



# Assessing amphibian disease risk across tropical streams while accounting for imperfect pathogen detection

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## Abstract

Ecologists studying emerging wildlife diseases need to confront the realism of imperfect pathogen detection across heterogeneous habitats to aid in conservation decisions. For example, spatial risk assessments of amphibian disease caused by *Batrachochytrium dendrobatidis* (*Bd*) has largely ignored imperfect pathogen detection across sampling sites. Because changes in pathogenicity and host susceptibility could trigger recurrent population declines, it is imperative to understand how pathogen prevalence and occupancy vary across environmental gradients. Here, we assessed how *Bd* occurrence, prevalence, and infection intensity in a diverse Neotropical landscape vary across streams in relation to abiotic and biotic predictors using a hierarchical Bayesian model that accounts for imperfect *Bd* detection caused by qPCR error. Our model indicated that the number of streams harboring *Bd*-infected frogs is higher than observed, with *Bd* likely being present at ~43% more streams than it was detected. We found that terrestrial-breeders captured along streams had higher *Bd* prevalence, but lower infection intensity, than aquatic-breeding species. We found a positive relationship between *Bd* occupancy probability and stream density, and a negative relationship between *Bd* occupancy probability and amphibian local richness. Forest cover was a weak predictor of *Bd* occurrence and infection intensity. Finally, we provide estimates for the minimum number of amphibian captures needed to determine the presence of *Bd* at a given site where *Bd* occurs, thus, providing guidance for cost-effective disease risk monitoring programs.

**Keywords** *Batrachochytrium dendrobatidis* · Atlantic forest · Amphibian disease · Tropical streams · Bayesian hierarchical model

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## Introduction

The number of emerging wildlife diseases has dramatically increased over the past several decades, causing population declines and species extinctions across a wide range of taxa (Daszak et al. 2000; Jones et al. 2008; Fisher et al. 2012).

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The amphibian pathogen *Batrachochytrium dendrobatidis* (hereafter *Bd*) is among the first described fungal pathogens to threaten hundreds of vertebrate species (Skerratt et al. 2007; DiRenzo et al. 2017; Scheele et al. 2019); *Bd* has been detected on over 700 species across all three extant orders of amphibians (Olson et al. 2013; Olson and Ronnenberg 2014; James et al. 2015; Becker et al. 2016a). In Brazil, the country with the highest amphibian diversity globally (> 1000 species), *Bd* is widely distributed across the Atlantic Forest biome (Becker and Zamudio 2011; James et al. 2015), where it threatens amphibians with a variety of life-history strategies (Jenkins et al. 2013; Greenspan et al. 2018). More specifically, *Bd* has been historically linked to amphibian declines and extinctions throughout the Atlantic Forest since the late 1970's (Carvalho et al. 2017).

Atlantic Forest frogs have a wide variety of reproductive modes (Haddad and Prado 2005), which likely impacts host exposure to the waterborne pathogen *Bd* (Gründler et al. 2012; Becker et al. 2016b; Mesquita et al. 2017; Greenspan et al. 2018). It has been hypothesized that terrestrial-breeding amphibians, typically referred to as direct-developers, evolved as a response to minimize the risk of pathogenic infections from aquatic environments (Todd 2007). In this case, terrestrial-breeding amphibians are less likely to be exposed to waterborne *Bd*, and thus, often have lower prevalence than species with aquatic larvae (Kriger and Hero 2007; Brem and Lips 2008). However, experimental studies with terrestrial-breeding species from the Atlantic Forest have shown that once exposed to *Bd*, terrestrial-breeders tend to have higher *Bd* infection intensity and are more susceptible to chytridiomycosis than species with aquatic larvae due to a lack of acquired resistance against the pathogen (Mesquita et al. 2017; Greenspan et al. 2018). Thus, host life history and water dependency are expected to influence *Bd* prevalence (i.e., proportion of infected hosts in a community) and infection intensity (Lips et al. 2003; Becker et al. 2014; Mesquita et al. 2017).

*Bd* is typically not randomly distributed within (i.e., prevalence) and across (i.e., occurrence) amphibian communities (Kriger and Hero 2007; Becker and Zamudio 2011). Survival, growth, reproduction and dispersal of *Bd* are strongly favored by particular microclimatic conditions, such as mild temperatures (optimal growth 17–25 °C) and high humidity (Piotrowski et al. 2004; Woodhams et al. 2008; Voyles et al. 2017). Thus, variation in topographic complexity, elevation, and latitude are expected to predict *Bd* infection dynamics locally because these gradients are often correlated with local microclimatic variation (Kriger et al. 2007; Brem and Lips 2008; James et al. 2015; Becker et al. 2016b). Similarly, highly forested areas are often linked to higher *Bd* occurrence, prevalence, and pathogen intensity because tree cover acts like a microclimate buffer, aiding in the *Bd* persistence (Becker and Zamudio 2011; Becker et al. 2012, 2017; Scheele et al. 2015).

In addition, forest cover is often correlated with species diversity, which can also influence disease transmission through several potential mechanisms. Although many empirical studies and theoretical models support the idea that biodiversity at the local scale often mitigates disease risk via the 'dilution effect' (Keesing et al. 2006; Becker et al. 2014; Civitello et al. 2015; Cohen et al. 2016), high host species diversity can also amplify disease risk when long-lived disease-resistant hosts maintain the pathogen in a population (Ostfeld and Keesing 2012). It is likely that forest cover also affects disease risk in these scenarios, affecting pathogen persistence and transmission dynamics among host species and populations. Additionally, amphibian disease risk may increase with the availability of water bodies in the landscape (e.g., drainage density; Ruggeri et al. 2018) because *Bd* is a waterborne pathogen and fungal zoospores can persist in water for several weeks, making perennial water sources an environmental reservoir for the pathogen (Longcore and Pessier 1999; Johnson and Speare 2003; Kilpatrick et al. 2010; Chestnut et al. 2014). Due to multiple abiotic and biotic factors influencing *Bd* prevalence, infection intensity and occurrence, it is important to disentangle their role in *Bd* dynamics to support pathogen mitigation in wild amphibian communities.

