EFFECTS OF CANOPY COVER ON THE LANDSCAPE EPIDEMIOLOGY OF AN AMPHIBIAN CHYTRID FUNGUS

BY

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THESIS

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ABSTRACT

Batrachochytrium dendrobatidis (Bd) is a globally distributed fungal pathogen of amphibians that causes the disease chytridiomycosis and is one of the major contributors to recent population declines of amphibians. The prevalence of Bd should vary spatially across landscapes because survival of the pathogen is influenced by environmental variables. In particular, wetland temperatures should vary with forest canopy cover in heterogeneous landscapes. However, few studies have examined the relationship between Bd prevalence and canopy cover.

I sampled anurans for Bd at 33 wetlands in east-central Illinois representing a gradient in canopy cover. I hypothesized that amphibians from closed canopy wetlands would have higher Bd prevalence than amphibians from open canopy wetlands because high canopy cover would prevent temperatures from exceeding Bd's upper critical maximum as often. My sampling was conducted in spring during the severe drought of 2012, and during more typical weather conditions in 2013. I used occupancy modeling to examine how Bd prevalence varied spatially in relation to canopy cover and other environmental covariates, and to determine if weather conditions altered such relationships. I analyzed all common species combined and Spring Peepers (*Pseudacris crucifer*) separately, which was the most abundant species.

Bd was widespread throughout the study wetlands. During the drought year of 2012, however, prevalence of Bd was substantially reduced compared to 2013 for common species combined and for Spring Peepers. As expected, air and water temperatures at closed canopy wetlands were lower than temperatures at open canopy wetlands. The influence of canopy cover on Bd was complex, however, and I found only limited support for my hypothesis. Bd prevalence for Spring Peepers during 2012 was related positively to canopy cover. In contrast,

Bd prevalence of Spring Peepers was not affected by canopy cover in 2013. Likewise, Bd prevalence for common species combined was not related positively to canopy cover in either year. Effects of other environmental covariates, such as water depth, differed between years and were probably influenced by the variable weather conditions. Lastly, connectivity to other wetlands was generally not an important predictor of spatial heterogeneity in Bd prevalence.

Weather conditions had a large impact on Bd prevalence patterns, which makes predicting pathogen dynamics difficult. In the Midwest region, climate change models predict more frequent droughts, which may generally inhibit Bd while also altering the effect of canopy cover on Bd prevalence for amphibians.

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GENERAL INTRODUCTION

Batrachochytrium dendrobatidis (Bd) is a pathogenic fungus affecting amphibians on every continent except Antarctica. To date, it has been detected in >500 species (Olson et al. 2013). First identified as a cause of amphibian declines in 1998, chytridiomycosis, the amphibian disease caused by Bd, has led to declines and extinctions of many amphibian species, mostly in tropical and subtropical areas (Berger et al. 1998; Longcore et al. 1999; Lips et al. 2006). Since the identification of Bd as the cause of chytridiomycosis, the fungus has been identified from museum specimens dating back to 1928 in the United States and 1894 in Brazil (Huss et al. 2014; Rodriguez et al. 2014).

Bd is a fungus of the family Chytridiomycota and is one of only two members of the family known to be pathogenic in vertebrate hosts (Longcore et al. 1999; Martel et al. 2013). Bd infects the keratinized skin of post-metamorphic amphibians and the mouthparts of larval anurans (frogs and toads) (Berger et al. 1998). As one part of its lifecycle, Bd has an aquatic, flagellated zoospore stage that allows the fungus to disperse through an aquatic environment to new hosts. After reaching a new amphibian host, the zoospores infect by attaching to keratinized tissue, reducing the ability of the host to osmoregulate. In lethal infections, osmoregulation is completely blocked and the individual dies from respiratory arrest due to an electrolyte imbalance. Death occurs when an individual's Bd load exceeds 10,000 zoospores (Voyles et al. 2009; Vredenburg et al. 2010).

While most of the declines in amphibian populations caused by Bd have been in the tropics and sub-tropics, Bd-related population declines have occurred in the United States with the most notable cases being in the mountain yellow-legged frog complex (*Rana muscosa* and *Rana sierrae*) in California, where southern populations have declined by 92.5% (Briggs et al.

