



Deforestation, host community structure, and amphibian disease risk

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Abstract

Habitat disturbances and the emergence of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) are linked to accelerated amphibian declines. Deforestation can directly alter amphibian community structure through abiotic and biotic mechanisms including shifts in local microclimates and species interactions. Changes in amphibian community attributes, in turn, potentially impact *Bd* transmission dynamics, and thus also have an indirect role in biodiversity persistence. Here, we tested whether deforestation influences *Bd* infections through shifts in amphibian community structure, including species richness, community composition, total host density, and host biomass. We surveyed 22 temperate and tropical amphibian communities across gradients of deforestation in the U.S. and Brazil, and we experimentally exposed a representative subsample of each amphibian community to standardized *Bd* zoospore loads in mesocosms under controlled microclimate. We found that denser temperate amphibian communities commonly found at pristine sites showed higher *Bd* loads when microclimates were held constant. In contrast, tropical amphibian communities found at pristine forest sites carried lower *Bd* infection loads in the absence of variable microclimates, likely due to their host species composition. Previous host exposure to the pathogen in tropical communities also played an important role in determining infection loads; we identified a negative association between *Bd* infection loads observed in the wild and in the laboratory. Our results highlight that deforestation can have cascading biotic effects on disease risk, and that quantifying the net contribution of host community attributes to *Bd* infections will help us identify specific drivers of disease and inform conservation strategies.

Zusammenfassung

Störungen des Lebensraumes und das Auftreten des Chytridpilzes, *Batrachochytrium dendrobatidis* (*Bd*), stehen mit dem beschleunigten Rückgang der Amphibien in Verbindung. Entwaldung kann die Struktur einer Amphibiengemeinschaft direkt durch abiotische (lokales Mikroklima) oder biotische (interspezifische Interaktionen) Mechanismen verändern. Diese Veränderungen der Amphibiengemeinschaft könnten ihrerseits die *Bd*-Übertragungsdynamik beeinflussen und so eine indirekte Rolle für den Erhalt der Biodiversität spielen. Wir untersuchten, ob Entwaldung über Änderungen der Struktur von Amphibiengemeinschaften die *Bd*-Infektionen beeinflusste. Wir erfassten dazu Artenreichtum, Artenzusammensetzung, gesamte Wirtsdichte

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und Wirtsbiomasse von 22 gemäßigten (USA) und tropischen (Brasilien) Amphibiengemeinschaften, die Gradienten von unterschiedlicher Bewaldung repräsentierten. In Mesokosmen mit kontrolliertem Mikroklima setzten wir repräsentative Proben aller Amphibiengemeinschaften standardisierten Bd-Zoosporen-Belastungen aus. Wir fanden, dass individuenreiche gemäßigte Gemeinschaften, die gewöhnlich an unberührten Standorten gefunden wurden, höheren Bd-Befall zeigten, wenn das Mikroklima konstant gehalten wurde. Dagegen waren tropische Gemeinschaften aus unberührten Habitaten bei konstantem Mikroklima geringer infiziert, vermutlich bedingt durch ihre Artenzusammensetzung. Frühere Exposition der Wirte in tropischen Gemeinschaften spielte eine wichtige Rolle: wir fanden eine negative Beziehung zwischen Bd-Infektion im Freiland und im Labor. Unsere Ergebnisse zeigen, dass Entwaldung kaskadierende biotische Effekte auf das Befallsrisiko haben kann und dass die Quantifizierung des Netto-Beitrags der Parameter der Wirtsgemeinschaften zur Bd-Infektion uns helfen wird, spezifische Steuerfaktoren zu identifizieren und Schutzstrategien zu begründen.

