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Effects of urbanization on the occurrence of Batrachochytrium dendrobatidis: do urban environments provide refuge from the amphibian chytrid fungus?

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Effects of urbanization on the occurrence of *Batrachochytrium dendrobatidis*: do urban environments provide refuge from the amphibian chytrid fungus?

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Abstract *Batrachochytrium dendrobatidis* (*Bd*) is a widespread pathogenic fungus that is known to cause the disease, chytridiomycosis, which can be lethal to many amphibians. We compared occurrence rates on spring peepers (*Pseudacris crucifer*) in urban and forested breeding sites in eastern Texas, USA. All study sites were at approximately the same latitude and altitude, and samples were collected at the same time of year to isolate differences in *Bd* infection rates between habitat types. We found significant differences ($p<0.001$) in the occurrence of *Bd* between habitats; with dramatically lower rates of occurrence at urban sites (19.5 %), compared to forested sites (62.9 %). The exact reason for the observed differences in the occurrence of *Bd* is not known, however, we suspect that warmer temperatures or lower population densities and lower species richness at urban sites all could play a role in our results. Our findings suggest that urban environments may provide a refuge for some amphibians from the pathogen.

Keywords Amphibians · Chytrid fungus · Urbanization · Habitat

Introduction

Most ecosystems are currently experiencing a loss of biodiversity, much of which is associated with human activity (Soule 1991; Naeem et al. 1994). Land-use change can facilitate local and regional extinction of populations by killing organisms, removing suitable habitat, and interfering

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with breeding activities (Collins and Storfer 2003). However, depending on the level of urbanization, some taxa may actually increase in richness due to factors such as spatial heterogeneity, intermediate disturbance, and scale (McKinney 2008).

Amphibian population declines, a global pattern that has garnered much interest in the past few decades, are certainly a major component of the overall loss of diversity (Wake 1991; Pechmann and Wilbur 1994; Houlahan et al. 2000, 2010). Along with population declines, amphibians have the highest global extinction rate among vertebrates (Stuart et al. 2004). Although habitat loss and urbanization are major threats to amphibians (Collins and Storfer 2003; Hamer and McDonnell 2008; McKinney 2008), it has become increasingly clear that many factors and combinations of these factors play important roles in the world-wide decline of amphibian populations (Alford and Richards 1999; Houlahan et al. 2000).

In addition to habitat loss and urbanization, the pathogenic fungus, *Batrachochytrium dendrobatidis* (*Bd*) is a significant threat to amphibians by causing the lethal skin disease, chytridiomycosis (Berger et al. 1998; Longcore et al. 1999; Lips et al. 2006). The pathogen is known to occur on every continent except Antarctica (Fisher et al. 2009) and has caused the decline or extinction of about 200 species of frog worldwide (Daszak et al. 2003; Skerratt et al. 2007). *Batrachochytrium dendrobatidis* may be the most serious infectious disease threat to biodiversity (Kilpatrick et al. 2010), and is now listed as an internationally notifiable disease by the World Organization for Animal Health as an epidemiologically important pathogen (Schloegel et al. 2010).

Occurrence and infectiousness of *Bd* are variable and known to be influenced by several factors. Higher altitude (Brem and Lips 2008), higher latitude (Kriger et al. 2007; Rodder et al. 2008), wetter habitats (Murphy et al. 2009, 2011), and cool seasons (Berger et al. 2004; Kriger and Hero 2008; Lannoo et al. 2011) all increase the incidence of *Bd* and decrease survival of infected amphibians. Temperature seems to be a primary factor that influences occurrence and infectiousness (Muths et al. 2008; Olson et al. 2013) but there is evidence that greater species richness increases the likelihood of infection and transmission of *Bd* (Olson et al. 2013).