Here, we used a hierarchical Bayesian model that accounts for imperfect pathogen detection caused by qPCR error to estimate how abiotic and biotic factors predict *Bd*. Our hierarchical model differs from traditional statistical approaches by allowing us to correct for false negatives in *Bd* detection/non-detection and measurement error of infection intensity across sampling sites (streams). Specifically, we tested the effects of (i) abiotic variables (stream density and native forest cover), and (ii) biotic variables (amphibian species richness and host life-history traits) on *Bd* occurrence, prevalence, and infection intensity in a diverse Neotropical landscape. We expected that *Bd* prevalence would be lower among direct-developing amphibian species than host species with aquatic larvae, because the former group does not exhibit breeding aggregation behavior and does not require water bodies to reproduce, thus reducing contact with infected individuals and *Bd* zoospores in aquatic environments. Conversely, we expected that direct-developing amphibians would show higher *Bd* infection intensity than aquatic-breeding frogs that are continuously exposed to *Bd* in water bodies and can be primed with tolerance through adaptive immunity to waterborne *Bd* (Mesquita et al. 2017; Greenspan et al. 2018). Based on previous studies of biotic and abiotic drivers of *Bd* (Johnson and Speare 2003; Piotrowski et al. 2004; Becker and Zamudio 2011; Scheele et al. 2015; Becker et al. 2017), we expected that high forest cover, high stream density and low local frog species richness would be positively related to *Bd* occurrence. Similarly, we expected that high forest cover would be positively related to prevalence and infection intensity (Becker and Zamudio 2011; Ruggeri et al. 2018; Becker et al. 2017). Combined, our results

highlight the importance of accounting for imperfect pathogen detection to predict how biotic and abiotic factors impact wild-life disease distribution.

## Materials and methods

### Study area

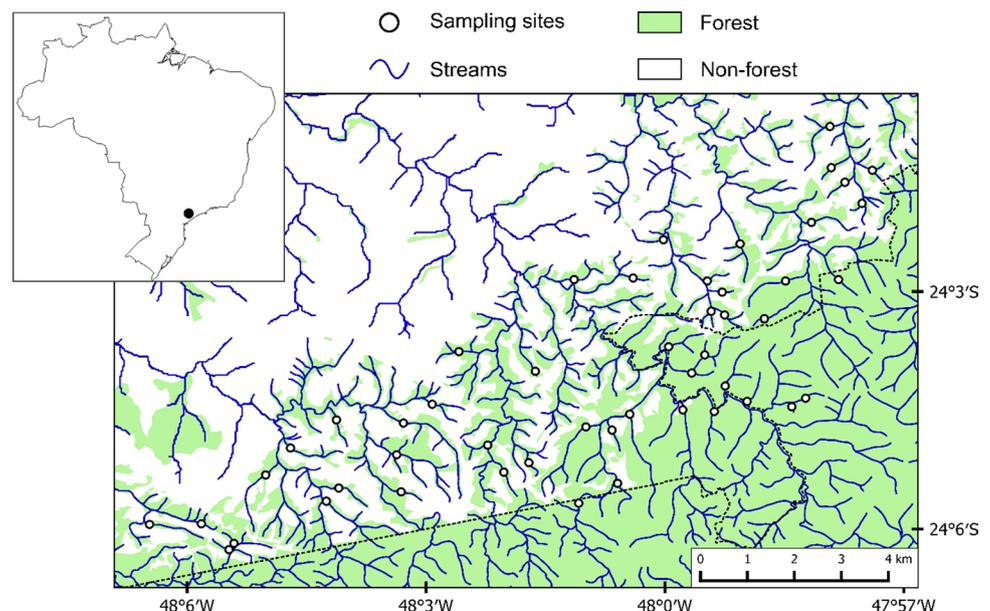
The study occurred at Carlos Botelho State Park and adjacent forest fragments in the Paranapiacaba plateau, State of São Paulo, Brazil (Fig. 1). The Park's headquarters ( $24^{\circ} 3' 22.94''$  S,  $47^{\circ} 59' 37.08''$  W) are located in the Upper Paranapanema basin, a region harboring one of the largest remaining areas of the Atlantic Forest with more than 35,000 hectares of protected forest (Kronka et al. 2005; Ribeiro et al. 2009). The vegetation is predominantly composed of Montane Ombrophilous dense forest (Kronka et al. 2005) surrounded by an exotic forest of pines and eucalyptus (i.e., silviculture), agricultural areas (i.e., vineyard, cattle, pasture, and orchards), and rural buildings. The region has humid subtropical hot summers and dry winters, but years without a dry winter occur periodically (Alvares et al. 2013). The average annual temperature is  $20^{\circ}\text{C}$  (ranging  $18\text{--}22^{\circ}\text{C}$ ) with annual precipitation over 1600 mm (Alvares et al. 2013). This region exhibits high biodiversity with more than 110 amphibian species; most of these species use riparian habitats for foraging and breeding (Araujo et al. 2013; Ribeiro et al. 2018).

### Bd data

We used land cover information and drainage networks to select 50 streams distributed across 10 watersheds with varying levels of native forest cover (23–99%). We established an upstream 100 m transect in each of our focal streams. Due to logistic challenges, we were unable to collect swab samples in one stream, reducing our final sample size to a total of 49 streams (Fig. 1). We surveyed amphibians using standardized acoustic and visual sampling protocols along each of the 100 m transects (Heyer et al. 1994; Ribeiro et al. 2018). Each transect was sampled at least once diurnally and nocturnally by two or three researchers that walked slowly along transects locating amphibians visually and acoustically in their microhabitats, such as stream banks, rocks, vegetation, leaf-litter, bromeliads, and burrows. Field sampling for *Bd* occurred during the rainy season, from Nov 2015 to Mar 2016 (total number of sampling days = 43), when most frogs were most active in the study region.

