

Seasonality, Environmental Factors, and Host Behavior Linked to Disease Risk in Stream-Dwelling Tadpoles

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ABSTRACT: The aquatic pathogen *Batrachochytrium dendrobatidis* (*Bd*) can cause declines in amphibian populations and species extinctions worldwide. In tadpoles, epizootic events have not been reported; however, there is a possibility for rapid spread of disease in water. Here, we quantified *Bd* infection dynamics in tadpoles from two streams in the northeastern Atlantic forest of Brazil. *Bd* prevalence varied seasonally and increased during the rainy season. *Aplastodiscus sibilatus* and *Proceratophrys renalis* tadpoles were *Bd* positive in both seasons, whereas *Agalychnis granulosa* tadpoles were *Bd* positive only during the rainy season. *Bd* prevalence was higher in *A. sibilatus* than in *A. granulosa* and *P. renalis*. We found that larger individuals have a higher probability of being *Bd* positive, independent of the developmental stage. Moreover, we found that canopy cover, water flow, water temperature, water depth, and pH were associated with *Bd* prevalence. Our results highlight the fact that tadpoles can serve as pathogen reservoirs; therefore, monitoring anuran larvae populations in these habitats can enhance the impact assessment of this pathogen in biodiversity hot spots.

Key words: Abiotic factors; Anura larvae; Atlantic forest; *Batrachochytrium dendrobatidis*; Disease ecology

HABITAT destruction and infectious diseases are major threats that lead to declines and extinctions of amphibian populations worldwide (Skerratt et al. 2007; Sodhi et al. 2008; Mann et al. 2009). Among diseases, chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*), has already infected over 600 amphibian species (Olson et al. 2013; James et al. 2015; Valencia-Aguilar et al. 2015), causing epizootic events in Australia (McDonald and Alford 1999), Costa Rica (Pounds et al. 1997; Lips 1998; Lips et al. 2003), Panama (Lips 1999; Lips et al. 2003), and Venezuela (La Marca et al. 2005). *Bd* is a pathogenic fungus with flagellated aquatic zoospores that infects keratinizing tissues—skin in adult frogs and oral structures in tadpoles (Berger et al. 1999; Altig 2007; Viera et al. 2013). Zoospores spread and infect amphibians by close or direct contact during mating, tadpole schooling, or other gregarious behavior (Berger et al. 1999; Catenazzi et al. 2013).

Susceptibility of adults, metamorphs, and tadpoles to the infection varies among species, populations, and sites (Blaustein et al. 2005; Fisher et al. 2009; Kilpatrick et al. 2010), indicating differences in the host–pathogen interaction (Woolhouse et al. 2001; Briggs et al. 2010). Within a population, the infection can persist in tadpole hosts, which are usually less susceptible to the pathogen and therefore could act as reservoirs (Woolhouse et al. 2001). In particular, tadpoles are a potentially important reservoir for the fungus in aquatic systems (Woodhams and Alford 2005; Smith et al. 2007; Conradie et al. 2011; Catenazzi et al. 2013), because *Bd* is directly transmitted through water and can survive for up to 7 wk in sterile lake water (Johnson and Speare 2003; Rachowicz and Vredenburg 2004). Although tadpoles have been considered important reservoir for *Bd* in wild populations (Rachowicz and Vredenburg 2004; Woodhams and Alford 2005; Catenazzi et al. 2013), little is known about the influence of environmental factors and life conditions of

host species on the virulence of *Bd* in wild populations (Conradie et al. 2011; Catenazzi et al. 2013).

Batrachochytrium dendrobatidis prevalence in amphibians is affected by temperature and humidity, factors that vary with site, elevation, or between seasons (Woodhams and Alford 2005; Fisher et al. 2009). Higher temperatures in low altitudes are associated with lower infection prevalence (Rödger et al. 2008) and, in some cases, tadpoles and adults at environmental temperatures >26°C are able to clear themselves of *Bd* infection (Woodhams et al. 2003; Geiger et al. 2011). In this sense, many terrestrial habitats are too warm or too dry for *Bd* survival (Ron 2005; Rödger et al. 2010; Gründler et al. 2012), and seasonal or even daily temperature fluctuations can affect infection prevalence (Woodhams and Alford 2005; Kriger and Hero 2007a; Ruggeri et al. 2015). Permanent waterbodies are typically cooler and more thermally stable (Brönmark and Hansson 2005), however, providing refuge for both *Bd* and tadpoles, and contributing to their survival (Kriger and Hero 2007a; Peterson et al. 2007; Raffel et al. 2010).

To determine *Bd* prevalence and its seasonal variation in stream-dwelling tadpoles of *Agalychnis granulosa*, *Aplastodiscus sibilatus*, and *Proceratophrys renalis*, we conducted a study in the northern Atlantic forest of Brazil, where awareness of this infection is incipient (Carnaval et al. 2006; Lisboa et al. 2013; Valencia-Aguilar et al. 2015). Environmental conditions differ between the southern and northern regions of the Atlantic forest (Ribeiro et al. 2009). Therefore, information regarding the geographic variation in prevalence and intensity of *Bd* in this forest would benefit the understanding of disease ecology (Keesing et al. 2006; Kriger and Hero 2008; Gallana et al. 2013). We quantified some environmental variables and life-history traits to determine which of these could affect *Bd* prevalence. Some amphibian clades show a greater susceptibility to *Bd* infection than others (Corey and Waite 2008); therefore we expect to find variation in *Bd* prevalence among the infected species.