Recent studies are beginning to shed light on the relationships between human impacts on the environment and *Bd*. Pauza et al. (2010) suggested that anthropomorphic activities promote the spread of *Bd*, but there is little evidence linking habitat loss with increases in the incidence of the pathogen. However, Van Sluys and Hero (2009) found higher densities of stony creek frogs (*Litoria wilcoxii*), and lower prevalence of *Bd* in open farmland compared to nearby forested habitat, suggesting that habitat strongly influenced *Bd* prevalence. Becker and Zamudio (2011) provided further direct evidence that the prevalence and infection intensity of *Bd* was inversely correlated to habitat loss in Costa Rica, Brazil, and Australia, possibly caused by increased temperatures resulting from habitat loss. Similarly, Lane and Burgin (2008) observed greater frog diversity and abundance in urban sites compared to non-urban sites in Australia but were only able to speculate that chemicals in urban wastewater provided protection from diseases, such as chytridiomycosis.

Given that habitat loss and chytridiomycosis are two major threats to biodiversity, and that current literature (Lane and Burgin 2008; Van Sluys and Hero 2009; Becker and Zamudio 2011) suggests likely interactions between the two factors, further explorations of these relationships is warranted. To date, no definitive evidence exists pertaining to the effects of urbanization on the relationship between the fungal pathogen and amphibian populations. Herein, we compare the incidence of *Bd* between amphibian populations from urban and forested habitats. Our findings provide insights for how habitat loss may affect host-pathogen interactions.

Methods

We chose the spring peeper (*Pseudacris crucifer*) as our focal species because it is known to occur in urban and forested wetlands and actively breeds in the cooler months of the year (Saenz et al. 2006) when the prevalence of *Bd* should be highest (Berger et al. 2004; Woodhams and Alford 2005; Kinney et al. 2011). In addition, spring peepers are abundant and known to have a relatively high incidence of *Bd* in our study area (Saenz et al. 2010).

We sampled frogs for *Bd* at six urban sites within the city of Nacogdoches, Texas, USA (31.6089° N, 94.6508° W); two forested sites in the Stephen F. Austin Experimental Forest (SFA EF) in Nacogdoches County, Texas (31.4667° N, 94.7833° W); and six forested sites in the Davy Crockett National Forest (DCNF) in Houston County, Texas (31.3333° N, 94.0833° W). The urban sites consisted of a variety of habitats including, drainage ditches, associated overflow areas, and a flooded soccer field (Fig. 1). These sites tended to be more exposed with less vegetative cover than forested sites. Roads, housing, and human structures were in close proximity to all urban sites (Fig. 1). The forested sites were all naturally occurring ephemeral wetlands in habitat dominated by secondary growth loblolly (*Pinus taeda*), and shortleaf (*P. echinata*) pines with a mixed deciduous hardwood component (Fig. 2).

We sampled individual spring peepers for *Bd* at 14 different sites on seven nights from 24 January to 16 March 2010. We collected as many spring peepers, by hand, as possible on a single trip to each site, to avoid resampling of individuals. Individual frogs were typically located by listening for calling activity then homing in on individual frogs. This technique was quite effective and we feel that we were able to capture over 95 % of the frogs that we observed calling in both habitat types. We sampled for *Bd* by rubbing a sterile cotton swab on the dorsum, ventral surfaces and feet of each frog for approximately 30 s. After swabbing, we immediately released the frogs at their place of capture. We used sterile techniques throughout the swabbing process; including using a new pair of gloves for handling each individual frog and autoclaving the cotton swabs prior to sampling, to prevent cross-contamination among samples. Swabs were placed into microcentrifuge tubes containing 1 ml of 70 % ethanol, stored in a freezer, and later analyzed for *Bd* DNA by polymerase chain reaction (PCR)

Fig. 1 Typical urban swabbing site, a drainage ditch behind a shopping center in Nacogdoches, Texas. (Site Nacogdoches-2)

Fig. 2 Typical forested swabbing site, an ephemeral wetland in the Davy Crockett National Forest, Houston County, Texas. (Site DCNF-3)

analysis (Pisces Molecular Laboratory, Boulder, Colorado,USA). Percent occurrence of *Bd* among frogs sampled was calculated per location, and Chi-square analyses were used to determine if *Bd* occurrence differed between the habitat types ($p<0.05$).

