Vol. 111: 51–60, 2014
doi: 10.3354/dao02760

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published August 21

Batrachochytrium dendrobatidis infection dynamics vary seasonally in upstate New York, USA

Melissa A. Lenker[1,2,*], Anna E. Savage[1,3], C. Guilherme Becker[1], David Rodriguez[1], Kelly R. Zamudio[1]

[1]Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY 14853, USA

[2]*Present address:* McGill University, Department of Natural Resource Sciences, Sainte-Anne-de-Bellevue, QC H9X 3V9, Canada

[3]*Present address:* Center for Conservation and Evolutionary Genetics, Smithsonian Institution, Washington, DC 20008, USA

ABSTRACT: The amphibian disease chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), is a major cause of worldwide amphibian declines and extinctions. Although several studies indicate that *Bd* prevalence and infection intensity vary seasonally, temporal variation of *Bd* at high-latitude sites, such as the northeastern USA, is still poorly characterized. We screened amphibians for *Bd* monthly at 2 study sites in New York State from April to October 2011 and used quantitative polymerase chain reaction (qPCR) to detect and quantify temporal variability in *Bd* infection prevalence and intensity. We found pronounced seasonal variation in both *Bd* infection prevalence and intensity at the community level, and our data indicate that this pattern is due to a few species (*Lithobates catesbeianus*, *L. clamitans*, and *Notophthalmus viridescens*) that drive temporal variability in disease dynamics. Amphibian body mass and sex were significant predictors of infection intensity but not infection prevalence. Understanding the temporal dynamics of *Bd* host–pathogen interactions provides important insight into regional, seasonal, and host-specific determinants of disease outbreaks. Further, our study elucidates the most relevant and informative timing for *Bd* surveys in temperate amphibian assemblages. Seasonal variation of infection dynamics suggests that *Bd* surveys from different sampling time points are not comparable, and summer surveys to evaluate chytridiomycosis may significantly underestimate *Bd* prevalence and intensity, leading to false conclusions about the severity of chytridiomycosis-induced amphibian mortality and population decline.

KEY WORDS: Chytrid · Fungus · Chytridiomycosis · Seasonal variation · *Lithobates* · *Notophthalmus* · Amphibian

INTRODUCTION

Chytridiomycosis, an amphibian disease caused by the chytrid fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), is responsible for worldwide amphibian mortality, morbidity, and extinction (Longcore et al. 1999, Lips et al. 2006). *Bd* infects at least 508 amphibian species (Olson et al. 2013) and is widespread in the Americas, Europe, Africa, and Australia (Kriger & Hero 2009, Olson et al. 2013). In the last 2 decades, amphibian extinction rates have increased 200 times over the extinction rate of the last 350 million years, due in large part to habitat conversion and emergent infectious diseases (Roelants et al. 2007). The most severe chytridiomycosis epidemics, resulting in mass mortalities of amphibians, have occurred in tropical regions that experience little seasonal variation in daily mean temperature (Berger et al. 1998, Lips et al. 2005); however, chytridiomycosis outbreaks also occur in highly seasonal temperate regions that experience significant temporal variation in daily mean temperature (Bosch et al. 2001, Muths et al. 2003,

*Corresponding author: mal299@cornell.edu

Briggs et al. 2005, Savage et al. 2011). *Bd* dynamics in seasonal temperate environments appear to be more variable than in the tropics, with some populations showing no observed negative fitness (Daszak et al. 2004, Longcore et al. 2007) and others succumbing to disease-driven population declines (Bosch et al. 2001, Muths et al. 2003, Kriger & Hero 2007).

Seasonal correlates of chytridiomycosis dynamics are expected in natural populations because temperature affects fungal growth and host immune response, both of which influence pathogen growth and transmission (Maniero & Carey 1997, Berger et al. 2004, Piotrowski et al. 2004, Kriger & Hero 2007). *Bd* maintains high fitness from 4 to 25°C through a series of life history tradeoffs. The fungus grows optimally and develops into zoosporangia faster in warm temperatures from 17 to 25°C, but develops greater numbers of zoospores per zoosporangium from 7 to 10°C (Woodhams et al. 2008). Growth is inhibited at temperatures as cold as 4°C (Piotrowski et al. 2004), but the pathogen maintains longer periods of zoospore activity (Voyles et al. 2012). Although *Bd* thrives in relatively cool weather, it perishes in prolonged temperatures above 30°C (Piotrowski et al. 2004). In contrast to the relative success of *Bd* at low temperatures, amphibians are more susceptible to infection during variable or cold weather (Maniero & Carey 1997, Raffel et al. 2006, Kriger & Hero 2007, Savage et al. 2011).

Low temperatures may promote chytrid infections in the early spring when the amphibian immune response is suppressed following hibernation (Maniero & Carey 1997), and cool but rising temperatures encourage *Bd* growth (Woodhams et al. 2008). Similarly, infection intensity and prevalence may be low in the summer because high temperatures strengthen the amphibian immune system and decrease fungal growth (Ribas et al. 2009), allowing individuals to clear infection (Woodhams et al. 2003, Rowley & Alford 2013). We therefore hypothesized that *Bd* infection dynamics in the northeastern US would be highly seasonal due to temperature effects on the amphibian immune response (Ribas et al. 2009) and/or optimal environmental conditions for *Bd* growth and survival (Piotrowski et al. 2004, Woodhams et al. 2008, Voyles et al. 2012).

