Infection Status as the Basis for Habitat Choices in a Wild Amphibian

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ABSTRACT: Animals challenged with disease may select specific habitat conditions that help prevent or reduce infection. Whereas preinfection avoidance of habitats with a high risk of disease exposure has been documented in both captive and free-ranging animals, evidence of switching habitats after infection to support the clearing of the infection is limited to laboratory experiments. The extent to which wild animals proximately modify habitat choices in response to infection status thus remains unclear. We investigated preinfection behavioral avoidance and postinfection habitat switching using wild, radio-tracked boreal toads (*Anaxyrus boreas boreas*) in a population challenged with *Batrachochytrium dendrobatidis* (*Bd*), a pathogenic fungus responsible for a catastrophic panzootic affecting hundreds of amphibian species worldwide. Boreal toads did not preemptively avoid microhabitats with conditions conducive to *Bd* growth. Infected individuals, however, selected warmer, more open habitats, which were associated with elevated body temperature and the subsequent clearing of infection. Our results suggest that disease can comprise an important selective pressure on animal habitat and space use. Habitat selection models, therefore, may be greatly improved by including variables that quantify infection risk and/or the infection status of individuals through time.

Keywords: habitat selection, behavioral fever, habitat switching, *Batrachochytrium dendrobatidis*, chytridiomycosis, behavioral flexibility.

Introduction

Infectious disease has traditionally been overlooked as an important evolutionary force shaping animal habitat and space use (Lozano 1991). Recent advances in the study of wildlife disease dynamics, however, suggest that some animals select specific habitat conditions to defend against disease (Parker et al. 2011). Defensive habitat choices may occur prior to infection in response to perceived infection risk (i.e., avoidance of habitat patches with high apparent pathogen prevalence; Weinstein et al. 2018). Conversely, following infection by a pathogen, animals can switch habitat patches to conditions that improve host immunity, for example, the way infected ectotherms seek out warm microclimates to induce behavioral fever (Elliot et al. 2002; Murphy et al. 2011). Whereas preinfection behavioral avoidance of habitats with a high risk of disease exposure has been well-documented in both captive and free-ranging animals (Sarabian et al. 2018), evidence of postinfection habitat switching to conditions that help reduce or clear infection is limited to laboratory experiments. Controlled laboratory conditions, however, can mask important trade-offs that organisms face when choosing habitat patches in natural ecosystems (e.g., predation risk, foraging efficiency). The extent to which wild animals behaviorally modify the use of habitats in response to infection status thus remains unclear. Understanding how, and the contexts under which, wild populations employ both defensive strategies will facilitate the integration of host-pathogen ecology and habitat selection theory.

Amphibian populations challenged with the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) provide an excellent system with which to test the ability of wild animals to select habitats that help combat disease. *Bd*, which causes chytridiomycosis, a skin disease responsible for a catastrophic panzootic impacting hundreds of amphibian species worldwide (Skerratt et al. 2007; Scheele et al. 2019), requires moist environments and grows optimally between 17° and 25°C (Piotrowski et al. 2004). Amphibians may preemptively avoid cool, moist, covered patches where infection risk is highest, and/or infected individuals may seek out warmer, drier, more open patches that hinder fungal growth following infection. For instance, following exposure to *Bd*, infected alpine newts (*Ichthyosaura alpestris*) switched from aquatic to terrestrial habitats in mesocosm

experiments, which may help fight infection by desiccating the fungus (Daversa et al. 2018). Furthermore, warmer amphibian body temperatures have been associated with lower infection rates in both field and laboratory settings (Murphy et al. 2011; Rowley and Alford 2013), and individuals held above 25°C for extended periods of time have cleared the infection (McMahon et al. 2014). Postinfection thermoregulatory behaviors that elevate body temperature, such as basking in open habitat patches, may therefore serve as important defense mechanisms against *Bd* (Richards-Zawacki 2010). To achieve a mechanistic understanding of correlations between host body temperature and *Bd* infection, however, postinfection habitat switching between disparate microclimates must be distinguished from preinfection behavioral avoidance of conditions favorable for *Bd* (Sauer et al. 2018). Teasing apart these alternatives necessitates concomitant measurements of habitat choices, body temperature, and infection status, which, to our knowledge, has not yet been done in any study of a wild population.

Our objective was to determine whether wild animals use habitat choices to defend against disease. We evaluated two hypotheses: behavioral avoidance (all individuals reduce the probability of infection by avoiding certain habitats) and habitat switching (infected individuals change infection status by seeking out certain habitats). We expected that were amphibians to use behavioral avoidance, individuals would avoid cool, wet, sheltered habitat patches that are favorable for *Bd* growth. We reasoned that if amphibians use habitat switching to defend against *Bd*, then (i) infected individuals would shift habitats to warmer, drier, more open patches; (ii) such environments would be associated with elevated host body temperature; and (iii) warmer body temperature and drier conditions would increase the probability of clearing infection.