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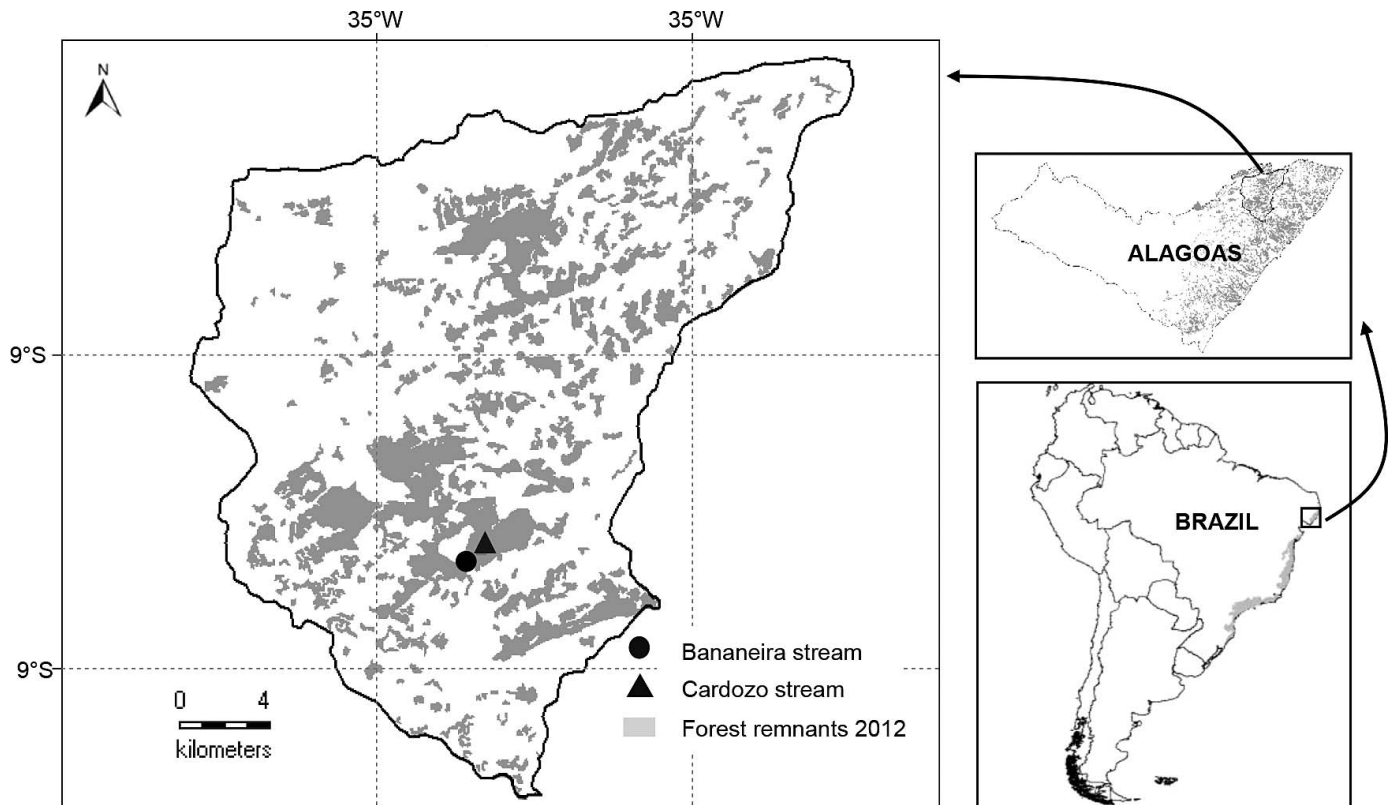


FIG. 1.—Study sites in the Pernambuco subregion of the Atlantic forest, Estação Ecológica de Murici, State of Alagoas (upper inset), Brazil (lower inset). Source for remaining forest cover: SOS Mata Atlântica/INPE (2012).

Because loss of oral structures in tadpoles leads to a reduction in the foraging efficiency (Venesky et al. 2010), we also expected that infected individuals would have smaller body lengths compared with uninfected individuals. Lastly, given that abiotic factors such as temperature affect *Bd* infection (Raffel et al. 2010; Bancroft et al. 2011), we expected a higher prevalence in areas with colder waters.

MATERIALS AND METHODS

Field Survey

Two permanent streams (Cardozo, 9°12'42.6"S, 35°51'52.5"W, 541 m above sea level [asl]; and Bananeira, 9°13'3.5"S, 35°52'32.1"W, 564 m asl; datum = WGS84) were studied, in the Estação Ecológica de Murici, of the Pernambuco subregion of the Atlantic forest (Fig. 1; Silva and Casteleti 2003). Because of differences among host breeding season, each stream was sampled monthly during the dry (November 2013–January 2014), onset (April–May 2014), and peak rainy (June–July 2014) seasons, to analyze as many species of tadpoles as possible. Tadpoles were collected in 23 different sites along each stream, by the dip-net survey method described by Shaffer et al. (2001). We standardized our per-site sampling method over a duration of 19 min (three sweeps of 5 min each interspersed by 2 min between sweeps).

