

DROUGHT REDUCES CHYTRID FUNGUS (*BATRACHOCHYTRIUM DENDROBATIDIS*) INFECTION INTENSITY AND MORTALITY BUT NOT PREVALENCE IN ADULT CRAWFISH FROGS (*LITHOBATES AREOLATUS*)

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ABSTRACT: To fully understand the impacts of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) on amphibians it is necessary to examine the interactions between populations and their environment. Ecologic variables can exacerbate or ameliorate *Bd* prevalence and infection intensity, factors that are positively related when *Bd* is acting on naive amphibian populations as an epidemic disease. In crawfish frogs (*Lithobates areolatus*), a North American species with a complex life history, we have shown that *Bd* acts as an endemic disease with impacts that vary seasonally; the highest infection prevalences and intensities and highest frog mortality occurred during late spring in postbreeding individuals. In this study, conducted between 28 February and 23 August 2011 in southwestern Indiana on the same population, we report an uncoupling of the previously observed relationship between *Bd* prevalence and intensity following an extreme drought. Specifically, there was a postdrought reduction in *Bd* infection intensity and mortality, but not in infection prevalence. This result suggests that the relationship between prevalence and intensity observed in *Bd* epidemics can be uncoupled in populations harboring endemic infections. Further, constant prevalence rates suggest either that crawfish frogs are being exposed to *Bd* sources independent of ambient moisture or that low-level infections below detection thresholds persist from year to year. Drought has several ecologically beneficial effects for amphibians with complex life histories, including eliminating fish and invertebrate populations that feed on larvae. To these ecologic benefits we suggest another, that drought can reduce the incidence of the severe skin disease (chytridiomycosis) due to *Bd* infection.

Key words: *Bd* load model, chytridiomycosis, disease, pathogen.

INTRODUCTION

The chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al. 1999), has been devastating amphibians globally and is propelling a wave of population extirpations and species extinctions (e.g., Lips et al. 2006). Variations in natural history, life history, behavioral, and immunologic traits among species can strongly affect the development and outcome of *Bd* infection (Voyles et al. 2011). In addition, intraspecific variation in ecology (e.g., Puschendorf et al. 2009), geography (e.g., Kriger et al. 2007), and season (e.g., Lannoo et al. 2011) can also influence infection prevalence, infection

intensity, and host mortality. *Batrachochytrium dendrobatidis* infections are generally thought to be most intense in cool, humid habitats (e.g., Woodhams et al. 2005) and in species that aggregate when threatened by dehydration (Longo et al. 2010).

The relationship between *Bd* infection prevalence (proportion of individuals affected) and infection intensity (expressed as zoospore equivalents per swab) has been most often examined in amphibian populations in which *Bd* is a novel pathogen causing epidemic disease (Berger et al. 1998). Briggs et al. (2010) have described this relationship mathematically as the *Bd* load model. The question arises

whether the *Bd* load model holds when populations have had a long history of exposure, and *Bd* is thus an endemic infection.

To fully understand the impacts of *Bd* on amphibians, it is necessary to examine the interactions between life history patterns and environmental variables (i.e., ambient moisture) that can exacerbate or ameliorate *Bd* prevalence and intensity (Lips et al. 2003). We have shown that *Bd* infection varies seasonally in crawfish frogs (*Lithobates areolatus*), a North American ranid with a complex life history (Kinney et al. 2011). When not breeding, individuals generally live alone in burrows dug by crayfish (Heemeyer et al. 2012). Adults clear the infection (at least to the point where *Bd* zoospores are undetectable using sensitive Taqman PCR techniques) during the summer, probably by being isolated from other frogs and by basking in sunlight on their feeding platforms (Kinney et al. 2011). During the winter, a subset of adults reacquires infection while overwintering at the base of their burrows. Infected adults then carry *Bd* to breeding wetlands, where infection prevalence and intensity increase, resulting in the development of the severe skin disease, chytridiomycosis, and the death of a subset of postbreeding adults (Kinney et al. 2011). However, newly metamorphosed juvenile crawfish frogs exhibit low *Bd* prevalences and intensities, despite having spent the previous 60–90 days as larvae in the same wetlands where *Bd* infections are exacerbated in adults (Kinney et al. 2011).