Results

Spring peeper population densities appeared to be higher in forested habitats than in urban sites, as evidenced by the number of samples taken from each habitat type, and the comparable sampling efforts per habitat. We collected 41 spring peepers from six urban wetlands and 89 individuals from eight sites in forested habitats. PCR results indicated that spring peepers from forested habitats had significantly higher incidence of *Bd* than urban frogs ($\chi^2=21.16$, df=1, $p<0.001$). Of the individuals collected in the city of Nacogdoches, 19.5 % tested *Bd*-positive (Table 1), whereas 56.5 and 77.8 % tested positive from the DCNF and the SFA EF (Table 2), respectively. All frogs collected in this study appeared to be healthy. No dead or dying frogs were observed at our study sites.

Table 1 *Batrachochytrium dendrobatidis* PCR results from swabs taken from spring peepers collected in and around urban wetlands in the city of Nacogdoches in Nacogdoches County, Texas

Urban sites	Date	+/Total	% Positive
Nacogdoches-1	24 Jan 2010	0/6	0 %
Nacogdoches-2	28 Jan 2010	0/4	0 %
Nacogdoches-3	28 Jan 2010	0/12	0 %
Nacogdoches-4	9 Mar 2010	2/6	33.3 %
Nacogdoches-5	9 Mar 2010	1/2	50 %
Nacogdoches-6	10 Mar 2010	5/11	45.45 %
Total urban		**8/41**	**19.51 %**

Table 2 *Batrachochytrium dendrobatidis* PCR results from swabs taken from spring peepers collected in the Stephen F. Austin Experimental Forest in Nacogdoches County, Texas and the Davy Crockett National Forest in Houston County, Texas

Forested sites	Date	+/Total	% Positive
SFA EF-1	21 Feb 2010	8/10	80 %
SFA EF-2	21 Feb 2010	13/17	76.47 %
	SFA EF total	**21/27**	**77.77 %**
DCNF-1	15 Mar 2010	18/21	85.71 %
DCNF-2	15 Mar 2010	0/2	0 %
DCNF-3	15 Mar 2010	4/7	57.14 %
DCNF-4	16 Mar 2010	11/21	52.38 %
DCNF-5	16 Mar 2010	0/1	0 %
DCNF-6	16 Mar 2010	2/10	20 %
	DCNF total	**35/62**	**56.45 %**
Forested total		**56/89**	**62.92 %**

Discussion

Unlike studies that sampled and compared forested and deforested non-urban sites, we compared forested sites with urban sites to provide new insights into the relationship between human activity and *Bd*. Our findings that frogs in forested sites were significantly more likely to test positive for *Bd* than frogs in urban sites seem congruent with recent studies concluding that the risk of *Bd* contraction is higher in natural habitats (Van Sluys and Hero 2009; Becker and Zamudio 2011). Van Sluys and Hero (2009) found the incidence of *Bd* in forested sites to be 56.7 % compared to 29.8 % at open sites. In our study, we found a similar incidence of *Bd* in forested sites (62.9 %) but an even lower rate in urban sites (19.5 %). In effect, the difference in the occurrence of *Bd* in their study was just below 2:1 while we found a greater than three-fold difference between urban and forested sites. We suggest that the addition of urbanization to a deforested habitat may augment the previously established effects of deforestation on *Bd* infectiousness; whereas deforested non-urban sites may be intermediate by comparison (between urban and forested). However, further investigation is warranted to determine if this trend continues across multiple urban settings.

