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ECOLOGY OF *BATRACHOCHYTRIUM DENDROBATIDIS* AND TADPOLE ASSEMBLAGES IN THE NORTHEASTERN ATLANTIC FOREST

Maceió – AL
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Dissertação apresentada ao Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos, Instituto de Ciências Biológicas e da Saúde da Universidade Federal de Alagoas, como requisito para obtenção do título de Mestre em CIÊNCIAS BIOLÓGICAS, área de concentração em Diversidade e ecologia de organismos tropicais

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ABSTRACT

Amphibians are very diverse and abundant in tropical ecosystems and they have been rapidly declining worldwide in the last decades. Declines have been associated mainly with habitat loss, climate alterations and diseases that have negative effects in the amphibian communities. In this study were evaluated the ecology of *Batrachochytrium dendrobatidis* on tadpoles in the Northeastern Atlantic forest and how biotic and abiotic descriptors are influencing tadpole assemblages. For the first goal we quantified *Bd* infection dynamics on tadpoles of two breeding hosts. *Bd* prevalence varied seasonally, and tadpoles of *Aplastodiscus sibilatus* and *Proceratophrys renalis* were *Bd* positive found in both seasons, while tadpoles of *Agalychnis granulosa* were *Bd* infected only during the rainy season. Larger individuals had a higher probability to be *Bd* positive independent of developmental stage. Besides, environmental factors such as canopy cover, stream flow, water temperature, stream depth, pH and host density were associated with the *Bd* prevalence. Both tadpoles and streams could be reservoirs of the pathogen, thus monitoring frog populations in these habitats is necessary to assess the impact of this pathogen in this biodiversity hotspot. For the second goal, were studied tadpole assemblages in temporary and permanent ponds to determine the variation on their composition and abundance in the ponds and to measure the influence of biotic and abiotic factors shaping. Tadpole species richness was explained by environmental and biological variables through General Regression Models. The relationships between the tadpole assemblages and predictors on their spatial variation were measured using a Canonical Correspondence Analysis (CCA). Tadpole richness differed between ponds, although abundance did not. Depth and water temperature were related to tadpole richness, while canopy cover, dissolved oxygen, richness of predators and water temperature were related to tadpole abundance. In the CCA analysis depth, dissolved oxygen, richness predator, canopy cover and water temperature explained the tadpole assemblage structure in permanent and temporary ponds.

Keywords: Amphibia. Lagoas state – Brazil. Disease ecology.

RESUMO

Os anfíbios são muito diversos e abundantes nos ecossistemas tropicais, no entanto têm declinado rapidamente em todo o mundo nas últimas décadas. Os declínios têm sido associados principalmente as alterações climáticas e doenças as quais têm efeitos negativos nas comunidades de anfíbios. Neste estudo, foi avaliada a ecologia do fungo *Batrachochytrium dendrobatidis* (*Bd*) nos girinos da Mata Atlântica nordestina e como fatores bióticos e abióticos estão influenciando as taxocenoses de girinos. Para o primeiro objetivo, a dinâmica da infecção do *Bd* em girinos de dois riachos foi avaliada. A prevalência de *Bd* variou sazonalmente, girinos infectados de *Aplastodiscus sibilatus* e *Proceratophrys renalis* foram encontrados em ambas as estações, enquanto girinos infectados de *Agalychnis granulosa* foram encontrados somente durante a estação chuvosa. Os indivíduos maiores apresentavam uma maior probabilidade de serem infectados, independentemente do seu estágio de desenvolvimento. Além disso, fatores ambientais, como a cobertura do dossel, velocidade da corrente, temperatura e profundidade da água, pH e densidade dos girinos foram associados com a prevalência do fungo. Ambos os girinos e os riachos poderiam estar atuando como reservatórios do fungo, assim, o monitoramento destas populações nesses habitats é fundamental para avaliar o impacto deste patógeno neste *hotspot*. Para o segundo objetivo, as taxocenoses de girinos em poças temporárias e permanentes foram analisadas, a fim de determinar a variação na composição e abundância e avaliar a influência dos fatores bióticos e abióticos na sua estruturação. A riqueza de espécies de girinos foi relacionada a variáveis ambientais e biológicas através dos modelos gerais de regressão. As relações entre as taxocenoses de girinos e seus preditores e sua variação espacial foram analisados com uma análise de correspondência canônica (CCA). A riqueza de girinos foi diferente entre as poças, porém a abundância não diferiu. A profundidade e temperatura da água foram relacionadas com a riqueza de girinos, enquanto a cobertura do dossel, oxigênio dissolvido, a riqueza de predadores e temperatura da água foram relacionadas com a abundância dos girinos. Na CCA, a profundidade, oxigênio dissolvido, a riqueza dos predadores, cobertura do dossel e temperatura da água explicou a estrutura das taxocenoses dos girinos nas poças permanentes e temporárias.

Palavras-chave: Amphibia. Estado de Alagoas – Brasil. Ecologia de doenças.

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1. GENERAL INTRODUCTION

Amphibians are a very diverse and abundant group in tropical ecosystems, where they play multiple functions that maintain and regulate ecological processes (Wells, 2007; Collins & Crump, 2009; Ruiz et al., *In press*). Thus their loss could have negative impacts for ecosystems functioning (Young et al., 2004). In the last decades, amphibians have been rapidly declining worldwide, becoming the more threatened taxa on the planet, with over 41% of species experiencing population declines or in the brink of extinction (Stuart et al., 2004; Bielby et al., 2008; Baillie et al., 2010). These declines have been associated mainly with habitat loss, diseases, climate change, and pollution (Mann et al., 2009). Climate alterations and diseases have negative effects in the structure and composition of anuran communities (Yanoviak, 2001; Parris, 2004; Strauß et al., 2013) at local as well as a global scales (Daszak et al., 2005; Pounds et al., 2006; Bielby et al., 2008; Catenazzi et al., 2010; Gallana et al., 2013; Loyola et al., 2014). Nonetheless, while in some areas amphibian declines have been associated with chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (Berger et al., 1998; 1999; Stuart et al., 2004; Lips et al., 2005; La Marca et al., 2005; Pounds et al., 2006; Skerratt et al., 2007; Catenazzi et al., 2010), in others, alterations on the physical habitat structure (hydroperiod, precipitation, temperature) at the breeding site have been pointed out as the main causes for their declines (Daszak et al., 2005; Becker et al., 2007).

In many areas of the world, baseline information on amphibian species abundances and distributions is not available, and consequently, it is not possible to assess the level of threat for most of these species (Stuart et al., 2004; Bielby et al., 2008). Therefore due to the high number of threatened amphibian species worldwide (Bielby et al., 2008), it is imperative to identify the factors as well as their synergic effects responsible for shaping the structure and composition of anuran communities (Shodi et al., 2008). Althouhg, in some areas chytridiomycosis leads to rapid species death (Australia, Berger et al. 1998; Costa Rica, Lips et al. 2003; Mexico, Lips et al. 2004; Panama, Lips et al. 1999; Spain, Bosch et al. 2001; United States, Vredenburg et al. 2010; Venezuela, La Marca et al. 2005), in other regions, *Bd* infection does not seem to have any effect on amphibian populations (Brazil, Rodriguez et al. 2014; Canada, Forzán et al. 2010; Cameroon, Doherty-Bone et al. 2013; Germany, Ohst et al. 2013). Therefore, evaluating the importance of environmental factors in population declines is necessary. Changes in rainfall patterns could alter hydroperiod dynamics causing changes in breeding habitats (like ponds) availability (Newton, 2007) and consequently in amphibian communities by disrupting their reproductive cycles (Daszak et al., 2005) or larvae development (Márquez-García et al., 2009). Particularly, natural assemblages of anuran larvae are good models to study the influence of environmental factors and diseases determining their structure and composition, because they are spatially restricted and are present in a wide variety of abiotic and biotic conditions (Ultsch, 1999).

This dissertation is divided in three chapters as follows; In the first chapter, new data were reported from poorly explored regions to extend current information on the distribution of *Bd* in the northern Atlantic forest region. In addition, was tested the hypothesis that *Bd* is a generalist pathogen in this forest. In the second chapter, was evaluated the ecology of infection of the fungus *Batrachochytrium denbrobatidis* in stream tadpoles. Here the *Bd* infection and seasonal variation of *Bd*-prevalence on the streams of the Northern Atlantic forest were assessed. Furthermore, were quantified environmental and life history variables to determine which of those could be affecting *Bd* prevalence. In the third chapter, the variation on the diversity of tadpoles and their predators from temporary and permanent ponds was assessed to examine how biotic and abiotic environmental variables influence the tadpole assemblage in these lentic systems. All chapters were conducted in the northern biogeographic region of the Atlantic forest, Alagoas state, Brazil. Furthermore, in the chapter one were also included data from Amazonian forest.

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2. LITERATURE REVIEW

2.1 Amphibian population declines

Amphibians are one of the most abundant and diverse vertebrate taxa in tropical ecosystems (Pough et al., 2004; Wells, 2007), where they play multiple functions maintaining and regulating ecological processes (Wells, 2007; Wake & Vredenburg, 2008; Collins & Crump, 2009; Ruiz et al., *In press*). The complex amphibian life-cycle involves generally aquatic larvae and terrestrial adults (Wake & Vredenburg, 2008), which exposes them to multiple threats (Pounds et al., 2006; Becker et al., 2007; Blaustein et al., 2007; Mann et al., 2009). Global assessments have suggested that about 41% of the species of this group have suffered declines and/or extinctions (Stuart et al., 2004; 2008; Bielby et al., 2008; Baillie et al., 2010; IUCN, 2014). Evidence indicates that declines began in the past decades in fast pace (Houlahan et al., 2000; Wake & Vredenburg, 2008), being more severe in tropical areas, such as Australia (McDonald & Alford, 1999), Puerto Rico (Burrowes et al., 2004), Mexico (Lips et al., 2004), Costa Rica (Pounds et al., 1997; Lips et al., 1998; 2003), Panama (Lips et al., 1999; 2003; 2005a), Venezuela (La Marca et al., 2005), Colombia (Lynch & Grant, 1998), Ecuador (Ron et al., 2003) and Brazil (Heyer et al., 1988; Eterovick et al., 2005), than in other regions (Stuart et al., 2004; Baillie et al., 2010).

Researchers argue that more than one factor may be determining the decline of amphibian populations (Young et al., 2004; Blaustein et al., 2007; Mann et al., 2009). However, complex synergistic interactions between factors such as alteration and loss of habitat, pollution, infectious disease, climate change, introduced species and human disturbance increase the risk of threat to this taxa (Young et al., 2001; 2004; Lips et al., 2005b; La Marca et al., 2005; Blaustein et al., 2007; Sodhi et al., 2008). While habitat alteration and deforestation can be directly linked to some declines (Tarvin et al., 2014), numerous disappearances of populations from high altitudes and small geographic ranges were associated in the past decade with susceptibility to the pathogen *Batrachochytrium dendrobatidis,* which cause chytridiomycosis, a fatal disease in amphibians (hereafter as *Bd,* Berger et al., 1998; 1999; Lips, 1999; Longcore et al., 1999; Young et al., 2001; Daszak et al., 2003; Burrowes et al., 2004).

2.2 Chytridiomycosis in amphibians

Since the first reports of chytridiomycosis in the late 90s (Berger et al., 1998; 1999; Longcore et al., 1999), wild amphibians have been found with *Bd* infection in Australia, Europe, Africa, North and Central America, where infection have been associated with mass mortalities and declines (Daszak et al., 2003; Brem & Lips, 2008; Baillie et al., 2010). This disease is caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Berger et al., 1999; Longcore et al., 1999). This fugus is found in aquatic systems primarily as parasites of algae, and in soil, or as saprotrophs of pollen (Letcher et al., 2006). However, it also degrades keratin and is the first member of the phylum Chytridiomycota to be recognized as a parasite of vertebrates, especially amphibians (Berger et al., 1998). Recentely, a second chytrid species, *Batrachochytrium salamandrivorans* was isolated from infected populations of European fire salamanders (*Salamandra salamandra*) (Martel et al., 2013). However, although this fungus is highly pathogenic and currently is causing biodiversity loss in naïve European amphibian populations, is restricted to salamanders and newts (Martel et al., 2014).

Batrachochytrium dendrobatidis has two main stages in their life cycle: *the motile*, waterborne, short-lived zoospore for dispersal, and *the stationary*, monocentric thallus, which develops into a zoosporangium for asexual amplification (Berger et al., 2005). Zoosporangia live inside epidermal cells, initially parasitizing cells a few layers deep, the flagellum is thus reabsorbed to initiate the formation of rhizoids and growth of the thallus which ripen between 4-5 days; thereafter zoospores formed by mitotic division are discharged from the zoosporangia when temperature and humidity conditions are appropriate initiating the cycle again (Berger et al., 1998).

The *Bd* fungus uses keratin as a nutrient source (although it is not the only one) for growth and development (Voyles et al., 2011). In most adults and postmetamorphic amphibians, this protein is present in the *stratum corneum* (Wells, 2007), therefore *Bd* zoospores can be found in the *stratum granulosum* and *corneum* in the superficial epidermis (Berger et al., 1998; 2005). In tadpoles, zoospores infect the oral apparatus, the only region containing keratin (Altig, 2007, Fellers et al., 2001). Although, *Bd* zoospores can infect multiple anuran species, host susceptibility vary widely among species (Woodhams & Alford, 2005). Several populations of adults and metamorphs have experienced massive mortality events, while tadpoles are apparently not affected by the pathogen (Berger et al., 1998; 1999; McDonald & Alford, 1999; Burrowes et al., 2004; Puschendorf et al., 2006; Schloegel et al., 2006; Smith et al., 2007; Conradie et al., 2011; Catenazzi et al., 2013). Fungal zoospores are able to kill their amphibian adult hosts, because *Bd* disrupts the normal cutaneous transport, causing an osmotic imbalance (Voyles et al., 2007). In infected individuals, more than 50% of the electrolyte transport across the epidermis is inhibited and both plasma sodium and potassium concentrations are reduced between 20-50%, resulting in an asystolic cardiac arrest and death (Voyles et al., 2009).

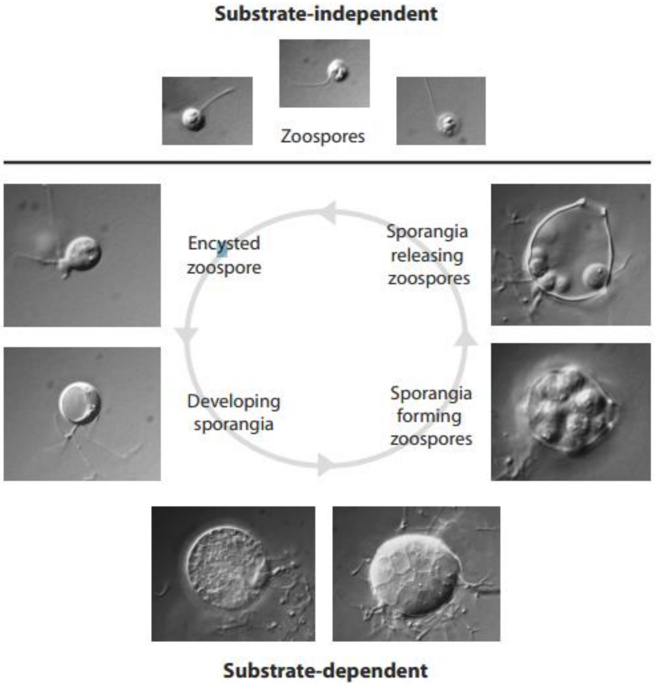

Figure 1. The life cycle of *Batrachochytrium dendrobatidis*. Adapted from Rosenblum et al. (2008).

Genetic evidence revealed three deeply divergent lineages of *Bd*, including the global panzootic lineage (GPL), a *Bd* isolate from Brazil (which seems to contain ancestral variation endemic to the Atlantic forest) and a *Bd*-Brazil hybrid (Farrer et al., 2011; Rosenblum et al., 2013; Rodriguez et al., 2014). This evidence also suggests that some *Bd* lineages are more ancient than previously thought (Rosenblum et al., 2013; Rodriguez et al., 2014), contrasting the “novel pathogen hypothesis” or “spreading pathogen hypothesis” that proposes a distributional spread of an emerging pathogen highly transmissible and virulent (Rachowicz et al., 2005; Skerratt et al., 2007; Fiser et al., 2009). Another hypothesis, the "endemic pathogen" argues that an environmental change altered amphibians-*Bd* dynamic converting the fungus in a highly virulent and transmissible pathogen (Rachowicz et al., 2005; Skerratt et al., 2007). This hypothesis has received support from studies revealing the complex evolutionary history of *Bd.* Although it is still missing the specific mechanisms that may have led to shifts in their virulence in systems where it is present (Rosenblum et al., 2013; Rodriguez et al., 2014).