To test for *Bd*, we captured each frog using fresh disposable gloves to avoid cross-contamination among samples. We used a sterile swab to sample frog skin following the protocol described by Kriger et al. (2006). Then, we released each frog at the original point of capture. We made sure each frog was sampled only once in transects where we sampled multiple times, by following this procedure: (1) we walked upstream along with the transect sampling individuals; (2) before moving to the following transect, we collected some individuals and released others in the current transect—this way, we never sampled the same individual in a downstream transect; (3) when we returned to a previously sampled stream in a different day, we only sampled individuals from species that had not been sampled before or from species

**Fig. 1** Study area in the southeastern state of São Paulo, Brazil (black dot in the top left panel), and distribution of the 49 sampling sites in the Atlantic Forest (white circles). The dotted line represents the northern limit of Carlos Botelho State Park. Green denotes native forested areas; the white denotes non-forest areas (i.e., silviculture, agricultural areas, and rural buildings); blue lines indicate stream network



of which all individuals had been collected before (i.e., not a single individual had been released). We sampled up to 10 individuals per sampling stream following Scheele et al. (2015). We sampled a total of 339 frogs from 27 species in our 49 focal streams. The number of individuals captured per stream ranged from 1 to 10 amphibians (mean = 6.9; sd = 3.25). The number of swabbed individuals per species was also variable, ranging from 1 to 85 individuals (see Supplementary Information), and in general, the number of swabs collected per species was representative of the relative abundance of these species at the sites (JWRJ pers. obs.).

We stored swabs in 1.5 ml polypropylene tubes and refrigerated samples until DNA extraction. We followed the protocol described by Boyle et al. (2004) and extracted *Bd* DNA from each swab using PrepMan Ultra® (Applied Biosystems). We quantified *Bd* infection loads (zoospore genomic equivalents: ZGE) in each swab using qPCR analysis with TaqMan assays (Applied Biosystems). Each plate contained *Bd* standards of 0.1, 1, 10, 100, and 1000 ZGE from strain CLFT 159. *Bd* strain CLFT 159 was isolated from the species *Hylodes cardosoi*, collected in the municipality of Morretes, State of Paraná, Brazil. This strain was isolated from a tadpole mouthpart (Vieira and Toledo 2012). From previous research, we know that the primers (ITS1-3 and 5.8S Chytr) and the probe (Chytr MGB2) used in our qPCR assay amplify both *Bd* lineages that occur in our study region (*Bd*-GPL and *Bd*-Brazil; see Greenspan et al. 2018). Although both strains seem to be successfully amplified using our TaqMan assay, variation in detection probability among strains under co-infection scenarios (using field data) remains to be investigated. We ran the samples in singlicate and samples that had *Bd* load equal to or greater than one were treated as *Bd*-positive (Kriger et al. 2006, 2007).

### Biotic and abiotic covariates

We calculated the proportion of native forest cover (%) within a 200 m buffer surrounding each sampled stream using aerial photographs with 1 m of spatial resolution (EMPLASA 2011). To estimate topographic complexity, we generated a relief map of the study area handling a digital elevation model with a pixel resolution of 30 m (Topodata project; <https://www.dsr.inpe.br/topodata>). We generated stream networks using digital elevation models and calibrated with a high-resolution topographic map produced by the Brazilian Institute of Geography and Statistics (1:50,000 scale; <https://www.ibge.gov.br/>). We extracted information on stream density, topographic complexity, forest edge, and forest cover within a 200 m buffer surrounding each sampled stream, adopting the standard deviation of the mountain slope as a measure of topographic complexity (Toledo et al. 2014), length of native forest edge (m) as an estimate of the forest edge, and defined stream density as the total

length of the stream network in meters within a buffer (m/buffer; as in Ruggeri et al. 2018). All geographic information data was extracted using ArcGIS version 10.3.1 (ESRI 2015). We used mean estimates of amphibian species richness published by Ribeiro et al. (2018)—a study carried out in the same focal streams at the same time as the present study—as a fine-scale measure of local amphibian diversity. We assessed collinearity among all covariates using Pearson's correlation coefficient ( $r$ ; Sokal and Rohlf 1994). We excluded topographic complexity and forest edge from the analysis because they were highly correlated with forest cover ( $r > 0.48$ ; Table 1). All other covariates retained were weakly correlated ( $r < 0.15$ ; Table 1). Although variation in altitude and microclimate are often confounded (and both may influence amphibian occurrence), our sampling sites had minimal variation in elevation (mean = 741.7; SD = 30.9).

### **Bd occurrence, prevalence, and infection intensity model**

We used a hierarchical Bayesian model that accounts for imperfect pathogen detection caused by qPCR error (Miller et al. 2012; DiRenzo et al. 2018) to estimate *Bd* occurrence, prevalence, and infection intensity. We sampled a subset of individuals from multiple stream communities and tested for *Bd* detection/non-detection and infection intensity once per individual captured. This model allows for the simultaneous estimation of *Bd* occurrence across the landscape, prevalence, and infection intensity while accounting for imperfect pathogen detection (i.e., false negatives).

The main assumptions of this model are: (i) the occupancy probability and infection intensity are constant during the study timeframe (i.e., the target species does not colonize, go extinct, reproduce or die AND no individual gains or losses infection) or the changes are random; (ii) all state variables are constant, or any heterogeneities are modeled

**Table 1** Correlation coefficients (*Pearson*) between biotic and landscape covariates

	Richness	Slope	Stream density	Forest	Edge
Richness	1.00	-0.46	0.06	-0.10	0.12
Slope		1.00	0.21	0.48	-0.46
Stream density			1.00	0.13	-0.09
Forest				1.00	-0.96
Edge					1.00

We removed topographic complexity and forest edge from downstream analyses to avoid multi-collinearity in our models

Richness = local frog species richness, Slope = topographic complexity, Stream density = the total length of the stream network in meters within a buffer, Forest = amount of native forest cover within a buffer, Edge = forest edge within a buffer

using covariates; (iii) individual detections are independent; and (iv) there are no false positives or double counts (MacKenzie et al. 2002; Royle 2004). We used a robust study design to meet these assumptions.