Although the pattern of differences in the occurrence of *Bd* between habitats appears to be consistent among recent studies, the proximate causes for these differences are still undetermined. Urban pollution may be a factor that can negatively affect *Bd*, as suggested by Lane and Burgin (2008). There are several chemical treatments that can clear *Bd* infections (Johnson et al. 2003; Berger et al. 2009; Woodhams et al. 2012); therefore, it is plausible that urban pollution may act in a similar manner. Depressional wetlands in urban areas can be sinks for sediments, nutrients, metals, greater nitrogen mineralization and nitrification (Faulkner 2004). In a recent study, Parris and Baud (2004) suggest that the synergistic effects of copper and *Bd* on amphibians may be negative, which would contradict the hypothesis that urban pollution is providing protection from *Bd*; however, sodium chloride has been shown to inhibit the growth of *Bd*, increase host survival, and has been suggested as a potentially useful antifungal agent for the prevention of chytridiomycosis (Stockwell et al. 2012). Runoff pollution from deicing salt could be a factor affecting wetlands in some regions (Sanso and Hecnar 2006; Karraker and Ruthig 2009), however, in our study region in the southern United States, deicing salt is not commonly used, therefore, it likely had no effect on the results of this study. Urban pollution may be a contributing factor in reducing the incidence of *Bd* in some cases but, to date, we have no evidence to support this assumption.

Density dependence and species richness could be other factors influencing the occurrence of *Bd* (Rachowics and Briggs 2007; Olson et al. 2013). We had a much higher incidence of *Bd* in forested sites and possibly higher densities or frogs compared to urban sites. In addition, species richness is likely to be higher in forested habitats as well (Saenz, personal observations). While our study was not designed to account for effect of host density and community complexity on the frequency of *Bd*, we cannot rule these out as factors and should be considered in future research.

While pollution and host density dependence are factors that warrant more investigation, temperature and moisture are well-accepted correlates of *Bd* infection rates (Schlaepfer et al. 2007; Murphy et al. 2009). Whitfield et al. (2012) found that despite small changes in temperature, *Bd* was much more prevalent in the cooler months, suggesting that small fluctuations in temperature may significantly affect *Bd* occurrence. Chatfield and Richards-Zawacki (2011) provided further evidence for the potential importance of temperature by using heat therapy to clear *Bd* from captive frogs. Hossack et al. (2013) found that boreal toads sampled in recently burned areas had a lower incidence of *Bd*, potentially due to a drier, warmer microclimate. This supports previous work implicating not only temperature, but moisture as an important factor influencing *Bd* infection rates as well (Murphy et al. 2009, 2011). Although we did not collect temperature and moisture data directly from the urban and forested sites, based on existing literature (Kim 1992), we expect city temperatures to be higher, and moisture levels lower in urban settings compared to forested sites; which would be consistent with the lower incidence of *Bd* observed in our study.

Furthermore, the effects of natural vegetation on microclimates are likely important in the context of this study. Raffel et al. (2010) suggest that shade may play an important role in *Bd* infection by lowering temperature. Becker et al. (2012) found that canopy density, natural vegetation, and daily average water temperature were the best predictors of *Bd* and differences in microclimate. Kim (1992) suggests that urban heating is attributed to rapidly heating urban surfaces, such as bare soil and short grasses, (see Fig. 2) which are less common in forested habitats and could lead to differences in the occurrence of *Bd* (Berger et al. 2004). We suggest that differences in temperature, generally resulting from land-use change are likely driving *Bd* occurrence rates, but we cannot rule out other factors at this point.

Although our study does not address the proximate causes of the observed differences in the occurrence rates, human-caused disturbances appear to have a clear effect on the incidence of *Bd*. We can conclude that, in our study in a temperate region, an inverse relationship with *Bd* and habitat disturbance does occur, as was observed in the tropics in previous studies (Van Sluys and Hero 2009; Becker and Zamudio 2011). Among human activities that cause habitat loss, urbanization produces some of the greatest extinction rates and eliminates a large majority of native species (McKinney 2002). However, our study adds to the paradox that disturbed habitat, including urban areas, may be acting as refugia from diseases, such as *Bd*, when species are able to tolerate urban environments and deforestation, as was suggested by Becker and Zamudio (2011).

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