Following our initial *Bd* surveys (Kinney et al. 2011) in late 2010, our field site experienced a 3-mo extreme drought, giving us the opportunity to examine the subsequent effects of dry conditions on *Bd* infection prevalence and intensity. During the spring of 2011 we sampled pre- and postbreeding crawfish frog adults entering and exiting wetlands, respectively; later in the summer we sampled postmetamorphic juveniles as they exited wetlands. We

compared these data to previously published data from the 2010 field season collected on the same population (and in 19 cases on the same individuals; Kinney et al. 2011). Given that *Bd* thrives in cool, high-humidity environments, following Kriger (2009), we hypothesized that infection prevalence and intensity, as well as observed mortality due to chytridiomycosis, would be lower than levels observed by Kinney et al. (2011).

MATERIALS AND METHODS

Breeding crawfish frog adults were captured along drift fences or in pitfall traps adjacent to drift fences (Gibbons and Bennett 1974) as they attempted to enter or exit two wetlands in southwestern Indiana. These wetlands (Nate's and Cattail ponds; 39°07'N, 87°13'W), described by Kinney et al. (2011), are approximately 0.9 km apart and crawfish frog adults and juveniles move between them. We present data from each wetland for completeness, but because the data between wetlands were similar within years, we combined data from both sites for our statistical analyses comparing infection prevalences and intensities between years. We use the 2010 dataset (which includes both prevalence and intensity; Kinney et al. 2011) to form a basis to compare against the 2011 dataset. Sampling and analytical techniques (below) were identical both years.

In 2011, breeding frogs were sampled 28 February–11 May. When first encountered, each frog was given a passive integrated transponder (PIT) tag inserted subcutaneously (Christy 1996), which allowed us to identify individuals. *Batrachochytrium dendrobatidis* samples were coded by PIT tag number and date obtained. All entering and exiting crawfish frog adults were sampled for *Bd* unless they were handled in any way that could have contaminated the sample. Because available evidence indicates all adult crawfish frogs breed (Heemeyer et al. 2012), we censused (detection probability=1.0) rather than sampled (detection probability<1.0) our populations (Mills 2007).

Newly metamorphosed juvenile crawfish frogs were captured along drift fences while exiting wetlands. Between 8 June and 23 August 2011, 3,152 crawfish frogs metamorphosed: 3,122 from Nate's Pond, 30 from Cattail Pond. During peak metamorphosis, there were often dozens of juvenile crawfish frogs in each pitfall trap. To avoid inflating

TABLE 1. Temperature and precipitation and Palmer Z-index (drought) values for August–October, including the 30-yr norm and 2008–2010 (NCDC 2013).

	Temperature (C)			Precipitation (cm)	Palmer Z-index			
	Mean	Max	Min		August	September	October	Mean
30-yr norm	19.0	25.9	12.0	25.4				
2008	18.3	24.9	11.6	13.0	-2.05	-0.17	-1.09	-1.10
2009	17.1	22.7	11.4	19.6	-0.50	3.60	7.14	3.41
2010	19.4	26.7	11.8	8.9	-2.47	-2.92	-2.63	-2.67

positive results due to cross-contamination, we swabbed the first animal processed from each trap.

Animals were swabbed according to the methods of Pessier and Mendelson (2010) and swabs were analyzed using real-time Taqman PCR (Hyatt et al. 2007) as previously described (Kinney et al. 2011). Prevalences were calculated as ratios of positive animals to all animals sampled. The intensity of infection in positive samples was expressed as the number of zoospore equivalents per swab (Vredenburg et al. 2010).

We determined mortality by direct observations in or near the breeding wetlands. Frogs with chytridiomycosis were tentatively characterized in the field by redness and occasional lesions on their ventral surface, as well as lethargy (Kinney et al. 2011). We confirmed these preliminary diagnoses by swabbing and testing for zoospores (see below) and, when mortality occurred, by necropsy (performed by A.P.P.). Our estimate of crawfish frog mortality should be considered a minimum count, as we could not determine cause of death once animals left a breeding wetland and were never again observed.

We used nonparametric statistics to compare 2010 and 2011 results. We used Pearson's χ^2 test and Fisher's exact test to examine differences in infection prevalence, and Mann-Whitney *U*-tests to examine differences in infection intensity. To run our analyses, we used SPSS® statistical software (SPSS 17.0, IBM® Corporation, Armonk, New York, USA).