Canopy cover of the stream was measured at each sampling site as the vertical projection of plant foliage (Fiala et al. 2006). The canopy was photographed (at chest height) at five points (one in each cardinal direction and one in the center) and photographs were converted to binary images

where the canopy is rendered as black and the sky as white. We projected eight vertical and six horizontal grid lines onto each photograph, and then calculated the proportions of black and white areas as estimates of percent canopy cover at each site. Water temperature and pH were measured 10 cm below the water surface at a single location within each sampling site ($n = 23$) of the streams, with the use of a multiparameter Hanna HI 9828. Stream depth was recorded in three locations within each sampling site (two at the ends of each sweep, and one in the center) with the use of a measuring tape. Water flow rate was calculated as displacement distance per unit time (m/s).

Batrachochytrium dendrobatidis Detection and Tadpole Measurements

Live tadpoles were transported to Laboratório de Biologia Celular in the Universidade Federal de Alagoas. Only tadpoles in the developmental Stages 25 to 40 (Gosner 1960) were analyzed because, after these stages, the adult mouth parts and tongue replace the oral disc of the tadpole, the only keratinizing epithelium during this period of the larval life history (Duellman and Trueb 1994; Altig and McDiarmid 1999). Subjects were decapitated and their mouthparts excised, mounted on microscope slides, and visually inspected following Lambertini et al. (2013). This technique consisted of examining the oral structures (jaw sheath and tooth rows) of tadpoles at $\times 40$ and $\times 100$ to detect the presence of *Bd* zoospores. An individual was considered *Bd*-positive when a spherical or septate sporangium (Longcore et al. 1999) was observed in the tissue (Fig. 2).

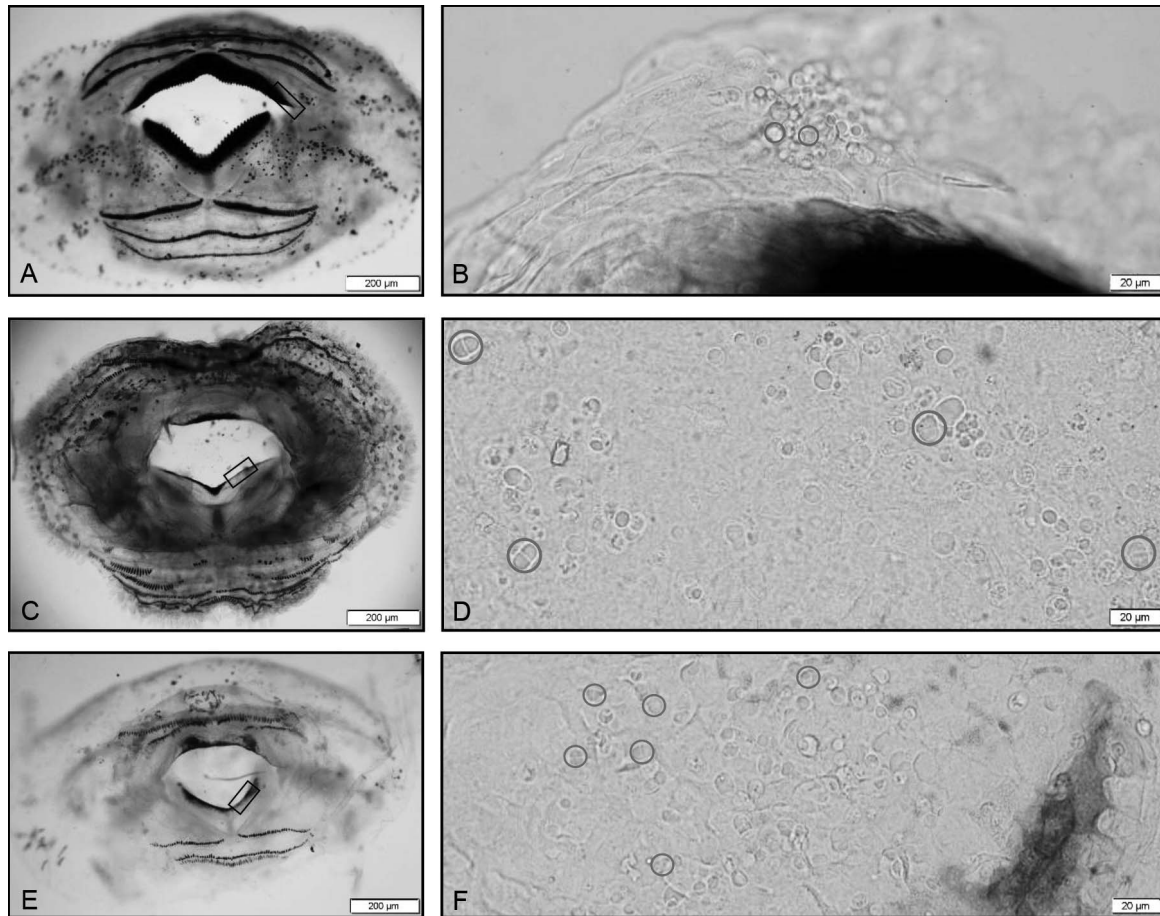


FIG. 2.—Photomicrographs of the larval mouthparts of *Agalychnis granulosa* (A), *Aplastodiscus sibilatus* (C), and *Proceratophrys renalis* (E) indicating focal areas (black rectangles) scanned for evidence of infection by *Batrachochytrium dendrobatidis*. Jaw sheath tissue of infected tadpoles of *Agalychnis granulosa* (B), *Aplastodiscus sibilatus* (D), and *P. renalis* (F) showing septate sporangia of the fungus (red circles).