To account for imperfect *Bd* detection and infection intensity measurement error, we use informative priors from Miller et al. (2012). Using these informative priors comes with assumptions, such as (i) the conditions between Miller et al. 2012 and our study (i.e., amphibian species, habitats, *Bd* strains, laboratories, and observers) are equivalent such that they both produce the same *Bd* detection probability and infection intensity measurement error estimates, and (ii) all heterogeneities in *Bd* detection probability and infection intensity measurement error are captured within the 95% credible interval reported by Miller et al. (2012). Ideally, we would have collected replicate swabs from each individual frog or performed triplicate qPCR runs; however, we had limited available funds to gather the amount of data needed to calculate *Bd* detection probability and infection intensity measurement error. Instead, we used informative *Bd* detection priors obtained using the same swab brand (Medical Wire®), swabbing protocol, qPCR assay (Hyatt et al. 2007), number of amplification cycles (50), and the same qPCR station (ABI 7300) as in Miller et al. (2012). The Bayesian paradigm supports the combined use of prior information with current data (e.g., Van Dongen 2006, Gelman et al. 2017), as long as scientists acknowledge the limitation of priors. Here, we assume that using informative priors for *Bd* detection probability and infection intensity measurement error from Miller et al. (2012) likely provides more reliable estimates of *Bd* prevalence and infection intensity than if we assumed perfect pathogen detection, which is highly unlikely given lower ITS detection/amplification at low concentrations (e.g., Miller et al. 2012; DiRenzo et al. 2018). Whenever possible, researchers should estimate *Bd* detection probability and infection intensity measurement error in their own study systems by either collecting replicate swabs from a single individual or running each swab in triplicate reactions.

We started by modeling the true, latent variable *Bd* occurrence ( $z_j$ ) across streams. We assume that *Bd* occurrence at the  $j$ th stream is a binary random variable, such that:  $z_j \sim \text{Bernoulli}(\Psi_j)$ ; where  $\Psi_j$  is *Bd* occupancy probability at the  $j$ th stream. Thus, if *Bd* was present at stream  $j$ , then  $z_j = 1$ , and  $z_j = 0$  if not. We related *Bd* occupancy probability ( $\Psi_j$ ) to forest cover, stream density, and species richness using a logit link function (Adams et al. 2010; Kéry and Schaub 2012), such that:  $\text{logit}(\Psi_j) = a_0 + a_1 * \text{Forestcover}_j + a_2 * \text{Streamdensity}_j + a_3 * \text{Speciesrichness}_j$ ; where  $a_0$  represents the intercept and  $a_1$  to  $a_3$  represent regression coefficients (i.e., the effect of the covariates on *Bd* occupancy probability) on the logit scale. Although forest cover and amphibian species diversity are often positively correlated,

we found a weakly negative relationship between forest cover and amphibian richness in this landscape ( $r = -0.096$ ). We standardized all continuous covariates by subtracting the mean and dividing by the standard deviation.

Adams et al. (2010) showed that if pathogen presence is tested only once on a host in a finite population, then the traditional occupancy probability parameter ( $\Psi$ ) is underestimated because it is a mixture proportion of true occupancy probability and the probability that at least one animal in a population has *Bd*. However, if population size is large enough ( $> 50$ ), then the difference between true occupancy probability and the mixture proportion is negligible. Here, most of the streams likely harbor more than 50 amphibian hosts (JWRJ pers. obs.); therefore, we interpret  $\Psi_j$  as *Bd* occupancy probability at a stream  $j$ , rather than a mixture proportion. If the latter case were true, then we would be underestimating the parameter, meaning our inference is conservative.

To estimate *Bd* prevalence within a community ( $p_{j,k}$ ), we treated each swabbed individual at each stream  $j$  as a replicate sample  $k$  (Adams et al. 2010; Mosher et al. 2019). If a sampled individual amphibian  $k$  tested positive for *Bd* at stream  $j$ , then  $y_{j,k} = 1$ , and 0 otherwise. Thus, each individual  $k$  at stream  $j$  is a Bernoulli random process conditional on true *Bd* occurrence at stream  $j$  (i.e.,  $z_j = 1$ ):  $y_{j,k} \sim \text{Bernoulli}(p_{j,k} * z_j)$ . We modeled *Bd* prevalence ( $p$ ) at each stream  $j$  as a function of the amphibian's aquatic index (AI, explained below), and forest cover on the logit scale, using:

$$\text{logit}(p_{j,k}) = b_0 * \text{AI-0}_{j,k} + b_1 * \text{AI-1}_{j,k} + b_2 * \text{AI-2}_{j,k} + b_3 * \text{Forestcover}_j$$

Since variation in temperature and humidity may be associated with a change in *Bd* prevalence, we included forest cover to account for this heterogeneity, given that these variables are typically correlated. Aquatic index is a measure of amphibian water dependency and has been used to relate the probability a species will be infected by *Bd* in amphibian communities (Lips et al. 2003; Becker et al. 2014; Mesquita et al. 2017). We classified species into three distinct groups: terrestrial species with terrestrial eggs (AI-0), arboreal species with an aquatic larval phase (AI-1), and terrestrial species with an aquatic larval phase (AI-2; Lips et al. 2003; Becker et al. 2014; Mesquita et al. 2017).

We corrected the observed *Bd* detection/non-detection ( $d_{j,k}$ ) and infection intensity ( $w_{j,k}$ ) data for false negatives and measurement error caused by the diagnostic method qPCR using informative priors from Miller et al. (2012). We considered the observed *Bd* detections/non-detections ( $d_{j,k}$ ) as a Bernoulli random variable that arises as the product of *Bd* detection probability ( $q_{j,k}$ ) at the  $j$ th stream from the  $k$ th individual and the diagnostic-corrected *Bd* detection/non-detection data ( $y_{j,k}$ ):  $d_{j,k} \sim \text{Bernoulli}(q_{j,k} * y_{j,k})$ . To

clarify,  $y_{j,k}$  is the observed *Bd* detection/non-detection ( $d_{j,k}$ ) corrected for imperfect *Bd* detection.