2005; Vredenburg et al. 2007, 2010; Piovia-Scott et al. 2011). Bd is widespread among amphibian species in North America and is now considered endemic across much of the continent (Lannoo et al. 2011), but not all species are equally susceptible to Bd infection, and infection may be present in a population asymptomatically (Blaustein et al. 2005; Rachowicz & Briggs 2007; Tennessen et al. 2009). Amphibians are classified as having the disease chytridiomycosis if they are exhibiting clinical or pathological signs of the disease (Berger et al. 1998; Pessier et al. 1999; Nichols et al. 2001). Still, even slight infections may disrupt the immune response of hosts and affect their ability to cope with other demands on their immune system.

Although the life cycle of Bd and the mechanistic cause of mortality resulting from chytridiomycosis are known, much of the epidemiology (i.e., Bd reproduction, initial infection and transmission among hosts) remains unknown. Hypotheses about how Bd is spread at the local scale include amphibian host dispersal and direct contact; movement by stream flow in water and sand; or by movements of birds, humans, or other animals (Johnson & Speare 2005; Morgan et al. 2007; Kilpatrick et al. 2010). Anuran populations in close proximity to Bd-positive populations are more likely to be infected than isolated populations (Padgett-Flohr & Hopkins 2010). The presence of Bd carrier species, those that transport Bd on their body without becoming infected, also affects the probability of Bd spreading to non-carrier species. In the Midwest, potential amphibian Bd carriers include American Bullfrogs (*Lithobates catesbeianus)* and aquatic salamanders (e.g., *Ambystoma tigrinum*) (Davidson et al. 2003; Daszak et al. 2004). However, with few species experiencing Bd-related symptoms, nearly all amphibian species in the Midwest can be considered carriers. Therefore, in this region, the most influential carriers are those species with high vagility and dense populations. Other amphibian carriers may owe

their resistance to Bd to peptides and antifungal bacteria present on their skin (Rollins-Smith et al. 2011). Non-amphibian hosts of Bd which also act as carriers include nematodes and crayfish (Shapard et al. 2012; McMahon et al. 2013).

The relationship between infection and temperatures, termed the "Chytrid-Thermal Optimum Hypothesis" (Pounds et al. 2006), led to a proposed link between the spread of chytridiomycosis and climate change. The hypothesis states that recent increases in environmental temperatures have facilitated chytridiomycosis outbreaks by changing temperatures to be within the optimum growth range for the Bd pathogen. The optimal temperature range for Bd is between 17°C and 25°C (Piotrowski et al. 2004). However, many researchers have refuted the climate change link and published evidence disproving this hypothesis (Lips et al. 2008; Rohr et al. 2008). In actuality, rising temperatures may help affected amphibians if they increase above the Bd thermal optimum range to temperatures that kill the fungus. However, climate change may have other detrimental effects on amphibians that may make them more susceptible to Bd infection. Bd may be able to acclimate to temperature fluctuations faster than anurans, and anuran resistance to Bd can be lower with fluctuating temperatures than with constant temperatures. Climate change models predict an increase in the severity of temperature fluctuations, which could therefore lead to more Bd infections in anurans (Raffel et al. 2012).

Although the distribution of Bd has been modeled at biogeographic scales, few studies have focused on predicting the occurrence of Bd at landscape scales based on spatial variation in biotic and abiotic variables (Padgett-Flohr and Hopkins 2010). In general, few studies have been conducted on the dynamics of Bd in the temperate areas of the Midwest United States. Research is needed on the role of temperate forested wetlands in the pathogenicity of the fungus and on the

potential for open canopy wetlands to serve as refuges from Bd. In addition to chytridiomycosis, amphibians around the world are facing a bombardment of problems affecting their ability to persist. Other diseases (e.g., ranavirus or trematode infections), climate change, chemical toxins, and habitat destruction are leading to a perilous existence for many species. Although debilitating Bd infections remain rare in the Midwest, changes in environmental conditions or introduction of a new disease could alter the system, allowing Bd to become lethal. With the future of amphibians in such a perilous predicament, understanding the landscape factors affecting occurrence of the disease is paramount to future disease management and protection of amphibian species.