We tested our hypotheses by assessing the habitat selection, thermal profiles, and disease status of wild boreal toads (*Anaxyrus boreas boreas*, a subspecies of the western toad) in western Wyoming. Boreal toads use a variety of habitats (Bartelt et al. 2004; Bull 2006; Long and Prepas 2012) and are tolerant of a wide range of environmental conditions (Guscio et al. 2007; Browne and Paszkowski 2014), including temperature (Lillywhite et al. 1973; Carey 1978). Thus, boreal toads have at least the physiological capacity to switch between cool, moist, sheltered habitats and warm, dry, open habitats. Furthermore, although the expectation that toads will avoid cool, wet, covered habitat patches seems unrealistic, in scenarios wherein the selective pressure from *Bd* is extreme (i.e., high pathogen virulence and/or high host susceptibility), pathogen avoidance may represent the only survival strategy. Indeed, severe population crashes have been attributed to *Bd* (Muths et al. 2003; Scherer et al. 2005; Pilliod et al. 2010), suggesting high susceptibility to chytridiomycosis in at least some boreal toad populations. Last, several investigations have linked the thermal ecology of boreal toads to *Bd* infection rates. For instance, boreal toads found in warmer, recently burned areas (Hossack et al. 2009) were significantly less likely to be infected than toads in cooler, unburned areas (Hossack et al. 2013). Taken together, boreal toads exposed to *Bd* emerge as an appealing biological model to evaluate both behavioral avoidance and habitat switching. Herein, we develop a novel approach to investigating the relationship between infection status and host habitat choice by temporally matching longitudinal disease histories with concomitant host habitat and thermal data to make inferences on the adaptive capacity of wild animals to select habitat conditions that help combat infectious disease.

Methods

Study Area

We studied boreal toads at six streams along the eastern flank of the northern Wyoming Range (~200 km^2) within the Bridger-Teton National Forest (BTNF; 42°59′N, 110°24′W). The area has a continental subarctic climate and receives a mean of 1,900 mm of precipitation annually, most of which (~1,600 mm) falls as snow between October and May. The average elevation is 2,500 m, and mean daily temperatures range from –13°C in January to 15°C in July (summarized 1982–2016 climate data from the Blind Bull Summit SNOTEL station, located within 10 miles of all study sites). The landscape is a matrix of mixed-conifer and aspen (*Populus tremuloides*) forests, sagebrush (*Artemisia tridentata*) and subalpine meadows, and riparian areas with willow complexes (*Salix* spp.). Study streams were selected based on previous surveys that identified active breeding sites of boreal toads. Focal streams were spatially isolated by mountain ridges, thereby composing distinct replicates, yet all were similar in terms of their environmental characteristics. Specifically, temperature and moisture variables were similar among study sites (table S1; tables S1–S4 are available online), both of which could influence *Bd* growth on host amphibians and *Bd* persistence in the environment (Raffel et al. 2010; Becker et al. 2012).

Animal Capture and Transmitter Attachment

We captured adult boreal toads by hand (*n* = 42; 17 females and 25 males) after individuals emerged from hibernacula in May and June of 2016. Adult toads were identified by size (snout-urostyle length: >55 mm), and males were distinguished from females based on the presence of dark nuptial pads on their thumbs. We affixed 1.8-g BD-2 radio transmitters (Holohil Systems, Carp, Ontario) around

the waist of each adult toad with a belt harness (Burow et al. 2012).

Radio Telemetry and Habitat Assessment

We tracked each individual toad every 2–3 days from May to September via radio telemetry using an R-1000 handheld receiver (Communications Specialists, Orange, CA) and a Yagi directional antenna. Over the 5-month study period, 90% of tracking (605 of 676 relocations) occurred during daylight hours (∼0800–1600). We include this information to acknowledge the possibility that we may have missed important nocturnal movements and habitat choices that could have influenced host thermoregulation and infection status (Bartelt et al. 2004; Long and Prepas 2012). We characterized habitat use at the exact position of the animal and at a paired, unused site available to the toad. Paired available sites were 20 m away in a random direction from the used site and constrained to fall within the same macrohabitat as the used site (i.e., riparian, sagebrush/subalpine meadow, forest). We selected a 20-m distance between each used and paired available location based on a 2015 pilot study in which adult toads were radio tracked and moved an average of ∼20 m per day (G. M. Barrile, unpublished manuscript). This distance, moreover, is relatively short compared with the typical home range of a boreal toad (e.g., median 95% adaptive kernel home range = 17,435 m^2; Goates et al. 2007), ensuring substantial interspersion among an individual's used and available points over time. Of total toad relocations, 94% (635 of 676) were in terrestrial versus aquatic habitats. Given the paucity of aquatic locations, we restricted analyses to terrestrial data.