RESULTS

In 2010, the August–October Palmer Z-index value for drought intensity (NCDC 2012) averaged -2.67 , compared to -1.10 in 2008, 3.41 in 2009, and a -0.36 average over the past 3 yr (2008–10; Table 1). Droughts are driven both by a lack of rainfall and by high ambient temperatures. The lack of rainfall was clearly the major

driver of the 2010 drought. Although rainfall during this period typically averages 25.4 cm (30-yr norm; NCDC 2013; Table 1), in 2010 only 8.9 cm fell, representing 35.0% of normal precipitation. August–October precipitation during the two previous years was 13.0 cm (2008) and 19.6 cm (2009; Table 1), also below normal but not considered extreme (see above Palmer Z-index values). Despite being dry, August–October 2010 temperatures were comparable to the 30-yr norm and to recent years. In 2010, temperatures averaged 19.4 C, nearly identical to the 30-yr norm (19.0 C) as well as the 2008 and 2009 averages (18.3 and 17.1 C, respectively). The 2010 drought conditions lowered the water table and dried both semipermanent wetlands and upland crayfish burrows. On 19 November 2010, we examined six occupied crawfish frog burrows with a V S72-10WD Digital Video Borescope® (Visual Optics, Wynnewood, Oklahoma, USA). Burrows were dry and frogs were sitting about a meter below the surface on damp soil.

We analyzed 111 *Bd* samples from crawfish frog adults breeding in 2011: 62 from frogs entering wetlands, 49 from frogs exiting (Table 2); 42 frogs were sampled both entering and exiting. Nineteen frogs had been previously sampled in 2010. A total of 430 swabs were taken from newly metamorphosed juvenile crawfish frogs in 2011. From this large sample, we analyzed a random subsample of 101 swabs: 77 from Nate's Pond and 24 from Cattail. In total, these samples represent 3.2% of juveniles encountered and 23.5% of swabs taken.

TABLE 2. Prevalence and intensity of *Batrachochytrium dendrobatidis* infection and mortality in crawfish frogs (*Lithobates areolatus*) following drought in 2011, compared with data on the same populations from 2010. Intensities are presented as means (\pm SE).

Year and stage	Prevalence (%)	Intensity (\pm SE)	Mortality
2010			
Prebreeding	17 (9/54)	481.5 (\pm 257.3)	0
Postbreeding	47 (23/49)	3,707.2 (\pm 1,666.8)	5
Juvenile	2 (2/99)	8.8 (\pm 6.8)	0
2011			
Prebreeding	21 (13/62)	2.6 (\pm 2.3)	0
Postbreeding	43 (21/49)	18.4 (\pm 9.7)	0
Juvenile	0 (0/101)	0	0

Prevalence

In 2011, 30.6% of all *Bd* samples from breeding crawfish frogs at our study site were positive. Specifically, 21% of samples from prebreeding frogs were positive (Table 2). At Nate's Pond, 22% (10/45) of prebreeding adult samples were positive, whereas at Cattail Pond, 18% (3/17) of prebreeding adults were positive. Among postbreeding frogs, over twice as many animals were positive for *Bd* (Table 2). At Nate's Pond, 45% (17/38) of postbreeding adults were positive, compared to 36% (4/11) at Cattail Pond.

Overall *Bd* prevalence rates did not differ significantly between 2011 and 2010 ($P=1.000$; Fig. 1A). Similarly, neither prebreeding nor postbreeding *Bd* prevalence rates were significantly different between 2011 and 2010 samples (prebreeding; $P=0.639$; postbreeding; $P=0.839$; Table 2).

In 2011, no samples taken from juveniles metamorphosing at either Nate's or Cattail ponds were positive for *Bd* (Table 2). This result was similar to data collected in 2010, when 2% of animals sampled were positive for *Bd* (Table 2).

Intensity and observed mortality

In 2011, *Bd* intensities were relatively low compared to 2010 samples (Fig. 1B; Table 2). Prebreeding crawfish frogs averaged 2.6 ± 2.3 (SE) zoospore equivalents (Table 2), with animals from Nate's Pond averaging 0.34 ± 0.30 zoospore equivalents and from Cattail Pond averaging 10.2 ± 10.0

zoospore equivalents. Postbreeding crawfish frogs averaged 18.4 ± 9.7 zoospore equivalents (Table 2), with Nate's Pond frogs averaging 8.8 ± 5.3 and Cattail Pond frogs averaging 59.3 ± 44.4 .

Among infected crawfish frogs, infection intensities were significantly lower in 2011 than they had been in 2010 ($Z=-5.431$; $P<0.001$; Fig. 1B) among both prebreeding frogs ($Z=-3.584$; $P<0.001$) and postbreeding frogs ($Z=-3.843$; $P<0.001$). In 2010, five crawfish frog deaths were attributed to chytridiomycosis; in 2011 no observed mortality was attributed to chytridiomycosis (Table 2).