2.3 Host-pathogen interactions

Host–pathogen interactions are amongst the most plastic and dynamic systems in nature (Keesing et al., 2006). Generally, pathogen induces alterations of host physiology, morphology, and behavior (Gómez-Díaz et al., 2012). Theoretical studies suggest that changes in abundance and behavior of pathogen can strongly influence disease prevalence and the potential for disease emergence across multiple host species (Holt, 2008). Besides, the availability of multiple hosts is predicted to decrease the likelihood of pathogen disappearance within a population (Power & Flecker, 2008). Pathogens can infect hosts by different routes, such as direct contact (physical contact or close proximity), indirect contact (contamination of food, contact with environmental reservoirs, and contact with free-living infectious stage), and vector-borne (via arthropods biting, including mechanical transmission) (Woolhouse, et al., 2001). Because of its low host specificity, *Bd* can infect a broad range of amphibian species (Daszak et al., 2003; Fisher et al., 2009; Olson et al., 2013). Species using water bodies for reproduction, with aquatic larvae and inhabiting mid and high elevations are at greatest risk (Lips et al., 2005a,b; La Marca et al., 2005; Bancroft et al., 2011), because aquatic phase of the fungus that have zoospores with flagella is easily spread in water (Berger et al., 2005). 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), suggesting differences in the host-pathogen interaction (Briggs et al., 2010; Woolhouse, et al., 2001). Within a population, the infection can persist in host as tadpoles, which are usually less susceptible to the pathogen and therefore could act as reservoirs (Woolhouse et al., 2001). Particularly, tadpoles are an important reservoir for the fungus in aquatic system such as streams (Woodhams & Alford, 2005; Smith et al., 2007; Conradie et al., 2011; Catenazzi et al., 2013) or ponds (Bosch et al., 2001; Raffel et al., 2010; Wolff et al., 2012), where they are often present throughout the year (Rodrigues et al., 2010).

Tadpoles can also transmit infection to uninfected adults, other tadpoles or postmetamorphic individuals (Rachowicz & Vredenburg, 2004). As tadpoles and adults tend to congregate in permanent or temporary waterbodies during certain periods (dry or breeding season), the risk of *Bd* transmission within and between life stages increases (Kriger & Hero, 2007; Russell et al., 2010; Catenazzi et al., 2013). Postmetamorphic individuals appear to be more susceptible to mortality (Marantelli et al., 2004; Blaustein et al., 2005), therefore maintaining the infection through development or becoming infected from less-developed conspecific tadpoles may have critical effects on species survival (Berger et al., 1998; Rachowicz & Vredenburg, 2004). These aspects of *Bd* host-pathogen ecology are important in understanding tadpole-adult disease dynamics (Kilpatrick et al., 2010).

2.4 Environmental variables and prevalence of *Bd*

Prevalence of chytridiomycosis in amphibians is affected by temperature and humidity, factors that vary with site, elevation, season, and through time (Woodhams & Alford, 2005; Fisher et al., 2009). Higher temperatures in low altitudes are associated with lower infection prevalence (Rödder et al., 2008). In this sense, many terrestrial habitats are too warm or to dry for *Bd* survival (Ron, 2005; Rödder et al., 2010) and seasonal or even daily temperature fluctuations can affect infection prevalence (Woodhams & Alford, 2005; Kriger & Hero, 2007). However, lentic waterbodies are typically cooler and more thermally stable (Brönmark & Hansson, 2005), providing both *Bd* and tadpoles refuge and protection, and thus contributing for their survival (Peterson et al., 2007; Kriger & Hero 2007; Raffel et al., 2010).

In some regions, *Bd* infection in amphibians inhabiting ponds is influenced by leaf litter, vegetation cover and temperature, likely because vegetation cover provides shade that can lower the temperature in the pond or perhaps because leaf litter might provide substrates for environmental growth of the fungus (Raffel et al., 2010). Conversely, at a large spatial scale habitat or vegetation loss is negatively associated with prevalence of *Bd* infection, because in altered habitats microclimatic conditions for the fungus are suboptimal (Becker & Zamudio, 2011).

2.5 *Bd* in Brazilian Atlantic forest

Bd is common in the Neotropics where several areas have favorable climatic conditions for its establishment - particularly temperate and tropical forests at altitudes above 1,000 m (Bosch et al., 2001; Ron, 2005; Fisher et al., 2009; Rödder, 2010; Gründler et al., 2012). The Brazilian Atlantic forest has highly heterogeneous environmental conditions (Ribeiro et al., 2009), a wide range of elevations (0-2,700 m) and diverse forest composition (Pinto & Brito 2003). This widespread heterogeneity has undoubtedly contributed to an impressively high level of amphibian species diversity (540 species) and endemism (80%) (Haddad et al., 2013). In the Atlantic forest, *Bd* studies began in 2005 with the first record of the fungus in tadpoles of *Hylodes magalhaesi* (Carnaval et al., 2005). A year later, morphological and histological screenings conducted on preserved specimens of four anuran families showed that the fungus was widely distributed in this forest, with 22 species (tadpoles, young and adults) infected and the oldest record dating from 1981 (Carnaval et al., 2006). Authors also claimed that although anuran populations from lowland (<100m) and mid to high elevations (500-2,400m) were infected there was no evidence that these species were declining. In fact, *Hylodes magalhaesi* populations were monitored by five years and no apparent decline was seen (Carnaval et al., 2006; Toledo et al., 2006a,b). In the last years, the list of infected host species in the Atlantic forest has increased to 130 species belonging to fourteen families (Aromobatidae, Brachycephalidae, Bufonidae, Centrolenidae, Ceratophrydae, Craugastoridae, Cycloramphidae, Hemiphractidae, Hylidae, Hylodidae, Leptodactylidae, Mycrohylidae, Odontophrynidae, Ranidae), being Hylidae and Hylodidae the families with the highest number of infected species; 55 (11 genera) and 23 (3 genera) respectively (Valencia-Aguilar et al., *in press*).

Recently, it was found that the pathogen has been present in the southern portion of Atlantic forest since 1897 (more than 100 years), where two lineages; *Bd*-Brazil (an endemic lineage) and the Global Pandemic Lineage (*Bd*GPL) are present in terrestrial and aquatic habitats (lentic and lotic) apparently without epizootic events on anuran populations (Rodriguez et al., 2014). In contrast, in regions such as Bahia and Permanbuco in the northern part of this biome only few reports of infection have been done (Carnaval et al., 2006; Lisboa et al., 2013; Valencia-Aguilar et al., *in press;* this study) and so far only the GLP strain has been found in this area (Toledo, personal communication). The altitudinal and latitudinal variations throughout the Atlantic forest extension cause differences in climatic regimes and in plant species composition (Câmara, 2003; Carnaval et al., 2014). Thus, although the dynamics of *Bd* are completely unknown, it may differ in such heterogeneous environment such as seen in the Atlantic forest extension. In other words, the dynamics of *Bd* may be different in south (Araucaria, Interior and Serra do Mar sub-regions) when compared to the northern portion (Bahia and Penambuco subregios) of the biome.

2.6 Tadpole assemblages and environmental factors

Many environmental, physiological and species-specific traits seem to influence *Bd* prevalence, affecting consequently anuran communities (Daszak et al., 2003; La Marca et al., 2005; Skerrat et al., 2007). However, community composition can also be influenced by interactions between organisms and their local environment, including immediate neighbors (Daszak et al., 2005; Agrawal et al., 2007). The spatial distribution of organisms may vary from aggregated (clustered) through a random pattern to the regular (uniform) case (Dray et al. 2012). Species spatial distributions are the result of population demography, behavioral traits, and species interactions in spatially heterogeneous environmental conditions. The presence and abundance of individual species vary through space in a nonrandom way, displaying spatial partitioning. Hence, the composition of species assemblages is an integrative response and its variability can be explained by the complex interplay among several structuring factors (Dray et al. 2012). Most anuran species require aquatic environments to complete their life-cycle and are inhabitants of streams, wetland, ponds or larger bodies of water (Duellman & Trueb, 1994; Wells, 2007). These water bodies may differ regarding temperature, dissolved oxygen, pH, canopy cover and so on (Sparling, 2010), characteristics that can affect survival, growth, maturation, physical development and consequentely viability of amphibian populations (Wells, 2007).

2.6.1 Temperature

Among the physical characteristics in the aquatic environment, temperature has probably the most dramatic effect on the physiology, ecology and behavior of anuran larvae (Ultsch et al., 1999). Anurans are ectothermic and have limited ability to regulate their body temperature and are greatly affected by the temperature on their surrounding environment. Water temperature, therefore, is extremely important by affecting metabolic rates, other physiological processess and behaviors. In general, between 10°C and 40°C, each 10°C increase in the ambient temperature, metabolism increases by 1.4-2.4 times (Rome et al., 1992). Higher metabolic rates require greater oxygen; however, oxygen concentrations decrease as water temperature increase. At high temperatures, above 30°C-35°C for some species but at 25°C-30°C for less tolerant ones, thermal stress can result in reduced mobility, abnormally high heart rates, and eventually death (Wells, 2007).

2.6.2 pH

For most aquatic organisms, pH ranges of 6.0-7.5 are generally considered to be circumneutral or within a range that should present no harm to them (Sparling, 2010). Sensitivity to low pH varies throughout the life stages, population and species levels

(Ultsch et al., 1999; Wells, 2007). Tadpoles are more tolerant to low pH than embryos (Freda, 1986; Pehek, 1995). In some species, embryonic development may cease entirely at pH values below 4.5, while at higher pH values (~4.5-5.0) development continues but hatching is reduced (Dunson & Connell, 1982). The primary mechanism of toxicity due to low pH is interference with ion transport (Ultsch et al., 1999; Wells, 2007). Other effects include debilitated immune systems (Brodkin et al., 2003), an inability of embryos to hatch, reduced growth, and delayed metamorphosis. However, tadpoles living in acidic environments may acquire tolerance to low pH and consequently, display higher tolerance to acidic pH than those inhabiting circumneutral waters (Andren et al., 1989).

2.6.3 Dissolved oxygen

Oxygen concentrations can vary widely through the course of a day, especially in warm, eutrophic bodies of water (Sparling, 2010). In ponds, a major source of oxygen comes from photosynthesis by algae and other green plants. Because photosynthesis is driven by water temperature and sunlight, dissolved oxygen concentrations are frequently lowest at dawn, increase several-fold during the course of the day, reach maximum concentrations in mid-afternoon, and decrease sharply during the night due to biological oxygen demand. In lotic conditions, especially in shallow headwater streams, dissolved oxygen concentrations are relatively uniform throughout the water column (Sparling, 2010). In lentic environments, in contrast, oxygen concentrations can fluctuate or persist at low concentrations, and there is often a declining gradient from the surface (where mixing with air is maximal) to the bottom (where anaerobic decay is greatest).

2.6.4 Canopy cover

Canopy illumination changes with the sun´s position and cloud cover. In the wet equatorial tropics, total monthly sunlight can vary by 50% between could-free and overcast months (Newton, 2007). Within rainforest there is a strong vertical gradient in light, varying from full daylight above the canopy to sometimes below 1% daylight on the forest floor (Ghazoul & Sheil, 2010). Predictability also varies: while the upper canopy is guaranteed high light, the shaded understory may experience occasional periods of strong sunlight as sunflecks pepper the forest floor, or may be suddenly opened by a tree fall with longer lasting impact on light conditions (Ghazoul & Sheil, 2010).

Forest cover influences many abiotic and biotic characteristics of ponds and these traits can influence the vital rates of tadpoles (Hawley, 2010). Compared to open habitats, rainforests provided cooler, more humid, darker, and in some aspects more varied and dynamic environments (Newton, 2007). Below the canopy, weather and vegetation interaction causes significant microclimatic variation in both time and space. During the day, canopy foliage often reaches temperatures several degrees higher than environment. Temperature declines and humidity increases with depth beneath the canopy. At night the understory becomes fractionally warmer than the canopy and overall climatic differences are less marked (Ghazoul & Sheil, 2010). Rates of tadpole growth and development tend to be faster at warmer water temperatures (Ultsch et al. 1999; Duellman & Trueb, 1994). In closed canopy, dissolved oxygen concentrations in ponds are lower compared with open canopy ponds, due to decreased in the primary productivity which is high in open canopy ponds (Sparling, 2010). Generally, the diversity and abundance of tadpoles in close canopy ponds is lower than in open canopy ponds, because the low concentrations of dissolved oxygen, which can reduce the growth and survival rates of larvae (Schiesari, 2006). Furthermore, nutrient availability in open-canopy ponds is higher and offer a better nutritional quality for some anuran tadpoles (Schiesari, 2006). This is mainly due to the high photosynthetic rate of periphyton, a common source of food for tadpoles (Ultsch et al. 1999; Wells, 2007).

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3 The chytrid fungus acts as a generalist pathogen that infects species-rich amphibian families in Brazilian rainforests

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Running page head: *Bd* as generalist pathogen in Brazil

ABSTRACT

The fungus *Batrachochytrium dendrobatidis* (*Bd*) is one of the main causes of declines in amphibian populations. This fungus is a generalist pathogen because it infects several species and spreads rapidly in the wild. To date, *Bd* has been detected in more than 100 anuran species in Brazil, mostly in the southern portion of the Atlantic forest. Here, we have reported survey data from some poorly explored regions; these data considerably extend current information on the distribution of *Bd* in the northern Atlantic forest region. In addition, we tested the hypothesis that *Bd* is a generalist pathogen in this biome. We also report the first positive record for *Bd* positive in an anuran caught in the wild in Amazonia. In total, we screened 27 anuran species (90 individuals) for *Bd*, 81.5% (22 species, 39 individuals) of which showed positive results. All samples collected in Bahia (two individuals), Pernambuco (three individuals), Pará (one individual), and Minas Gerais (one individual) showed positive results for *Bd.* We found a positive correlation between anuran richness per family and the number of infected species in the Atlantic forest, supporting previous observations that *Bd* lacks strong host specificity; of 38% of the anuran species in the Atlantic forest that were tested for *Bd* infection, 25% showed positive results. The results of our study may partly explain the pandemic and widespread nature of *Bd* infection in amphibians.

Keywords: Pathogen, *Batrachochytrium dendrobatidis*, geographic distribution, Atlantic forest, Amazon forest, Anurans

3.1 Introduction

Many anuran populations worldwide have experienced severe declines (Williams & Hero 1998, Bustamante et al. 2005), and anurans are justly considered among the most threatened vertebrates (Baillie et al. 2004, 2010). Infectious diseases are considered one of the main causes of these population declines (Baillie et al. 2004, Fisher et al. 2012). Particularly, the aquatic chytrid fungus *Batrachochytrium dendrobatidis* (hereafter referred to as *Bd*) has led to the decline of several amphibian populations and species worldwide (Stuart et al. 2004, Lips et al. 2005, La Marca et al. 2005, Pounds et al. 2006, Skerratt et al. 2007, Fisher et al. 2009, 2012), and it has mostly affected species with a high dependence on water (Bielby et al. 2008, Catenazzi et al. 2010). Previous studies have suggested that *Bd* is a generalist pathogen (Morgan et al. 2007, Walker et al. 2010, Olson et al. 2013) as it has infected more than 500 amphibian species in a wide variety of aquatic and terrestrial habitats (Gründler et al. 2012). *Bd* also has high environmental tolerance, and it is found in diverse locations throughout Africa, Asia, Europe, and the Americas (Fisher et al. 2009, Olson et al. 2013).

Species diversity may affect the prevalence of infectious diseases because of increased interactions among the pathogen and potential hosts (Keesing et al. 2006). Data from Global Amphibian Assessment suggest that amphibian declines associated with *Bd* infection are greater in regions with higher species richness (Olson et al. 2013). However, at local scales, higher diversity tends to attenuate infection indices (Becker et al. 2014). Moreover, the effects of *Bd* vary among species and populations worldwide (Berger et al. 1999, Briggs et al. 2010, Kilpatrick et al. 2010, Searle et al. 2011), probably because of a combination of environmental factors and host traits that impacts the spread and susceptibility of species to *Bd* (Walker et al. 2010, Bancroft et al. 2011). Life history traits have also been associated with higher *Bd* infection rates (Bancroft et al. 2011, Guayasamin et al. 2014) among families with high diversity, broad distributions, and with both aquatic and terrestrial stages (Corey & Waite 2008, Olson et al. 2013).