In anuran larvae, *Bd* infection has been associated with the oral structure loss (Fellers et al. 2001; Knapp and Morgan 2006; Smith et al. 2007; Vieira et al. 2013). Because these oral structures are related to the feeding efficiency in some species (Alford 1999), we collected data on body length (± 1 mm; Altig and McDiarmid 1999), development stage (Gosner 1960) and oral disc condition in each sampled individual. We used these data to determine if the oral structure loss associated with *Bd* infection led to decreased the tadpole growth. We also isolated the fungus from infected tadpoles from the Cardozo stream in the Universidade Federal de Alagoas, and sent the isolates to the Universidade Estadual de Campinas (UNICAMP) where the fungus was sequenced and identified as the Global Pandemic Lineage (*Bd*-GPL).

Statistical Analyses

Of the collected species, only *Agalychnis granulosa*, *Aplastodiscus sibilatus*, and *P. renalis* were included in the analyses because they were infected and available in adequate sample sizes (Table 1). *Bd* prevalence for each species was calculated as the number of infected tadpoles, divided by the total number of tadpoles collected. We used a Kruskal–Wallis (KW) test to examine the seasonal variation in *Bd* prevalence within and between Cardozo and Bananeira streams.

We evaluated if body size differed between infected and uninfected tadpoles with the use of a Mann–Whitney test.

We categorized the sampled tadpoles ($n = 100$ per species) into three developmental classes—I (Gosner 25–30), II (Gosner 31–35), and III (Gosner 36–40)—to determine if body size predicted the probability of infection in a particular ontogenetic stage. To test this hypothesis, a logistic regression was used to examine the effects of body size, with developmental class as factor and the probability of infection as predictor variable. We used nonparametric tests because our data did not satisfy the assumptions for parametric analyses, even after we log-transformed body size.

To minimize the probability of incurring a Type I error in our analyses, we examined the data from the measured habitat variables for evidence of spatial autocorrelation (Legendre et al. 2004). In the Bananeira stream, canopy cover, water flow and depth were autocorrelated during the dry season and at the onset of the rainy season. In the Cardozo stream, water depth and pH were autocorrelated during the peak rainy season. Prior to any further analyses, we removed these autocorrelation effects using a spatial filtering model (Diniz-Filho and Bini 2005). The distance between sampling locations and the data from each of those locations were used to construct a pairwise matrix of physical distance. The matrix was subjected to a principal components analysis (PCA) to extract the eigenvectors from the distance matrix among the sample units (each location). The first two eigenvectors represented a broad variation, so they were used in multiple regressions with each environmental

TABLE 1.—*Batrachochytrium dendrobatidis* prevalence of the collected species during three seasonal periods (dry, onset of rainy, and peak of rainy) in the Cardozo and Bananeira streams. Total of tadpoles collected in parentheses; spaces marked with a dash (–) indicate that species was not found.

| Family | Species | Stream/season | | | | | |
|-----------------|--------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | | Cardozo | | | Bananeira | | |
| | | Dry | Rainy onset | Rainy peak | Dry | Rainy onset | Rainy peak |
| Hylidae | <i>Agalychnis granulosa</i> | 0% (<i>n</i> = 26) | 13.33% (<i>n</i> = 15) | 0% (<i>n</i> = 40) | 0% (<i>n</i> = 94) | 8.65% (<i>n</i> = 104) | 6.15% (<i>n</i> = 65) |
| | <i>Aplastodiscus sibilatus</i> | 41.77% (<i>n</i> = 79) | 53.33% (<i>n</i> = 75) | 54.54% (<i>n</i> = 66) | 8.49% (<i>n</i> = 106) | 53.06% (<i>n</i> = 49) | 43.85% (<i>n</i> = 57) |
| | <i>Dendropsophus haddadi</i> | – | – | – | 0% (<i>n</i> = 1) | 0% (<i>n</i> = 2) | 0% (<i>n</i> = 2) |
| | <i>Hypsiboas exastis</i> | – | – | – | 0% (<i>n</i> = 4) | 0% (<i>n</i> = 1) | – |
| | <i>Hypsiboas freicanecae</i> | 50% (<i>n</i> = 8) | – | – | – | – | – |
| Hylodidae | <i>Crossodactylus dantei</i> | 16.66% (<i>n</i> = 6) | 14.28% (<i>n</i> = 7) | 0% (<i>n</i> = 4) | – | – | – |
| Odontophrynidae | <i>Proceratophrys renalis</i> | 10.71% (<i>n</i> = 28) | 3.57% (<i>n</i> = 56) | 4.76% (<i>n</i> = 84) | – | – | – |

variable. Residuals of these regressions were used as the independent variables in the regression models. The dependent variable (*Bd* prevalence) and the independent variables (canopy cover, dissolved oxygen, pH, water temperature, water depth, and flow rate) were used in a multiple regression model (performed only using data from the species with the largest sample size) that examined the influence of each variable on *Bd* prevalence within each stream. The best model was selected with the use of stepwise analysis with Akaike information criteria (AIC). Multicollinearity structure among predictive variables was analyzed with the use of the variance inflation factors (VIF). Variables with a VIF >3 were removed from the model to avoid problems associated with multicollinearity (Zuur et al. 2010). All statistical tests were performed using R (v3.1.1, R Development Core Team 2014).