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INTRODUCTION

Batrachochytrium dendrobatidis (Bd) is a globally distributed fungal pathogen of amphibians that causes the disease chytridiomycosis and is one of the major contributors to recent population declines of amphibians (Berger et al. 1998; Crawford et al. 2010). Bd is widespread among amphibian species in North America and is now considered endemic across much of the continent (Lannoo et al. 2011). However, susceptibility to Bd infection varies among populations and species (Retallick et al. 2004; Woodhams & Alford 2005; Blaustein et al. 2005), and infection prevalence varies spatially across landscapes (Woodhams & Alford 2005; Muths et al. 2008; Pearl et al. 2009; Adams et al. 2010; Phillott et al. 2013).

The ability of Bd to infect hosts is strongly influenced by environmental conditions, including temperature and humidity. Bd survives and reproduces optimally with cool temperatures and requires a moist environment. In these conditions, zoospores can survive without a host for months (Johnson & Speare 2005). In the laboratory, the optimal temperature range for Bd is between 17°C and 25°C and spore death begins to occur above 28°C (Piotrowski et al. 2004). Some frogs are able to clear themselves of Bd infection in the laboratory when environmental temperatures exceed 30°C (Woodhams et al. 2003). Temperature is an important factor to consider when investigating patterns of Bd infections because temperature affects both Bd growth and amphibian immune responses (Russell et al. 2010).

Interspecific and intraspecific patterns in infection prevalence and intensity of Bd can depend on species traits, environmental conditions, and landscape structure. Interspecific variation has been attributed to differences in behavior and life-history traits (Rowley & Alford 2007; Bancroft et al. 2011). Also, amphibians that are mostly aquatic tend to have higher Bd prevalence than more terrestrial species (Pearl et al. 2007). For intraspecific differences, the

impact of Bd is less in warmer areas (Kriger et al. 2007; Forrest & Schlaepfer 2011), in dry areas (Puschendorf et al. 2011), at low versus high elevations and latitudes (Woodhams & Alford 2005; Kriger et al. 2007), and in summer versus winter or spring (Berger et al. 2004; Woodhams & Alford 2005; Kriger & Hero 2007). Bd infections can also vary between years as environmental conditions change. For instance, Bd infection intensity in Crawfish Frogs (*Lithobates areolatus*) was significantly reduced following a drought (Terrell et al. 2014). A heterogeneous landscape may create substantial variability in infection prevalence in populations even within a small area (Van Sluys & Hero 2009). Wetlands with higher temperatures or less humidity could therefore serve as environmental refuges from the disease during warmer seasons. Moreover, the probability of Bd infection has been correlated negatively with distance to a wetland with high Bd prevalence (Padgett-Flohr & Hopkins 2010), indicating landscape connectivity can be consequential for disease dynamics.

The amount of forest canopy covering a wetland may have an impact on Bd infections of resident amphibians. Canopy cover can reduce the temperature of shallow wetlands and prevent large fluctuations in water temperature (Werner & Glennemeier 1999). Conversely, a lack of canopy cover can allow water or ambient air temperatures to exceed the maximum temperature threshold of Bd spore tolerance. Open wetlands may therefore serve as environmental refuges from Bd infection by having temperatures above 28°C more than closed wetlands. For instance, Green Frogs (*Lithobates clamitans)* in New York had higher Bd prevalence in a population from a forested wetland than in populations from open wetlands due to lower water temperature in the forested wetland (Becker et al. 2012). In Australia, *Litoria wilcoxii* sampled along streams had higher Bd prevalence at upstream closed canopy sites than they did at downstream open canopy sites (Van Sluys & Hero 2009). However, the effect of canopy cover on Bd infections may

change between drought years and non-drought years. Specifically, the role of open wetlands as refuges for amphibians might be strongest during warm and dry years.

Landscape structure and connectivity among sites should affect disease transmission and spatial patterns of infections (Ostfeld et al. 2005). Padgett-Flohr & Hopkins (2010) applied this idea to Bd infections across the landscape and found in central California that perennial ponds in close proximity to sites deemed Bd hotspots were more likely to have Bd than more isolated ponds. Likewise, *Litoria rheocola* populations from low elevation sites that were connected by a stream to high elevation sites had higher Bd prevalence than low elevation sites that were not connected to other sites (Sapsford et al. 2013). Therefore, landscape connectivity may influence the Bd prevalence for wetlands with the expectation that wetlands in close proximity to one another will have higher Bd prevalence than isolated wetlands.