The Neotropical region of South and Central America contains the highest diversity of amphibians (Baillie et al. 2010). This area includes the Brazilian Atlantic forest and Cerrado (an open grassland physiognomy): two of the most endangered, species-rich (Myers et al. 2000) biomes and centers of endemism in the world (Haddad et al. 2013, Valdujo et al. 2013). Three distinct genotype lineages (including the most ancestral) of the *Bd* pathogen have been found in the Atlantic forest, and this biome may represent the center of origin for *Bd* (Schloegel et al. 2012; Rosenblum et al. 2013). To date, two anuran species in Cerrado (Ramalho et al. 2013) and 110 species in several habitats of the Atlantic forest (Carnaval et al. 2005, 2006, Toledo et al. 2006a, 2006b, Sluys et al. 2007, Ferreira et al. 2008, Brito-Gitirana et al. 2009, Becker & Zamudio 2011, De Paula 2011, De Paula & Catão-Dias 2011, Gründler et al. 2012, Vieira et al. 2012, 2013, Lisboa et al. 2013, Rodriguez et al. 2014) have been recorded as *Bd*-positive (Table S1), with records dating back to more than 100 years ago (Rodriguez et al. 2014). However, the vast majority of these reports are from the south and southeast Atlantic forest (Lisboa et al. 2013, Rodriguez et al. 2014). In the northern portion of the biome, the distribution and prevalence of *Bd* are poorly documented (Carnaval et al. 2006, Verdade et al. 2012, Lisboa et al. 2013).

Here, we have provided current information on *Bd* distribution in north and northeastern Brazil. We have included the first record of *Bd* in the state of Bahia, as well as the first record in an anuran caught in the wild in Brazilian Amazonia. We also used data from previous chytrid studies in the Atlantic forest (south, southeast, and northern regions) to test the hypothesis that *Bd* is a generalist pathogen in this biome. If *Bd* is a generalist, we would expect that the number of species per family is positively correlated with the number of infected species in the family.

3.2 Methods

We conducted fieldwork in five Brazilian localities: four in the Atlantic forest in the states of Pernambuco, Alagoas (June to December 2013 and February to May 2014), Bahia (July 2010), and Minas Gerais (August 2010) and one in the Amazon forest in the state of Pará (February 2013). At each location, we swabbed individuals from terrestrial (forest and open areas) and aquatic habitats (ponds and streams) during diurnal and nocturnal visual encounter surveys, as per the methods of Crump & Scott (2001).

To avoid cross contamination, each collected anuran was immediately and individually maintained in a plastic bag. Individuals were swabbed for *Bd* detection by using sterile cotton swabs and wiping the forelimbs, hindlimbs, and pelvic region five times for each individual, as per the method described by Hyatt et al. (2007). DNA extraction was performed using 50 µL of PrepMan® ULTRA for each swab, followed by Taqman® qPCR assays for detection and quantification of *Bd* loads, according to the method described by Lambertini et al. (2013). *Bd* prevalence was estimated as the number of *Bd*-positive frogs divided by the total number of sampled frogs. Infection intensity was estimated as the number of zoospore equivalents obtained using qPCR (see Lambertini et al. 2013). The strain used for the standard curve was CLFT 023 (from Monte Verde, Camanducaia, Minas Gerais), allowing for comparisons with other localities worldwide (Longo et al. 2013).

To test the hypothesis that *Bd* is a generalist pathogen (showing low species-specificity), we combined our data with records of previous Atlantic forest studies (Carnaval et al. 2005, 2006, Toledo et al. 2006a, 2006b, Sluys et al. 2007, Ferreira et al. 2008, Brito-Gitirana et al. 2009, Becker & Zamudio 2011, De Paula 2011, De Paula & Catão-Dias 2011, Gründler et al. 2012, Vieira et al. 2012, 2013, Lisboa et al. 2013, Rodriguez et al. 2014) to calculate the number of infected species. We then used a linear regression to test the correlation between the number of infected species and the total number of species registered in the biome (from Haddad et al. 2013). Differences between *Bd* prevalence and load among families were tested with analyses of variance (ANOVA). Analyses were performed using the R statistical computing environment (R Development Core Team 2013) after normalizing data through a log transformation.

3.3 Results

Out of the 27 sampled species from eight anuran families, 22 (81.5%) tested positive for *Bd* (Table 1). Out of the 90 sampled individuals from five localities (Figure 1), 39 (43.3%) tested positive for *Bd* infection. All the samples collected in Bahia (n = 2), Pernambuco (n = 3), Pará (n = 1) and Minas Gerias (n = 1) tested positive for *Bd* presence (Table 1).

Among families with the greatest number of samples, *Bd*-prevalence was higher in Craugastoridae (56%; 2 species; 16 individuals) and Hylidae (42%; 15 species; 52 individuals) and was lower in Leptodactylidae (38%; 3 species; 8 individuals) and Bufonidae (25%; 3 species; 8 individuals). *Bd* prevalence among families did not differ (F = 0.80, df = 3, p = 0.50). Infection load was the highest in Craugastoridae (log 1.39GE ±1.51, n = 9 individuals) and Hylidae (log 1.05GE ±0.87, n = 21 individuals), although loads did not differed between families (F= 0.92, df = 5, p = 0.47). The highest intensity of infection was detected in *Crossodactylus dantei* and *Phyllodytes gyrinaetes* followed by *Phyllomedusa nordestina, Frostius pernambucensis, Phyllodytes edelmoi, Hypsiboas crepitans* and *Pristimantis ramagii.* The following species had the lowest loads: *Rhinella crucifer, Scinax nebulosus, Phyllodytes tuberculosus, Pristimantis vinhai, Scinax pachycrus, Scinax* cf. *eurydice, Adelphobates galactonotus* and *Hypsiboas albomarginatus* (log 0 – 1.40). *Bd* was not detected in *Rhinella hoogmoedii*, *Gastrotheca* sp., *Aplastodiscus sibilatus*, *Hypsiboas semilineatus* and *Leptodactylus natalensis* (only one individual was tested for all these species). The sampled individual of *Adelphobates galactonotus* collected in the Pará, in the Amazonia, was infected.

Anuran richness per family was positively correlated with the number of infected species in the Atlantic forest ($r^2 = 0.753$, $p < 0.001$, df = 14, Figure 2). The richest families in the Atlantic forest (Hylidae, Hylodidae, and Leptodactylidae) had greater numbers of infected species (Table S2).

3.4 Discussion

Among the six main Brazilian biomes, three tested positive for *Bd* infection: Amazonia (present study), Cerrado (Ramalho et al., 2013), and the Atlantic forest (Toledo et al. 2006a, Lisboa et al. 2013, Rodriguez et al. 2014, present study). Our record is the first report of Bd infection in a frog caught in the wild; the previous record of *Bd* in Amazonia was in the tadpoles of an exotic bullfrog (*Lithobates catesbeianus*) obtained from a commercial frog farm (Schloegel et al. 2009). Although bullfrogs have been suggested as potential reservoirs and vectors of *Bd* (Schloegel et al. 2009), *Bd* was present in Brazil long before the introduction of bullfrogs (Rodriguez et al. 2014).

Bd has been, and is still, broadly studied in the Atlantic forest, where the number of infected species has increased to 131 (Table S1); majority of these reports are from the southern region, where two divergent strains of *Bd* (*Bd*-GPL and *Bd*-Brazil) have been present at least since 1897 (Rodriguez et al. 2014) in terrestrial and aquatic habitats without lethal effects on anuran populations (Toledo et al. 2006b, Gründler et al. 2012, Rodriguez et al. 2014). In the northern region, information on *Bd* has been limited to reports of anuran infection from the states of Alagoas, Bahia, and Pernambuco (Carnaval et al. 2006, Lisboa et al. 2013, present study). Our findings of *Bd* in Bahia fill a gap of 1,600 km between Minas Gerais and Alagoas, indicating that *Bd* is widespread across a broad host range throughout the Atlantic forest. *Bd* prevalence and load (Table 1) among anurans in the northern region may be regarded as moderate. However, there is a clear need for more surveys in this region, since small sample sizes and differences in sampling effort may lead to a bias in estimating prevalence and pathogen load (Ohst et al. 2013). Climatic and forest characteristics can affect *Bd* infection (Rödder et al.

2008, Fisher et al. 2009, Gründler et al. 2012), and the differing climate dynamics in northern and southern parts of the Atlantic Forest may therefore affect *Bd* dynamics in these regions (Câmara 2003, Carnaval et al. 2014).

Our *Bd* data for the northern region of the Atlantic forest are consistent with an enzootic pattern where there is no detectable effect of *Bd* on anuran survival (Briggs et al. 2010). The same pattern was found in the south Atlantic forest (Rodriguez et al. 2014) and other areas (Canada Forzán et al. 2010, Cameroon Doherty-Bone et al. 2013, Germany Ohst et al. 2013). These findings contradict epizootic events reported in Australia (Berger et al. 1998), Costa Rica (Lips et al. 2003), Mexico (Lips et al. 2004), Panama (Lips et al. 1999), Spain (Bosch et al. 2001), United States (Vredenburg et al. 2010), and Venezuela (La Marca et al. 2005), where high prevalence (Lips et al. 2005) and high *Bd* infection loads (Vredenburg et al. 2010) have been associated with local mass extinctions and fatal declines.

The Atlantic forest is one of the most threatened forests in the world, where more than 80% of the remaining fragments are smaller than 0.5 km2 (Ribeiro et al. 2009). Nevertheless, this biome has high anuran species richness (522 species) in Brazil, presumably because of its diverse geographical and climatic characteristics (Ribeiro et al. 2009, Toledo & Batista 2012, Haddad et al. 2013, Toledo et al. 2014). This high diversity of species and habitats may favor *Bd* infections by providing diverse hosts and microhabitats (Becker & Zamudio 2011). As in Costa Rica and Australia (Becker & Zamudio 2011), the number of species per family was positively correlated with the number of infected species in the Atlantic forest (Figure 2). Even though several anuran species in the Atlantic forest still need to be screened for *Bd* infection, our data support the hypothesis that this fungus is a generalist pathogen (Fisher et al. 2009) that spreads rapidly (Morgan et al. 2007) and infects many different types of host species (Walker et al. 2010, Crawford et al. 2010). In contrast, *Bd* infections in Europe and the United States are associated with amphibian families that display similar life-history traits (Corey & Waite 208, Bancroft et al. 2011, Baláž et al. 2013). For example, species of the family Ranidae are more susceptible to *Bd* infection because of their similarities in body shape and habitat use for breeding (Bancroft et al. 2011).

We found that Hylidae and Leptodactylidae have the greatest number of *Bd*-positive species in the Atlantic forest (Table S2). These families exhibit the greatest diversity of species and reproductive modes in this biome and include species that spawn aquatic and terrestrial eggs and use different types of lotic, lentic, and terrestrial habitats during the breeding season (Haddad & Prado 2005). In south and southeast Brazil, species that breed in lentic and terrestrial habitats are characterized by a higher *Bd* prevalence when compared with species that breed in lotic habitats (Gründler et al. 2012, Rodriguez et al. 2014). Likewise, North American ranids (another species-rich family) that are dependent on water bodies (i.e., lentic habitats) for laying eggs and tadpole development seem to be more prone to *Bd* infection than strictly terrestrial or direct-development species (see also Becker et al. 2014).

The Atlantic Forest extends from 3°S to 31°S and 35°W to 60°W in Brazil (Ribeiro et al. 2009); to date, only 38% of the anuran species present in 10 states have been screened for *Bd* infection, of which 25% tested positive. Several taxa (mainly in the north and northeastern regions) have not been sampled, and long-term monitoring of anurans in the Brazilian biomes is clearly needed to fill geographical, taxonomic, and ecological gaps. These data reveal infection trends in the wild, providing *sine qua non* information that can be directly integrated into amphibian conservation plans.

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Table 1. Anuran species sampled for *Batrachochytrium dendrobatidis* presence in the Amazonia and in the Atlantic forest. Zoospore equivalents (mean and standard deviation [SD] was calculated only for positive individuals). All sampled individuals were adults; juveniles were sampled only for *Proceratophrys renalis.*

Taxa	**Locality/State**	**Zoospore equivalents**			**Prevalence (Infected/Analyzed individuals)**
		Mean	**Min-Max**	**SD**	
Bufonidae					
Frostius pernambucensis	Murici/Alagoas	62.17	-	-	1/2
Rhinella crucifer	Murici/Alagoas	4.79	-	-	1/3
Rhinella hoogmoedi	Murici/Alagoas	-	-	-	0/3
Craugastoridae					
Pristimantis vinhai	Itacaré/Bahia	2.37	-	-	1/1
Pristimantis ramagii	Murici/Alagoas	25.51	3.53-135.72	48.71	7/14
Dendrobatidae					
Adelphobates galactonotus	Santa Cruz Dos Martírios/Pará	1.56	-	-	1/1
Hemiphractidae					
Gastrotheca sp.	Murici/Alagoas	-	-	-	0/1
Hylidae					
Aplastodiscus sibilatus	Murici/Alagoas	-	-	-	0/1
Dendropsophus minutus	Murici/Alagoas	12.4	-	-	1/2
Hypsiboas albomarginatus	Murici/Alagoas	1.05	-	-	1/1
Hypsiboas crepitans	Poção/Pernambuco	34.62	2.2-67.05	45.85	2/2
Hypsiboas exastis	Murici/Alagoas	7.88	-	-	1/1
Hypsiboas semilineatuss	Murici/Alagoas	-	-	-	0/1
Phyllodytes cf. *acuminatus*	Murici/Alagoas	16.33	-	-	1/1
Phyllodytes cf. *edelmoi*	Murici/Alagoas	25.56	-	-	1/1
Phyllodytes edelmoi	Murici/Alagoas	60.22	2.95-227.27	111.37	4/12
Phyllodytes gyrinaethes	Murici/Alagoas	359.16	1.72-1276.49	555.53	5/24
Phyllodytes tuberculosus	Itacaré/Bahia	2.93	-	-	1/1
Phyllomedusa nordestina	Murici/Alagoas	72.63	3.30-141.95	98.04	2/2
Scinax cf. *eurydice*	Carangola/Minas Gerais	1.64	-	-	1/1
Scinax nebulosus	Murici/Alagoas	4.32	-	-	1/2
Scinax pachycrus	Poção/Pernambuco	1.88	-	-	1/1
Hylodidae					
Crossodactylus dantei	Murici/Alagoas	2793.2	264.98-5321.50	3575.55	2/2
Leptodactylidae					
Physalaemus cf. *cuvieri*	Murici/Alagoas	7.91	4.27-11.55	5.14	2/6
Leptodactylus natalensis	Murici/Alagoas	-	-	-	0/1
Leptodactylus cf. *mystaceus*	Murici/Alagoas	5.1	-	-	1/1
Odontophrynidae					
Proceratophrys renalis	Murici/Alagoas	6.01	-	-	1/2

Figures

Figure 1. Positive records of *Batrachochytrium dendrobatidis* infection reported in the present study for the Atlantic forest, in the states of Pernambuco (PE), Alagoas (AL), Bahia (BA) and Minas Gerais (MG), and for the Amazon forest, in the state of Pará (PA). In parentheses the number of individuals tested positive out of total sampled. Source for remaining forest cover: SOS Mata Atlântica/INPE 2012.

Figure 2. Linear regression of the number of species per family reported in the Atlantic forest on the number of infected species ($r^2 = 0.753$, $p < 0.001$, $df = 14$). Dotted lines represent upper and lower 95 % confidence intervals. Aro: Aromobatidae, Bra: Brachycephalidae, Buf: Bufonidae, Cen: Centrolenidae, Cer: Ceratoprhyidae, Cra: Craugastoridae, Cyc: Cycloramphidae, Ele: Eleutherodactylidae, Hem: Hemiphractidae, Hyl: Hylidae, Hlo: Hylodidae, Lep: Leptodactylidae, Mic: Microhylidae, Odo: Odontophrynidae, Pip: Pipidae, Ran: Ranidae.