We related *Bd* detection probability ( $q_{j,k}$ ) to individual-level *Bd* infection intensity ( $N_{j,k}$ ; modeled below) using the logit link function as:  $\text{logit}(q_{j,k}) = c_0 + c_1 * N_{j,k}$ . We modeled true stream-level *Bd* infection intensity ( $x_j$ ) at the  $j$ th stream using a lognormal distribution given that *Bd* was present (i.e.,  $z_j = 1$ ):  $x_j \sim \text{Lognormal}(\mu_j, \sigma^2)$  with mean  $\mu_j$  and standard deviation  $\sigma^2$  on the log scale. We related mean stream-level *Bd* infection intensity at the  $j$ th stream to species richness and forest cover using the identity link function:  $\mu_j = \gamma_0 + \gamma_1 * \text{Forestcover}_j + \gamma_2 * \text{Streamdensity}_j$ , where  $\gamma_0$  is the intercept and  $\gamma_1, \gamma_2$  are regression coefficients. Then, given that *Bd* was present in stream  $j$  and individual  $k$  (i.e.,  $y_{j,k} = 1$ ), we modeled individual-level variation in *Bd* infection intensity at the  $j$ th stream ( $N_{j,k}$ ) as a lognormal distribution:  $N_{j,k} \sim \text{Lognormal}(x_j, \sigma_1^2)$ , with average stream-level infection intensity  $x_j$  and standard deviation  $\sigma_1^2$  on the log scale. Lastly, we corrected for *Bd* measurement error in infection intensity caused by the diagnostic test qPCR using informative priors from Miller et al. (2012). We indicated that the observed *Bd* infection intensity ( $w_{j,k}$ ) at the  $j$ th stream on the  $k$ th individual was lognormally distributed:  $w_{j,k} \sim \text{Lognormal}(N_{j,k}, \sigma_e^2)$ , with individual-level mean infection intensity  $N_{j,k}$  and measurement error,  $\sigma_e^2$  on the log scale. We used informative priors from Miller et al. (2012) for the measurement error of *Bd* infection intensity  $\sigma_e^2$ . We log transformed the observed *Bd* infection intensity prior to modeling; therefore, all infection intensity estimates are on the scale of  $\log(w_{j,k})$ , not  $w_{j,k}$ .

To quantify the importance of aquatic indices to prevalence, we calculated the differences between pairs of parameters at each MCMC iteration following Ruiz-Gutiérrez et al. (2010). We computed the proportion of iterations where one parameter was greater than the other. We report these values as the probability that parameter  $a$  is greater than parameter  $b$ , written as  $\text{Pr}(a > b)$ . We considered effects with small posterior means or with a large degree of parameter overlap to be either unimportant to the process being modeled or to have been estimated too imprecisely to draw the conclusive inference. We use the same approach described for comparing parameters in the case of comparing *Bd* infection intensity between aquatic indices. However, we note that these estimates are on the scale of  $\log(w_{j,k})$  and not  $w_{j,k}$ .

We estimated the model parameters using a Bayesian framework and non-informative priors for most of the parameters following the suggestions by Lunn et al. (2012); normal distribution with a mean of zero and standard deviation of 3.68. We used the following informative priors:  $\sigma_e^2 \sim \text{Unif}(1.47, 1.97)$ ,  $c_0 \sim \text{Unif}(0.25, 1.32)$ , and  $c_1 \sim \text{Unif}(0.14, 0.51)$  from Miller et al. (2012). We ran the model in JAGS version 4.2.0 (Plummer 2003) using R version 3.3.1 (R Core Team 2016) with

“jagsUI” package version 1.4.4 (Kellner 2016). We fit the model with three Markov chain Monte Carlo, 55,000 iterations, a burn-in of 5,000 iterations, thinning by 50, and an adaptive phase of 100,000 iterations. We checked the convergence of all the parameters using the R-hat statistic, where values  $< 1.10$  indicate convergence, and we visually inspected MCMC chains for well-mixing behavior (data and code to run the model are available at: [https://github.com/Xuletajr/amphibian\\_disease\\_risk\\_across\\_streams\\_brazil](https://github.com/Xuletajr/amphibian_disease_risk_across_streams_brazil)).

## Sampling recommendations

We calculated the cumulative probability of detecting a *Bd* infected individual for each of the three aquatic index groups in an area with 30% and 100% forest cover given that *Bd* occurs at the site as the number of samples collected increases. We used the binomial argument with  $n$  identical and independent amphibian captured and swabbed:

$$P^* = 1 - (1 - p)^n$$

where  $p$  is *Bd* prevalence modeled and outlined above, and  $n$  is the number of amphibians captured and swabbed (Adams et al. 2010; Kéry and Schaub 2012; DiRenzo et al. 2018). Our recommendations were based on the minimum number of amphibian captured and swabbed needed to be 95% certain that if an infected amphibian occurred in a stream then *Bd* would be detected.

## Results

### Summary

We sampled a total of 27 anuran species in 10 families. The total number of swabbed individuals per species was widely variable (see Supporting Information). Approximately 75% of swabs were collected from seven species, and the most representative species were *Crossodactylus caramaschii* ( $n=85$  individuals; Hylodidae), *Bokermannohyla circumdata* ( $n=35$ ; Hylidae), *Proceratophrys boiei* ( $n=33$ ; Odontophrynidae), *Bokermannohyla hylax* ( $n=31$ ; Hylidae), *Ischnocnema guentheri* ( $n=27$ ; Brachycephalidae), *Paratelmatobius* sp. ( $n=22$ ; Leptodactylidae), and *Oloolygon brieni* ( $n=18$ ; Hylidae). Of the 339 swabs, 46 frogs were infected with *Bd*, resulting in a naïve *Bd* prevalence (proportion of detected infected hosts) of 13.6%.

Observed *Bd* infection intensity was highly variable ranging from 1 to 18,846 ZGE (overall median = 7.4; interquartile range [IQR] = 37.7). Terrestrial-breeding species had higher observed *Bd* infection intensities (AI-0 overall median = 64.8; IQR = 361.7), followed by arboreal species with aquatic larvae (AI-1 overall median = 9.2; IQR = 15.2), and terrestrial species with aquatic larvae (AI-2 overall median = 5.2; IQR = 19.2).