We measured five variables related to temperature and moisture that could be important for boreal toad habitat selection and *Bd* growth (Guscio et al. 2007; Becker et al. 2012). We recorded ground temperature and relative humidity using a digital psychrometer (Extech Instruments, Waltham, MA), soil moisture using a soil probe (General Tools and Instruments, Secaucus, NJ), and functional canopy cover using a spherical densiometer held at toad height. We defined functional canopy cover as any vegetation or debris that obstructed sunlight from reaching the toad's location. Because boreal toads commonly take refuge in shrubs (e.g., riparian willows), we visually estimated percent shrub cover within a 1-m^2 quadrat centered on each relocation site.

Preinfection Behavioral Avoidance

To determine whether toads avoided habitats with conditions conducive to *Bd* growth, we first generally characterized toad habitat selection by comparing used microhabitats (i.e., relocation sites) to paired available microhabitats

using conditional logistic regression (response variable = used microhabitats [coded as 1] and paired available microhabitats [coded as 0]). We parameterized a full model with all five of the measured habitat variables. In model specification, a stratum included the used microhabitat and its associated paired available microhabitat (i.e., 635 relocations = 635 strata). To account for spatiotemporal autocorrelation and pseudoreplication among an individual's telemetry locations, we calculated robust standard errors and 95% confidence intervals (CIs) of parameters using generalized estimating equations (Craiu et al. 2008; Merkle et al. 2014). All strata for a given individual were assigned a unique cluster (i.e., 42 individuals = 42 clusters). Model parameters were estimated using maximum likelihood in the survival package (clogit function) in R version 3.4.2 (R Core Team 2017). We verified that there was no multicollinearity among covariates by confirming all variance inflation factors were less than two and then centered (by subtracting variable means) and scaled (by dividing centered variables by their standard deviations) variables to better compare their relative importance with the response (Schielzeth 2010).

Postinfection Habitat Switching

Disease status. We collected five disease samples from each individual (42 toads × 5 = 210 samples) using standardized protocols to swab ventral skin surfaces of host amphibians for *Bd* (Brem et al. 2007). We swabbed toads on average once every 18 days, though swabs did not occur on regular intervals due to logistical constraints (e.g., because individuals were located deep within refugia). Disease samples were analyzed by Pisces Molecular (Boulder, CO) via a quantitative polymerase chain reaction to detect *Bd* (Annis et al. 2004, as modified by J. Wood, personal communication), which has the advantage of high sensitivity and a low false-negative rate (Skerratt et al. 2011). To examine whether diseased individuals changed infection status by seeking out certain habitat conditions, we included only data from individual toads that tested both positive and negative for *Bd* during the study period (*n* = 24 of 42 toads). From the 391 unique observations (i.e., relocations) of the 24 toads that tested both *Bd* positive and negative, we further truncated the data to include only observations for which we had disease information (five disease samples per individual × 24 toads = 120 observations) to avoid the presumption of infection status for toads between disease sampling events.

Habitat switching. To determine whether toads altered habitat choices when infected with *Bd*, we compared microhabitats used by individuals when infected to microhabitats used by the same individuals when uninfected, using

conditional logistic regression (response variable = *Bd* positive [coded as 1] and *Bd* negative [coded as 0]). Similar to the habitat selection model described above to assess behavioral avoidance, we parameterized a full model with all five field-measured variables. Ambient temperature influences ground temperatures, so we standardized ground temperatures by subtracting ambient temperature from ground temperature. In model specification, a stratum included all five observations from each individual. Strata, therefore, could differ in the response (i.e., the number of positive and negative *Bd* results), an inconsistency that is permitted in conditional logistic regression (Hosmer et al. 2013). Model parameters were estimated as described above.

Behavioral thermoregulation. We measured body temperatures of toads at each relocation using an Extech Infra-Red Thermometer (Extech, Nashua, NH; Rowley and Alford 2007). We first determined whether infected individuals had elevated body temperatures by comparing average body temperatures of individuals when infected to average body temperatures of those same individuals when uninfected using a paired *t*-test. As with ground temperatures discussed above, we accounted for the influence of ambient temperature on ectotherm body temperature by subtracting ambient temperatures from body temperature values. We then determined whether habitats used by infected individuals were responsible for elevated body temperatures by modeling body temperature as a function of the five field-measured habitat variables. We parameterized a linear mixed effects model, specifying individual as a random intercept. Model parameters were estimated using restricted maximum likelihood in the lme4 package (lmer function) in R (R Core Team 2017).