To better understand how Bd prevalence varies spatially among wetlands in a heterogeneous landscape, I sampled anurans in wetlands with variable canopy cover. I hypothesized that the prevalence of Bd infection would be higher in populations from closed canopy wetlands versus populations from open canopy wetlands because temperatures in open wetlands will exceed Bd's upper temperature threshold of 28°C more often. I expanded on Becker et al.'s (2012) study by sampling from a greater number of wetlands representing a more complete gradient of forest canopy cover. I also conducted sampling during a warm, dry year (severe drought of 2012) and a more typical year (2013) to determine how weather might interact with canopy cover, and other environmental factors, to affect Bd infection. Such interactions should be important for understanding how future climate change could influence amphibian disease dynamics (Lafferty 2009a; Altizer et al. 2013). Finally, I asked whether landscape connectivity was an important predictor of spatial patterns of Bd prevalence.

METHODS

Study area and weather conditions

My study was conducted in eastern Illinois within the Middle Fork State Fish and Wildlife Area and the Kickapoo State Recreation Area (40.19°N, 87.75°W; area = 25 km^2). I sampled anurans from 33 freshwater wetlands with variable hydroperiods and a substantial range of canopy cover. Wetlands ranged in size from 47 m^2 to 3613 m^2 (median = 142 m^2 in 2012, median = 154 m^2 in 2013). The median nearest-neighbor distances between wetlands was 80 m (range = 15 – 596 m).

I sampled anurans for Bd during spring in 2012 and 2013. Each wetland was surveyed once between 6 March and 17 May 2012 and once between 15 March and 30 May 2013. In spring 2012, two of the 33 wetlands were dry and could not be sampled. In spring 2013, one of the wetlands was dry and not sampled.

Weather conditions varied considerably between sampling years with 2012 being dry and warm and 2013 being wetter and cooler. For my region, the 30-year average for spring (March – May) precipitation was 29.6 cm, and the 30-year average for spring temperature was 11.7°C (Illinois State Water Survey, Danville, IL). During spring 2012, the area received only 16.3 cm of rain and had an average temperature of 15.6°C. This severe drought of 2012 represented the lowest spring precipitation total during the past 30 years. During spring 2013, conditions were more typical and the area received 29.7 cm of rain and had an average temperature of 10.5°C.

Sampling for chytrid fungus

Surveys were conducted in the evenings starting around sunset. Each site was surveyed by searching the wetland plus an area of ~2 m away from the water's edge for anurans for one

hour, collecting all visible anurans using sterilized dip nets or plastic bags inverted over a hand. The most common species collected were Spring Peepers (*Pseudacris crucifer*), Blanchard's Cricket Frogs (*Acris blanchardi*), American Bullfrogs (*Lithobates catesbeianus*), and Green Frogs (*Lithobates clamitans*). I placed individuals in clean, individual plastic bags following capture. I then stored bags in a cool location until all individuals were captured. By collecting all individuals at a site before processing, I avoided capturing individuals twice. I then swabbed the captured anurans for Bd using established sampling protocols (Brem et al. 2007). I swabbed individuals with a sterile swab (MW100; Medical Wire & Equipment) on the ventral abdomen, inner thighs, feet, and underarms for a total of 27 sweeps. Swabs were then placed back in their original containers, kept in a cooler in the field, and then placed in a -20°C freezer within eight hours of sampling. Along with swabbing, I recorded species, life stage (adult or juvenile), snout-to-vent length (SVL), and any physical abnormalities of each individual. Most sampled individuals were adults (2012: 88.4 % adults; 2013: 92.5 % adults) but Bullfrogs and Green Frogs included a larger percentage of juvenile samples (2012: 69.6% juvenile; 2013: 29.0% juvenile). After processing, I released individuals back along the water edge. All equipment, including boots, was sterilized after sampling using a 10% bleach solution made with water from the wetland. All bleach water was disposed of on graveled paths or roads, away from the wetlands.