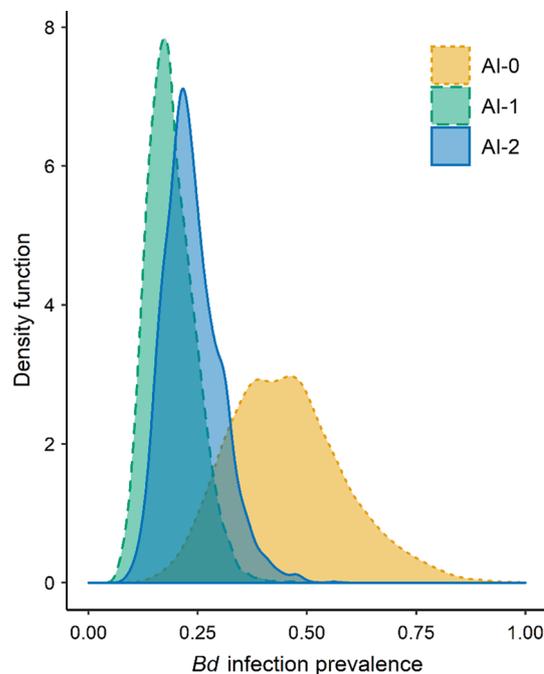
### Stream-level results

We detected *Bd*-positive frogs in 26 streams, with the naïve occupancy (i.e., the proportion of streams *Bd* was detected) as 0.53. However, our hierarchical Bayesian model estimated that *Bd* occurred at 37.1 out of 49 streams (95% credible interval [CI<sub>95%</sub>] = 30–45), and *Bd* occupancy probability was 0.793 on average (CI<sub>95%</sub> = 0.569–0.966; all continuous explanatory variables were fixed to their means). We found a positive relationship between *Bd* occupancy probability and stream density (mean of the posterior distribution for parameter  $a_2 = 0.52$ , CI<sub>95%</sub> = -0.44–1.66; Fig. 2), with 85.2% of parameter posterior distribution including positive values. We also found a negative relationship between *Bd* occupancy probability and amphibian species richness (mean of the posterior distribution for parameter  $a_3 = -0.68$ , CI<sub>95%</sub> = -1.79–0.37; Fig. 2), with 89.7% of parameter posterior distribution containing negative values. There was a positive association between forest cover and *Bd* occupancy probability, but this relationship was weak, with 57.3% of posterior distribution containing positive values (Fig. 2; mean of the posterior distribution for parameter  $a_2 = 0.08$ , CI<sub>95%</sub> = -1.29–1.35).

We found a weak positive relationship between mean stream-level *Bd* infection intensity and forest cover (mean of the posterior distribution for  $\gamma_1 = 0.279$ , CI<sub>95%</sub> = -0.739–1.389), with 70.7% of posterior distribution including positive values. Similarly, we found a weak positive relationship between mean stream-level *Bd* infection intensity and stream density (mean of the posterior distribution for  $\gamma_2 = 0.102$ , CI<sub>95%</sub> = -0.799–0.985), with 59.7% of posterior distribution including positive values.

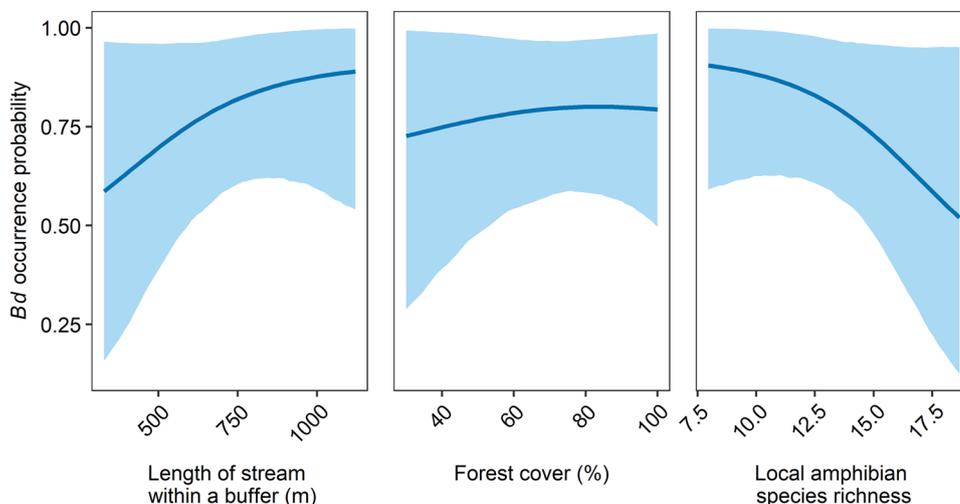
### Individual-level results

We found that *Bd* prevalence differed among host species depending on their aquatic index. Contrary to our predictions, *Bd* prevalence of terrestrial-breeding species (AI-0; mean of the posterior distribution of all individuals that fit this life history category = 45.4%, CI<sub>95%</sub> = 23.4–74.5%) was around two-times higher than both the mean *Bd* prevalence of arboreal species with aquatic larvae (AI-1; mean of the



**Fig. 3** Kernel density estimation plot of the posterior distribution for *Bd* infection prevalence (Aquatic Index groups) across Atlantic Forest streams. Terrestrial species with terrestrial eggs (AI-0, dotted line); arboreal species with aquatic larvae (AI-1, dashed line); terrestrial species with aquatic larvae (AI-2, solid line)