Behavioral fever and clearing of infection. To test whether body temperature influenced switching from an infected to an uninfected state by an individual toad, we fit our longitudinal *Bd* and host thermal data to a multistate Markov model (Jackson 2011). The model described a process wherein individual toads moved through a series of states (*Bd* positive/negative) in continuous time, with observations recorded at arbitrary times because exact times of state changes were unknown. Models were predicated on the Markov assumption, in which future state changes depended solely on the current state. Model parameters were estimated using maximum likelihood in the msm package (msm function) in R (R Core Team 2017), which provides functions to fit multistate models by calculating the likelihood from a transition probability matrix.

Habitat choice, body temperature, and infection status. Given the correlational nature of the analyses described in previous sections, we conducted additional analyses to bolster our inference on the interplay among host habitat choice, thermal profile, and infection status, particularly the relative importance of host body temperature in the clearing of infection. If individuals used certain habitat variables differentially when infected versus when uninfected (i.e., habitat switching), we isolated those variables and, along with body temperature, inserted them into a path analysis. The path analysis took the form of a structural equation model and was parameterized in the piecewiseSEM package (psem function) in R. The model consisted of two linear mixed effects models, both specifying individual as a random intercept (fit using the lmer function in the lme4 package in R). Model coefficients were standardized (placed in units of standard deviations of the mean via the scaling of coefficients by the ratio of the standard deviation of *x* over the standard deviation of *y*) such that the relative strength of predictors could be compared across multiple responses. Although we hypothesized that the influence of habitat switching on the clearing of infection would be mediated through body temperature, habitat choices may also influence the clearing of infection more directly. We therefore fit additional multistate models with the same field-derived microhabitat variables (as covariates) used to assess habitat choice. Model parameters in multistate models were estimated as described above. We combined the structural equation model with relevant multistate models into one figure to illustrate the relationships among habitat switching, body temperature, and the probability of clearing infection.

Results

Preinfection Behavioral Avoidance

We tracked toads for an average of 77 days (range 32–136) during May–September 2016. During the 5-month study period, the average number of unique relocations per individual was 16 (range 9–26; table S2). Toads selected microhabitats with higher humidity, soil moisture, functional canopy cover, and shrub cover compared with what was available (table 1). Habitat selection was unrelated to ground temperature, as indicated by the model-derived 95% CI of the coefficient overlapping zero. Habitat selection results did not differ between male and female toads (fig. S1, available online).

Postinfection Habitat Switching

Disease status. Fifteen toads were uninfected throughout the study (all five disease samples were negative), 12 tested positive once, nine tested positive twice, one tested positive three times, two tested positive four times, and three tested

Table 1: Standardized parameter estimates (β) and robust standard errors (SE) from a habitat selection model for adult boreal toads

Variable	β	SE
Ground temperature	−.016	.185
Relative humidity	.910[a]	.126
Soil moisture	.623[a]	.165
Functional canopy cover	.287[a]	.091
Shrub cover	.606[a]	.108

Notes: The toads ($n = 42$) were tracked via radio telemetry during May–September 2016 in the Bridger-Teton National Forest, Wyoming. The model was parameterized using conditional logistic regression (response variable = used microhabitats [coded as 1] and paired available microhabitats [coded as 0]) and provided inference on the toads' preinfection behavioral avoidance of habitats with conditions conducive to the growth of the amphibian chytrid fungus.

[a] 95% confidence interval of β did not overlap zero.

positive five times (table S2). The three toads that were infected throughout the study exhibited signs of chytridiomycosis (lethargy, excessive skin shedding) and were ultimately found deceased.

Habitat switching. Toads used warmer habitats with less shrub cover when infected with *Bd* compared with when those same individuals were uninfected (table 2). Selection for humidity, soil moisture, and canopy cover was not associated with individual infection status (table 2).

Behavioral thermoregulation. The average body temperature of individuals when infected versus uninfected was 21°C and 20°C, respectively. Relative to ambient temperature, however, individual toads had significantly elevated body temperatures when infected with *Bd* compared with when uninfected ($t_{23} = 7.786$, $P < .001$; fig. 1). Elevated body temperatures were associated with habitats containing higher ground temperature ($\beta_{\text{ground (−ambient) temperature}} = 0.616$, SE = 0.110) and lower shrub cover ($\beta_{\text{shrub cover}} = -0.033$, SE = 0.017; fig. 2). Relative humidity was also correlated with body temperature (table S3).