Infection rates in the same animals between years

Nineteen crawfish frogs sampled in 2011 had been sampled in 2010. Of these, seven were negative both years, six were negative in 2010 and positive in 2011, four were positive in 2010 and negative in 2011, and two were positive both years. A χ^2 test demonstrated no differences in prevalence among these groups (Pearson $\chi^2=0.12$; $P=0.599$). In 2010, the overall mean infection intensities among *Bd*-positive animals was 301.2 ± 244.4 , with a high of 1,505.2 zoospore equivalents, compared to a mean of 9.8 ± 9.4 and a high of only 76.2 zoospore equivalents in 2011.

DISCUSSION

We tracked *Bd* infection prevalences and intensities in a population (and in 19

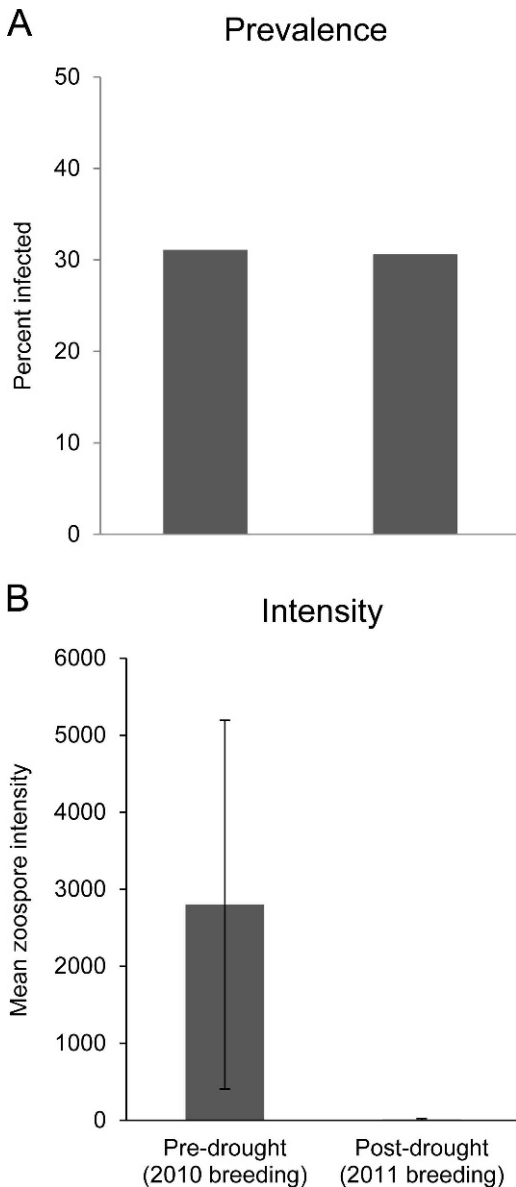


FIGURE 1. A comparisons of prevalence and intensity of positive samples (mean zoospore equivalents) of *Batrachochytrium dendrobatidis* (*Bd*) infection in breeding crawfish frog (*Lithobates areolatus*) adults in 2010 and following an extreme drought in 2011. (A) *Bd* prevalences were not significantly different ($P=1.000$) between 2010 (31.1%; 32/103) and 2011 (30.6%; 34/111). (B) *Bd* intensity in 2010 ($\bar{x}=2800.0 \pm 1220.6$ SE) and 2011 ($\bar{x}=2.4 \pm 6.1$).

cases the same individuals) of crawfish frogs through a severe drought, and found that while pre- and postdrought prevalences were similar, postdrought infection intensities and observed mortalities were significantly reduced in the following breeding season (Fig. 1; Table 2). This result suggests that in crawfish frogs, and perhaps in other species of upland-dwelling amphibians with complex life histories, *Bd* infection prevalence may not be greatly influenced by recent precipitation levels, whereas infection intensity and incidence of chytridiomycosis are influenced by recent precipitation levels. This further suggests that crawfish frogs are either exposed to novel *Bd* sources less dependent on surface moisture levels—perhaps to the feces of *Bd*-infected crayfish (McMahon et al. 2013)—or that low-level infections below the detection threshold of the test are persisting from year to year.