Amphibian ontogenetic variation is also highly seasonal. Amphibian metamorphs frequently exhibit higher infection intensity and prevalence than their adult counterparts (Longo & Burrowes 2010, Russell et al. 2010). Increased infection in juveniles is not completely understood and may signal either increased (Russell et al. 2010) or decreased (McCallum 2005) susceptibility of metamorphs to chytridiomycosis. If increased susceptibility to the epithelial disease is the cause, high infection prevalence may originate in part from an undeveloped immune system (Rollins-Smith 1998) and greater surface area-to-volume ratio (Longo & Burrowes 2010). Juveniles typically emerge in late summer and gain body mass as they age, causing a correlation between season and mean population body mass. Therefore, amphibian body mass is a potentially confounding factor linking host–pathogen dynamics and seasonality. The effect of sex on infection prevalence is still unclear. Some studies have found that males show higher infection prevalence than females (Kriger & Hero 2007), whereas other studies have found that females show higher infection prevalence than males (Russell et al. 2010).

The amphibian chytrid fungus is widespread in the northeastern USA (Longcore et al. 2007, Becker et al. 2012), but with a few exceptions, seasonal dynamics of *Bd* in high-latitude, highly seasonal temperate regions have not been well studied (but see Groner & Relyea 2010, Russell et al. 2010). In this study, we characterized temporal *Bd* dynamics in upstate New York. We investigated the role of host body mass, host identity, sex, and environmental temperature on *Bd* prevalence and infection intensity in 2 amphibian communities. Characterizing seasonal *Bd* dynamics across species and habitats clarifies factors that lead to disease outbreaks or sub-lethal *Bd* impacts on temperate amphibian populations, and provides insights into regional, seasonal, and host-specific determinants of chytridiomycosis-induced declines. Finally, understanding seasonal *Bd* dynamics helps target the most effective sampling times for future surveys to quantify *Bd* impacts on amphibian assemblages in the temperate northeastern USA and regions of similar climatic conditions.

MATERIALS AND METHODS

Study site

We quantified *Bd* infection intensity and prevalence across seasons in amphibian communities at 2 ponds in Ithaca, New York, USA. The first study site comprised a vernal pool with substantial canopy cover located at Ringwood Preserve (RP; 42.451° N, 76.365° W). The preserve is managed by Cornell University and experiences low levels of both human influence and environmental degradation. The second study site comprised an open pond located in Sapsucker Woods (SW) at the Cornell University Lab

of Ornithology (42.481° N, 76.451° W). SW is a relatively disturbed habitat with limited canopy cover and higher human habitation density. The study pond at RP was approximately 450 m^2, whereas the study pond at SW was considerably larger at approximately 4000 m^2. We expected the ponds to exhibit different amphibian compositions due to discrepancy in size and vegetation cover, and thus sampled both ponds to discern infection patterns more similar to the region as a whole than could be determined at a single site. We surveyed each study site once a month from April 2011 to October 2011, for a total of 7 consecutive months of sampling at each pond. Sampling did not take place from November through March because of snow cover and the absence of amphibians. Although *Notophthalmus viridescens* can be active beneath winter ice (George et al. 1977), we felt that retrieving an adequate sample size would have been difficult and less informative than focusing on larger samples during the months of highest activity.

To survey each population, species were caught by hand or dip net opportunistically through visual encounter surveys around the perimeter of each pond. Sampled animals occurred both at the water's edge and submerged within several feet of water. We swabbed the skin of captured individuals with sterile fine-tip swabs following standardized *Bd* protocols (Hyatt et al. 2007). We handled each amphibian with a fresh pair of latex gloves to prevent *Bd* transmission between individuals. In total, the survey included 13 species of amphibians: *Lithobates* (*Rana*) *catesbeianus* (American bullfrog), *L. clamitans* (green frog), *L. palustris* (pickerel frog), *L. sylvatica* (wood frog), *L. pipiens* (northern leopard frog), *L. septentrionalis* (mink frog), *Ambystoma jeffersonianum* (Jefferson salamander), *A. maculatum* (spotted salamander), *Anaxyrus americanus* (American toad), *N. viridescens* (eastern newt), *Plethodon cinereus* (redbacked salamander), *Pseudacris crucifer* (spring peeper), and *Hyla versicolor* (gray treefrog). For every individual captured, we recorded body mass to the nearest 0.1 g. We also recorded sex for adult *L. catesbeianus* and *L. clamitans* to test for the influence of host sex on infection prevalence and intensity.

Laboratory methods

Following a protocol specific for *Bd* (Hyatt et al. 2007), we extracted DNA from swabs using Prepman Ultra (Applied Biosystems) and measured infection intensity for each individual using real-time quantitative polymerase chain reaction (qPCR; Boyle et al. 2004). We calculated infection intensity as the number of zoospores present on each swab, and prevalence as the percentage of individuals in a population infected with chytridiomycosis. We calculated standard error confidence intervals for infection load estimates and 95% binomial confidence intervals for monthly infection prevalence estimates. The ITS copy number of the Ithaca, NY, *Bd* strain is unknown, but likely similar to JEL404 (New Hampshire, USA) which contains 39 ITS copies; the JEL427 strain used in qPCR analysis contains 65 ITS copies (Longo et al. 2013). Given the difference in ITS copies between strains, we considered an individual *Bd*-positive if its swab sample exhibited successful qPCR replication relative to negative controls, even when zoospore genome equivalents were <1, to prevent underestimation of *Bd* load (Longo et al. 2013).

Statistical analysis

The Northeast Regional Climate Center Game Farm Road weather station in Ithaca, NY, provided air temperature data for the study (Northeast Regional Climate Center 2011). We averaged daily air temperature (calculated by averaging high and low daily temperatures for all 30 days prior to each amphibian sampling date) to include seasonal temperature in the statistical analysis of community infection prevalence and intensity. We tested for seasonal changes in *Bd* infection prevalence and intensity over time for all species combined (community level) and independently for *L. catesbeianus*, *L. clamitans*, and *N. viridescens*. These species were chosen for individual analysis because they had a large, consistent sample size throughout our survey period (Table 1).