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Introduction

Amphibian biodiversity is declining worldwide at unprecedented rates (IUCN et al. 2014). Two important factors implicated in population declines and extinctions are habitat loss (Becker, Fonseca, Haddad, Batista, & Prado, 2007; Cushman 2006) and chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium* (Martel et al., 2014; Skerratt et al., 2007). Loss of natural vegetation changes amphibian community structure by increasing population isolation (Arens et al., 2007), inbreeding (Andersen, Fog, & Damgaard, 2004), edge effects, and discontinuity between terrestrial and aquatic habitats (Becker, Fonseca, Haddad, & Prado, 2010; Rittenhouse & Semlitsch 2006). Disturbances to natural habitats also shift both macro (Costa & Foley 2000) and microclimates (Kapos 1989). Therefore, habitat loss can have potentially large effects on amphibian susceptibility to disease by altering host community attributes and optimum microclimates for both amphibian hosts and pathogens (Becker, Rodriguez, Longo-Berrios, Talaba, & Zamudio, 2012; Raffel, Michel, Sites, & Rohr, 2010).

The frog killing fungus *Batrachochytrium dendrobatidis* (*Bd*) is a waterborne epidermal pathogen with a broad host range among anurans (Fisher, Garner, & Walker, 2009). Because many amphibian species share aquatic breeding sites (e.g., ponds, streams), inter-host transmission is common (Searle, Biga, Spatafora, & Blaustein, 2011). Free-swimming zoospores shed by infected individuals colonize the skin of other hosts and proliferate, causing chytridiomycosis (Longcore, Pessier, & Nichols, 1999). Shifts in amphibian community attributes likely influence *Bd* transmission dynamics (Becker et al., 2014; Searle, Biga, Spatafora, & Blaustein, 2011; Venesky et al., 2014a), because host species vary in their susceptibility to infection (Gervasi, Gondhalekar, Olson, & Blaustein, 2013) and possibly shed zoospores into aquatic habitats at different rates. Likewise, density-dependent transmission (Searle et al., 2011; Venesky et al., 2014a) will also cause potential changes in host–pathogen dynamics as populations and communities shrink or expand in modified habitats. Therefore, we predict

that following anthropogenic habitat alteration, shifts in host community composition and density will have potentially large impacts on pathogen burden and disease risk.

Host community attributes such as species richness, species composition, total host density, and biomass can be precisely quantified across amphibian communities in the wild. However, their effects on host–pathogen dynamics are often confounded by the influence of abiotic factors that independently regulate pathogen abundance or host exposure. For instance, the influence of microclimate on likelihood of amphibian infection by *Bd* is unequivocal. Mounting evidence points to direct effects of temperature and humidity on *Bd* growth and persistence both in the laboratory and in the wild (Becker et al., 2012; Becker & Zamudio, 2011; Raffel, Rohr, Kiesecker, & Hudson, 2006; Raffel, Michel, Sites, & Rohr, 2010; Raffel et al., 2015). Because deforestation drastically changes local microclimates (Kapos 1989), along with the aforementioned host community attributes (Chapin et al., 2000), integrative field and laboratory studies of host–*Bd* interactions are necessary if we are to understand the contribution of biotic variables to disease dynamics.

Here, we tested whether anthropogenic changes to natural vegetation influence *Bd* infection dynamics through shifts in amphibian community attributes. Specifically, we used a combination of field surveys and mesocosm experiments to address hypotheses about the role of habitat change in mediating disease risk through shifts in host species richness, community composition, total host density and biomass. We surveyed and *Bd*-screened 22 natural amphibian communities in temperate and tropical landscapes and quantified the effect of deforestation on host species composition and relative abundance across sampling sites. We then experimentally exposed a representative sample of each host community to standardized *Bd* zoospore loads in mesocosms to test the effect of host community attributes on *Bd* infection dynamics under controlled microclimates. Combined, our field surveys and mesocosm experiments quantify the net contribution of host community structure to host–pathogen dynamics. Experimentally testing how anthropogenic habitat change influences *Bd* infection intensity in ecologically

realistic communities is critical for the development of appropriate conservation efforts in the wake of accelerated habitat destruction.