Comparison with other *Bd* infection scenarios

Working with mountain yellow-legged frogs (*Rana sierrae*), Briggs et al. (2010) developed a *Bd* load model consisting of a set of linear ordinary differential equations based on the number of zoospores in a zoospore pool (i.e., wetland or lake) and the number of sporangia on each frog. The two assumptions of this model are that frogs have no resistance to this pathogen, or no behavioral/environmental mechanisms to clear it, and frogs are constantly aggregated. In this model, *Bd* prevalence and infection intensity are linked, as may be the case when *Bd* infection is epidemic (Lips 1999). For example, working on the same populations studied by Briggs et al. (2010), Vredenburg et al. (2010) found that *Bd* recently appeared in mountain yellow-legged frog populations as a novel pathogen and arrived as an epidemic. Both infection prevalence and intensity increased in parallel, and once individuals reached zoospore intensities of about 10,000 zoospore equivalents, mass mortality began. Tadpoles survived, but newly

metamorphosed juveniles quickly succumbed. Eventually, exposed populations became extirpated.

The biology of crawfish frogs violates the broad assumptions of the Briggs et al. (2010) *Bd* load model in two ways. First, when not breeding, crawfish frogs inhabit upland breeding sites consisting of an abandoned crayfish burrow and associated feeding platform (Heemeyer et al. 2012). This feeding platform also serves as a basking site, where frogs have the opportunity to behaviorally increase their body temperature by basking and are able to clear sporangia (Kinney et al. 2011). Second, when not breeding, crawfish frogs live in isolation and rarely, if ever, share burrows with other crawfish frogs (Heemeyer et al. 2012). However, the Briggs et al. (2010) model likely applies to crawfish frogs during their breeding season when they are in wetlands and the observed increase in *Bd* prevalence and intensity in postbreeding animals likely follows the model predictions. For females, exposure in breeding wetlands can last from as little as one night to a week or more; for males, exposure can last for a week to a month (Kinney et al. 2011). Nevertheless, because of the differences between crawfish frog and mountain yellow-legged frog life histories, crawfish frogs violate the assumptions of the *Bd* load model for most of the year and prevalence and intensity can become decoupled in ways not accounted for by the Briggs et al. (2010) model.

Longo et al. (2010), working in Puerto Rico, also found an uncoupling of *Bd* prevalence and intensity due to a species-specific drought response in coquis (*Eleutherodactylus coqui*). They found, as we did, that desiccating environments do not affect *Bd* prevalence. However, they also found that animals infected at low intensities under ordinary environmental conditions could develop chytridiomycosis and suffer mortality under desiccating conditions. This conclusion is not only the opposite of ours, but appears to be

contrary to the generalization that cool, moist conditions promote *Bd* infection prevalence and intensity (Kriger 2009). It turns out it is not. When facing desiccation, coquis aggregate in moist microhabitats, which promotes *Bd* intensity and transmission rates. In contrast, when not breeding and facing desiccation, crawfish frogs conserve moisture not by aggregating in moist microhabitats, but by moving deeper into their individual burrows.

Implications for reductions in *Bd* intensity by drought

Despite the fact that amphibians with complex life histories depend on freshwater for reproduction and larval growth, and the fact that drought can have many deleterious effects, including prematurely drying wetlands and eliminating entire cohorts of eggs and tadpoles (Lake 2011), drought also has beneficial effects for amphibian populations. One is that wetland drying following metamorphosis eliminates predatory fish populations, which will reduce or eliminate the larvae of many species of pond-breeding amphibians (Petranka et al. 1987). A second is that wetland drying reduces the numbers of predatory invertebrates, which also feed on amphibian eggs and larvae (Caldwell et al. 1980). To these two ecologic benefits we suggest a third: drought can potentially reduce chytridiomycosis in amphibians that live as adults in upland habitats.

ACKNOWLEDGMENTS

We thank K. Smith, R. Ronk, and B. Feaster with the Indiana Department of Natural Resources (DNR) for enabling this project and J. Heemeyer, S. Lannoo, T. Wheat, J. Klemish, R. Stiles, P. Lannoo, A. May, A. Preston, J. Burchell, and R. Arndt for assistance with data collection, processing, and assistance with Figure 1. All research described here was conducted under IACUC 3-24-2008 issued by Indiana State University, and Scientific Purposes License Permit 09-0084 issued by Indiana DNR. This project was supported by a State Wildlife Grant (SWIG), grant number E2-08-WDS13, awarded by the Indiana DNR.

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Submitted for publication 15 January 2013.

Accepted 22 March 2013.