Behavioral fever and clearing of infection. Elevated body temperatures increased the probability of transitioning from an infected to an uninfected state ($\bar{X}$ odds ratio [95% CI]; $\beta_{\text{body (−ambient) temperature}} = 1.385$ [1.032, 1.859]; fig. 3a). Higher absolute body temperatures (unadjusted for ambient temperature) were also associated with toads transitioning from *Bd* positive to *Bd* negative ($\bar{X}$ odds ratio [95% CI]; $\beta_{\text{body temperature}} = 1.169$ [1.057, 1.293]; fig. 3b).

Habitat choice, body temperature, and infection status. Given that toads used habitats with less shrub cover and higher ground temperature when infected compared with when

those same individuals were uninfected (table 2), we inserted those two variables (along with body temperature) into our structural equation model. Shrub cover significantly influenced ground temperature ($\beta = -0.058$, $P < .001$), and both shrub cover ($\beta = -0.035$, $P = .032$) and ground temperature ($\beta = 0.554$, $P < .001$) significantly influenced body temperature. However, standardized estimates suggested shrub cover indirectly affected body temperature through its effect on ground temperature (fig. 4). Furthermore, whereas higher relative ground temperature at microhabitats used by infected individuals significantly increased the probability of a toad transitioning from an infected to an uninfected state, shrub cover did not significantly affect the clearing of infection (table S4). Taken together, the influence of shrub cover on the ability of an individual to clear infection was indirect (i.e., mediated through its effect on ground and body temperature; fig. 4).

Discussion

Choices animals make about which habitats to use in heterogeneous environments strongly influence individual fitness and scale up to affect population dynamics and community interactions. Traditionally, habitat selection was treated as a process shaped by selective forces primarily associated with foraging, competition, reproduction, predation risk, and physiology (MacArthur and Levins 1964; Charnov 1976; Lima and Dill 1990; Huey 1991). Our study suggests that infection status also can form the basis for habitat choices in wild animals. When infected with the

Table 2: Standardized parameter estimates (β) with standard errors (SE) from an individual habitat use model for adult boreal toads when infected versus uninfected with *Batrachochytrium dendrobatidis* (*Bd*)

Variable	β	SE
Ground − ambient temperature	.448[a]	.252
Relative humidity	.018	.190
Soil moisture	.001	.236
Functional canopy cover	.169	.214
Shrub cover	−.632[a]	.257

Note: The model was parameterized using conditional logistic regression (response variable = *Bd* positive [coded as 1] and *Bd* negative [coded as 0]) and provided inference on the extent of the toads' postinfection habitat switching in response to chytridiomycosis. The 24 toads used in this analysis represent a subset of the 42 toads tracked via radio telemetry during May–September 2016 in the Bridger-Teton National Forest, Wyoming, that tested both positive and negative for *Bd* (of the remaining 18 toads, 15 were uninfected and three remained infected throughout the duration of the study). The subset of 24 that tested both *Bd* positive and *Bd* negative was used in this analysis because the focus was on within-individual comparisons (i.e., habitat conditions used by an individual when infected compared with habitat conditions used by the same individual when uninfected).

[a] 90% confidence interval of β did not overlap zero.
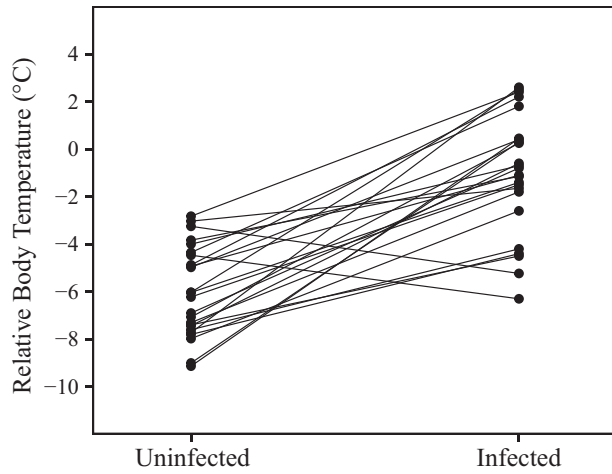
Figure 1: Changes in mean relative body temperature (body temperature − ambient temperature) in adult boreal toads (*n* = 24) when infected versus uninfected with *Batrachochytrium dendrobatidis*. Measurements were taken from May to September of 2016 in the Bridger-Teton National Forest in Wyoming. Each line represents an individual toad.

pathogenic fungus *Bd*, boreal toads exhibited habitat switching, whereby infected individuals selected warmer, more open habitats. This state-dependent habitat selection, moreover, appeared to be adaptive, as increased warmth was as-

sociated with the clearing of infection, likely via the elevation of host body temperature. Infection with *Bd* carried fitness costs, as the three toads that remained infected throughout our study all died with apparent signs of chytridiomycosis. Despite these costs, toads generally did not avoid but rather selected habitats with conditions conducive to *Bd* growth (moist, sheltered habitats), potentially due to stronger pressures from predation and biophysical demands when uninfected. Only after infection did individuals display defensive tactics in the form of shifts in habitat choices.