Community and species-specific seasonal variation in infection prevalence was examined using generalized linear models (GLMs) with binominal distribution and logit link. Standard least squares residuals of log community infection load by month ($W = 0.640$, $p < 0.0001$), and log community infection load regressed on multiple explanatory variables ($W = 0.764$, $p < 0.0001$) were both non-normal according to the Shapiro Wilk *W*-test for non-normality. Thus, we used GLMs with Poisson distribution and log link to test for seasonal variation in infection intensity. Zoospore genome equivalent *Bd* load was rounded up to integers to fit a Poisson distribution. Although the Poisson distribution appears zero-inflated from the inclusion of uninfected individuals, exclusion of uninfected individuals did not significantly change

Table 1. Number of individuals separated by species caught each month during the sampling period and the total number of individuals sampled each month. (+): number of *Batrachochytrium dendrobatidis*-positive individuals; R: number of individuals; from Ringwood Preserve; S: number of individuals from Sapsucker Woods

Species	April			May			June			July			August			September			October			Total		
	n (+)	R	S	n (+)	R	S	n (+)	R	S	n (+)	R	S	n (+)	R	S	n (+)	R	S	n (+)	R	S	n (+)	R	S
Lithobates catesbeianus	1 (1)	0	1	3 (3)	0	3	11 (7)	0	11	19 (8)	3	16	22 (6)	2	20	12 (6)	2	10	7 (7)	0	7	75 (38)	7	68
L. clamitans	4	0	4	4 (2)	0	4	17 (4)	0	17	47 (13)	28	19	20 (7)	5	15	8 (5)	3	5	0	0	0	100 (31)	36	64
L. palustris	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
L. sylvatica	15 (5)	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15 (5)	15	0
L. pipiens	1 (1)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1)	0	1
L. septentrionalis	0	0	0	0	0	0	1 (1)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1)	1	0
Ambystoma jeffersonianum	11 (3)	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11 (3)	0	0
A. maculatum	21 (6)	21	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	22 (6)	22	0
Anaxyrus americanus	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Notophthalmus viridescens	14 (2)	14	0	8 (2)	8	0	6 (4)	6	0	5	5	0	8 (3)	8	0	4 (1)	4	0	8 (6)	8	0	53 (18)	53	0
Plethodon cinereus	2	2	0	1	1	0	2 (1)	2	0	3	3	0	1 (1)	1	0	12	12	0	19 (3)	19	0	40 (5)	40	0
Pseudacris crucifer	41 (19)	14	27	20 (10)	11	9	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	62 (29)	25	37
Hyla versicolor	0	0	0	16 (7)	2	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16 (7)	2	14
Total	112 (37)	79	33	52 (24)	22	30	37 (17)	9	28	75 (21)	42	35	51 (17)	16	35	37 (12)	21	16	34 (16)	27	7	398 (144)	214	184

Table 2. Generalized linear models (GLMs) simultaneously testing the effects of species identity, month, location (pond), body mass (g), and 30 d mean air temperature (°C) on *Batrachochytrium dendrobatidis* infection prevalence in 2 ponds in upstate New York, USA. (A) Binomial GLM of prevalence. (B,C) Poisson GLMs of intensity with uninfected individuals (B) included or (C) excluded

Factor	β	df	χ^2	p
(A) Prevalence				
Species identity	–	12	25.00	0.0148
Month	–	6	21.52	0.0015
Location	−1.108	1	24.76	<0.0001
Body mass	0.007	1	2.86	0.0909
30 d mean temp	−0.128	1	3.09	0.0788
Full model test		21	77.1	<0.0001
(B) Intensity: uninfected included				
Species identity	–	12	15400.35	<0.0001
Month	–	6	6340.05	<0.0001
Location	−2.997	1	5881.61	<0.0001
Body mass	−0.027	1	1990.57	<0.0001
30 d mean temp	0.092	1	152.25	<0.0001
Full model test		21	220075.7	<0.0001
(C) Intensity: uninfected excluded				
Species identity	–	10	10303.24	<0.0001
Month	–	6	3044.33	<0.0001
Location	−3.244	1	4043.73	<0.0001
Body mass	−0.030	1	2235.68	0.0001
30 d mean temp	−0.025	1	9.11	0.0025
Full model test		19	176366.5	<0.0001

model results (Table 2). At the community level, models testing seasonal variation in infection prevalence and intensity also included the fixed effects of sampling site, species, body mass, and temperature. Addition of the fixed effect sampling site controls for spatial heterogeneity in all analyses containing individuals from both RP and SW.

In the prevalence and infection intensity GLMs for *L. catesbeianus* and *L. clamitans*, we independently tested the effects of sex and mass, and controlled for month and site as fixed effects. We define μ as mean infection intensity when comparing *Bd*-load between sexes. In the prevalence and infection intensity GLMs for *N. viridescens*, we tested the effect of month and mass but did not control for sampling site or sex because this species was present in only 1 pond and we were unable to sex individuals.