Materials and methods

Study sites and GIS analyses

We surveyed amphibians from ten permanent ponds (mean circumference = $114.75\text{m} \pm 49.47$ SD) in Eastern Forest-Boreal Transition (Adirondack Park) of the northeastern U.S. ($43^{\circ}15'N$; $74^{\circ}35'W$) and 12 permanent ponds (mean circumference = $130.90\text{m} \pm 53.49$ SD) in the Serra do Mar Coastal Forest in southeastern Brazil ($23^{\circ}13'S$; $45^{\circ}20'W$). We quantified undisturbed forest cover for each sampling site based on high-resolution orthophotos from 2008 to 2009 (15 and 30 cm resolution; [USGS, 2010]) for the U.S. and high-resolution satellite images from 2010 (SPOT, 2-m resolution) for Brazil. For both temperate and tropical landscapes, study sites were chosen along a vegetation gradient from disturbed/open to pristine/closed-canopy vegetation cover. At each sampling site, we quantified the percentage of undisturbed forest cover in a radius of 30 m from the edge of the pond using ArcGIS v.10 (ESRI, 2013). In our study, we classified primary or secondary mature forests as undisturbed forest cover. We classified urban, pasture, agriculture, silviculture, recreational land (e.g., golf-courses, soccer fields), and natural vegetation in early successional stages as disturbed land-cover types. We recorded surface water temperatures for each of our sampling ponds using waterproof pendant data loggers (Hobo UA-002-64; $0.1\text{u}^{\circ}\text{C}$ resolution). We placed three data loggers (spaced equidistantly) in each pond at 10 cm depth and used 30 min interval records taken simultaneously at all ponds to calculate daily average temperatures at each site. To avoid confounding effects of elevation and macroclimate across our sampling ponds, we explicitly chose landscapes with low climatic and topographic variability (Becker et al., 2012; Becker and Zamudio 2011).

Host community survey and *Bd* screening in the wild

We conducted visual encounter surveys in ponds in the Adirondack Park during the boreal summer (June), and in ponds in Brazil during the austral summer (November) of 2012. At each sampling pond, we surveyed amphibian communities over three consecutive nights to obtain comparable data on amphibian species composition and relative abundance among sites in our disturbance gradients. We performed visual surveys within a 3 m buffer from the water line. Surveys at all ponds in each landscape occurred within a window of seven or six days. In each landscape, we standardized survey effort (person hours) across our focal ponds (150 person hours and 280 person hours in temperate and

tropical landscapes, respectively). Using these pond survey data, we estimated amphibian species composition and relative abundance across ponds. After this initial survey we collected (and swabbed) a representative subsample of individual amphibians from each pond mimicking host species richness, density, and total biomass from natural communities for posterior mesocosm experiments (see more details below).

This short time period for surveys reduces the potential bias of variable meteorological or seasonal conditions that occurs over longer sampling periods. The potential disadvantage of our survey is that it did not capture early-breeding species that may contribute to transmission dynamics from earlier reproductive bouts. Likewise, although tadpoles can serve as pathogen reservoirs, and most certainly play an important role in *Bd* dynamics (Briggs, Knapp, & Vredenburg, 2010), our study focused on post-metamorphic amphibians. Although these life stages and early breeders were not quantified, our survey data capture the main adult species contributing to transmission and persistence of *Bd* at that point in time.

Mesocosm host communities

We brought to the laboratory representative subsamples of amphibian communities for a total of 10 temperate and 12 tropical mesocosm communities. We used our initial survey data on host relative abundance (by species and among sampling ponds) to create mesocosm assemblages that mimicked host community attributes (density and species relative abundance) observed in the field. For our temperate amphibian assemblages, we established a limit of 20 individuals maximum per experimental tank, resulting in assemblages that ranged from 3 to 20 individuals based on capture rates observed among natural ponds. Our field surveys in Brazil showed a much greater variation in amphibian numbers among sampling ponds. For our tropical amphibian assemblages, we established a maximum of 29 individuals per experimental tank, resulting in assemblages that ranged from 2 to 29 individuals based on capture rates observed among natural ponds (see Appendix A). Thus, we included a total of five temperate amphibian species and 14 tropical amphibian species in our experiments; species richness in mesocosms varied from one to three species in temperate assemblages and two to five species in tropical assemblages.

Each experimental unit consisted of a large water tank (120 cm diameter \times 100 cm height) with terrestrial habitat covering one half of the container (i.e., autoclaved moist *Sphagnum* moss) and aquatic habitat on the other half. We covered each experimental unit with plastic mesh to prevent escapes. We maintained all tanks indoors at a constant room temperature of 20°C to control for microclimatic variation.