Defensive responses to infection status, but not infection risk, may evolve if the cost of using habitat patches with low infection risk (e.g., high predation risk) is comparable to the cost of becoming infected (Parker et al. 2011). Boreal toads commonly used shrubs and small mammal burrows as refuge sites—habitats characterized by moist, sheltered conditions (i.e., favorable for *Bd* growth; Long and Prepas 2012). When boreal toads are uninfected, costs associated with increased predation risk and evaporative water loss in open habitats away from refuge sites (Bartelt and Peterson 2005) likely outweigh the benefits of avoiding *Bd* infection. When toads are infected, however, the cost of a progressing disease likely overrides the cost of moving to open, riskier habitats, thereby favoring postinfection habitat switching. Boreal toads also face a trade-off between
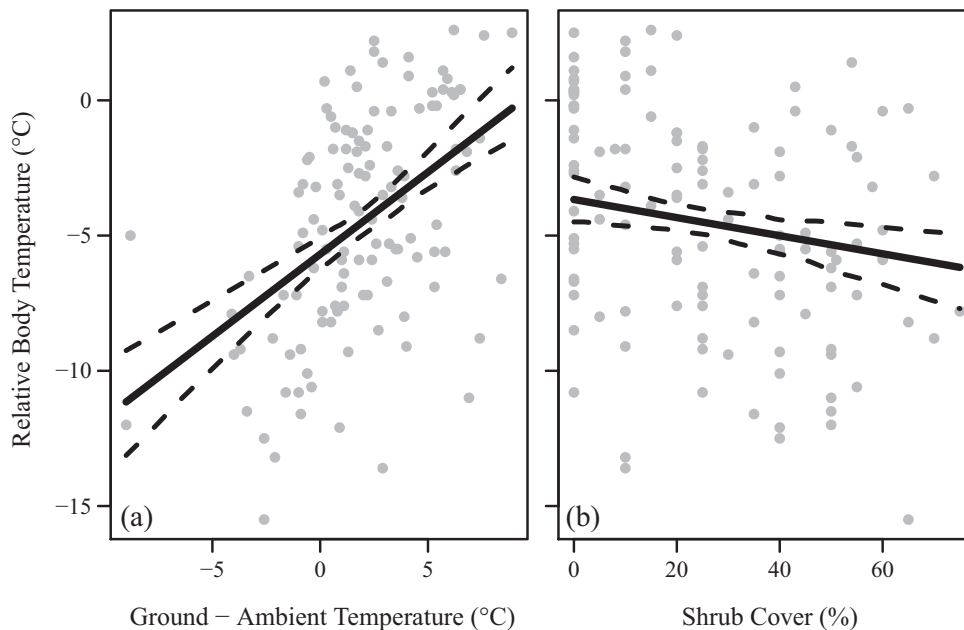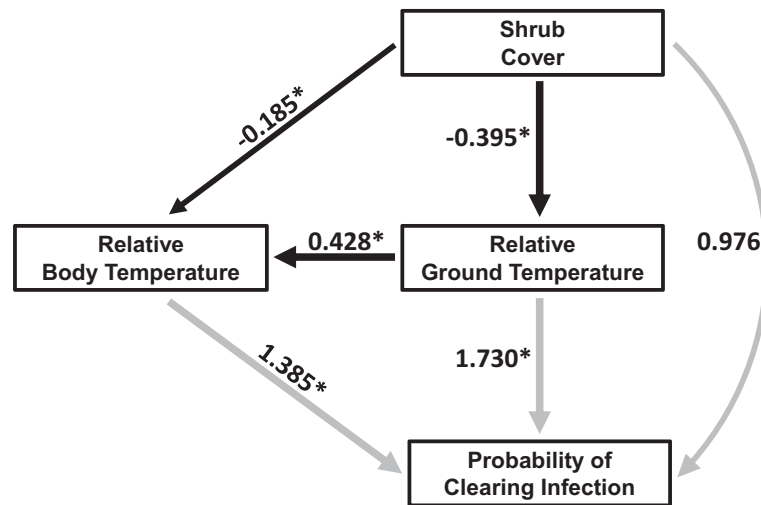