RESULTS

Seasonal patterns

We detected *Bd* every month at 2 focal study sites in upstate New York. The mean 30 d air temperature

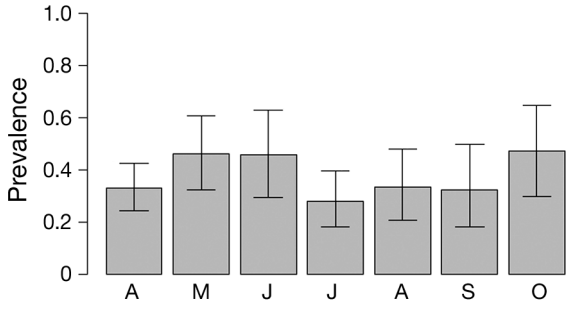

Fig. 1. Monthly *Batrachochytrium dendrobatidis* (*Bd*) infection prevalence for 2 amphibian assemblages in upstate New York, USA, exhibiting seasonal variation in community prevalence through time. Prevalence refers to the proportion of *Bd*-infected individuals in the amphibian community. Error bars represent 95% confidence interval. Total sample sizes as in Table 1

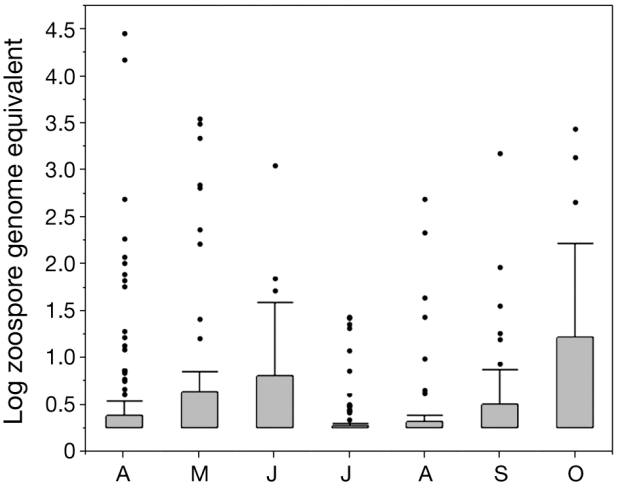

Fig. 2. Monthly *Batrachochytrium dendrobatidis* infection intensity for 2 amphibian assemblages in upstate New York, USA, exhibiting monthly variation in community level infection intensity. Infection intensity refers to the zoospore genome equivalents present on sampled individuals. Box: 25th–75th percentiles; whiskers: 5th–95th percentiles; dots: outliers. Total sample sizes as in Table 1

ranged from 0.7 to 22.0°C. Temperature in the optimal range of *Bd* growth (17 to 25°C; Piotrowski et al. 2004) occurred from June to September. All days meeting or exceeding 29°C, i.e. the temperature at which amphibians may clear chytridiomycosis infections (Piotrowski et al. 2004, Rowley & Alford 2013) and therefore the minimum air temperature at which amphibian body temperatures could approach *Bd*-killing levels, also occurred from June to September. The majority of days that met or exceeded 29°C occurred in July (N = 18 out of 31 total days; 58%), which was also the month with the lowest community infection prevalence and intensity (Figs. 1 & 2).

Spring and fall supported the highest community infection prevalence, which peaked in May (46.2%; CI 32.2–60.6%) and October (47.1%; CI 29.8–64.9%), respectively (χ^2 = 21.52, df = 6, p < 0.0015). Community infection prevalence decreased during the summer, reaching its lowest point in July (28.0%; CI 18.2–39.6%; Table 3, Fig. 1).

At the species level, *Lithobates catesbeianus* and *L. clamitans* followed seasonal prevalence patterns similar to that of community infection. *L. catesbeianus* infection prevalence peaked in the spring and hit its lowest level in August (27.3%; CI 10.7–50.2%). Although April and May exhibited 100% prevalence, this pattern was not significant due to small sample size (χ^2 = 17.12, df = 5, p < 0.0043; Table 3). *L. clamitans* infection prevalence peaked in May (50.0%; CI 6.8–93.2%) and September (62.5%; CI 24.5–91.5%) and decreased during the summer, with the lowest infection prevalence during June (23.5%; CI 6.8–49.9%; χ^2 = 15.20, df = 5, p < 0.0095; Table 3). In contrast, *Bd* prevalence among *Notophthalmus viridescens* populations increased steadily from April (14.3%; CI 1.8–42.8%) until June (66.7%; CI 22.3–95.7%), followed by the disappearance of infections in this species in July (0.0%; CI 0.0–52.5%; n = 5; Table 3). Infection prevalence in *N. viridescens* then increased again from July to October (75.0%; CI 34.9–96.8%; χ^2 = 15.75, df = 6, p < 0.0152; Table 3).

Community infection intensity also varied significantly across months, generally following the same seasonal pattern as prevalence (χ^2 = 6340.05, df = 6, p < 0.0001). Excluding load outliers in April, the highest average infection intensities occurred in May and October, whereas the lowest average infection intensity occurred in July (Table 3, Fig. 2). *L. catesbeianus* (χ^2 = 11768.89, df = 5, p < 0.0001) exhibited significant variation in infection intensity across months, with load decreasing from May to July and increasing thereafter through October (Table 3). *L. clamitans* (χ^2 = 2758.93, df = 5, p < 0.0001) and *N. viridescens* (χ^2 = 2784.47, df = 6, p < 0.0001) also exhibited statistically significant seasonal variation in infection intensity, with load increasing in the spring and fall, and decreasing in the summer (Table 3).

Community: combined effects of species identity, month, location, mass, and temperature

In the community GLM using infection prevalence as a dependent variable, species identity, month, and

Table 3. Monthly *Batrachochytrium dendrobatidis* (*Bd*) infection prevalence and intensity for the amphibian community and *Lithobates catesbeianus*, *L. clamitans*, and *Notophthalmus viridescens* populations at 2 ponds in upstate New York, USA. Prevalence refers to the percent of *Bd* infected individuals in the species under consideration (errors are 95% binomial confidence intervals). Infection intensity refers to the zoospore genome equivalents present on sampled individuals from qCPR analysis (confidence intervals are ±1 SE from the mean). NA: not applicable