RESULTS

Stream Tadpole Assemblages and *Bd* Prevalence

We collected tadpoles from seven species and three families. Hylid frogs were the most diverse (five species) and abundant (dry season *n* = 318; rainy season *n* = 476), and Odontophrynidae (dry season *n* = 28; rainy season *n* = 140) and Hylodidae (dry season *n* = 6; rainy season *n* = 11) were each represented by one species (Table 1). We screened all species for *Bd*; *Agalychnis sibilatus* and *P. renalis* tested positive for *Bd* during the dry and rainy seasons, whereas tadpoles of *Agalychnis granulosa* were *Bd* positive in only the rainy season (Table 1). We did not find dead or dying tadpoles in the streams. Nevertheless, *Bd* prevalence was higher in the rainy season (Fig. 3) and varied between seasons (KW test, $H = 18.24$, *df* = 2, $P < 0.001$, *n* = 176) and species (KW test, $H = 51.83$, *df* = 2, $P < 0.001$, *n* = 176).

Body Size and *Bd* Presence

Body size of the tadpoles was positively correlated with developmental class of tadpoles of *Agalychnis granulosa* ($r = 0.62$, $P < 0.001$), *Aplastodiscus sibilatus* ($r = 0.46$, $P < 0.001$), and *P. renalis* ($r = 0.66$, $P < 0.001$). The incidence of *Bd* infection increased with increasing tadpole body size in *Agalychnis granulosa* (Mann–Whitney test, $W = 422.5$, $P < 0.001$, *n* = 344) and *Aplastodiscus sibilatus* ($F = 89.14$, *df* = 1, $P < 0.001$, *n* = 432; Fig. 4). When classes were included as a factor in the analysis to control for developmental stage, there was no interaction between *Bd* prevalence and classes (I, II, III). Thus, larger individuals of

Agalychnis granulosa ($B = 0.84$, $P < 0.001$) and *Aplastodiscus sibilatus* ($B = 0.47$, $P < 0.001$) have a higher probability to be *Bd* infected, independent of the developmental stage. Furthermore, in *P. renalis*, incidence of *Bd* among tadpoles did not differ in relation to body size ($W = 642$, $P = 0.60$, *n* = 171). We observed a greater proportion of loss of the oral structures in the infected tadpoles compared with uninfected tadpoles ($P < 0.001$).

Influence of Habitat Variables in *Aplastodiscus sibilatus*

The flow rate, temperature, depth, and pH of the water varied between streams and seasons (Table 2). Water flow ($F = 4.74$, *df* = 12, $P < 0.001$, rainy peak season), temperature ($F = 85.35$, *df* = 12, $P < 0.001$, dry season), and depth ($F = 4.35$, *df* = 12, $P = 0.001$, rainy season) were higher in the Bananeira stream, whereas pH ($F = 75.58$, *df* = 12, $P < 0.001$, dry and rainy seasons) was higher in the Cardozo stream. Correlation analyses showed that the density of *Aplastodiscus sibilatus* tadpoles decreased with increasing depth in both streams (Bananeira, $r = -0.63$, $P < 0.001$; Cardozo, $r = -0.60$, $P < 0.001$). Canopy cover and pH, temperature, depth, and flow rate of the water were significant predictors of *Bd* prevalence in *Aplastodiscus sibilatus* (Table 3). At the Cardozo stream, *Bd* prevalence was associated with low pH values in the dry season ($\bar{X} = 6.35$,

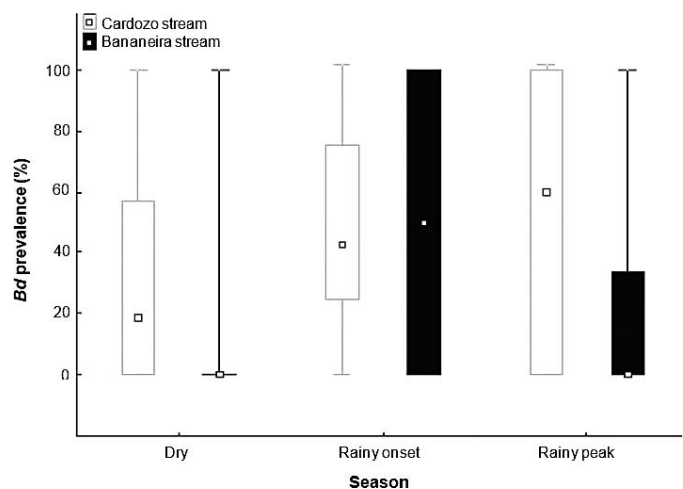


FIG. 3.—*Batrachochytrium dendrobatidis* prevalence (infected tadpoles as a proportion of total collected) between seasons in the Cardozo and Bananeira streams. Box-whisker plots depict median values, quartiles, and ranges. Prevalence of *Bd* varied between streams ($P = 0.002$), and between seasons in Bananeira stream ($P < 0.001$).