Host community attributes in mesocosms

We define host species richness as the number of amphibian species in each experimental unit. As *Bd* potentially

infects all host species used in this study (Becker et al., 2012; Becker & Zamudio, 2011), we quantified total host density as the total number of individual amphibians within each experimental unit. Similarly, we measured biomass as the sum of body mass across all individual hosts within each experimental unit. In addition to these community attributes, we measured two variables (community similarity and host aquatic index) that quantify sampled communities in terms of species composition and life-histories, both factors potentially influencing the likelihood of *Bd* infection in the community. We consolidated data on species composition and relative abundance across sampling ponds employing Non-Metric Multidimensional Scaling (NMDS) using Bray-Curtis dissimilarity matrices. We performed NMDS analyses independently for temperate and tropical assemblages, using NMDS axes 1 and 2 as a metric of community similarity. Ecologically similar communities tend to have similar NMDS values, and therefore, NMDS axes are to some extent a proxy for host identity and relative abundance among communities. The second variable is an index representing how much each species uses aquatic habitats during their lifetime (aquatic index; AI; modified from Lips, Reeve, & Witters, 2003), thus this index quantifies the degree of *Bd* exposure and transmission (Lips et al., 2003). Arboreal species (i.e., Hylidae) that spend less time in water were coded AI = 1 and species occupying aquatic vegetation and moist terrestrial microhabitats around ponds (i.e., Leptodactylidae, Ranidae, Bufonidae, Cycloramphidae) were coded as AI = 2. We then averaged AI across all individuals in each experimental unit, reaching a weighted average AI for each community (see Appendix A).

Experimental infections

Prior to the experimental infections with temperate amphibians, we cleared frogs of *Bd* in the laboratory by keeping all animals housed at a temperature of 31 °C for 12 consecutive days and changing the water every three days (Woodhams, Alford, & Marantelli, 2003). Prior to the experimental infections in Brazil, we administered a 7-day disinfection treatment using Itraconazole at 0.01% solution (Pessier & Mendelson, 2010). In both cases, we verified clearance of pathogens from all frogs using qPCR and allowed a four-day post-treatment recovery time prior to the beginning of the experimental infection.

For experimental infections, we inoculated temperate and tropical amphibians using two locally isolated *Bd* strains. We cultured global panzootic *Bd* strains JEL404-Maine-U.S. (used for temperate amphibians; Gahl, Longcore, & Houlahan, 2012) and CLFT023-MinasGerais-Brazil (used for tropical amphibians; Schloegel et al., 2012) at 19 °C for 7 d. We harvested *Bd* by flooding plates with distilled water and waiting ~3 h for zoospore release. We then pooled water with zoospores from multiple plates, quantified zoospores with a hemocytometer, and added ~10⁷ zoospores in 6 L of dechlorinated water to each experimental unit. This protocol

guaranteed comparable infection regimes across replicates with *Bd* strains naturally experienced by the hosts in the wild. We added *Bd*-negative amphibians to the terrestrial habitat of each experimental unit and kept temperatures at 20 °C on a 12 h day-night light cycle. We monitored amphibians daily and fed them pinhead crickets and wingless flies *ad libitum*.

We swabbed all individuals prior to the experimental infection and upon termination of the experiment on the 18th day post-infection. This period encompasses approximately five replication cycles of the pathogen (Longcore et al., 1999) and is sufficient for *Bd* to reach high infections in susceptible captive amphibians (Savage & Zamudio, 2011). In case of disease, we swabbed dead or dying animals and removed them from the experimental units. We tested swabs for *Bd* using Taqman qPCR (Boyle, Boyle, Olsen, Morgan, & Hyatt, 2004) with *Bd* strain-specific standards of 0.1, 1, 10, 100, and 1000 zoospore genomic equivalents (GE) to determine the infection intensity of *Bd* in each sample. At the end of the experiment, we euthanized all animals using Benzocaine 4% (Cornell IACUC #2010-0069).