Month	No. infected/ Total	Prevalence (%)	Prevalence 95% CI	Intensity Mean ± SE	Intensity CI
Community (all)					
April	37/112	33.0	24.4–42.6	376.2 ± 273.4	102.8–649.6
May	24/52	46.2	32.2–60.6	173.4 ± 83.8	89.6–257.2
June	17/37	45.9	29.5–63.1	28.2 ± 24.2	3.9–52.4
July	21/75	28.0	18.2–39.6	1.2 ± 0.4	0.8–1.6
August	17/51	33.3	20.8–47.9	11.8 ± 8.0	3.8–19.8
September	12/37	32.4	18.0–49.8	36.9 ± 33.6	3.4–70.5
October	16/34	47.1	29.8–64.9	120.1 ± 74.7	45.4–194.8
L. catesbeianus					
April	1/1	100.0	2.5–100.0	377.0	NA
May	3/3	100.0	29.2–100.0	44.7 ± 36.4	8.2–81.1
June	7/11	63.6	30.8–89.1	3.7 ± 2.2	1.5–5.9
July	8/19	42.1	20.3–66.5	1.1 ± 0.7	0.4–1.7
August	6/22	27.3	10.7–50.2	8.2 ± 7.1	1.1–15.3
September	6/12	50.0	21.1–78.9	7.0 ± 5.3	1.7–12.3
October	7/7	100.0	59.0–100.0	567.4 ± 326.9	240.6–894.3
L. clamitans					
April	0/4	0.0	0.0–60.2	0.0 ± 0.0	0.0–0.0
May	2/4	50.0	6.8–93.2	29.5 ± 28.8	0.7–58.3
June	4/17	23.5	6.8–49.9	3.2 ± 2.8	0.4–6.0
July	13/47	27.7	15.6–42.6	1.4 ± 0.6	0.9–2.0
August	7/20	35.0	15.4–59.2	20.7 ± 18.9	1.7–39.6
September	5/8	62.5	24.5–91.5	160.1 ± 154.7	5.4–314.8
October	0/0	NA	NA	NA	NA
N. viridescens					
April	2/14	14.3	1.8–42.8	9.7 ± 6.8	2.9–16.5
May	2/8	25.0	3.2–65.1	21.8 ± 21.2	0.6–42.9
June	4/6	66.7	22.3–95.7	151.8 ± 149.4	2.4–301.3
July	0/5	0.0	0.0–52.2	0.0 ± 0.0	NA
August	3/8	37.5	8.5–75.5	1.1 ± 0.7	0.4–1.9
September	1/4	25.0	0.6–80.6	0.3 ± 0.3	0.0–0.5
October	6/8	75.0	34.9–96.8	13.5 ± 7.3	6.2–20.8

location (pond) strongly predicted *Bd* infection prevalence within communities while amphibian body mass and 30 d average air temperature did not (Table 2). The infection intensity GLM showed a different pattern, with species identity, month, location (pond), body mass, and average 30 d temperature all strongly predicting community *Bd* load (Table 2).

Species: combined effects of month, mass, location, and sex

In the species-specific GLMs, month significantly predicted infection prevalence and intensity for *L. catesbeianus*, *L. clamitans*, and *N. viridescens* (see 'Seasonal patterns' above). Body mass failed to predict infection prevalence in *L. catesbeianus* (χ^2 = 2.69, df = 1, p = 0.1010), *L. clamitans* (χ^2 = 2.12, df = 1, p = 0.1459), and *N. viridescens* (χ^2 = 1.13, df = 1, p = 0.2885), but significantly predicted infection intensity in all 3 species (*L. catesbeianus*: χ^2 = 2166.76, df = 1, p < 0.0001; *L. clamitans*: χ^2 = 98.59, df = 1, p < 0.0001; *N. viridescens*: χ^2 = 132.27, df = 1, p < 0.0001). Location significantly predicted *L. catesbeianus* infection intensity (χ^2 = 31.15, df = 1, p < 0.0001) but not infection prevalence (χ^2 = 1.92, df = 1, p = 0.1659). Location also significantly predicted infection prevalence (χ^2 = 38.76, df = 1, p < 0.0001) and load (χ^2 = 2293.25, df = 1, p < 0.0001) in *L. clamitans*.

Sex was not a significant predictor of infection prevalence for *L. catesbeianus* (χ^2 = 0.72, df = 2, p = 0.6985) or *L. clamitans* (χ^2 = 4.34, df = 2, p = 0.1140). However, sex was a significant predictor of infection intensity for *L. catesbeianus* (χ^2 = 3909.72, df = 2, p < 0.0001) and *L. clamitans* (χ^2 = 1138.67, df = 2, p < 0.0001). Female *L. catesbeianus* (μ = 68.4; CI 9.1–127.6; n = 39) and *L. clamitans* (μ = 4.6; CI 1.4–7.9; n = 36) exhibited higher average infection loads than males of the same species (*L. catesbeianus*: μ = 1.0; CI 0.3–1.7, n = 5; *L. clamitans*: μ = 2.3; CI 0.7–3.9, n = 30).

DISCUSSION

Seasonal variation in wildlife diseases is common in both tropical and temperate regions (Grassly & Fraser 2006). Causes of disease seasonality include temporally driven changes in amphibian host immunity (Maniero & Carey 1997, Raffel et al. 2006), variable pathogen growth (Piotrowski et al. 2004), and seasonal host life history traits such as breeding, aggregation (Kinney et al. 2011), and development (Rollins-Smith 1998). In this study, we found that variation in *Bd* dynamics was highly seasonal in 2 amphibian assemblages in the northeastern USA. Our results corroborate field and laboratory studies in which *Bd* infections increased under seasonally

(Kriger & Hero 2007, Savage et al. 2011) and environmentally cool conditions (Raffel et al. 2010), and decreased under high temperature exposure (Woodhams et al. 2003, Chatfield & Richards-Zawacki 2011, Rowley & Alford 2013). The temperature-dependent dynamics of *Bd* point to 3 distinct periods of community infection prevalence and intensity in the northeast: spring and fall generally support high population-wide *Bd* infections, in contrast to the summer when *Bd* infections are typically low.