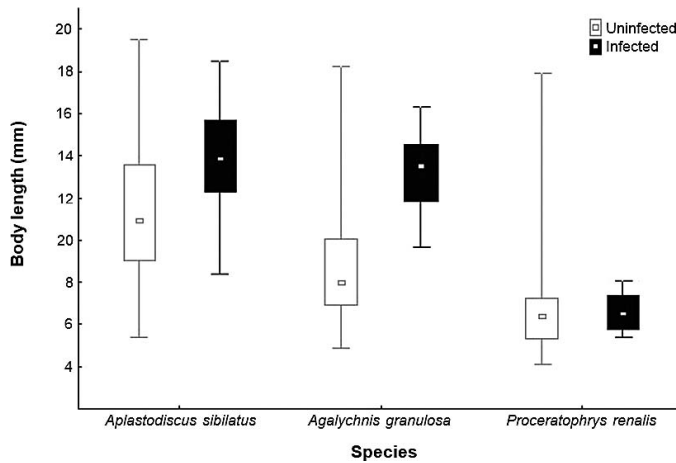


FIG. 4.—Body lengths of infected and uninfected tadpoles of *Aplastodiscus sibilatus* (infected $n = 169$; uninfected $n = 263$), *Agalychnis granulosa* (infected $n = 15$; uninfected $n = 330$), and *Proceratophrys renalis* (infected $n = 10$; uninfected $n = 159$) in the Cardozo and Bananeira streams. Box-whisker plots depict median values, quartiles, and ranges. Values for body size differed as function *Bd* presence in tadpoles of *Aplastodiscus sibilatus* ($P < 0.001$) and *Agalychnis granulosa* ($P < 0.001$), but not for *P. renalis* ($P = 0.60$).

$P = 0.01$) and onset of the rainy season ($\bar{X} = 5.95$, $P = 0.03$). Moreover, in the peak of the rainy season, *Bd* prevalence was associated with a high percentage of canopy cover ($\bar{X} = 80.77\%$, $P = 0.005$) and shallow water ($\bar{X} = 17.62$ cm, $P = 0.04$). In the Bananeira stream, *Bd* prevalence was associated with a high percentage of canopy cover (onset of the rainy season, $\bar{X} = 80\%$, $P < 0.001$), shallow water (onset of rainy season, $\bar{X} = 19.41$ cm, $P = 0.02$; peak of rainy season, $\bar{X} = 18.39$ cm, $P = 0.01$), warmer water (onset of rainy season, $\bar{X} = 21.9^\circ\text{C}$, $P = 0.02$) and low flow rate (peak of rainy season, $\bar{X} = 0.17$ m/s, $P = 0.001$).

DISCUSSION

Stream Tadpole Assemblages and *Bd* Prevalence

Our results show that during the rainy season *Agalychnis granulosa*, *Aplastodiscus sibilatus*, and *P. renalis* experience high rates of *Bd* incidence. During the dry season, however, the *Bd* prevalence in *Aplastodiscus sibilatus* is low, and the infection rate apparently dissipates in *Agalychnis granulosa*. Specific characteristics or variances in life-history traits can also be related to species' susceptibility to infections (Kriger and Hero 2007a; Bancroft et al. 2011). As examples, females of *Aplastodiscus* spp. lay eggs in subterranean chambers in the margins of lotic water bodies (Faivovich et al. 2005; Haddad et al. 2013), and females of *Proceratophrys* spp. lay eggs on water surface and submerged rocks (Dias et al.

2013). In contrast, females of *Agalychnis* spp. lay eggs on a variety of substrates such as aquatic vegetation, tree trunks and leaves, or bromeliads (Haddad et al. 2013; Vilela et al. 2014). As a result, *Agalychnis granulosa* tadpoles have a lower probability of acquiring a *Bd* infection because the larvae can develop in microhabitats where conditions are inappropriate for *Bd* growth (Bancroft et al. 2011).

Aplastodiscus sibilatus and *P. renalis* are likely to experience a similar risk of infection during the early stages of development because of their dependence on water as breeding habitat. We found a high *Bd* prevalence in *Aplastodiscus sibilatus*, and a low prevalence in *P. renalis*. Higher levels of the *Bd* prevalence might be correlated with an increase in tadpole social behavior (Rowley and Alford 2007; Venesky et al. 2011). In fact, although we observed aggregations in both *Aplastodiscus sibilatus* and *P. renalis* (usually under leaf-litter), the proximity was closer, and rate of contact was higher, among *Aplastodiscus sibilatus* tadpoles. The high sociability and benthic ecology of this species might facilitate *Bd* transmission between infected and uninfected tadpoles, increasing the *Bd* prevalence in *Aplastodiscus sibilatus* (the highest rate of the three species having adequate sample sizes; Rachowicz and Briggs 2007; Rowley and Alford 2007; Raffel et al. 2010; Venesky et al. 2011).

In the case of *Agalychnis granulosa*, only a low percentage of tadpoles were infected during the rainy season. In contrast to the other species, *Agalychnis granulosa* tadpoles appeared to avoid contact with conspecific or heterospecific tadpoles, instead remaining in the middle of the water column. This behavior could explain the low *Bd* prevalence in this host species (see Becker et al. 2014). Moreover, mid-column and surface water temperatures were higher than those at the bottom of the stream channel (the latter of which were within the optimal range for *Bd* growth; Poole and Berman 2001), possibly allowing tadpoles to clear any acquired infection (Woodhams et al. 2003; Geiger et al. 2011), or reducing the chance of infection by avoiding contact with other infected individuals (Venesky et al. 2011). Here, and in the southern region of the Atlantic forest (James et al. 2015), the *Bd*-GPL strain was found in several anuran populations. Although this strain has been considered as the more virulent (Fisher et al. 2009; Farrer et al. 2011), the *Bd*-GPL strain apparently does not have lethal effects for the anuran species in the Atlantic forest. Differences in the *Bd*-GPL virulence might also be attributed to geographic variations (Smith et al. 2007). Based on our results, we suggest that *Agalychnis granulosa*, *Aplastodiscus sibilatus*, and *P. renalis* could be acting as *Bd* reservoirs in streams. Because infected tadpoles do not seem to develop the disease despite carrying *Bd* zoospores, they can infect other individuals or species

TABLE 2.—Environmental variables measured during dry and rainy (onset, peak) seasons in two Atlantic forest streams. Values are shown as mean ± 1 SD (min–max).