Statistical analyses

Studies addressing disease dynamics in wild host communities must take into account the tradeoff between biological reality and statistical power. In previous work, we used a factorial experimental design to investigate the effects of host diversity on disease without taking the direct effect of deforestation into account (Becker et al., 2014). Our goal here was to mimic the impact of deforestation on the structure of natural communities and quantify the consequences for host–pathogen interactions. Due to limitations in the number of frogs per pond that could be captured and brought to the laboratory, it was not feasible to have statistical replication within ponds in our mesocosm tanks. Therefore, we employed a regression-based approach that allowed us to estimate the responses of unique host assemblages to *Bd*. We employed a stepwise GLM procedure, using host community attributes as biological explanatory variables, to find the combinations of variables that best explained *Bd* infection intensity (log₁₀ transformed) at the end of the experiment. Because prior infection by *Bd* may influence susceptibility of hosts to reinfection (Raffel et al., 2010), we included *Bd* infection intensity at the moment of capture in the wild as a control variable for previous exposure and potential acquired resistance. We used daily average pond water temperatures to account for the impact of temperature shifts and host thermal acclimation in our experimental infections (Raffel et al., 2013; Raffel et al., 2015). Specifically, we calculated the deviation of field temperatures at each pond from the constant temperature in mesocosms (20 °C), as a metric of temperature shift in the analyses. We placed temperature data loggers in 12 ponds in Brazil, but lost devices in four ponds due to unforeseen circumstances. Finally, we included data on pond circumference in the analyses.

Our stepwise selection of variables was based on Bayesian Information Criterion (BIC), using minimum BIC values as a cutoff for the inclusion of additional parameters (i.e., forward method), thus avoiding over-fitted complex models. We did not analyze prevalence data because >96% of the hosts became infected with *Bd* during the experiments and because infection intensity, measured as mean pathogen load in the community, is a better indicator of infection dynamics in mixed populations. Mortality was negligible across tropical amphibian communities (i.e., only a single frog died), and thus we only analyzed mortality for temperate amphibian communities. We used the same stepwise approach to find the combination of biotic and abiotic variables that best explain community-level *Bd* infection intensity observed in the wild (\log_{10} transformed). Finally, we quantified the effect of percent undisturbed forest surrounding sampling ponds on host community attributes using a regression-based analyses [General Linear Models with standard least squares (GLMs)], and we used Pearson correlations to cross pond circumference with percent undisturbed forest. We performed all analyses using JMP v. 10.0 (SAS 2010) and R (R Core Team 2014; Oksanen et al., 2014).

Results

In temperate amphibian communities we found marginally higher host capture rates in ponds surrounded by undisturbed forest habitats than in ponds in disturbed habitats ($F_{[1,8]} = 4.645$, $\beta = 0.087$, $P = 0.064$; Fig. 1A), but the amount of natural vegetation around sampling ponds did not predict host species richness, NMDS, average AI, and biomass. Temperate host communities were dominated by *Lithobates clamitans* across the gradient of habitat alteration. Higher host density (i.e., capture rates as a proxy) found in ponds surrounded by undisturbed forest habitats predicted higher average *Bd* infection intensity in mesocosm amphibian assemblages ($F_{[1,8]} = 6.410$, $\beta = 0.067$, $P = 0.035$; Fig. 1B). However, assemblages with high host densities did not show higher mortality rates during the course of the experiment ($F_{[1,8]} = 2.005$, $P = 0.194$). Looking simultaneously at all explanatory biological variables controlling *Bd* infection intensity in our mesocosm, our stepwise selection of variables included total host density as the single positive predictor of *Bd*. These findings were not associated with host acquired resistance, as mean *Bd* infection loads observed in the laboratory were unrelated to loads observed in the wild prior to the experimental infection ($F_{[1,8]} = 0.069$, $P = 0.798$).