Although amphibian assemblages exhibit similar overall seasonal patterns of infection prevalence and intensity, monthly *Bd* infection dynamics differ across species, even within the same pond. Species identity significantly predicted *Bd* infection intensity and prevalence in the community GLMs, suggesting that different amphibian species and populations contribute variably to community infection dynamics and exhibit species-specific seasonal patterns of infection (Gervasi et al. 2013). Differences in infection dynamics may be due to variation in host resistance or tolerance, which determine their susceptibility to chytridiomycosis (Woodhams et al. 2007, Tobler & Schmidt 2010, Savage & Zamudio 2011). For instance, tolerance to *Bd* infection has been characterized in *Lithobates catesbeianus* (Daszak et al. 2004, Gahl et al. 2012) and hypothesized in *L. clamitans* (Gahl et al. 2012), both thought to be carriers of *Bd*. Possible mechanisms of resistance include anti-fungal microbial interactions (Harris et al. 2009, Lam et al. 2010), acquired immune defenses (Richmond et al. 2009, Savage & Zamudio 2011), and antimicrobial peptide defenses (Rollins-Smith & Conlon 2005). Species density and composition may also partially determine differences in *Bd* seasonality among species by influencing the perpetuation of *Bd*–host transmissions (Becker & Zamudio 2011, Becker et al. 2012, Venesky et al. 2014) and affecting disease transmission through individual contact rates (Rachowicz & Briggs 2007). Future studies sampling pond diameters or transects rather than only pond edges may reveal additional within-pond *Bd* dynamics.

Species tested individually for seasonal variation were chosen on the basis of a large, consistent sampling size across months (Table 1). Although we only collected enough data to analyze 3 species independently, all 3 species exhibited similar seasonal patterns of infection dynamics, despite substantial taxonomic and life history differences. The most prevalent species (*L. catesbeianus*, *L. clamitans*, and *Notophthalmus viridescens*) comprised the majority of potentially infected individuals, and therefore likely drove the overall infection dynamics we characterized. Community-level analysis including all species captured portrayed seasonal patterns similar to the 3 most abundant species, emphasizing that species of small sample size either follow the dominant pattern of seasonality, or contribute minimally to overall infection dynamics if exhibiting another pattern of seasonality.

Seasonal variation in species composition meant that the variables month and species identity were not independent; however, we conclude that month remains a significant predictor of infection dynamics independent of species because month significantly predicted infection dynamics in the single-species models. Although species composition is confounded with sampling site, we used 2 separate ponds in the analysis precisely because we were expecting the ponds to exhibit different amphibian compositions due to their discrepancy in size and vegetation cover. Analysis of a single site may have reported patterns specific to that site rather than the region overall. Addition of the fixed effect sampling site also controls for spatial heterogeneity in all analyses containing individuals from both RP and SW. For these reasons, we find it appropriate to retain our analyses with individuals captured from both sampling locations.

Host body mass failed to predict infection prevalence, but predicted infection intensity in the community and species-specific GLMs. If one assumes that smaller and/or younger amphibians generally weigh less than larger and/or adult counterparts, our results corroborate studies that found a negative correlation between amphibian body mass (Garner et al. 2009), size (Kriger et al. 2007, Pearl et al. 2009), and age class in comparison to infection intensity (Longo & Burrowes 2010, Russell et al. 2010). In contrast to other temperate study systems, host sex failed to predict infection prevalence in our study (Kriger & Hero 2007, Russell et al. 2010), although it significantly predicted infection intensity. To our knowledge, we are among the first to discern a relationship between sex and infection intensity, an intriguing pattern suggesting that sex-specific physiological or immunological differences may influence disease progression.

Air temperature in the 30 d prior to sampling significantly predicted infection intensity, but surprisingly failed to predict infection prevalence. Absence of a significant pattern highlights the possibility that seasonal correlates unrelated to temperature, or another measure of temperature, such as temperature averaged over a shorter time interval or daily highs/lows, better track seasonal changes in *Bd* prevalence than the average 30 d temperature prior to sampling. Our results may also indicate that sea-

sonal fluctuation in environmental temperature affects infection intensity more strongly than infection prevalence. Despite evidence of environmental temperature effects on *Bd* infection prevalence and intensity (Kriger & Hero 2007, Savage et al. 2011), the effect of a warming climate on infection dynamics in high-latitude, low-altitude temperate regions remains unclear; increasing temperatures may both help amphibians clear infection during warm summer months (Woodhams et al. 2003, Rowley & Alford 2013) and increase infection during winter months as rising temperatures approach growth and survival optima of *Bd* (Piotrowski et al. 2004, Woodhams et al. 2008), possibly resulting in enhanced seasonal fluctuations of infection prevalence and intensity. In general, the contribution of climate change to *Bd*-driven amphibian declines remains uncertain (Lips et al. 2008, Li et al. 2013).

Bd is widespread in the northeastern USA, but amphibian populations persist in an enzootic state, with no evidence of population declines to date. However, even an enzootic state of *Bd* may produce low level effects on the population, such as smaller body size and reduced reproductive rates (Burrowes et al. 2008). Despite persisting with *Bd*, many enzootic populations experience declines, particularly in juveniles (Longo & Burrowes 2010) and in cooler seasons (Savage et al. 2011). If amphibian decline exists in the northeastern USA and infection intensity predicts an individual's susceptibility, then May and October are likely the critical months of frog decline, while June would bear the highest rate of salamander decline. However, species level variation in temporal *Bd* dynamics somewhat diminishes the notion of critical months of decline in an ecosystem. Further, the absence of high-latitude amphibian die-offs may be attributed to environmental temperatures during the warmest and coolest months; winter might suppress *Bd* infection through low temperatures and low amphibian abundance, whereas summer could suppress infection due to temperatures higher than those at which *Bd* is able to grow and/or survive.