| Stream | Season | Variable | | | | |
|-----------|-------------|-----------------------------|-----------------------------|--|--------------------------|-----------------------------|
| | | Water depth (cm) | Water flow (m/s) | Water temperature ($^\circ\text{C}$) | Canopy cover | pH |
| Cardozo | Dry | 15.20 \pm 6.98 (7–34) | 0.06 \pm 0.05 (0–0.20) | 21.95 \pm 0.38 (21.5–22.80) | 80.45 \pm 7.22 (75–90) | 6.40 \pm 0.53 (5.5–7.45) |
| | Rainy onset | 18.96 \pm 8.97 (6–39) | 0.08 \pm 0.06 (0.02–0.20) | 22.66 \pm 0.27 (22.3–23.20) | 81.90 \pm 8.13 (70–90) | 5.91 \pm 0.29 (5.41–6.51) |
| | Rainy peak | 18.31 \pm 8.22 (8–40) | 0.10 \pm 0.06 (0.02–0.20) | 21.72 \pm 0.13 (21.4–21.90) | 82.72 \pm 7.02 (80–90) | 5.31 \pm 0.17 (5.03–5.98) |
| Bananeira | Dry | 14.95 \pm 5.32 (8–30) | 0.03 \pm 0.03 (0–0.10) | 23.02 \pm 0.39 (22.28–23.57) | 79.54 \pm 7.85 (70–90) | 4.62 \pm 0.55 (3.96–5.92) |
| | Rainy onset | 21.87 \pm 6.56 (13–34.50) | 0.08 \pm 0.05 (0.02–0.20) | 21.86 \pm 0.31 (21–22.20) | 81.30 \pm 8.14 (70–90) | 4.49 \pm 0.37 (4.11–5.42) |
| | Rainy peak | 21.04 \pm 5.04 (14–31) | 0.13 \pm 0.11 (0.02–0.50) | 21.56 \pm 0.13 (21.16–21.78) | 81.73 \pm 7.16 (75–90) | 4.42 \pm 0.48 (3.32–5.70) |

TABLE 3.—Summary of multiple comparison analyses for overall effects of the environmental variables on *Batrachochytrium dendrobatidis* prevalence in *Aplastodiscus sibilatus*, as a function of stream and season. Models were obtained with the use of a forward stepwise selection process. Bold values indicate significant results ($\alpha < 0.05$). Spaces marked with an asterisk (*) indicate variables with a variance inflation factor >3 , which were excluded from the model.

| Source of variation | Cardozo stream | | | Bananeira stream | | |
|-----------------------------------|---------------------|----------------|--------------|---------------------|----------------|------------------|
| | Estimate \pm 1 SE | <i>t</i> value | <i>P</i> | Estimate \pm 1 SE | <i>t</i> value | <i>P</i> |
| Dry season | | | | | | |
| Intercept | 14.03 \pm 7.23 | 1.94 | 0.07 | 53.53 \pm 56.49 | 0.95 | 0.36 |
| Canopy cover | * | * | * | -0.16 \pm 0.23 | -0.68 | 0.51 |
| Water flow (m/s) | 9.82 \pm 9.14 | 1.08 | 0.30 | -29.12 \pm 26.16 | -1.11 | 0.29 |
| Water temperature ($^{\circ}$ C) | * | * | * | -2.15 \pm 2.34 | -0.92 | 0.38 |
| Water depth (cm) | * | * | * | -0.29 \pm 0.24 | -1.22 | 0.25 |
| Dissolved oxygen (%) | 0.21 \pm 0.30 | 0.71 | 0.49 | 0.19 \pm 1.04 | 0.19 | 0.86 |
| pH | -2.55 \pm 0.92 | -2.78 | 0.01 | -1.13 \pm 1.49 | -0.76 | 0.46 |
| Onset of rainy season | | | | | | |
| Intercept | -48.21 \pm 19.23 | -2.51 | 0.03 | -41.80 \pm 22.60 | -1.85 | 0.09 |
| Canopy cover | * | * | * | 0.26 \pm 0.04 | -7.48 | <0.001 |
| Water flow (m/s) | * | * | * | 7.80 \pm 6.29 | 1.24 | 0.24 |
| Water temperature ($^{\circ}$ C) | * | * | * | 2.73 \pm 1.04 | 2.63 | 0.02 |
| Water depth (cm) | 0.25 \pm 0.14 | 1.82 | 0.09 | 0.12 \pm 0.05 | 2.66 | 0.02 |
| Dissolved oxygen (%) | * | * | * | * | * | * |
| pH | -7.30 \pm 3.18 | 2.30 | 0.03 | * | * | * |
| Peak of rainy season | | | | | | |
| Intercept | -19.81 \pm 6.25 | -3.17 | 0.01 | -6.95 \pm 2.48 | -2.80 | 0.02 |
| Canopy cover | 0.24 \pm 0.08 | 3.18 | 0.005 | * | * | * |
| Water flow (m/s) | * | * | * | 19.40 \pm 4.53 | 4.28 | 0.001 |
| Water temperature ($^{\circ}$ C) | * | * | * | * | * | * |
| Water depth (cm) | -0.17 \pm 0.08 | -2.17 | 0.04 | 0.37 \pm 0.13 | 2.87 | 0.01 |
| Dissolved oxygen (%) | * | * | * | * | * | * |
| pH | * | * | * | * | * | * |

within the stream (Rachowicz and Vredenburg 2004; Raffel et al. 2010; Catenazzi et al. 2013; Narayan et al. 2014).