In our diverse tropical communities from Brazil, we found the same pattern of higher host capture rates in ponds surrounded by natural vegetation ($F_{[1,10]} = 7.208$, $\beta = 0.181$, $P = 0.023$). In these communities, we also found a significant positive effect of natural vegetation on host species richness ($F_{[1,10]} = 5.469$, $\beta = 0.024$, $P = 0.041$). None of the explanatory host community attributes we measured significantly predicted *Bd* loads in our mesocosm experimental infection

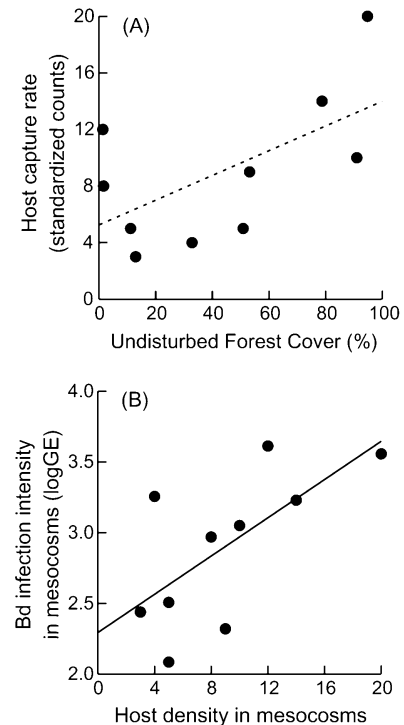


Fig. 1. Linear relationships between undisturbed forest cover surrounding temperate ponds in the U.S. and host capture rate (A), and between host density and average *Bd* infection intensities across mesocosm amphibian assemblages (B). Dashed line depicts a marginally significant relationship.

when regressed independently. Contrary to the pattern we observed in temperate amphibians, we found that higher *Bd* exposure in natural tropical communities significantly predicted lower *Bd* loads in mesocosms ($F_{[1,10]} = 9.476$, $\beta = -0.556$, $P = 0.011$; Fig. 2). In addition to this strong effect of previous exposure, our stepwise selection of variables identified other important factors potentially explaining *Bd* infection intensity: NMDS, average AI, and host capture

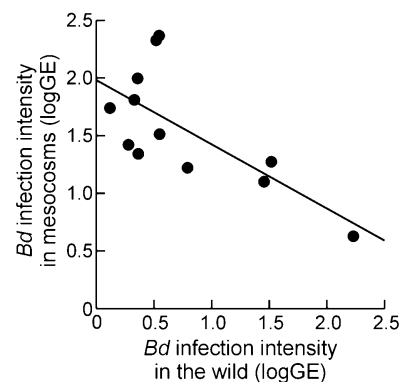


Fig. 2. Effect of previous *Bd* exposure on infection intensities in tropical amphibians. Linear regression shows a negative relationship between *Bd* infection intensities observed in the wild and in the posterior laboratory controlled experiment across 12 amphibian assemblages in Brazil.

Table 1. Generalized Linear Model (GLM) simultaneously testing the effects of previous host infection in the wild, host capture rate, average host aquatic index, and NMDS axes depicting host community composition on *Bd* infection intensity across tropical amphibian communities in Brazil, under controlled microclimates in the laboratory. Variance inflation factor (VIF) denotes colinearity in the model when higher than 10.

Variable	Std Beta	F	VIF	P
<i>Bd</i> infection loads in the wild (logGE)	−0.917	26.679	1.429	0.002*
Host capture rate	−1.175	14.887	4.202	0.008*
Average host aquatic index (AI)	−1.649	13.029	9.461	0.011*
NMDS axis 2	0.778	10.949	2.505	0.016*
NMDS axis 1	0.437	2.112	4.094	0.196

Full model statistics: $F_{[5,6]} = 7.862$, $r^2 = 0.867$, $P = 0.013$.

*denotes statistically significant variables.

rate (Table 1). Specifically, several assemblages of species typically found in disturbed habitats showed higher *Bd* infection loads following experimental infection in the laboratory when the effect of previous exposure was controlled for. Surprisingly, mesocosms dominated by hyloid species (AI=1) showed higher infection loads than mesocosms dominated by riparian/terrestrial species (AI=2) (Table 1).