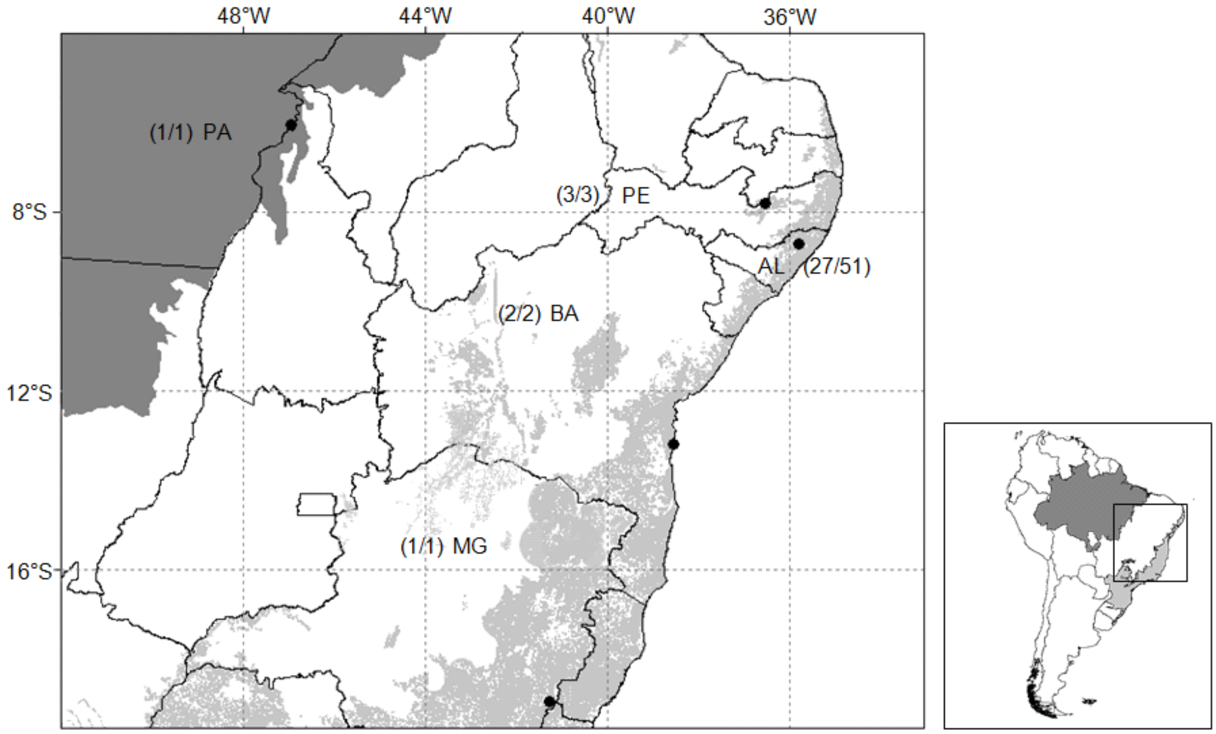
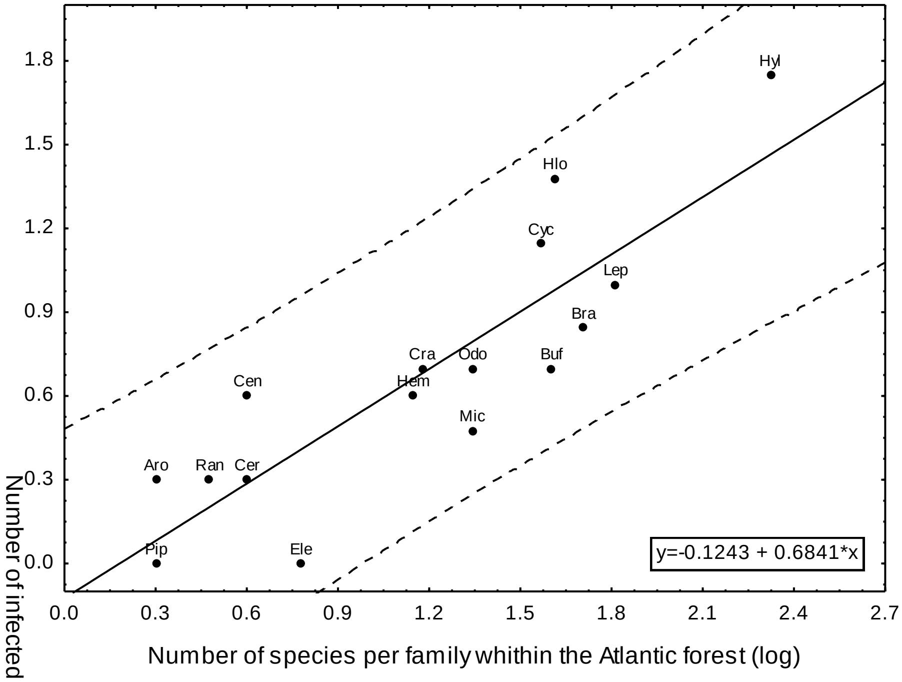


Figure 1


Hyl
Hlo
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Odo
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Hem
Mic
Aro
Ran
Cer
Pip
Ele
y=-0.1243 + 0.6841*x
1.8
1.5
1.2
0.9
0.6
0.3
0.0
0.0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
2.4
2.7
Number of infected
Number of species per family whithin the Atlantic forest (log)

Figure 2

4 Seasonality, environmental factors, and host behavioral linked to disease risk in stream-dweller tadpoles

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Running page head: *Bd* ecology in stream-dweller tadpoles

ABSTRACT

The aquatic pathogen *Batrachochytrium dendrobatidis* (*Bd*) causes amphibian population declines and species extinction worldwide. In post-metamorphic anurans mortality can be high, however, epizootic events have not been reported in tadpoles, in spite of the possibility of rapid disease spread in the water. Here, we quantified the dynamics of *Bd* infection in tadpoles of two streams of northeastern Atlantic forest. *Bd* prevalence varied seasonally, being higher in the rainy season. Tadpoles of *Aplastodiscus sibilatus* and *Proceratophrys renalis* were *Bd* positive in both seasons, while tadpoles of *Agalychnis granulosa* 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 to be *Bd* positive indepent of the developmental stage. Moreover, we found that canopy cover, stream flow, water temperature, stream depth, pH and host density were associated with *Bd* prevalence. Our results underscore that both hosts and streams could be reservoirs of the pathogen, thus monitoring populations of anuran larvae from these habitats will enhance to assess the impact of this pathogen in this biodiversity hotspot.

Key words: Anura, larvae, ontogeny, disease ecology, abiotic factors, *Batrachochytrium dendrobatidis*, Atlantic forest

4.1 Introduction

Habitat destruction and infectious diseases are the major threats leading to amphibian declines and extinctions worldwide (Skerratt et al. 2007, Wake & Vredenburg 2008, Sodhi et al. 2008, Mann et al. 2009). Chytridiomycosis can be caused by the fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*), an emerging infectious disease of amphibians that has already infected over 500 species (Olson et al., 2013). Climatic and microhabitat variables, such as temperature, precipitation, tree cover, pH, and substrate seem to influence *Bd* prevalence (Berger et al. 2004, Piotrowski et al. 2004, Kriger et al. 2007, Raffel et al. 2010, Rohr &Raffel 2010). Likewise, host density, diversity, behavior, water dependence and other host life-history traits are also related to *Bd* infection (Woodhams et al. 2003, Bancroft et al. 2011, Becker et al. 2014).

Batrachochytrium dendrobatidis is a pathogenic fungus with flagellated aquatic zoospores that spread among amphibians by close or direct contact during mating, schooling of tadpoles or other gregarious behaviors (Berger et al. 1999, Catenazzi et al. 2013). The ecology and behavior of a host species can affect the prevalence, intensity, transmission and seasonality of infection (Keesing et al. 2006). Amphibian life cycles involve aquatic development of larvae and terrestrial activity of post-metamorphic (Wells 2007). Because pathogens may infect their hosts at various life stages (Blaustein et al. 2005), data on *Bd* prevalence in both anura life-stages are necessary to understand the infection dynamics (Wake & Vredenburg 2008). In this sense, tadpoles can effectively be used to study seasonal *Bd* dynamics (Conradie et al. 2011), because they are present in many streams throughout the year (Alford 1999, Narayan et al. 2014) and interspecific differences in larval susceptibility to *Bd* were already detected (Parris & Cornelius 2004, Blaustein et al. 2005, Andre et al. 2008).

The pathogen infects the keratinizing tissue, tooth rows and jaw sheaths of tadpoles (Altig 2007, Viera et al. 2013). In laboratory *Bd* infection can cause mortality in tadpoles and reduce larval growth and developmental rates (Parris & Cornelius 2004, Blaustein et al. 2005, Andre et al. 2008, Venesky et al. 2009, 2010, 2011). However, in natural conditions *Bd* does not seem to have any lethal effect on the tadpoles (Berger et al. 1999, Peterson et al. 2007, Smith et al. 2007, Symonds et al. 2007, Conradie et al. 2011, Catenazzi et al. 2013). Therefore, wild tadpoles can act as reservoirs of *Bd*, infecting other tadpoles or other post-metamorphic individuals, keeping infection within and between seasons and habitats (Blaustein et al. 2005, Rachowicz & Vredenburg 2004, Catenazzi et al. 2013, Narayan et al. 2014). Likewise, terrestrial or aquatic habitats may serve as reservoirs for the pathogen in diverse amphibian communities (Kriger & Hero 2007a, Gründler et al. 2012, Longo et al. 2013, Guayasamin et al. 2014).

In some regions *Bd* infection varies among breeding habitats, with a higher risk of infection in lotic habitats (Kriger & Hero 2007, Sluys & Hero 2009). Nevertheless, in the southern Brazilian Atlantic forest where *Bd* seems to be an endemic pathogen, with the earliest date of detection (1894), there is a higher prevalence and intensity in lentic and terrestrial breeding habitats than in lotic habitats (Gründler et al. 2012, Rodriguez et al. 2014). This extremely fragmented and dynamic forest has highly heterogeneous environmental conditions (Ribeiro et al. 2009) including a wide range of elevations (0-2700 m) and diverse forest composition (Pinto & Brito 2003) which may influence the dynamics and evolutionary history of *Bd* (Kriger et al. 2007, Kriger & Hero 2008, Raffel et al. 2013, Rosenblum et al. 2013). Over 100 anuran species of South and southeastern regions of the Atlantic forest are *Bd*-positive (Valencia-Aguilar et al. *in press*). However, in the northern region the knowledge about this infection is incipient (Carnaval et al. 2006, Lisboa et al. 2013). Environmental conditions differ between the south and north regions of the Atlantic forest (Ribeiro et al. 2009). So, information about how *Bd* prevalence and intensity varies geographically in this forest would help to understand the disease ecology (Keesing et al. 2006, Kriger & Hero 2008, Gallana et al. 2013). Here, we assessed the *Bd* infection status of tadpoles present in two streams and the seasonal variation of *Bd*-prevalence in Northern Atlantic forest. Moreover, we quantified environmental and life-history variables to determine which of those could be affecting *Bd* prevalence. Some amphibian lineages show a greater susceptibility to *Bd* infection than others (Corey & Waite 2008), thus we expect to find a *Bd* prevalence variation in the infected species. Infected tadpoles loss their oral structures which reduces foraging efficiency (Venesky et al. 2010) in consequence we predicted smaller body lengths in infected individuals. Moreover, abiotic factors like temperature affect *Bd* infection (Raffel et al. 2010, Bancroft et al. 2011) and we expect a higher prevalence in areas with optimal water temperatures for *Bd* growth.

4.2 Material and methods

4.2.1 Field survey

Two permanent streams (9°12’42.6’’S 35°51’52.5’’W, 541 mamsl Cardozo; and 9°13’3.5’’S 35°52’32.1’’W, 564 mamsl Bananeira) were studied, in the Estação Ecológica de Murici, In the Pernambuco sub-region of the Atlantic forest (Figure 1) (Silva and Casteleti 2003). Due to difference among host breeding season, each stream was sampled during the dry (15 November 2013-31 January 2014), onset and peak rainy (17 May-21 July 2014) seasons, to analyze as many species of tadpoles as possible. Tadpoles were captured in 23 sampling points in each stream, which were sampled once in each season. Dip net survey method for collecting tadpoles was employed following Shaffer et al. (2001) and was standardized at 15 minutes per site (three sweeps of 5 minutes each interspersed by 2 minutes between sweeps).

In the Cardozo stream were collected tadpoles of *Agalychnis granulosa* (n = 81), *Aplastodiscus sibilatus* (n = 229), *Hypsiboas freicanecae* (n = 8), *Crossodactylus dantei* (n = 18) and *Proceratophrys renalis* (n = 176). In the Bananeira stream, *Agalychnis granulosa* (n = 266), *Aplastodiscus sibilatus* (n = 226), *Dendropsophus haddadi* (n = 6) and *Hypsiboas exastis* (n = 5). *Agalychnis. granulosa*, *A. sibilatus*, *H. freicanecae*, *C. dantei* and *P. renalis* were *Bd* positive. Besides, *D. haddadi* and *H. exastis* were excluded from the analyses because they were *Bd* non positive, and *H. freicanecae* and *C. dantei* due to small sample sizes (less than 50 individuals).

Canopy cover in each segment of the stream was measured as the vertical projection of plant foliage (Fiala et al. 2006). Canopy was photographed at chest level in five points (one in each cardinal point and one in the center) and photographs were converted to binary images where the canopy becomes black and the sky white. The proportion of black and white areas was calculated projecting eight vertical and six horizontal grid lines onto the photograph, and this was used to estimate the percent canopy cover. Water temperature and pH were measured 10 cm below the water surface at a single point in each segment (23 points) of the streams, using a multi-parameter Hanna HI 9828. Depth was taken in three points (two in each end and one in the center) in each segment of the stream using a 3 m measuring tape. Water flow was calculated as the distance in relation to time.

4.2.2 *Batrachochytrium dendrobatidis* detection and tadpole measurements

Tadpoles were brought alive to Laboratório de Biologia Celular the Universidade Federal de Alagoas. Only tadpoles in the developmental stages 25-40 (Gosner 1960) were analyzed because after this stage the oral disc of the tadpole (the only keratinizing epithelium at this stage) is replaced by the adult mouth jaws and tongue (Duellman & Trueb 1994, Altig & McDiarmid 1999). These animals were decapitated and their mouthparts were excised, prepared on slides, and microscopically examined for the presence of *Bd* following the technique proposed by Lambertini et al. (2013). The individual was considered *Bd*-positive when spherical or septate sporangium (Longcore et al. 1999) was observed in the tissue. Since loss of oral structures in tadpoles have been associated with the presence of *Bd* (Fellers et al. 2001, Knapp & Morgan 2006, Smith et al. 2007, Vieira et al. 2013), and because the oral structures are related with the feeding efficiency in some species (Alford 1999), the oral disc condition in each individual was assessed visually under a stereoscope. Loss of each oral structure (jaw sheath and tooth rows) was categorized as 0% loss, <25% loss, 25-50% loss, 51-75% loss and 76-100% loss. Body length (mm) was measured with a digital caliper (0.1 mm precision) following Altig & McDiarmid (1999) definition. Host density and social behavior have been associated with the *Bd* transmission and prevalence (Rachowicz & Briggs 2007, Briggs et al. 2010, Venesky et al. 2011). In this sense, tadpole density (abundance per area in each point of the stream) was measured to evaluate if *Bd* prevalence is affected (for example, by facilitating infection) for this variable.

4.2.3 Statistical analysis

Bd prevalence was calculated as the number of tadpoles tested positive for *Bd,* divided by the total number of tadpoles. Seasonal variation in *Bd*-prevalence within and between Cardozo and Bananeira streams and in tadpoles was tested with a Kruskal-Wallis (KW) nonparametric ANOVA.

Although in laboratory conditions *Bd*-infected tadpoles were smaller and less developed, in natural conditions body size was positively associated with infection (Smith et al. 2007, Conradie et al. 2011, Catenazzi et al. 2013). We evaluated if body size differed between *Bd* positive and non positive tadpoles using a Mann-Whitney test. However, because growth and developmental stage are correlated (Ultsch et al. 1999), tadpoles were categorized in three classes of developmental stage to determine if differences in body size between *Bd* positive and non positive individuals were the result of developmental changes or if they are restricted to a particular class. To test this hypothesis, a logistic regression was used to examine the effects of body size with developmental class as factor and pairwise interactions on the probability of infection. Finally, to assess whether loss of oral structures would be affecting the growth rate of tadpoles in each species a Kruskal-Wallis (KW) was performed. It was not possible to meet assumptions of parametric test, even after log transformed body size, for that reason, non-parametric tests were used.

A spatial filtering model approach (see Diniz-Filho & Bini 2005) was used to verify and remove the correlation among variables (tadpole density, canopy cover, water temperature, pH, depth and water flow) per point. Distance between points and data from each one were used to construct a pairwise matrix of physical distance. Then the matrix was submitted to a Principal Components Analysis (PCA) to extract the eigenvectors from the distance matrix among the sample units (each point). The first two eigenvectors represented a broad variation, so they were used in multiple regressions with each environmental variable. Residuals of these regressions without the effect of the spatial autocorrelation were considered as new variables and used in regression models. The dependent variable prevalence and the independent variables tadpole density, canopy cover, water temperature, pH depth, and water flow were used in a multiple regression model (performed just for the species with the largest sample size) to test influence of the explanatory variables on prevalence within each stream. The best model was selected using stepwise procedure with Akaike information criterion (AIC). For each analysis (stream x season) highly correlated ($r>0.7$) variables were excluded. All statistical tests were performed by using R v 3.1.1 (R Development Core Team, 2014).