**Fig. 2** Response of occupancy probability rate of *Bd* to stream density (length of stream network (m) within a 200 m buffer), proportion of forest cover within a 200 m buffer (%), and local amphibian species richness (number of species) in Atlantic Forest streams. We fixed other covariates to their mean when exploring the relationship in occupancy probability and the covariate of interest



posterior distribution = 18.9%,  $CI_{95\%} = 10\text{--}31\%$ ) and terrestrial species with aquatic larvae (AI-2; mean of the posterior distribution = 23.8%,  $CI_{95\%} = 13.5\text{--}38.4\%$ ). Terrestrial-breeding species had higher *Bd* prevalence than arboreal species [ $\Pr(\text{Prev}_{\text{AI-0}} > \text{Prev}_{\text{AI-1}}) = 0.986$ ] and aquatic-breeders dwelling in the forest floor [ $\Pr(\text{Prev}_{\text{AI-0}} > \text{Prev}_{\text{AI-2}}) = 0.97$ ; Fig. 3]. There was high overlap in *Bd* prevalence among the two groups of species with aquatic larval development [ $\Pr(\text{Prev}_{\text{AI-2}} > \text{Prev}_{\text{AI-1}}) = 0.768$ ; Fig. 3]. The mean posterior distribution for the forest cover parameter showed a positive relationship with *Bd* prevalence (mean of the posterior distribution for parameter  $b_3 = 0.28$ ,  $CI_{95\%} = -0.204\text{--}0.747$ ), with 87.7% of posterior distribution including positive values.

On the other hand, *Bd* infection intensity (log scale) of terrestrial-breeding species (AI-0; mean of the posterior distribution = 0.199,  $CI_{95\%} = -0.039\text{--}0.418$ ) was three-times lower than both the mean *Bd* infection intensity of arboreal species with aquatic larvae (AI-1; mean of the posterior distribution = 0.609,  $CI_{95\%} = -0.019\text{--}1.153$ ) and terrestrial species with aquatic larvae (AI-2; mean of the posterior distribution = 0.808,  $CI_{95\%} = 0.115\text{--}1.37$ ). Terrestrial-breeding species had lower *Bd* infection intensity than arboreal species [ $\Pr(\text{Int}_{\text{AI-0}} < \text{Int}_{\text{AI-1}}) = 0.934$ ] and terrestrial species with aquatic larvae [ $\Pr(\text{Int}_{\text{AI-0}} < \text{Int}_{\text{AI-2}}) = 0.973$ ]. There was high overlap of *Bd* infection intensity between species with aquatic larval development [ $\Pr(\text{Int}_{\text{AI-2}} > \text{Int}_{\text{AI-1}}) = 0.749$ ].

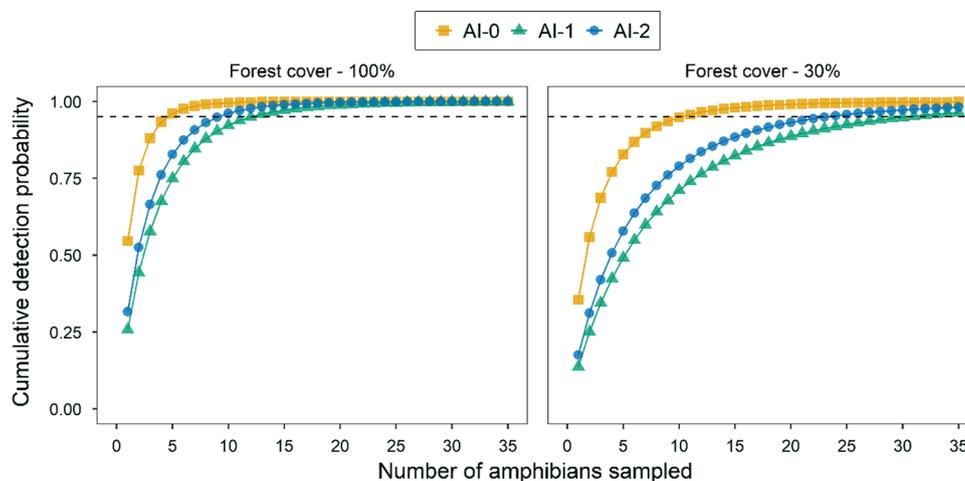
## Sampling recommendations

We used the posterior mean of  $p$  to predict the number of amphibians required to be 95% certain that a positive sample is detected in a stream where *Bd* occurs for the three aquatic index groups in two distinct levels of native forest cover: 100% and 30% of forest cover. Our results indicated that 12 swabs are needed to be at least 95% certain that an infected individual is captured in an area where *Bd* occurs at a stream with 100% forest cover, whereas at least 30 swabs are needed to reach the same level of certainty at a stream with 30% forest cover (Fig. 4).

## Discussion

Monitoring wildlife diseases across landscapes is key for implementing successful conservation strategies in response to disease-related declines (Mörner et al. 2002). Our hierarchical Bayesian model showed that the number of streams harboring *Bd* infected frogs was largely underestimated, with *Bd* likely being present at 43% more streams than the naïve occurrence estimate. Although there was support for widespread *Bd* distribution across streams (at least 75% of sampling sites), our results point to non-random *Bd* occurrence throughout the landscape, with a positive association of *Bd* occupancy probability with stream density, and a negative association with species diversity.

Perennial water bodies are natural environmental reservoirs of *Bd* and, thus, their higher availability (measured as stream density in our study) increases *Bd* disease risk



**Fig. 4** We used the average *Bd* prevalence to predict the number of amphibians required to be 95% certain that if *Bd* occurs at a site then it will be detected on an individual for each of the three aquatic index groups in two distinct levels of native forest cover: the left panel is a sampling site with 100% of forest cover and the right panel a sam-

pling site with 30% of forest cover. Dashed lines represent the 95% probability of detecting *Bd* when it is present in a stream. A more or less conservative estimate for  $p$  may be assumed, for instance using the upper or lower bound of the 95% credible interval

(Ruggeri et al. 2018). *Bd* zoospores can remain viable in water bodies for several weeks and on moist sand creek beds for up to three months (Johnson and Speare 2005, 2003). Therefore, landscapes with dense hydrological networks, such as those in the Atlantic Forest coastal mountains, can facilitate spillover among host species, because the movement of infected frogs from breeding grounds to overwintering sites potentially carries the fungus to new environments. In temperate systems, Scheele et al. (2015) showed that *Bd* occupancy probability in temporary ponds increases with proximity from perennial water bodies, such as streams, highlighting the importance of riparian zones to spatial patterns of *Bd* transmission.