Thermal acclimation was not a significant factor in our experiment, as *Bd* infection loads in the laboratory were unrelated to temperature shifts for both temperate ($F_{[1,8]} = 0.321$, $P = 0.586$) and tropical amphibian assemblages ($F_{[1,6]} = 0.013$, $P = 0.912$). Furthermore, vegetation cover and host community attributes were not significant predictors of community-level *Bd* loads observed in the wild for both temperate and tropical amphibian assemblages. Finally, pond circumference was not correlated with undisturbed forest cover (temperate: $r = 0.164$, $N = 10$, $P = 0.719$; tropical: $r = 0.155$, $N = 12$, $P = 0.669$) and was not a significant predictor of host density (temperate: $F_{[1,8]} = 2.638$, $P = 0.143$; tropical $F_{[1,10]} = 1.317$, $P = 0.277$) and species richness (temperate: $F_{[1,8]} = 0.178$, $P = 0.685$; tropical $F_{[1,10]} = 0.708$, $P = 0.419$).

Discussion

The mechanisms controlling disease dynamics in wildlife can be affected by a myriad of biotic and abiotic forces, which are often difficult to disentangle (Ostfeld & Keesing, 2012). Deforestation raises local temperatures (Kapos, 1989), which can suppress *Bd* growth and persistence in disturbed habitats (Becker et al., 2012; Raffel et al., 2010). However, the impact of microclimate on *Bd* infection loads is expected to vary across space and time (Kriger, Pereoglou, & Hero, 2007; Lenker, Savage, Becker, Rodriguez, & Zamudio, 2014), and thus may interact with biotic forces synergistically or antagonistically. Despite the well-known influence of deforestation on *Bd* spatial epidemiology at the population level (Becker et al., 2012; Becker & Zamudio, 2011), we did not detect land cover effects on community-level *Bd* infections in field-collected data. This is perhaps not surprising given the number of variables affecting community-level

disease dynamics in the wild. When controlling for the influence of microclimate, we detected significant effects of host community attributes on *Bd* infection dynamics, even after accounting for the effects of temperature shifts and previous host exposure.

Our mesocosm experiments confirmed the prediction that shifts in host community attributes arising from changes in natural forest cover play an important role in amphibian host–pathogen interactions. Our results indicate that higher-density temperate amphibian assemblages from forested closed-canopy sites are associated with elevated *Bd* loads under constant microclimate, supporting previous findings of elevated disease risk in natural habitats (Becker et al., 2012). In contrast, amphibian assemblages from ponds in closed-canopy forests of Brazil carry proportionally lower *Bd* infection loads when microclimate is held constant, due in part to changes in host species composition. Our combined results highlight that disturbances to natural vegetation could either increase or decrease the risk of chytridiomycosis through shifts in host community attributes, but that the effects vary depending on the host species pool and how these communities respond to deforestation.

Density-dependent transmission in temperate amphibians

If host communities do not vary significantly in species composition, diversity, or genetic factors to resist infection, then higher infection intensities are more likely to occur in denser host populations that promote continuous reinfection of hosts (Briggs et al., 2010). Indeed, laboratory studies show that *Bd* transmission rates in temperate amphibians increase with the density of infected hosts (Rachowicz & Briggs 2007; Searle et al., 2011; Venesky et al., 2014a). Furthermore, our previous work has showed that denser amphibian populations are more likely to carry higher *Bd* infection loads in the wild, although microclimate effects are often stronger than density-dependent mechanisms shaping *Bd* infections (Becker et al. 2012). Specifically, host population density positively predicted *Bd* infection intensity in wild-caught *L. clamitans* when analyzed independently in a simple linear

regression, but its effect became non-significant when considered together with other environmental factors such as water temperature and degree of shade at natural sites (Becker et al., 2012). The fact that *Bd* host–pathogen dynamics in climate-controlled mesocosms are strongly density-dependent corroborates earlier population level studies. This result shows that density-dependent transmission can occur at the community level and can be mitigated by microclimatic effects in the wild.