4.3 Results

4.3.1 *Bd* prevalence in streams, seasons and tadpoles

Bd prevalence was higher in the rainy season (onset and peak) in both streams (Figure 2) and varied between (Kruskal-Wallis Test, $H = 12.12$, $df = 2$, $P = 0.002$, $n = 943$ tadpoles) and within streams (Kruskal-Wallis Test, $H = 16.66$, $df = 2$, $P < 0.001$, $n = 475$ tadpoles; only Bananeira). Tadpoles of *A. sibilatus* and *P. renalis* were *Bd* positive during the dry and rainy season (onset and peak), while tadpoles of *A. granulosa* were *Bd* positive only in the rainy season (onset and peak) (Figure 3). Differences in prevalence between seasons were detected only in *A. sibilatus* (Kruskal-Wallis Test, H = 8.57, df = 2, P = 0.01, Cardozo stream).

4.3.2 Influence of *Bd* infection in the body size

Contrary to our hypothesis, *Bd* positive individuals of *A. granulosa* (Mann-Whitney test, W = 422.5, P < 0.001; n = 344) and *A. sibilatus* (F = 89.14, df = 1, P < 0.001; n = 432) were larger than *Bd* non positive ones. In *P. renalis*, body size between *Bd* positive and non positive tadpoles did not differ (W = 642, P = 0.60; n=171). When classes were included as factor in the analysis to control for developmental stage, there was no significant interaction between prevalence and classes (I, II, III), which suggests that larger individuals of *A. granulosa* (logistic regression, classes as factor, coef. = 0.84, P < 0.001) and *A. sibilatus* (logistic regression, classes as factor, coef. = 0.47, P < 0.001) have a higher probability to be *Bd* positive independent of the developmental stage.

4.3.3 Jaw sheath loss

Oral disc inspections indicated that in the Cardozo stream 27.16% (n = 132) of tadpoles had partial or complete loss of jaw sheath, of which 12.87% (n = 17) were negative for *Bd*. Furthermore, 1.41% (n = 5) of the 72.83% (n = 354) tadpoles without loss of jaw sheath were positive for *Bd*. Likewise, in the Bananeira stream 14.32% (n = 53) of tadpoles with partial or complete loss of jaw sheath, 22.64% (n = 12) were negative for *Bd*. Furthermore, 1% (n = 3) of the 85.67% (n = 317) tadpoles without loss of jaw sheath were positive for *Bd*. At similar developmental stages. *Bd* positive tadpoles of *A.*

granulosa (Kruskal–Wallis Test, H = 4.42, df = 3, P = 0.21), *A. sibilatus* (Kruskal–Wallis Test, H = 6.31, df = 4, P = 0.17) and *P. renalis* (Kruskal–Wallis Test, H = 5.86, df = 4, P = 0.20) did not show differences in body length as a result of jaw sheath loss.

4.3.4 Potential predictors of *Bd*-prevalence in *Aplastodiscus sibilatus*

Flow, temperature, depth and pH varied between streams and seasons (Table 1). 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. In the Bananeira stream canopy cover, depth water and host density were spatially autocorrelated during the dry and onset rainy seasons. While in the Cardozo stream water flow and depth, density host and pH were spatially autocorrelated during the dry and peak seasons. This autocorrelation effect was removed using the spatial autocorrelation analysis. All six variables were significant predictors of *Bd*-prevalence in *A. sibilatus* in the final models following stepwise selection (Table 2). At the Cardozo stream *Bd*-prevalence was associated to low pH values in the dry ($\bar{x}$ = 6.35, P = 0.023) and rainy onset season ($\bar{x}$ = 5.95, P = 0.037). Moreover, in the rainy peak season *Bd*-prevalence was associated with high canopy cover ($\bar{x}$ = 80.77, P = 0.002) and shallow water ($\bar{x}$ = 17.62, P = 0.017). In the Bananeira stream *Bd*-prevalence was associated with low host density ($\bar{x}$ = 0.24, P = 0.021, dry season), shallow water ($\bar{x}$ = 11.50, P = 0.017, dry season; $\bar{x}$ = 19.41, P = 0.019, onset rainy season), high canopy cover ($\bar{x}$ = 80, P < 0.001, onset rainy season), an optimal water temperature for growth ($\bar{x}$ = 21.92, P = 0.023, onset rainy season) (see Piotrowski et al. 2004) and low water flow ($\bar{x}$ = 0.17, P = 0.007, peak rainy season).

4.4 DISCUSSION

4.4.1 *Bd* prevalence in tadpoles

Alterations in seasonality host–pathogen interactions such as host behavior, host abundance, or alterations in host immune defenses (Altizer et al. 2006) affect the prevalence, intensity and transmission of infection between hosts (Woolhouse et al. 2006, Rowley & Alford 2007, Kilpatrick et al. 2011, Venesky et al. 2011, Gervasi et al. 2013). Our results show that *Bd* prevalence varied seasonally among streams and species. We found the highest values of prevalence in *A. sibilatus, A. granulosa,* and *P. renalis* during the rainy season, while during the dry season not only we found lower values but also infection apparently disappeared in *A. granulosa*. Some species are more susceptible to infection than others due to differences in life-history traits or specific traits (Kriger & Hero 2007, Bancroft et al. 2011). For example, females of *Aplastodiscus* spp. lay eggs in subterranean chambers in the margins of lotic water bodies (Haddad et al. 2013, Faivovich et al. 2005), and females of *Proceratophrys* spp lay eggs in water surface and submerged rocks (Santos Dias et al. 2013). Moreover, females of *Agalychnis* spp. lay eggs in a variety of substrates such as aquatic or surrounding vegetation, tree trunks, leaves or bromeliads (Haddad et al. 2013, Vilela et al. 2014). Consequently, tadpoles of *A. granulosa* have a lower infection probability, because the eggs of this species are laid in a variety of habitats allowing the larvae to avoid areas with individuals *Bd* positive or where conditions are appropriate for *Bd* growth (Bancroft et al. 2011). Althoug, *A. sibilatus* and *P. renalis* could have 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 *A. sibilatus* and a low prevalence in *P. renalis*, which could possibly be related to a phylogenetic component (Corey & Waite 2008) with some anuran families (like Hylidae) showing greater susceptibility to *Bd* infection than others.

Likewise, *Bd* transmission and prevalence might increase proportionally with the increase in the social behavior of tadpoles (Rowley & Alford 2007, Venesky et al. 2011). Individuals of *A. sibilatus* and *P. renalis* were observed aggregate or in close contact on the bottom of the stream usually under the leaf-litter. This high sociability and benthic ecology probably facilitated *Bd* transmission between *Bd* positive and non positive tadpoles of *A. sibilatus*, which showed the highest prevalence (Rachowicz & Briggs 2007, Rowley & Alford 2007, Raffel et al. 2010, Venesky et al. 2011). Instead, *A. granulosa* only was *Bd* positive during the rainy season and in a low percentage. Contrary to the other species, tadpoles of *A. granulosa* seem to avoid close contact with conspecifics or heterospecifics remaining in the middle of the water column. This behavior could explain the low *Bd* prevalence in this host species. Moreover, in the water column and surface temperature are higher as compared with the bottom (Poole & Berman 2001), therefore tadpoles probably clear their own infection (Woodhams et al. 2003), or reducing the chance of infection by avoiding contact with other infected individuals (Venesky et al. 2011). Our results shows that *A. granulosa, A. sibilatus* and *P. renalis* could be acting as reservoirs in streams, as they are not declining (personal observation), carry *Bd* zoospores during the development time and have the potential to infect other individuals or species within the stream (Berger et al. 1999, Rachowicz & Vredenburg 2004, Raffel et al. 2010, Catenazzi et al. 2013, Narayan et al. 2014).

4.4.2 Influence body size in likehood of *Bd* infection

Our results suggest that the larval body size of *A. granulosa* and *A. sibilatus* have a strong relationship with the probability of being *Bd* positive. In other words, individuals with a larger body size have a greater chance of becoming *Bd* positive. These data are consistent with previous studies for wild tadpoles with extended developmental periods (Smith et al. 2007, Symonds et al. 2007, Conradie et al. 2011, Searle et al. 2011, Catenazzi et al. 2013, Vieira et al. 2013). However, *A. granulosa* and *A. sibilatus* have a short developmental period; probably less than five months (see Borges 2007, Vilela et al. 2014). Developmental rate can respond to changes in growth rate throughout all of the larval period, or could remain at a constant rate throughout this period independent of changes in growth rate (Alford & Harris 1988). Since we found individuals with similar body sizes at different developmental stages, we argue that this variation in individual growth could explain the fact that larger individuals are *Bd* positive independent of their developmental stage. Older and larger individuals are probably more susceptible to *Bd* infection, because they are exposed for a longer period of time to zoospores, and they have larger available substrate area (larger mouthparts) for the pathogen (Bancroft et al. 2011, Vieira et al. 2013, Rachowicz & Vredenburg 2004, Smith et al. 2007, Rowley & Alford 2007, Raffel et al. 2010, Venesky et al. 2011). On the other hand, *P. renalis* (even after controlling for the developmental stage) did not show difference in body size between *Bd* positive and non positive tadpoles, similar as observed to *Strongylopus* *hymenopus* (Smith et al. 2007). Therefore, we suggest that tadpoles of *P. renalis* have the same probability of being *Bd* positive even at different developmental stage.

In tadpoles *Bd* infects keratinizing structures causing a disruption in the basal mitotic tissue, leading to the partial or complete loss of the oral structure (Altig 2007). This loss of oral structures affects foraging behavior of tadpoles and reduces the efficient to obtain food, which affects larval growth and developmental rates in laboratory conditions (Parris & Cornelius 2004, Blaustein et al. 2005, Andre et al. 2008, Venesky et al. 2009, 2010, 2011). In contrast in natural environments, *Bd* infection does not seem to show lethal effects on tadpoles (Berger et al. 1999, Peterson et al. 2007, Smith et al. 2007, Conradie et al. 2011, Catenazzi et al. 2013). We did not either find a high percentage of jaw sheath loss in analyzed tadpoles or find evidence of reduction in growth or development rates in *A. granulosa, A. sibilatus* and *P. renalis* due to *Bd* presence. This suggests that infected individuals can compensate for oral structure loss in some way. The position and morphology of the oral disc allow tadpoles to exploit different resources present in the aquatic habitats (Altig & McDiarmid 1999). For example, benthic tadpoles, such as *A. sibilatus* and *P. renalis* feed removing diatoms or sediments from substrate (Wells 2007). Moreover, tadpoles feed efficiently on a wide variety of particle sizes, generally obtained from the incoming water during filtration (Alford 1999, Wells 2007). Thus, it is possible that after the oral structures are loss, tadpoles of *A. granulosa, A. sibilatus* and *P. renalis*, continue feeding on suspended material apparently without any effect due to *Bd* infection.

4.4.3 Influence of environmental variables in *Bd* prevalence

In fast-flowing streams of Peru and South Africa high *Bd* prevalence has been documented during the dry season, when water level and flow decrease (Conradie et al. 2011, Catenazzi et al. 2013). Although *Bd* zoospores are aquatic and can swim long (2 cm) distances before encysting (Piotrowski et al. 2004), an increase in water flow during rainy season could reduce contact between hosts and affect zoospores dispersal. Nonetheless, we found a high *Bd* prevalence in the rainy season, probably because flow water 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 may be the result of changes in the environmental variables and subsequently in the host traits (Berger et al. 2004, Kriger & Hero 2007b, Conradie et al. 2011). Both temperature and pH affect the life-cycle (reproduction, growth, behavior, physiology) of amphibians and *Bd* (Ultsch et al. 1999, Piotrowski et al. 2004, Wells 2007, Woodhams et al. 2008, Knapp et al. 2011). Temperatures and pH recorded in our studied streams (21 - 23°C in the dry season and 21 - 22°C in the rainy season; 4 - 7 in the dry season and 4 - 5 in the rainy season) fell within the optimal range for *Bd* growth and persistence (Piotrowski et al. 2004, Woodhams et al. 2008). Nonetheless, as in other natural systems (ponds with pH 9) (Bosch et al. 2001) we found a high *Bd*-prevalence at low pH values, suggesting that in natural conditions *Bd* can grow and develop in broader ranges of pH (4 - 9) than those reported in laboratory (6 - 7) (Piotrowski et al. 2004). Depth and canopy cover also influenced *Bd* prevalence probably because of their negative relationship with temperature. An increase in depth and canopy cover would cause a decrease in temperatures in the stream bottom (Sparling 2010), where the leaf-litter will provide shade and shelter to the zoospores serving as environmental reservoirs of the pathogen (Rowley & Alford 2007, Raffel et al.

2010). Although it is expected that an increase in host density would increase the disease prevalence (Keesing et al. 2006), some studies have found no evidence for host density effects (Woodhams & Alford 2005, Raffel et al. 2010), while our results suggest that host density (in the case of *A. sibilatus*) is negatively associated with the *Bd*-prevalence. Since, transmission did not require direct contact with infected individuals (Rachowicz & Briggs 2007) and because non-host species can alter the probability that a contact results in infection (Keesing et al. 2006) it is possible that the high density of *A. sibilatus* and *P. renalis* (ecologically similar species) in streams would interfere with disease transmission (Rachowicz & Briggs 2007).

In the northern Atlantic forest, the climate and topography are characterized by rainy winters and lowland and mid-elevation, whereas at southern and southeastern there are cooler and higher elevation forests with rainy summers (Carnaval et al. 2014). In Alagoas (northern forest) where climate is classified as tropical with dry summers and annual temperatures and rainfall of 22 - 26°C and 1,300 – 1,600mm, respectively (Alvares et al. 2013), we observed a higher *Bd* prevalence during the rainy season. In the southern region *Bd* infections are high in lentic and terrestrial habitats during the rainy summer, when the anuran breeding activity begins (Becker & Zamudio 2011, Gründler et al. 2012, Rodriguez et al. 2014) and the environmental conditions are optimal for *Bd* growth (Berger et al. 2004, Piotrowski et al. 2004, Alvares et al. 2013). We showed that there is a seasonal variation in *Bd* prevalence in the northeast Atlantic forest. However, because there are no studies of seasonal variation of *Bd* dynamic in the southeast of this biome, we can not assert if the observed patterns for the Atlantic forest are completely different. Nevertheless, both northern and southern Atlantic forest, *Bd* infections seem to have a positive relationship with rainfall (Kriger et al. 2007), as the highest values of prevalence were observed in the onset of rains. A similar pattern was also found in eastern Australia where latitudinal variation in 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). Warmer and drier areas in the Atlantic forest at the northern region (Alvares et al. 2013) are also the less study one in this forest. *Bd* infection data from these not sampled 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.

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Table 1. Environmental variables measured during dry and rainy (onset, peak) seasons in two Atlantic forest streams. Results are shown as mean ± standard deviation (min-max)

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

Table 2. Summary of multiple comparations analyses for overall effects by seasons of the environmental variables on *Batrachochytrium dendrobatidis* prevalence in *Aplastodiscus sibilatus*, in the Bananeira and Cardozo streams by seasons. Models obtained by forward stepwise selection deletion. Bold numbers indicate significant results when *P* < 0.05 (one asterisk), or when *P* < 0.01 (two asterisks). Spaces marked with a dash (–) indicate variables highly correlated (r > 0.7) and excluded from the model.

	Cardozo stream				**Bananeira stream**			
Source of variation	Estimate	Std. Error	t value	Pr(>|t|)	Estimate	Std. Error	t value	Pr(>|t|)
Aplastodiscus sibilatus								
Dry season								
Intercept	28.00	44.50	0.63	0.54	51.40	38.31	1.34	0.20
Tadpole density	-0.43	3.00	-0.14	0.89	-8.64	3.30	-2.62	**0.021***
Canopy cover	0.02	0.07	0.30	0.77	-	-	-	-
Water flow (m/s)	4.45	19.05	0.23	0.82	-36.61	19.34	-1.89	0.080.
Water temperature (°C)	-0.55	1.88	-0.29	0.78	-2.10	1.65	-1.28	0.22
Water depth (cm)	-0.08	0.13	-0.59	0.56	-0.58	0.21	-2.73	**0.017***
pH	-2.53	0.96	-2.64	**0.023***	-	-	-	-
Rainy onset season								
Intercept	-48.21	19.23	-2.51	**0.025***	-41.38	22.46	-1.84	0.092.
Tadpole density	-	-	-	-	-	-	-	-
Canopy cover	-	-	-	-	-0.26	0.03	-7.51	**< 0.001***
Water flow (m/s)	-	-	-	-	7.67	6.25	1.23	0.25
Water temperature (°C)	-	-	-	-	2.70	1.03	2.63	**0.023***
Water depth (cm)	0.25	0.14	1.82	0.089.	0.13	0.05	2.74	**0.019***
pH	7.30	3.18	2.30	**0.037***	-	-	-	-
Rainy peak season								
Intercept	-21.21	6.09	-3.49	**0.003***	-8.17	3.03	-2.70	**0.022***
Tadpole density	-5.38	3.49	-1.54	0.14	-	-	-	-
Canopy cover	0.26	0.07	3.50	**0.002***	-	-	-	-
Water flow (m/s)	-	-	-	-	16.56	4.90	3.38	**0.007***
Water temperature (°C)	-	-	-	-	-	-	-	-
Water depth (cm)	-0.22	0.08	-2.65	**0.017***	0.30	0.14	2.08	0.063.
pH	-	-	-	-	-	-	-	-

Figures

Figure 1. Study sites in the Pernambuco sub-region of the Atlantic forest, Estação Ecológica de Murici, state of Alagoas, Brazil. Source for remaining forest cover: SOS Mata Atlântica/INPE 2012.

