ψ^{IC} (.), Ψ^{CI}(.), S(ΔMASS + BD)	965.60	112.38	0.00	5	955.51
Ψ^{IC} (.), Ψ^{CI}(.), S(TRT + ΔMASS * REG)	967.26	114.04	0.00	12	942.80
Ψ^{IC} (.), Ψ^{CI}(.), S(ΔMASS^{I} + REG)	967.78	114.56	0.00	7	953.62
Ψ^{IC} (.), Ψ^{CI}(.), S(ΔMASS * REG)	968.34	115.12	0.00	8	952.13
Ψ^{IC} (.), Ψ^{CI}(.), S(TRT + ΔMASS * POP)	968.61	115.39	0.00	20	927.38
Ψ^{IC} (.), Ψ^{CI}(.), S(ΔMASS^{IC} + REG)	969.63	116.41	0.00	8	953.42
Ψ^{IC} (.), Ψ^{CI}(.), S(TRT + ΔMASS)	971.07	117.85	0.00	8	954.86
Ψ^{IC} (.), Ψ^{CI}(.), S(BD)	973.16	119.94	0.00	4	965.10
Ψ^{IC} (.), Ψ^{CI}(.), S(ΔMASS)	978.06	124.84	0.00	5	967.97
Ψ^{IC} (.), Ψ^{CI}(.), S(TRT + POP)	981.33	128.11	0.00	10	961.02
Ψ^{IC} (.), Ψ^{CI}(.), S(TRT + REG)	981.71	128.49	0.00	8	965.50
Ψ^{IC} (.), Ψ^{CI}(.), S(TRT * REG)	984.07	130.85	0.00	10	963.76
Ψ^{IC} (.), Ψ^{CI}(.), S(POP)	989.21	135.99	0.00	8	973.01
Ψ^{IC} (.), Ψ^{CI}(.), S(TRT * POP)	990.38	137.16	0.00	16	957.59
Ψ^{IC} (.), Ψ^{CI}(.), S(REG)	990.54	137.32	0.00	6	978.42
Ψ^{IC} (.), Ψ^{CI}(.), S(TRT)	991.97	138.75	0.00	6	979.85
Ψ^{IC} (.), Ψ^{CI}(.), S(.)	999.25	146.03	0.00	3	993.21
Ψ^{IC} (.), Ψ^{CI}(.), S(I/C)	1001.08	147.86	0.00	4	993.02