Complex responses of diverse tropical amphibians to *Bd*

Deforestation in southeastern Brazil significantly modifies host community attributes (Becker et al., 2007) and disease risk (Becker & Zamudio 2011), and our results indicate that tropical amphibian species commonly found in disturbed habitats carry higher *Bd* infection loads in the laboratory. Host composition alone could explain the observed pattern, but deforestation can have other detrimental downstream effects on many host parameters that were not quantified in this study such as host immune responses (Carey, Cohen, & Rollins-Smith, 1999; Carey 2005) and genetic diversity (Savage & Zamudio, 2011). Additionally, our results could be partially explained by the mesocosm environment. For example, we unexpectedly found a negative relationship between AI and *Bd* infection intensities across our experimental communities. This result is primarily due to infections in hylids, which have a low aquatic index (lower exposure to *Bd* in aquatic reservoirs), yet showed disproportionately high pathogen infection loads in mesocosms. Wild hylids may avoid exposure due to arboreal lifestyles, and perhaps were not able to do so as effectively in mesocosms, thus increasing their pathogen burden. Alternatively, owing to their lower natural exposure to *Bd*, hylids may not have evolved strong immune responses and are therefore more susceptible to *Bd* in artificial environments.

We detected a strong effect of previous exposure to *Bd*, in that assemblages carrying high infection loads in the wild showed significantly lower infections in the experimental trials. We now know that a number of species can evolve resistance or tolerance to *Bd* after laboratory infection challenges (Ellison et al., 2014; McMahon et al., 2014; Venesky et al., 2014b). Whether acquired immunity evolved differently in the tropics and temperate zones is unknown. However, exposure varies significantly in the two regions. In the tropics, *Bd* is present in the environment year round (Longo, Burrowes, & Joglar, 2010). In contrast, at temperate latitudes *Bd* cycles seasonally (Lenker et al., 2014; Savage, Becker, & Zamudio, 2015), and during much of the year climatic conditions are outside the optimum climatic envelope of *Bd* (Lenker et al., 2014). This shorter exposure to the pathogen could partially explain the overall lower effect of acquired resistance in our temperate host assemblages.

Finally, our data show that host density and diversity – two factors known to affect *Bd* infection dynamics – may play different roles in the two regions we examined. All else being equal, density should enhance transmission probabilities, and thus will be positively correlated with disease risk (Briggs et al., 2010). In contrast, high host diversity often reduces pathogen burden, a mechanism known as the dilution effect (Keeseing, Holt, & Ostfeld, 2006; LoGiudice, Ostfeld, Schmidt, & Keeseing, 2003). Recent studies reported a dilution effect in host-*Bd* systems, such that an increased number of amphibian species reduced infection loads in tadpoles (Han et al., 2015; Searle et al., 2011; Venesky et al., 2014a;) and post-metamorphic amphibians (Becker et al., 2014). Therefore, we hypothesized that our mesocosm assemblages with high host diversity would show lower *Bd* infection loads. However, total host density and species richness were positively correlated in our tropical host assemblages. Assemblages with high amphibian density, presumably carrying higher *Bd* infection loads (Searle et al., 2011; Venesky et al., 2014a), also showed elevated host diversity, likely decreasing the odds of infection. These two antagonistic mechanisms likely interact, and therefore it is possible that the unexpected negative density-dependent relationship observed in our tropical amphibian assemblages could be actually a signal of host diversity (dilution effect).

Conclusions

With the high rate of anthropogenic modification affecting temperate and tropical forests (Chapin et al., 2000), understanding how deforestation influences disease risk is critical for predicting *Bd* spread and developing management tools for wild populations. The present study is the first to employ challenge infection experiments linking deforestation with disease risk in mimic amphibian assemblages that differ in species composition and relative abundance, and thus more closely represent natural amphibian communities. Our results highlight that deforestation can lead to an increase or decrease in amphibian disease risk depending on how habitat disturbances change host community structure. Therefore, understanding community-level disease dynamics will require first quantifying the consequences of deforestation for host community structure and then the cascading effects of altered host community attributes on exposure and subsequent pathogen infections. Regional differences in standing host species diversity will result in distinct and unique pathways when natural systems are disturbed. Understanding these pathways will help us identify case-specific drivers of wildlife epidemics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.baae.2015.08.004>.

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