Understanding *Bd* seasonality is critical to designing and implementing sampling protocols. For surveys that test the presence or absence of chytridiomycosis in a region, we recommend sampling during the coolest month(s) of the year that amphibians remain active (typically late spring and early fall) when *Bd* infections reach peak prevalence and intensity levels. Seasonality of *Bd* infection indicates that chytridiomycosis surveys from different sampling time points may not be comparable. Past chytrid sampling efforts from climatic conditions that discourage *Bd* growth may have underestimated chytridiomycosis prevalence and intensity or failed to detect disease presence (see Kriger & Hero 2007).

In conclusion, our results show that chytridiomycosis infection load varies seasonally in temperate climates: low infection intensity and prevalence occur in warm months (summer, specifically July) and high infection intensity and prevalence occur in cold months (fall and spring). We have also shown that amphibian mass and sex are poor indicators of infection prevalence, but species identity and season are important factors affecting disease dynamics. Characterizing the seasonal fluctuation of *Bd* dynamics in a highly seasonal environment further clarifies the optimal sampling periods to assess *Bd* presence and impact. Our study also indicates that summer surveys to evaluate chytridiomycosis may drastically underestimate *Bd* infection prevalence and intensity, leading to false conclusions about the severity of chytridiomycosis-induced mortality and decline.

Acknowledgements. We thank Amanda Talaba, Maggie Guthrie, and members of the Zamudio Lab for sampling assistance. Our work was funded by the Cornell University Hunter R. Rawlings III Presidential Research Scholars program and a Morley Student Research Grant to M.A.L., and by a National Science Foundation Research Experience for Undergraduates (DEB-1112738) award to the Zamudio Lab.

LITERATURE CITED

Becker CG, Zamudio KR (2011) Tropical amphibian populations experience higher disease risk in natural habitats. Proc Natl Acad Sci USA 108:9893–9898

Becker CG, Rodriguez D, Longo AV, Talaba AL, Zamudio KR (2012) Disease risk in temperate amphibian populations is higher at closed-canopy sites. PLoS ONE 7: e48205

Berger L, Speare R, Daszak P, Green DE and others (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proc Natl Acad Sci USA 95: 9031–9036

Berger L, Speare R, Hines HB, Marantelli G and others (2004) Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Aust Vet J 82: 434–439

Bosch J, Martínez-Solano I, García-Paris M (2001) Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. Biol Conserv 97:331–337

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD (2004) Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis Aquat Org 60:141–148

Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ (2005) Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. Ecology 86:3149–3159

Burrowes PA, Longo AV, Rodriguez CA (2008) Potential fitness cost of *Batrachochytrium dendrobatidis* in *Eleutherodactylus coqui*, and comments on environment-related risk of infection. Herpetotropicos 4:51–57

Chatfield MWH, Richards-Zawacki CL (2011) Elevated temperature as a treatment for *Batrachochytrium dendrobatidis* infection in captive frogs. Dis Aquat Org 94:235–238

Daszak P, Strieby A, Cunningham AA, Longcore JE, Brown CC, Porter D (2004) Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetol J 14:201–207

Gahl MK, Longcore JE, Houlahan JE (2012) Varying responses of northeastern North American amphibians to the chytrid pathogen *Batrachochytrium dendrobatidis.* Conserv Biol 26:135–141

Garner TWJ, Walker S, Bosch J, Leech S, Rowcliffe JM, Cunningham AA, Fisher MC (2009) Life history tradeoffs influence mass mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. Oikos 118:783–791

George CJ, Boylen CW, Sheldon RB (1977) The presence of the red-spotted newt, *Notophthalmus viridescens* Rafinesque (Amphibia, Urodela, Salamandridae), in waters exceeding 12 meters in Lake George, New York. J Herpetol 11:87–90

Gervasi S, Gondhalekar C, Olson DH, Blaustein AR (2013) Host identity matters in the amphibian-*Batrachochytrium dendrobatidis* system: fine-scale patterns of variation in responses to a multi-host pathogen. PLoS ONE 8: e54490

Grassly NC, Fraser C (2006) Seasonal infectious disease epidemiology. Proc R Soc Lond B Biol Sci 273:2541–2550

Groner ML, Relyea RA (2010) *Batrachochytrium dendrobatidis* is present in northwest Pennsylvania, USA, with high prevalence in *Notophthalmus viridescens*. Herpetol Rev 41:462–465

Harris RN, Brucker RM, Walke JB, Becker MH and others (2009) Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. ISME J 3: 818–824

Hyatt AD, Boyle DG, Olsen V, Boyle DB and others (2007) Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis Aquat Org 73:175–192

Kinney VC, Heemeyer JL, Pessier AP, Lannoo MJ (2011) Seasonal pattern of *Batrachochytrium dendrobatidis* infection and mortality in *Lithobates areolatus*: affirmation of Vredenburg's "10,000 Zoospore Rule". PLoS ONE 6:e16708

Kriger KM, Hero JM (2007) Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. J Zool 271:352–359

Kriger KM, Hero JM (2009) Chytridiomycosis, amphibian extinctions, and lessons for the prevention of future panzootics. EcoHealth 6:6–10

Kriger KM, Pereoglou F, Hero JM (2007) Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in Eastern Australia. Conserv Biol 21:1280–1290

Lam BA, Walke JB, Vredenburg VT, Harris RN (2010) Proportion of individuals with anti-*Batrachochytrium dendrobatidis* skin bacteria is associated with population persistence in the frog *Rana muscosa*. Biol Conserv 143: 529–531

Li Y, Cohen JM, Rohr JR (2013) Review and synthesis of the effects of climate change on amphibians. Integr Zool 8: 145–161