Figure 2: Boreal toad (*n* = 24) relative body temperature (body temperature − ambient temperature) increased in warmer microhabitats (*left*) with less shrub cover (*right*). Individuals were tracked via radio telemetry from May to September of 2016 in the Bridger-Teton National Forest, Wyoming. Mean predicted values (solid lines) and 90% confidence intervals (dashed lines) were derived from a linear mixed effects model specifying individual as a random intercept. The model also included relative humidity, soil moisture, and functional canopy cover (table S3), which were held at their mean values for prediction.
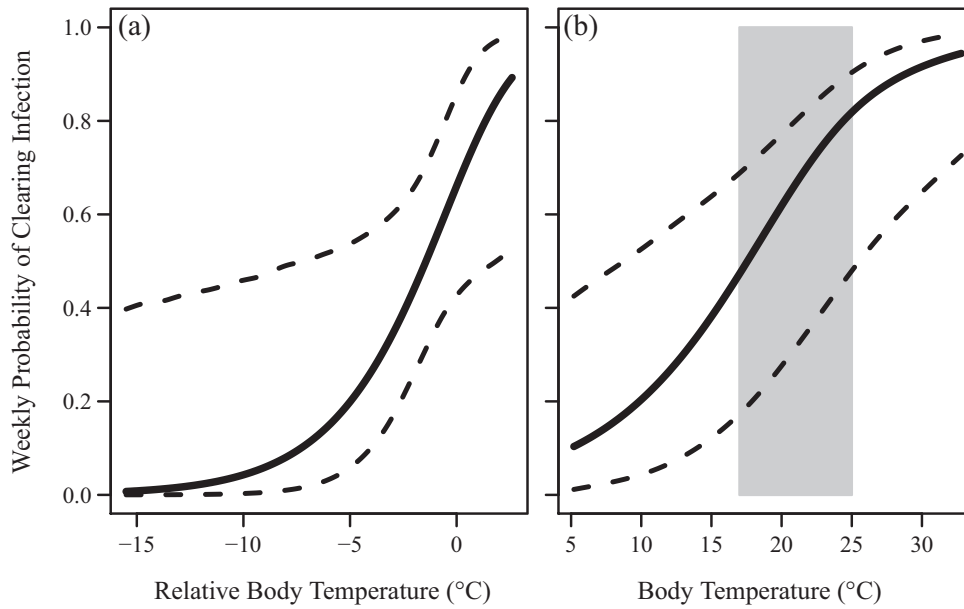
Figure 3: Weekly probability of clearing *Batrachochytrium dendrobatidis* (*Bd*) infection increased when relative body temperature – ambient temperature (*a*) and absolute host body temperature (*b*) were elevated in boreal toads (*n* = 24) radio tracked during May–September 2016 in the Bridger-Teton National Forest, Wyoming. Mean predicted relationships (solid black lines) and 95% confidence intervals (dashed lines) were derived from a multistate Markov model. The shaded region in panel *b* represents the optimal growth range for *Bd*.

reproduction and avoiding *Bd* exposure. Although *Bd* can persist in moist terrestrial habitats, contact with fungal zoospores occurs primarily at ponds during the breeding season (Corn 2007; Pilliod et al. 2010). To avoid zoo-spores, individuals would have to avoid breeding sites and forgo reproduction. Thus, preinfection avoidance behaviors are unlikely to evolve in boreal toads and in other pond-breeding amphibians, whereas habitat switching appears

Figure 4: Relationships among host habitat choice, body temperature, and infection status in adult boreal toads (*n* = 24) tracked via radio telemetry during May–September 2016 in the Bridger-Teton National Forest, Wyoming. Black arrows represent standardized estimates from a structural equation model, while gray arrows represent odds ratios from multistate models. Asterisks indicate significance (i.e., *P* < .05). Numbers above black arrows are standardized path coefficients, with the relative strength of each indicated by line width. Values above the black arrows are not comparable to those above the gray arrows, as they were derived from separate analyses (all estimates appear on the same figure purely for visualization purposes).

evolutionarily advantageous, depending on the costs of maintaining the mechanisms underlying behavioral flexibility in habitat choice (e.g., sensory machinery, information acquisition).

The use of preinfection behavioral avoidance or postinfection habitat switching (or both) can result in differences in animal space use. We demonstrate that wild boreal toads used moist, sheltered habitats when free of disease but moved to warmer, more open, but exposed habitats when infected. These results support laboratory findings that boreal toads display a strong behavioral response to *Bd* infection (Murphy et al. 2011), which likely plays a role in population persistence in western Wyoming. The maintenance of environmental heterogeneity, particularly with respect to temperature, moisture, and cover, will therefore be critical for the persistence of boreal toads in our study area. Furthermore, in areas where boreal toads have declined severely due to chytridiomycosis (Muths et al. 2003; Scherer et al. 2005), assessments of habitat heterogeneity could help determine the ability of those landscapes to support defensive strategies against *Bd*.