Figure 2. *Batrachochytrium dendrobatidis* prevalence between seasons in the Cardozo and Bananeira streams. Squares represent median, boxes quartiles and whiskers minimum to maximum. Significant *Bd* prevalence variation was detected between streams (P = 0.002), and within Bananeira stream (P < 0.001).

Figure 3. *Batrachochytrium dendrobatidis* prevalence in three stream-dweller amphibians species in the dry and rainy (onset, peak) seasons in two Atlantic forest streams.

Figure 4. Mean body length of infected and uninfected tadpoles of *Aplastodiscus sibilatus, Agalychnis granulosa* and *Proceratophrys renalis* in the Cardozo and Bananeira streams. Squares represent median, boxes quartiles and whiskers minimum to maximum. Significant variation on body size was observed for *A. sibilatus* (P < 0.001) and *A. granulosa* (P < 0.001), but not for *P. renalis* (P = 0.60).

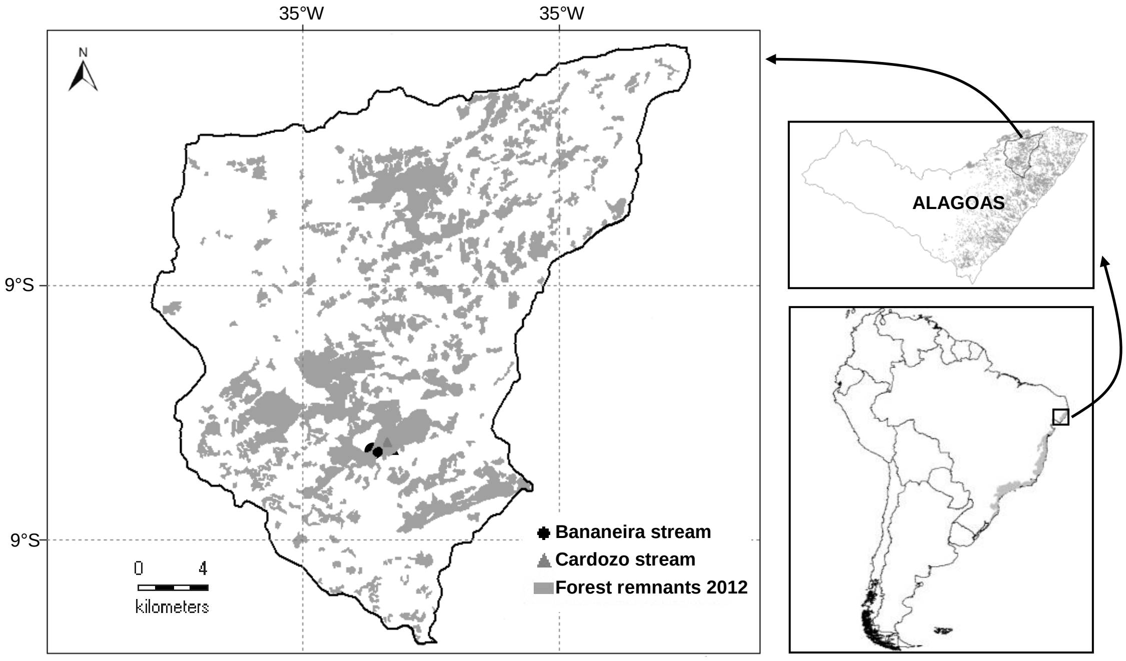
35°W
35°W
9°S
9°S
N
0
4
kilometers
● Bananeira stream
▲ Cardozo stream
▨ Forest remnants 2012
ALAGOAS

Figure 1

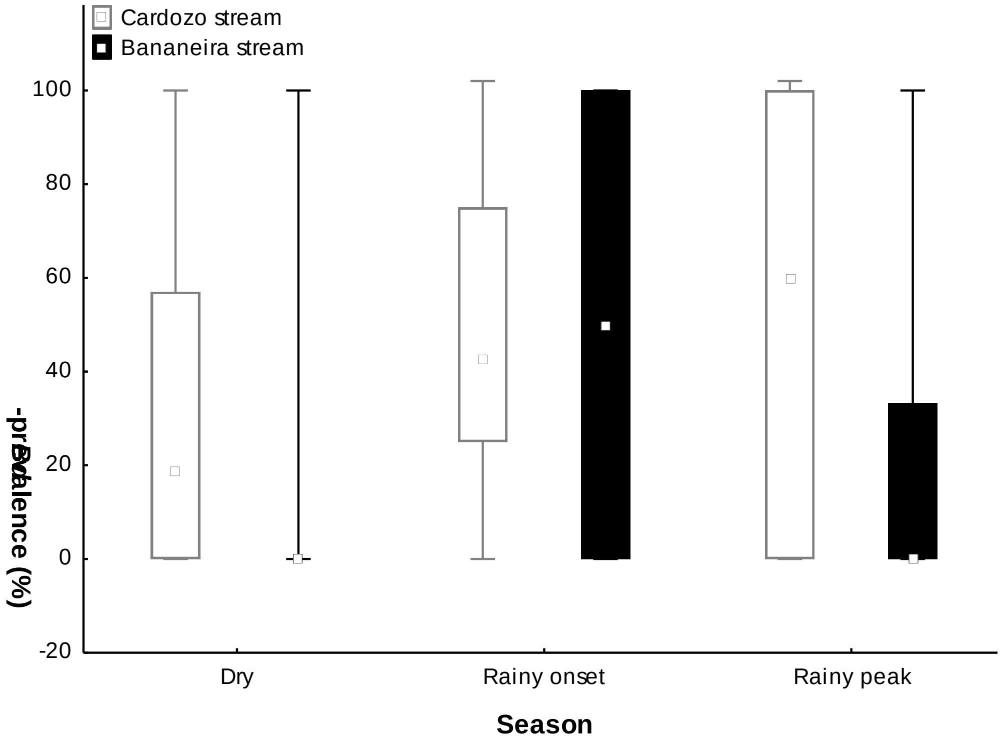
Cardozo stream
Bananeira stream
100
80
60
40
20
0
-20
Bd-prevalence (%)
Dry
Rainy onset
Rainy peak
Season

Figure 2

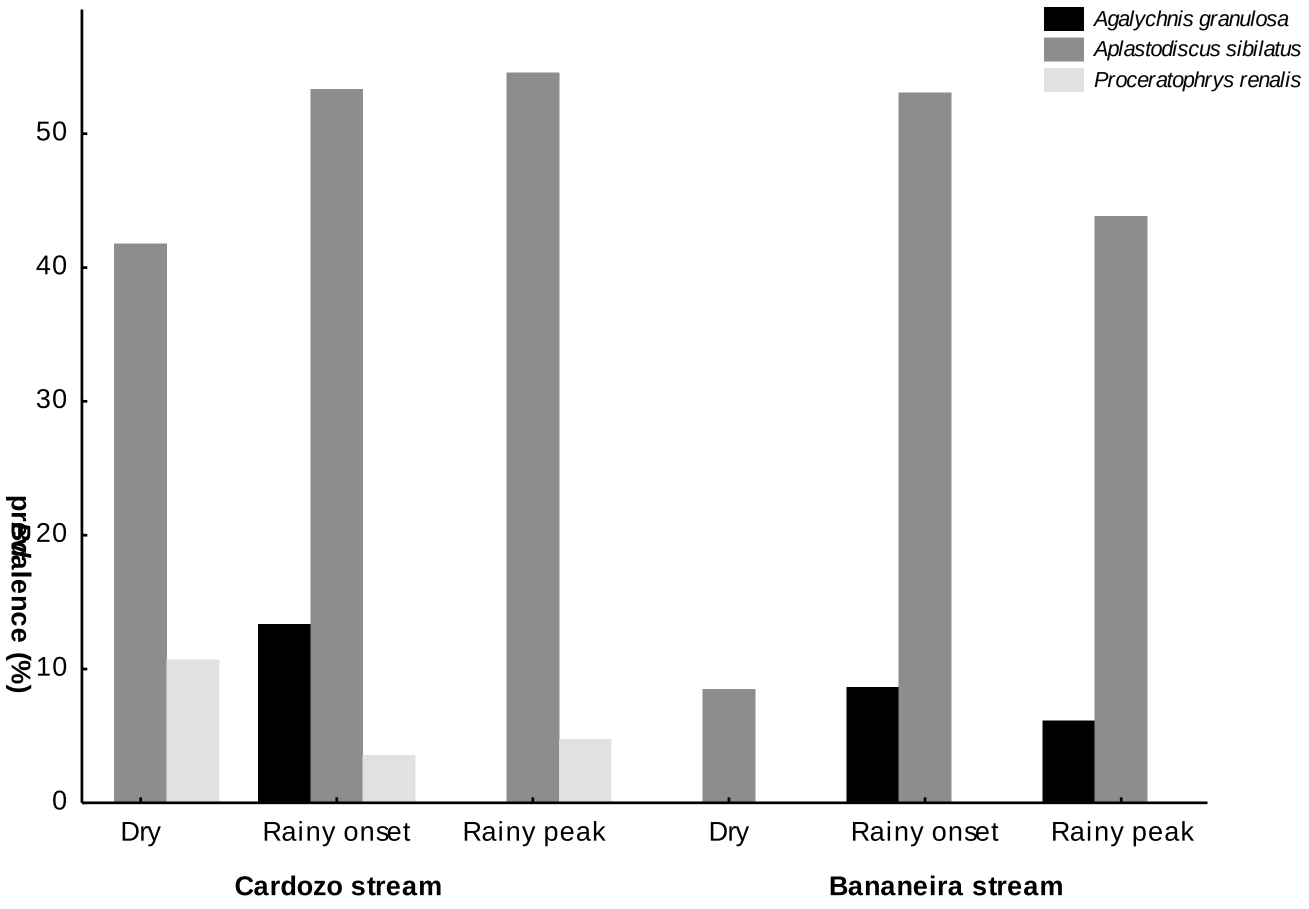
Agalychnis granulosa
Aplastodiscus sibilatus
Proceratophrys renalis
Bd prevalence (%)
0
10
20
30
40
50
Dry
Rainy onset
Rainy peak
Dry
Rainy onset
Rainy peak
Cardozo stream
Bananeira stream

Figure 3

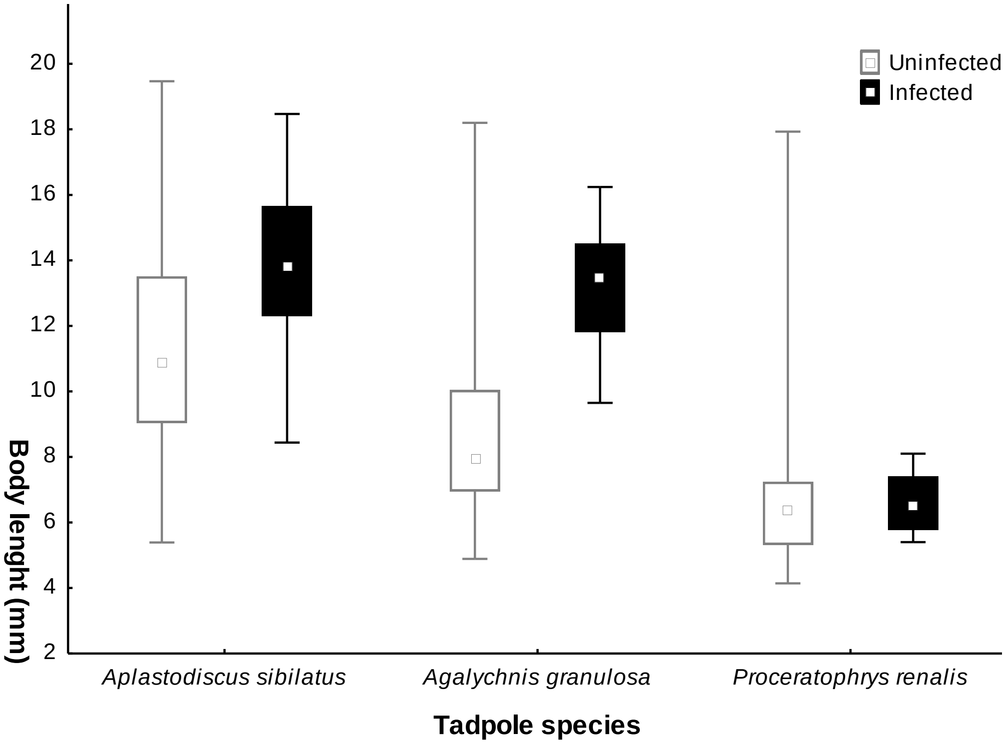
20
18
16
14
12
10
8
6
4
2
Body lenght (mm)
□ Uninfected
■ Infected
Aplastodiscus sibilatus
Agalychnis granulosa
Proceratophrys renalis
Tadpole species

Figure 4

5 Biotic and abiotic factors shaping anuran larval assemblages in the Brazilian Atlantic forest

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ABSTRACT

Species composition and spatial distributions can be explained by the complex interplay among several structuring factors. Tadpole assemblages were evaluated in temporary and permanent ponds to determine the variation in their species composition and to assess the influence of biotic and abiotic factors shaping them. Four temporary and four permanent ponds were sampled in fragments of Atlantic forest located in four municipalities in the Alagoas state, northeastern Brazil. Tadpole species richness was related to environmental and biological variables through General Regression Models. Relationships between tadpole assemblages and exploratory spatial predictors were measured using a partial Canonical Correspondence Analysis (CCA). Tadpole species richness was significantly different between temporary and permanent ponds, however abundance did not differ among them. Depth and water temperature were related to tadpole richness, while canopy cover, dissolved oxygen, richness of predators and water temperature were related to tadpole abundance. The most important predictors explaining tadpole assemblage structure in permanent and temporary ponds were depth, dissolved oxygen, predator richness, canopy cover and water temperature. Our results suggest that the synergy between abiotic and biotic forces operating in temporary and permanent ponds contribute in shaping species diversity of pond-breeding anurans in northern Atlantic forest.

Keywords: Species diversity, ponds, environmental conditions, Atlantic forest, tadpoles

5.1 Introduction

The presence and abundance of species vary through space in a nonrandom way (Dray et al., 2012), thus species assemblages in a given community are governed by population demography, behavioral traits, spatial and temporal interactions between organisms and environmental factors (Parris, 2004; Agrawal et al., 2007; Dray et al., 2012; Borges & Rocha, 2013). In the tropics, assemblages of tadpoles are very diverse (Alford, 1999; Wells, 2007), and many species of anurans usually use the same breeding sites with different temporal and spatial patterns of reproduction (Borges & Rocha, 2012). Consequently, most sites exhibit a staggered introduction of larvae into the community, and the number of species present varies through time (Wells, 2007).

Pond-breeding anurans may use permanent and temporary water bodies (Werner & McPeek, 1994), exploiting resources efficiently within a temporal partitioning (Duellman & Trueb, 1994). As a result of this temporal asynchrony in reproduction, several tadpole species experience different environmental conditions (Semlitsch et al., 1996; Wells, 2007). Climate can greatly influence both biodiversity distribution and breeding patterns in amphibians (Wells, 2007; Provete et al., 2014; Silva et al., 2014). Pond-breeding anurans are often tied to seasonal temperature and rainfall conditions (Babbitt et al., 2010) but the ideal condictions may vary among species. Thus the spatial distribution of tadpoles among ponds and their temporal patterns of occurrence depend on the spatial and temporal distribution of adults (Alford, 1999; Wells, 2007; Provete et al., 2014).