Some studies have shown that *Bd* occurrence, prevalence and intensity are positively correlated with natural forest cover (Becker and Zamudio 2011; Scheele et al. 2015; Becker et al. 2017), as highly forested areas provide optimal micro-climate characteristics (e.g., mild temperatures, high humidity) for *Bd* growth, development and dispersal (Piotrowski et al. 2004). We found no evidence that forest cover influenced *Bd* occurrence and infection intensity at stream-level in Atlantic Forest (see Fig. 2). A lack of association between forest cover and *Bd* occurrence could be attributed to the continuous dissemination of *Bd* from continuous to fragmented forests by infected amphibians; all streams we sampled are within or close to a large protected forest (see Fig. 1). Although *Bd* may persist across gradients of natural forest, disease risk should indeed increase towards highly forested areas, as suggested by previous studies (Becker and Zamudio 2011; Scheele et al. 2015; Becker et al. 2017), and our results (higher prevalence in more forested areas). Because higher amphibian diversity is often associated with highly forested areas (Becker et al. 2007; Ficetola et al. 2009), the effect of forest cover on *Bd* could also be confounded by host diversity (Becker et al. 2016b); although the observed weak correlation between land cover and amphibian species richness reduces the likelihood of this potentially confounding effect in our system (Table 1).

Theoretical and empirical studies have shown that local host biodiversity may positively (i.e., amplification effect) or negatively (i.e., dilution effect) affect disease risk in wildlife populations (Keesing et al. 2006; Ostfeld and Keesing 2012; Cohen et al. 2016). In agreement with the dilution effect prediction, we found lower *Bd* occupancy probability in more diverse amphibian communities. The dilution effect has shown to be a widespread pattern in a variety of disease systems (Ostfeld and Keesing 2012), including amphibian-*Bd* dynamic (Becker et al. 2014). Many recent experimental studies have supported the role of biodiversity in minimizing the risk of chytridiomycosis in amphibian communities at the mesocosm and microcosm scales (Searle et al. 2011; Becker et al. 2014; Venesky et al. 2014; Han et al. 2015). Therefore, anthropogenic pressures

that reduce amphibian diversity (e.g., deforestation, pollution and overexploitation) may indirectly favor disease, further augmenting threats for the persistence of tropical amphibians. This effect should be more prominent if low diversity communities are dominated by disease-tolerant host species that could act as super spreaders, increasing *Bd* spillover for susceptible species (DiRenzo et al. 2014; Scheele et al. 2017).

There is strong evidence supporting the hypothesis that terrestrial-breeding amphibians have lower *Bd* prevalence than aquatic-breeding species (e.g., Kriger and Hero 2007; Brem and Lips 2008; Mesquita et al. 2017). This is mostly associated with terrestrial-breeders having less contact with aquatic environments (i.e., environmental reservoirs of *Bd* zoospores) and dying quickly after coming into contact with *Bd* (Mesquita et al. 2017). But contrary to this expectation, we found higher *Bd* prevalence and lower infection intensity in terrestrial-breeding species compared to aquatic-breeding species. The primary difference between our study and others is that our sampling was strictly conducted along riparian zones and streams, which are environments expected to favor exposure to waterborne chytrids, whereas other studies sampled direct-developers predominately away from riparian habitats. Thus, it is likely that riparian environments play a central role in host-pathogen dynamics of direct-developing amphibian species. Because terrestrial-breeding amphibians in the Atlantic Forest have a higher susceptibility to *Bd* than species with aquatic larvae (Mesquita et al. 2017), climatic and anthropogenic changes (e.g., extreme climatic events and deforestation) that influence these frogs to disperse to riparian areas may pose a significant risk for this group. For instance, droughts have been linked to outbreaks of chytridiomycosis by increasing aggregations of amphibian hosts along streams (Adams et al. 2017). Future studies are needed to investigate whether terrestrial-breeding amphibians exhibit strong seasonal and fine-scale spatial variations in disease risk.

Our hierarchical Bayesian model accounted for imperfect *Bd* detection and measurement error to provide inference on *Bd* spatial occurrence, prevalence and infection intensity. Our model indicated that: (i) the number of streams in which *Bd* is present was largely underestimated; (ii) high local amphibian diversity decreases *Bd* occurrence; (iii) high concentration of streams within buffer increases *Bd* occurrence; and (iv) terrestrial-breeders have higher *Bd* prevalence than aquatic-breeding frogs along riparian zones and streams. Although we used informative priors to account for imperfect *Bd* detection and infection intensity measurement error, we urge other studies in the future to either collect multiple swabs per individual or run multiple qPCR runs on a single swab to estimate these parameters. When using informative priors for detection probabilities, there is the strong assumption that the probabilities are constant (i.e.,

not heterogeneous) across qPCR assays, focal host species, pathogen strains, etc., which is highly unlikely. This boils down to considering the tradeoffs in using informative priors from other studies versus assuming perfect detection. We also underscore that the Bayesian hierarchical modeling approach used here can be modified to deal with different levels of uncertainty, which are inherent to studying disease, provide more reliable estimates of *Bd* occurrence, prevalence and infection intensity (Miller et al. 2012; DiRenzo et al. 2018, 2019). For example, future studies can be designed to address how different *Bd* strains under co-infection scenarios in wild frogs influence disease detection in qPCR assays and can skew the estimates of infection parameters. Finally, our hierarchical Bayesian model aids in the estimation of the minimum sampling effort (i.e., the number of frogs to swab) to accurately detect *Bd* in a given site, guiding cost-efficient disease risk monitoring programs.

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**Author contribution statement** JWRJ, TS and CGB conceived and designed the study. JWRJ led the fieldwork. JWRJ, CL, MLL and LFT performed the molecular analyses. JWRJ, TS, GVD and CGB conducted the statistical analyses and developed the Bayesian hierarchical model. JWRJ led manuscript writing, with important contributions from all coauthors. JWRJ, TS, GVD, CL, MLL, LFT, CFBH and CGB contributed critically to the drafts and reviews.

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