Body Size and *Bd* Presence

We showed that *Bd* infection rates vary as a function of larval body size in *Agalychnis granulosa* and *Aplastodiscus sibilatus*, and that infected tadpoles experience great rates of loss of oral structures. We suggest that individuals with a larger body size have greater chances of becoming infected, as shown by previous studies (Smith et al. 2007; Symonds et al. 2007; Catenazzi et al. 2013; Vieira et al. 2013). This greater chance of infection in larger tadpoles probably occurs as a result of increased surface area (Rachowicz and Vredenburg 2004; Rowley and Alford 2007; Smith et al. 2007; Vieira et al. 2013) or behavioral changes such as behavioral fever (Woodhams et al. 2003). Another possible explanation is an alteration in host immune function, which can also affect incidence or transmission of infections (Altizer et al. 2006). Woodhams et al. (2007) reported that species with better adaptive immune responses had lower *Bd* infection intensity and higher survival rates. In contrast, species with suppressed immune systems are more susceptible to *Bd* infection (Ramsey et al. 2010). *Proceratophrys renalis* did not show any relationship between body sizes and infection rates, which indicates that tadpoles have the same probability of being infected with *Bd* at different developmental stages (similar to that observed in the stream-dweller, *Amietia hymenopus*; Smith et al. 2007).

Influence of Habitat Variables in *Aplastodiscus sibilatus*

In fast-flowing streams of Peru and South Africa, high *Bd* prevalence has been documented during the dry season, when water level and flow rate both decrease (Conradie et al. 2011; Catenazzi et al. 2013). Although *Bd* zoospores are aquatic and can swim long distances (2 cm) before encysting

(Piotrowski et al. 2004), an increase in water flow during rainy season could reduce contact between hosts and affect zoospore dispersal. Nonetheless, we found a high *Bd* prevalence in the rainy season, probably because the rate of flow in the Bananeira and Cardozo streams was not strong enough to affect zoospore mobility and consequently to reduce their chance of finding a host. Seasonal fluctuations of *Bd* infections might result from changes in environmental variables, and subsequently host traits (Berger et al. 2004; Kriger and Hero 2007b; Conradie et al. 2011; Ruggeri et al. 2015). Both temperature and pH affect the life-histories of amphibians and *Bd* (Ultsch et al. 1999; Piotrowski et al. 2004; Woodhams et al. 2008; Knapp et al. 2011). The temperatures and pH recorded in our study streams fell within the optimal range for *Bd* growth and persistence (Table 2; Piotrowski et al. 2004; Woodhams et al. 2008). We found a high *Bd* prevalence at low pH values, indicating that, in natural conditions, *Bd* can survive in broader pH ranges (4–9) than those reported in laboratory (6–7; Bosch et al. 2001; Piotrowski et al. 2004). Depth and canopy cover also influenced *Bd* prevalence, probably resulting from their negative relationship with temperature (Sparling 2010). Leaf litter at the bottom of the water column provides shade and might shelter the zoospores, thereby serving as a reservoir for this environmental pathogen (Rowley and Alford 2007; Raffel et al. 2010).

In the northern Atlantic forest, the climate and topography are low- to mid-elevation and characterized by rainy winters; in contrast with the southern and southeastern forests have higher elevations that experience cooler rainy summers (Carnaval et al. 2014). In Alagoas (northern forest), where the climate is classified as tropical, with dry summers and annual temperatures and rainfall are 22–26 $^{\circ}$ C and 1300–1600 mm, respectively (Alvares et al. 2013), we observed a higher *Bd* prevalence during the rainy season. Similarly in the

southern region, *Bd* prevalence is higher during winter (Ruggeri et al. 2015). There is a seasonal variation in *Bd* prevalence in the Atlantic forest (Ruggeri et al. 2015; this study), and there seems to be an infection pattern related to climatic changes. In the northern region, *Bd* infections appear to have a positive relationship with rainfall (Kriger et al. 2007), as the highest values for disease prevalence were observed at the onset of rains. In the southern region, *Bd* infections seem to have associated with environmental fluctuations (temperature, precipitation; Ruggeri et al. 2015). A similar pattern was also found in eastern Australia, where latitudinal variation in *Bd* prevalence was associated with rainfall and thermal regimes, and where climatic factors limited infection in the dry regions close to the equator (Kriger et al. 2007). The warmer and drier areas in the northern Atlantic forest (Alvares et al. 2013) are also less studied than the other regions. Data on *Bd* infection rates from these unsampled regions (between Minas Gerais and Ceará) are necessary to support our hypothesis that *Bd* prevalence and intensity are influenced by the wide latitudinal variation in the Atlantic forest.

Our findings have demonstrated a variation in the prevalence of *Bd* infection among tadpoles of syntopic frog species, even among those that are ecologically similar. Our data support previous results showing no lethal effects of *Bd* infection in wild tadpoles, even after loss of the oral structures (jaw sheath and tooth rows). This finding raises questions concerning the mechanisms of defense used by the larval, postmetamorphic, and adult stages against *Bd* infection (e.g., immune, behavioral response; Salla et al. 2015). Finally, whereas water temperature is typically identified as being the driver of *Bd* infection dynamics (Woodhams and Alford 2005; Ruggeri et al. 2015), we found that multiple environmental factors can influence the dynamics of *Bd* infections in populations that occupy specific microhabitats.

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