Lips KR, Burrowes PA, Mendelson JR, Parra-Olea G (2005) Amphibian population declines in Latin America: a synthesis. Biotropica 37:222–226

Lips KR, Brem F, Brenes R, Reeve JD and others (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc Natl Acad Sci USA 103:3165–3170

Lips KR, Diffendorfer J, Mendelson JR III, Sears MW (2008) Riding the wave: reconciling the roles of disease and climate change in amphibian declines. PLoS Biol 6:e72

Longcore JE, Pessier AP, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227

Longcore JR, Longcore JE, Pessier AP, Halteman WA (2007) Chytridiomycosis widespread in anurans of northeastern United States. J Wildl Manag 71:435–445

Longo AV, Burrowes PA (2010) Persistence with chytridiomycosis does not assure survival of direct-developing frogs. EcoHealth 7:185–195

Longo AV, Rodriguez D, da Silva Leite D, Toledo LF, Mendoza Almeralla C, Burrowes PA, Zamudio KR (2013) ITS1 copy number varies among *Batrachochytrium dendrobatidis* strains: implications for qPCR estimates of infection intensity from field-collected amphibian skin swabs. PLoS ONE 8:e59499

Maniero GD, Carey C (1997) Changes in selected aspects of immune function in the leopard frog, *Rana pipiens*, associated with exposure to the cold. J Comp Physiol B Biochem Syst Environ Physiol 167:256–263

McCallum H (2005) Inconclusiveness of chytridiomycosis as the agent in widespread frog declines. Conserv Biol 19: 1421–1430

Muths E, Corn PS, Pessier AP, Green DE (2003) Evidence for disease-related amphibian decline in Colorado. Biol Conserv 110:357–365

Northeast Regional Climate Center (2011) The Ithaca climate page. Available at www.nrcc.cornell.edu/climate/ithaca/ (accessed 7 October 2012)

Olson DH, Aanensen DM, Ronnenberg KL, Powell CI and others (2013) Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. PLoS ONE 8:e56802

Pearl CA, Bowerman J, Adams MJ, Chelgren ND (2009) Widespread occurrence of the chytrid fungus *Batrachochytrium dendrobatidis* on Oregon spotted frogs (*Rana pretiosa*). EcoHealth 6:209–218

Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15

Rachowicz LJ, Briggs CJ (2007) Quantifying the disease transmission function: effects of density on *Batrachochytrium dendrobatidis* transmission in the mountain yellow-legged frog *Rana muscosa*. J Anim Ecol 76:711–721

Raffel TR, Rohr JR, Kiesecker JM, Hudson PJ (2006) Negative effects of changing temperature on amphibian immunity under field conditions. Funct Ecol 20:819–828

Raffel TR, Michel PJ, Sites EW, Rohr JR (2010) What drives chytrid infections in newt populations? Associations with substrate, temperature, and shade. EcoHealth 7:526–536

Ribas L, Li MS, Doddington BJ, Robert J and others (2009) Expression profiling the temperature-dependent amphibian response to infection by *Batrachochytrium dendro-*

batidis. PLoS ONE 4:e8408
Richmond JQ, Savage AE, Zamudio KR, Rosenblum EB (2009) Toward immunogenetic studies of amphibian chytridiomycosis: linking innate and acquired immunity. Bioscience 59:311–320
Roelants K, Gower DJ, Wilkinson M, Loader SP and others (2007) Global patterns of diversification in the history of modern amphibians. Proc Natl Acad Sci USA 104: 887–892
Rollins-Smith LA (1998) Metamorphosis and the amphibian immune system. Immunol Rev 166:221–230
Rollins-Smith LA, Conlon JM (2005) Antimicrobial peptide defenses against chytridiomycosis, an emerging infectious disease of amphibian populations. Dev Comp Immunol 29:589–598
Rowley JJL, Alford RA (2013) Hot bodies protect amphibians against chytrid infection in nature. Sci Rep 3:1515
Russell DM, Goldberg CS, Waits LP, Rosenblum EB (2010) *Batrachochytrium dendrobatidis* infection dynamics in the Columbia spotted frog *Rana luteiventris* in north Idaho, USA. Dis Aquat Org 92:223–230
Savage AE, Zamudio KR (2011) MHC genotypes associate with resistance to a frog-killing fungus. Proc Natl Acad Sci USA 108:16705–16710
Savage AE, Sredi MJ, Zamudio KR (2011) Disease dynamics vary spatially and temporally in a North American amphibian. Biol Conserv 144:1910–1915
Tobler U, Schmidt BR (2010) Within- and among-population variation in chytridiomycosis-induced mortality in the toad *Alytes obstetricians*. PLoS ONE 5:e10927
Venesky MD, Liu X, Sauer EL, Rohr JR (2014) Linking manipulative experiments to field data to test the dilution effect. J Anim Ecol 83:557–565
Voyles J, Johnson LR, Briggs CJ, Cashins SD and others (2012) Temperature alters reproductive life history patterns in *Batrachochytrium dendrobatidis*, a lethal pathogen associated with the global loss of amphibians. Ecol Evol 2:2241–2249
Woodhams DC, Alford RA, Marantelli G (2003) Emerging disease of amphibians cured by elevated body temperature. Dis Aquat Org 55:65–67
Woodhams DC, Ardipradja K, Alford RA, Marantelli G, Reinert LK, Rollins-Smith LA (2007) Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Anim Conserv 10: 409–417
Woodhams DC, Alford RA, Briggs CJ, Johnson M, Rollins-Smith LA (2008) Life-history trade-offs influence disease in changing climates: strategies of an amphibian pathogen. Ecology 89:1627–1639

Editorial responsibility: Louise Rollins-Smith, Nashville, Tennessee, USA

Submitted: July 22, 2013; Accepted: May 6, 2014
Proofs received from author(s): July 18, 2014