Main environmental variables affecting anuran assemblages are precipitation, temperature, availability of nutrients, physical structure of the habitat (Parris, 2004;

Strauß et al., 2013) and biotic processes such as predation, competition, dispersal, disturbance and disease (Yanoviak, 2001; Vieira et al., 2012). Such factors often vary over time and certainly influence the spatial and temporal distribution of tadpoles (Wilbur, 1974; 1980; Heyer et al., 1975; McDiarmid & Altig, 1999; Provete et al., 2014). Particularly, tadpole assemblages in ponds are strongly influenced by hydroperiod gradients, canopy cover, predation and life history of species (Heyer et al., 1975; Both et al., 2009; Hawley, 2010). Temporary and permanent ponds often differ in species composition, which is caused by high variation in hydroperiod (Collinson et al., 1995; Both et al., 2009). While in the wet months species diversity tends to be higher in temporary ponds, during the dry months this pattern changes, and permanent ponds show a greater diversity (Wellborn et al., 1996). Likewise, canopy gradient and potential tadpole predator communities also vary along the hydroperiod (Heyer et al., 1975), influencing tadpole assemblages (Yanoviak, 2001).

Ephemeral habitats are generally free of large predators (Gunzburger & Travis 2004), such as fishes but often have large invertebrate predator communities (Wellborn et al., 1996; Azevedo-Ramos et al. 1999). Permanent habitats are more complex and can support larger invertebrate or vertebrate predators, which can exert strong pressure on tadpoles (Heyer et al., 1975; Wilbur 1997). As a result, it is expected that the richness and abundance of tadpole species would be greater in temporary ponds than in permanent ponds, due to differences in predation pressure (Hero et al., 2001; Both et al., 2009). Canopy cover is also an important factor that impacts directly or indirectly the temperature, pH, conductivity, and dissolved oxygen of water, which consequently influence larval development and survival in ponds (Ultsch et al., 1999; Skelly et al., 2002; Hawlet, 2010).

In tropical forests, ponds are a good model for understanding factors that influence anuran assemblage structure because they are: i) spatially circumscribed; ii) abiotically heterogeneous; iii) species rich; iv) easy to sample; v) vary in their temporal stability from permanent to temporary and vi) due to limited size may be the most important for determining coexistence between species. Here, we compared the variation in the diversity of tadpoles and predators in temporary and permanent ponds between seasons, and analyzed how biotic and abiotic environmental variables influence tadpole assemblages in these ponds.

5.2 Material and Methods

5.2.1 Study area

The study was conducted in four municipalities of Alagoas state, northeastern Brazil: Boca da Mata (9°39’48.5’’S 36°11’ 45.8’’W, 160 masl), Ibateguara (8°59’54.1’’S 35°52’44.3’’W, 415 masl), Maceió (9°36’51.6’’S 35°45’32.6’’W, 20masl), and Paripueira (9°26’48.3’’S 35°32’52.3’’W, 39 masl) (Figure 1). These localities are situated in the Pernambuco sub-region of the Atlantic forest, sensu Silva & Casteleti (2003) north of the São Francisco river, with highly heterogeneous environmental conditions (Ribeiro et al., 2009) and altitudinal variation between 0-600m (Tabarelli et al., 2010). The climate is classified as tropical with dry summer with annual temperature and rainfall of 22-26°C and 1,300-1,600mm, respectively (Alvares et al., 2013) with vegetation composed of

Ombrophilous (pluvial), Mixed Ombrophilous and Semi-deciduous forests (Assis, 2000; Câmara, 2003).

In these areas four temporal and four permanent ponds (Table 1) were sampled during twelve field trips during the rainy (August-October 2013) and dry (January-March 2014) seasons. Each pond was sampled twice during the rainy season and once (only permanent ponds) during the dry season. These ponds were natural or man-made water bodies with areas from 10m^2 to 1,000m^2, diverse shapes, and depth between 0.15-2m (Table 1). Permanent ponds were considered as lentic water bodies that hold water throughout the year, and temporary ponds were considered as water bodies that dry completely for long or short periods, holding water mainly during the rainy season (Brönmark & Hansson, 2005; Gascón et al., 2008). During sampling in 2014, temporary ponds were completely dry and consequently only permanent ponds were sampled in this year.

5.2.2 Data collection: environmental and biological descriptors

Tadpoles and insects were collected following the methodology proposed by Shaffer et al. (2001). Sweeps through the pond (borders, center, bottom) were made for 45 minutes only during the morning period (8:00-12:00) with a steel dip net of 26 x 50cm. Tadpoles and insects were placed in separated plastic bags with water and carried alive to the Coleção Herpetológica do Museu de História Natural da Universidade Federal de Alagoas (MUFAL). In the laboratory, tadpoles were euthanized and fixed in 10% formalin. For identification of tadpoles were used original descriptions of each species (Bokerman, 1963; Cruz, 1982; Peixoto & Cruz, 1983; Gomes & Peixoto, 1991; Rossa-

Feres & Nomura, 2006; Vieira et al., 2007; Nascimento et al., 2009; Lourenço-de-Morais et al., 2012). When identification of tadpoles was not possible, 3-7 individuals (of unidentified species) were kept in captivity until reach an appropriate stage for posterior identification. In the field, all insects captured were classified as potential predators or not (based on studies of tadpoles predation Heyer et al., 1975; Gascon, 1992; Toledo, 2005). Individuals classified as non-predators were released in the same place, and the potential predators were carried alive to MUFAL and preserved in 70% ethanol. Three types of insects were considered as potential predators; dragonfly larvae (considering the sub-orders Anisoptera and Zigoptera odonate naiads), giant water bugs (Hemiptera) and diving beetles (Coleoptera). The identification of these insects to sub-orden and family levels was made using Bouchard (2004), Costa et al. (2004), Souza et al. (2007) and Segura et al. (2011).

Besides biotic variable (predators), abiotic variables were also measured to assess the influence on these in tadpole assemblages. All measurements were taken in five points (cardinal points and one in the center). Each pond was georeferenced and bounded to estimate its area. Canopy cover was measured as the vertical projection of plant foliage (Fiala et al., 2006) over the stream surface. Canopy was photographed at chest level within the pond keeping the camera in a vertical position. Photographs were converted to binary images whereas the canopy becomes black and the sky white. The proportion of black and white areas was calculated projecting eight vertical and six horizontal grid lines onto the photograph, then the percentage of canopy cover was estimated. Water temperature (daily mean), dissolved oxygen (DO) and pH were measured 10 cm below the water in the water column using a multiparameter Hanna HI 9828. Depth was taken using a 3m measuring tape. Because these variables present variations throughout a day, all measurements were recorded in similar times of the day and sunlight for comparison among ponds (Sparling, 2010).

5.2.3 Statistical analysis

Richness and abundance were defined as the total number of species and the total number of individuals of each species in each pond, respectively (Gotelli & Chao, 2013). Differences in the richness and abundance of tadpoles and predators from temporary and permanent ponds were tested using an Analysis of Variance (ANOVA). Because temporary ponds dry out during the dry season, we only compared the richness and abundance of tadpoles between seasons in permanent ponds. We tested the effect of environmental and biotic variables on tadpole richness and abundance using General Regression Model (GRM). All variables were log (x+1) transformed to remove heteroscedasticity and make them comparable (Borcard et al., 2011). The best model was selected using stepwise procedure with Akaike information criterion (AIC).

Canonical Correspondence Analysis (CCA) was used to test the relationships between tadpole assemblages in the ponds and the biotic (predator richness and abundance) and abiotic factors (canopy cover, depth, temperature, pH and dissolved oxygen). CCA can simultaneously represent the spatial organization of the sampled species following their ecological optima (Borcard et al., 2011). This test explains a greater amount of the total variation in the species abundance data and allows an easy ecological interpretation of species assemblages (Legendre et al., 2005; Borcard et al., 2011). Variables were submitted to a forward procedure and then to a permutation tests with

1,000 iterations to test the significance of CCA results. After these tests, canopy cover and predator abundance were retained in the analysis. All statistics and graphics were performed using R ver. 3.1.1 software (R Development Core Team, 2014).

5.3 Results

5.3.1 Tadpoles and predators diversity

In total, 1,534 tadpoles of 20 species belonging to seven genera and three families were collected: Bufonidae (one species/genus), Hylidae (14 species/ four genera) and Leptodactylidae (five species/ three genera) (Table 2). Tadpole richness and abundance in permanent ponds ranged from 1 to 10 species ($\bar{x}$ = 1.87, SD = ± 0.83) and from 1 to 339 ($\bar{x}$ = 38.33, SD = ± 86.35), respectively. In temporary ponds, tadpole richness and abundance ranged from 2 to 13 species ($\bar{x}$ = 3.75, SD = ± 1.70) and from 1 to 547 ($\bar{x}$ = 63.26, SD = ± 136.76), respectively. Hylidae was the most species diverse (14 species, 73.6%) and abundant (984 individuals, 64%) family. *Phyllomedusa nordestina* was the most abundant species (606 individuals) followed by *Leptodactylus* sp. (339 individuals), *Rhinella* sp. (94 individuals), *Hypsiboas semilineatus* (81 individuals) and *Physalaemus cuvieri* (81 individuals). Furthermore, *Dendropsophus branneri* was the most common species (50% of ponds), followed by *D. haddadi, H. albomarginatus, H. atlanticus, P. nordestina* and *P. cuvieri* which were present in 25% of ponds (Table 2). Tadpoles of *D. branneri* and *H. albomarginatus* occurred in both types of ponds. *Rhinella* sp., *D. soaresi, Hypsiboas* spp., *P. nordestina, Scinax* spp., *L. latrans* and *P. cuvieri*, were found only in temporary ponds. Tadpoles of *D*. cf. *minutus, D. haddadi, H. atlanticus, H. semilineatus, Leptodactylus* sp., *L. vastus* and *Pseudopaludicola mystacalis* were found only in permanent ponds. Richness in temporary ponds was higher (F = 9.67, df = 16, p = 0.006) than in permanent ponds during the rainy season, although abundance between ponds did not differ (F = 0.72, df = 16, p = 0.40). In permanent ponds, richness (F = 0.15, df = 6, p = 0.70) and abundance (F = 2.41, df = 6, p = 0.17) did not differ between rainy and dry seasons.

In total, 403 predators, belonging to three orders, two suborders and five families were collected; Coleoptera (Dysticidae and Hydrophilidae), Hemiptera (Belostomatidae, Nepidae and Notonectidae) and Odonata (Anisoptera and Zygoptera) (Table 3). Odonatae larvae was more abundant in temporary (F = 5.15, df = 1, p = 0.03) than in permanent ponds, however abundance of Coleoptera (F = 1.10, df = 1, p = 0.33) and Hemiptera (F = 2.94, df = 1, p = 0.12) did not differ between the ponds.

5.3.2 Relationships between tadpole assemblages with both environmental and biological descriptors

Temporary ponds had on average higher air ($\overline{x}$ = 27.27, SD = ±1.58) and water temperatures ($\overline{x}$ = 28.04, SD = ±2.09) and dissolved oxygen ($\overline{x}$ = 6.34, SD = ±2.45) than permanent ponds. Permanent ponds had a greater canopy cover (closed-canopy) and on average higher depth ($\overline{x}$ = 32.97, SD = ±14.76) and relative humidity ($\overline{x}$ = 83.25, SD = ±8.31) than temporary ponds. Ponds with small areas, little canopy cover, shallow waters and a high number of predators and temperatures, were related to increased richness of tadpoles (R^2 = 0.85, F = 7, df = 6, p = 0.01). Furthermore, smaller areas and higher temperatures were related to increased abundance of tadpoles in ponds (R^2 = 0.85, F = 10.48, df = 7, p = 0.004).

The first three axes in the CCA analysis, explained 43.6% of the total variation in tadpole assemblages in the ponds (Table 3). The first axis was negatively correlated to area (r = -0.89). Depth (r = 0.38), water temperature (r = -0.41), pH (r = 0.44) and dissolved oxygen (r = 0.57) also showed a weak correlation in the second axis, whereas the third was positively correlated with canopy (r = 0.85) and negatively weak correlation with predator abundance (r = -0.21) (Table 3). Both, permanent and temporary ponds did show a strong association neither with any variable nor with a defined group (Fig. 2). *Hypsiboas semilineatus* and *H. atlanticus* were associated with deeper ponds and closer canopy cover.

5.4 Discussion

Environmental and biological descriptors were related with tadpole assemblage variation in permanent and temporary ponds. We found that temporary ponds supported both more tadpole and insect species (richness and abundance) than permanent ones. Even though temporary ponds were somehow dried up during months without rain, the tadpole richness and abundance in permanent ponds did not increase during this period, suggesting a possible preference for temporary ponds of anuran adults. This habitat selection might be related with the seasonal breeding activity (Babbitt et al., 2010), which generally coincides with the beginning of the rains (Haddad et al. 2013), when availability of breeding habitats increases (Wells, 2007). Adults may respond to the ecological requirements of their larvae (Alford, 1999). Therefore, the selection of temporary ponds would reduce predation pressure on tadpoles, which is higher in permanent ponds (Heyer et al., 1975; Alford, 1999; Azevedo-Ramos et al., 1999; Both et al., 2009).

Species composition varied between temporary and permanent ponds, which would be the expected pattern considering the differences in structure, biotic and abiotic characteristics of the ponds (Gascon, 1991, Both et al., 2009). In 38% of the reproductive modes found in the Atlantic forest, many anuran species use ponds as oviposition site or to complete the larvae development (Haddad & Prado, 2005). Among these anurans, Hylidae and Leptodactylidae families have the greatest diversification of reproductive mode (Haddad & Prado, 2005), besides high environmental plasticity to exploit a wide variety of habitats (Wells, 2007; Haddad & Prado, 2005; Camurugi et al., 2010). Indeed, foam nests used by several leptodactylids help eggs to resist drying-out and may be deposited in environments with variations in water availability such as temporary ponds (Bastos et al., 2010; Tippl, 2011). Although, *D. haddadi, D.* cf*. minutus* and *L. vastus* were associated with permanent ponds tadpoles of these species can also occur in temporary ponds (Vieira et al., 2007; Both et al., 2009; Lourenço-de-Moraes et al., 2012). Likewise tadpoles of *L. latrans* can also occur in permanent ponds, since adults use both types of ponds as breeding habitats (Camurugi et al., 2010). Only *H. albomarginatus* was found using both types of ponds during the rainy months, and also permanent ponds during the dry months. Thus it is possible that this species has a pattern of continuous reproduction in the northern Atlantic forest as reported in the southern (Giasson & Haddad, 2007).

Permanent ponds were deeper and had a closer canopy cover, lower mean temperatures and DO than temporary ponds. Moreover, we found a greater abundance of tadpoles and predatory insects in temporary ponds, as shown in other studies (Gascon, 1991; Azevedo-Ramos et al., 1999; Hero et al., 2001; Both et al., 2009; Rodrigues et al., 2010), where Odonate larvae were more abundant in temporary ponds than in permanent ponds, and without differences among Coleoptera and Hemiptera abundance (Azevedo-Ramos et al., 1999). Anderson et al. (2001) found that at higher temperatures tadpoles grew faster, and consequently, they exceeded the size limitation of their insect predators faster than tadpoles at lower temperatures. Hence, high temperatures recorded in our temporary ponds would allow a faster growth of the tadpoles and consequently their larger sizes could avoid high predation pressure, which could explain the higher abundance of tadpoles and predators in these ponds. Besides, tadpoles could display some defensive tactics like behavioral changes, unpalatability, selecting more suitable microhabitats or reducing their activity level (Heyer et al., 1975; Gascon, 1991; Alford et al., 1999; Eterovick & Barata, 2006; Haddad et al., 2013) to cohexist with predators.

Richness and abundance of tadpoles were correlated with both environmental and biological descriptors in temporary and permanent ponds. Canopy cover showed a weak negative correlation with tadpole assemblages, while water temperature was positively correlated. An increase in depth and canopy cover will cause a decrease in temperature and oxygen (Sparling, 2010); parameters that affect deeply the physiology, ecology, and behavior of tadpoles (Ultsch et al., 1999; Sparling, 2010). Therefore, it would be expected that larval growth and development were slower in closed-canopy ponds with lower temperatures than in open-canopy ponds (Schiesari, 2006; Hawley, 2010). An experimental study with *Rana pipiens* showed that tadpoles reared at shallow hypoxic conditions developed and metamorphosed faster than tadpoles in the deep hypoxic treatment; even when both were fed *ad libitum* (Feder & Moran, 1985). In this context, avoiding deeper microhabitats could be an advantage (Eterovick & Barata, 2006). However under environmental pressures (eg. torrential streams), selection of the deepest habitats could be a better strategy (Gascon, 1991). Our results show that environmental and biological descriptors are shaping tadpole assemblages of temporary and permanent ponds in the study area. Nonetheless, due to the particularities of each area these descriptors operate differently at broad and local scales (Both et al., 2009; Provete et al., 2014).