Our work contributes to the growing understanding of the role of host behavior and thermal profile in the amphibian-*Bd* system. Although the importance of host habitat use (Raffel et al. 2010; Daversa et al. 2018) and thermoregulation (Richards-Zawacki 2010; Rowley and Alford 2013) is well-documented in amphibian-*Bd* interactions, a mechanistic link between the interrelated processes was lacking, especially in wild populations. To our knowledge, we are the first to identify habitats used by wild amphibians to defend against *Bd* while also providing strong support for the mechanism (temperature) by which those habitats mediate host-pathogen interactions. Boreal toads exhibited behavioral fever in warm, open habitats used by individuals when infected with *Bd*. Elevated body temperatures may have directly killed *Bd* zoospores on host skin when temperatures exceeded the critical thermal maxima of *Bd* (~28°C; Piotrowski et al. 2004; Stevenson et al. 2013). However, given that the probability of clearing infection was relatively high at temperatures within the optimal growth range of *Bd* (fig. 3*b*) and that only 22% (nine of 41 observations) of body temperature recordings exceeded 25°C for the toads that cleared the infection, it may be more likely that increased warmth improved immune function and the antifungal activity of host skin microbiota (Daskin et al. 2014). Factors other than temperature, such as greater intensity of ultraviolet radiation and wind exposure in open habitats, may also have facilitated the clearing of infection (Bartelt and Peterson 2005; Walker et al. 2010). Habitats with less shrub cover (i.e., more open environments) did not directly influence the clearing of infection, however, suggesting that infected individuals selected open areas because those habitats offered warmer microclimates

(fig. 4), further indicating that temperature was indeed the mechanism through which habitat choice modulated infection status.

The observed temperature relations between boreal toads and *Bd* may be generalizable in the context of the thermal mismatch hypothesis, which is well-supported in the amphibian-*Bd* system (Sauer et al. 2018; Cohen et al. 2019). The hypothesis posits that pathogens should outperform their hosts when environmental conditions shift away from the thermal optima of hosts (e.g., warm-adapted hosts should be more susceptible at relatively cool temperatures), such that hosts are more susceptible under abnormal but not extreme conditions (Cohen et al. 2017). Although boreal toads inhabit high latitudes and elevations (i.e., cold environments), this species performs optimally at relatively warm temperatures (e.g., maximal growth and energy ingestion at ~27°C; Lillywhite et al. 1973). Therefore, because boreal toads prefer warmer microclimates, the ability to clear infection in warmer habitats is consistent with the thermal mismatch hypothesis. Persistence of populations challenged by *Bd*, however, is likely contingent on the availability of warm, open habitats that allow infected boreal toads to reach preferred temperatures and subsequently clear infection.

Bd has already caused the extinction of more than 100 amphibian species and remains a major threat to global biodiversity (Scheele et al. 2019). Hettyey et al. (2019) recently argued that current mitigation methods are not yet suitable for in situ application and proposed that the creation of microhabitats with elevated temperatures may be the best countermeasure to *Bd*, in terms of practicality and minimization of collateral damage to ecosystems. Microhabitat manipulation, however, hinges on the assumption that amphibians will use warm spots if provided them. We provide key empirical evidence to test this assumption and report that at least some wild amphibians have the capacity to actively seek warmer, more open microhabitats in a facultative manner when infected with *Bd*. Small-scale microhabitat manipulation to create warm patches, therefore, comprises a potentially effective mitigation action against *Bd* (for warm-adapted hosts) and possibly other amphibian diseases. For example, southern toads (*Anaxyrus terrestris*) provided with thermal gradients were able to decrease ranaviral load by adjusting temperature preference to achieve behavioral fever (Sauer et al. 2019).

Emerging infectious diseases pose a serious threat to global biodiversity (Smith et al. 2006; Jones et al. 2008). We demonstrate that behavioral flexibility in habitat choices may provide animals with a defense mechanism to persist in the face of infection. Our study represents an important step toward recognizing disease as an evolutionary force shaping patterns of animal habitat and space use. Finally, in some contexts, habitat selection models could be greatly

improved by including variables that quantify infection risk among habitats and incorporating the infection status of individuals.

Statement of Authorship

G.M.B. wrote the original draft, reviewed and edited subsequent drafts, and contributed to conceptualization, experimental design, data collection, data analysis, and data visualization. A.D.C. and A.W.W. contributed to conceptualization and funding acquisition, provided resources and supervision, and oversaw review and editing of the manuscript.

Data and Code Availability

Data supporting the results have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.d2547d81f; Barrile et al. 2020).

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