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Table 1. Sampled ponds for tadpoles and insect-predators in four municipalities of Alagoas state, Pernambuco sub-region of the Atlantic forest, Brazil.

Pond	Municipality	Altitude	Area (m²)	Length (m)	Width (m)	Hydroperiod
T1	Boca da Mata	180	82	17.5	6.7	Temporary
P2		160	918	44.5	20	Permanent
T3	Ibateguara	415	37	7.2	4.45	Temporary
T4	Maceió	20	399	29	20.5	Temporary
P5		35	126	9	3.3	Permanent
P6		45	226	25	14	Permanent
P7		55	141	25	13.8	Permanent
T8	Paripueira	9	12	9.35	3.35	Temporary

Table 2. Tadpoles species sampled in four permanent and four temporal ponds between August 2013 and March 2014, in four municipalities of Alagoas state, Pernambuco sub-region of the Atlantic forest, Brazil.

Taxa	**Temporary ponds**				**Permanent ponds**			
	T1	**T3**	**T4**	**T8**	**P2**	**P5**	**P6**	**P7**
Bufonidae								
Rhinella sp.	-	94	-	-	-	-	-	-
Hylidae								
Dendropsophus branneri	-	-	20	-	-		16	-
Dendropsophus cf. *minutus*	-	-	-	-	-	5	-	-
Dendropsophus haddadi	-	-	-	-	-	2	-	-
Dendropsophus soaresi	13	-	-	-	-	-	-	-
Hypsiboas albomarginatus	-	51	-	-	-	-	-	26
Hypsiboas atlanticus	-	-	-	-	-	42	-	-
Hypsiboas semilineatus	-	-	-	-	-	-	81	-
Hypsiboas sp. 1	-	-	1	-	-	-	-	-
Hypsiboas sp. 2	-	-	1	-	-	-	-	-
Hypsiboas sp. 3	-	-	1	-	-	-	-	-
Phyllomedusa nordestina	606	-	-	-	-	-	-	-
Scinax sp. 1	4	-	-	-	-	-	-	-
Scinax sp. 2	-	-	69	-	-	-	-	-
Scinax sp. 3	-	-	27	-	-	-	-	-
Leptodactylidae								
Leptodactylus latrans	-	-	-	35	-	-	-	-
Leptodactylus sp.	-	-	-	-	-	-	-	339
Leptodactylus vastus	-	-	-	-	-	-	-	1
Physalaemus cuvieri	27	-	-	-	-	-	-	-
*Pseudopaludicol*a *mystacalis*	-	-	-	-	56	-	-	-

Table 3. Insect-predators species sampled in four permanent and four temporal ponds between August 2013 and March 2014, in four municipalities of Alagoas state, Pernambuco sub-region of the Atlantic forest, Brazil.

Taxa	**Temporary ponds**				**Permanent ponds**			
	T1	**T3**	**T4**	**T8**	**P2**	**P5**	**P6**	**P7**
Coleoptera								
Dysticidae	1	-	1	-	3	-	-	-
Hydrophilidae	24	-	2	12	4	-	-	2
Hemiptera	-	-	-	-	-	-	-	-
Belostomatidae	9	-	17	-	5	6	7	-
Nepidae	-	-	2	1	1	5	11	1
Notonectidae	45	-	20	-	-	-	-	-
Odonata	-	-	-	-	-	-	-	-
Anisoptera	9	8	84	28	16	11	30	1
Zygoptera	-	-	28	1	8	-	-	-

Table 4. Summary of the CCA performed for environmental and biological factors used to explain tadpole assemblages among temporary and permanent ponds in four municipalities in Alagoas state, Pernambuco sub-region of the Atlantic forest, Brazil.

	Axis 1	**Axis 2**	**Axis 3**
Correlations (biplot scores)			
Predator abundance	0.90	0.12	-0.13
Predator richness	0.85	0.38	0.14
Canopy cover	-0.37	-0.25	0.40
Water depth (cm)	-0.003	-0.02	-0.64
Water temperature (°C)	0.07	-0.17	0.59
pH	0.65	0.34	-0.14
Dissolved oxygen	0.79	0.07	0.34
Summary statistics for ordination axes			
Cumulative percentage variance species–variables	15.86	29.53	42.14

Figures

Figure 1. Distribution of sampled sites in the Pernambuco sub-region of the Atlantic forest, Brazil. Sampling in the municipalities **1**. Boca da Mata, **2**. Ibateguara, **3**. Maceió and **4**. Paripueira in the Alagoas state, Brazil. Source for remaining forest cover: SOS Mata Atlântica/INPE 2012.

Figure 2. Canonical Correspondence Analysis (CCA) triplot of tadpole species and the environmental and biological descriptors. Variables are represented by lines. Black and gray circles represent temporary and permanent ponds respectively. Species abbreviations: *R*sp= *Rhinella* sp., *Dbr = Dendropsophus branneri, Dso= D. soaresi, Hal = Hypsiboas albomarginatus, Hat = H. atlanticus, Hse = H. semilineatus, Pno= Phyllomedusa nordestina, S*sp = *Scinax* sp.1, *Sc*p = *Scinax* sp.2, *Lla = Leptodactylus latrans, L*sp = *Leptodactylus* sp., *Lva = L. vastus, Pcu = Physalaemus cuvieri.*

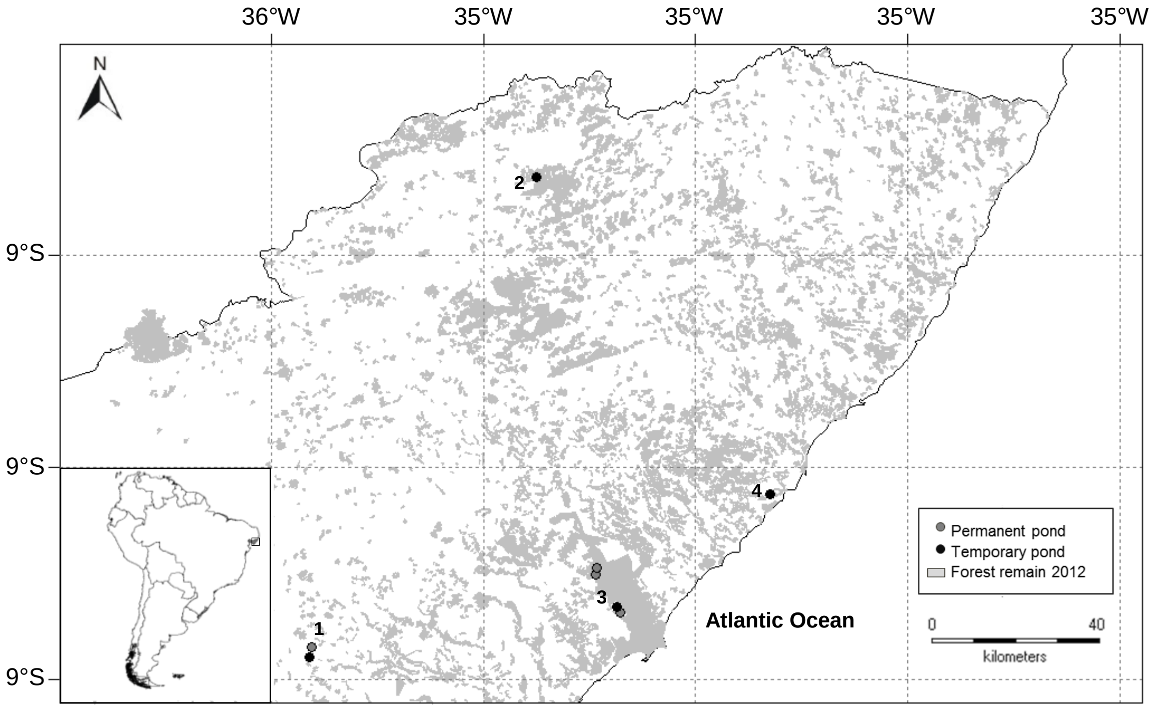
36°W
35°W
35°W
35°W
35°W
9°S
9°S
9°S
N
1
2
3
4
Atlantic Ocean
● Permanent pond
● Temporary pond
▭ Forest remain 2012
0
40
kilometers

Figure 1

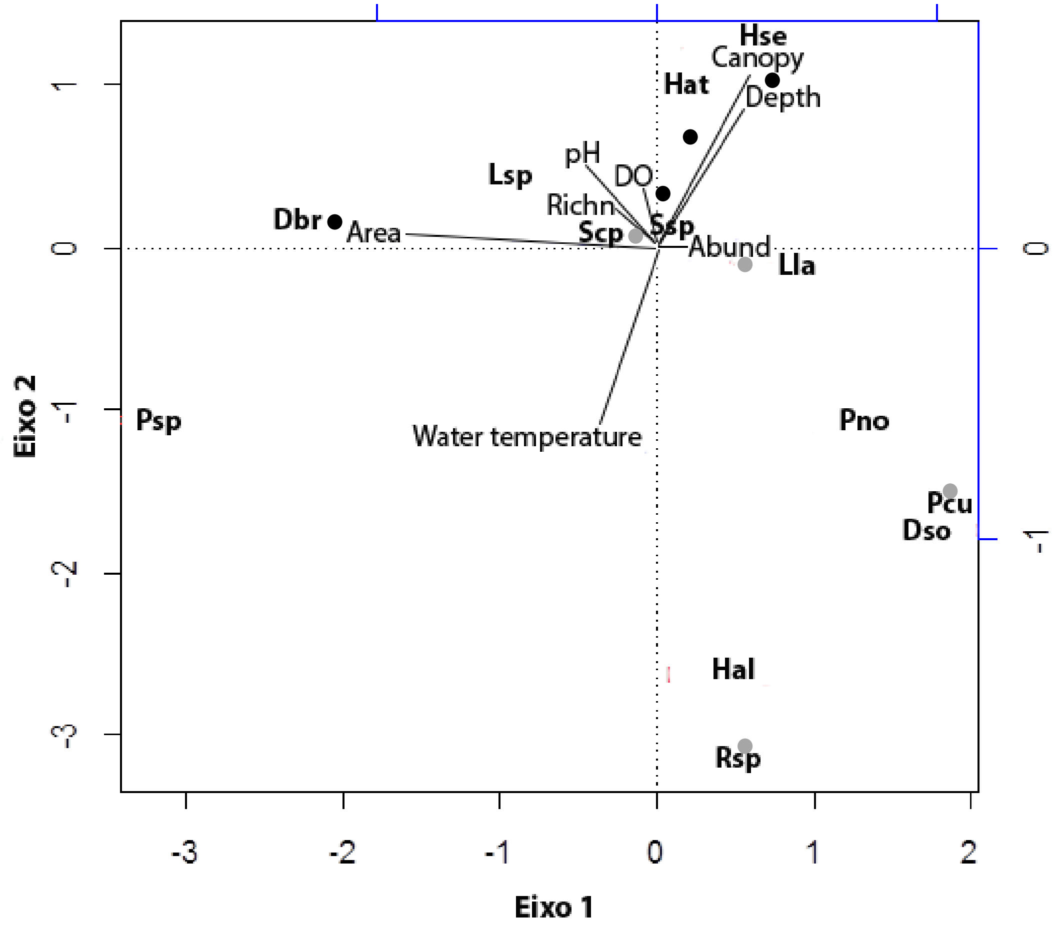
Hse
Canopy
Hat
Depth
pH
Lsp
DO
Richn
Dbr
Area
Scp
Ssp
Abund
Lla
Psp
Water temperature
Pno
Pcu
Dso
Hal
Rsp
Eixo 1
Eixo 2
-3
-2
-1
0
1
2
1
0
-1
-2
-3
0
-1

Figure 2

6. General discussion

The Atlantic forest is one of the most threatened forests in the world (Ribeiro et al. 2009), with a high diversity of species and habitats (Ribeiro et al. 2009, Toledo & Batista 2012, Haddad et al. 2013). However, some characterists of this forest may be favoring *Bd* spread (Becker & Zamudio 2011). We found that *Bd* is widely distributed in the Atlantic forest, with 131 *Bd* positive species, supporting the hypothesis that this fungus is a generalist pathogen (Fisher et al. 2009) in this forest.

We found similarities in *Bd* prevalence between the northern and southern Atlantic forest. In Alagoas, we observed a higher *Bd* prevalence during the rainy winter season. The same pattern was found in the southern region where *Bd* infection was higher during the rainy summer (Becker & Zamudio 2011, Gründler et al. 2012). However, since our results are restricted to a small area in Alagoas state, we can not generalize this pattern for the northern region of Atlantic forest. Although we found *Bd* infection only in the streams, our results are not conclusive because some species were exclusive for one type of these aquatic habitats (streams or ponds). Thus, we do not know if *Bd* prevalence could be affected by the host species identity, conditions in the habitat type or both. Likewise, our study was restricted by few sampled habitats, since we could not find an equal number of streams and ponds, or even similar species in these habitats to compare *Bd* infection status between places. Different environments (streams and ponds) and species can affect *Bd* prevalence in many ways therefore this interaction must be evaluate at local-fine, regional and global scale.

Some studies have evidenced a relationship between habitats and *Bd* prevalence (Kriger & Hero, 2007; Gründler et al., 2012). In Australia, *Bd* was detected in anurans breeding in permanent ponds and streams, while no evidence of infection in temporary ponds was found (Kriger & Hero, 2007). Conversely, in the Atlantic forest a higher *Bd* infection was found in lentic breeding species compared with lotic breeding (Gründler et al., 2012). We found that the temperature and pH recorded in our focal streams (21–23°C in the dry season and 21–22°C in the rainy season; 4-7 in the dry season and 4-5 in the rainy season) were lower than those measured in the ponds (26-30°C; 6-7 temporary and 25-28°C; 6-7 permanent ponds), which could explain the lack of *Bd* in the ponds, since there would not be conditions for *Bd* growth in these habitats. *Bd* growth is limited by temperature and desiccation (Johnson et al., 2003; Berger et al., 2004), thus the stress of high temperatures in temporary ponds could influence the fungal performance (Piotrowski et al., 2004, Woodhams et al., 2008) in the studied area.

Batrachochytrium dendrobatidis zoospores cannot survive in high temperatures (Johnson et al., 2003; Piotrowski et al., 2004). However, higher pond temperatures may stimulate amphibian metabolic processes and immune responses to pathogens; consequently accelerate the growth rate and metamorphosis (Ultsch et al., 1999). Thus, high temperatures in temporary ponds in Alagoas state could be not only promoting the rapid development of tadpoles but also avoiding *Bd* infection in the individuals.

Tadpole assemblages can be explained by differences on environmental and biological descriptors (Borges & Rocha, 2013). For the southern of the Atlantic forest, tadpole richness has been correlated with rainfall (Vasconcelos & Rossa-Feres, 2005). For Alagoas, we believe that this pattern could be the same, since we found greater tadpole richness during the rainy season on temporary ponds.

7. General conclusion

Although, the Atlantic Forest extends along 17 states; to date, only 38% of the anuran species present in 10 states have been screened for *Bd* infection, of which 25% tested positive. Therefore, long-term monitoring of anurans in the Atlantic forest (mainly in the north and northeastern regions) is clearly needed to fill geographical, taxonomic, and ecological gaps. Data that might provide useful information to be directly integrated into amphibian conservation plans.

Amphibian conservation is a global challenge and due to the multiple causes associate to amphibian declines, it is necessary to know and understand how species will respond to potential risks. Individuals of *Agalychnis granulosa*, *Aplastodiscus sibilatus*, *Hypsiboas freicanecae*, and *Crossodactylus dantei* in Alagoas state tested positive for *Bd*, though this pathogen does not seem to affect these anuran populations. Alhough, tadpoles of streams and ponds were tested for *Bd* presence, only in the streams infected tadpoles were found, when apparently the environmental conditions are favourable for both amphibians and *Bd*.

Macro and microclimate variation across aquatic habitats could be influencing *Bd* infection loads and prevalence. In the northern Atlantic forest *Bd* prevalence in lotic and lenthic habitats appears to be related to the local conditions and host traits. Thus, in lenthic habitats the biotic (host) and abiotic (temperature or pH) conditions may be changing rapidly, hindering the growth and maintenance of the fungus.

There are several forces acting on the tadpole assemblages, however, each species responds differently to these elements. We found the highest host species richness in temporary ponds during the rainy season. Still, our data are not conclusive the temporal dimension and hydroperiod could be playing an important role in structuring the studied tadpoles communities.

Water temperature was another important descriptor of tadpole assemblages. The high temperatures recorded in temporary ponds could accelerate tadpole growth rates, reduce predation pressure, and facilitate the coexistence of both tadpoles and predators.

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