Laboratory Methods

I extracted Bd DNA from swab samples using Qiagen DNeasy Blood and Tissue extraction kits using the included extraction protocol, with the exception of using 100µL AE buffer instead of 200µL to maximize the concentration of Bd DNA in the supernatant (Qiagen

Inc., Valencia, CA). Samples were then diluted 1:10 with water to reduce the probability of inhibition during quantification (Tobler et al. 2012). I quantified the samples at the W.M. Keck Center on the University of Illinois campus using a Taqman ABI 7900 real time PCR machine and the qPCR methods described by Boyle et al. (2004) with the modifications described by Kriger et al. (2006) and Ruthig & DeRidder (2012). Samples were run on 364-well plates and used 10μL reaction volumes. A standard curve was created using a Bd positive control. Water was used as the negative control. Each sample was run in duplicate on the same plate and was scored as positive only if amplification occurred in both wells.

Environmental Temperatures

To characterize temperature conditions experienced by amphibians at wetlands, and to verify that canopy cover decreased environmental temperatures, I deployed Thermochron iButton (Dallas Semiconductors) temperature data loggers at wetlands. All iButtons were coated with rubber (Plasti Dip, Plasti Dip International, Blaine, MN, USA) and placed in plastic bags prior to deployment to prevent water damage from occurring (Roznik & Alford 2012). Two iButtons were used at each wetland. One was anchored to a PVC pipe under water at least 1 m from the edge and approximately 6 cm from the bottom. The other was tied near ground level to woody vegetation, out of direct sunlight, within 10 m of the water's edge. The iButtons recorded water or air temperature measurements automatically every two hours during the anuran sampling season and every four hours between sampling seasons. However, because of failure of some iButtons, I have data for a sample of representative wetlands for each time period. For air temperatures, I collected data from 20 wetlands for spring 2012, 21 wetlands between

sampling seasons, and 22 wetlands for spring 2013. For water temperatures, I collected data from 18 wetlands during spring 2012 and 19 wetlands during spring 2013.

Environmental covariates for Bd prevalence

To determine environmental effects on Bd prevalence (i.e., proportion of infected individuals at a wetland), I measured a number of environmental factors expected to affect the fungus. I measured canopy cover with a spherical densiometer at each wetland during June-July 2012 following leaf-out. Measurements were taken from five points at each wetland, including the wetland center and from the north, south, east, and west edges. For large wetlands (>500 m^2), measurements were taken from four additional points on the northeast, northwest, southeast, and southwest edges. If the water level of the wetland exceeded chest height, the center measurement was excluded. At each sampling point, canopy was measured in each of the four cardinal directions and then averaged to give a point measurement. All point measurements were then averaged to obtain a single estimate of canopy cover per wetland. I re-measured canopy cover in July 2013 for wetlands that had changed in size between years. During June-July 2012, I also recorded the maximum water depth and repeated these measurements in July 2013. Wetland area was measured using GPS locations taken in the field in 2012 and 2013 and overlaid on 2011 digital images in ArcGIS 10 (ESRI, Redlands, CA, USA). Precipitation data were obtained from the Danville, IL, weather station (40.2°N, 87.6°W) located 13.2 km east of the center of my study area. I used both precipitation amounts occurring on the day of sampling and precipitation recorded in the 30 days prior to sampling. Precipitation occurring on the day of sampling may influence the density of anurans breeding at the wetland during sampling because

rainfall is known to initiate breeding in many temperate species (Duellman & Trueb 1994). I used 30-day precipitation levels to reflect the water depth at the wetlands and soil moisture.

Connectivity to other wetlands, especially those that could be Bd hotspots, may affect Bd occurrence in a wetland (Padgett-Flohr & Hopkins 2010). I measured spatial connectivity of wetlands using two simple, nearest-neighbor metrics: distance to nearest wetland, and distance to nearest Bd-positive wetland (Padgett-Flohr & Hopkins 2010). I also assessed connectivity using a more complex incidence function model (IFM) connectivity metric often applied to metapopulations of wetland species in patchy landscapes (Werner et al. 2009; Schooley & Branch 2009; Cosentino et al. 2010). The IFM metric considers both distances to and areas of multiple wetlands (Prugh 2009), and potential sources can be weighted by other factors that could affect relevant dispersal rates (Schooley & Branch 2009; Cosentino et al. 2010). Here, I weighted source populations by their estimated Bd prevalence because prevalence rate, combined with wetland area, should indicate the number of infected individuals available for dispersal. The connectivity (C_i) of wetland i, weighted by area and Bd prevalence, was defined as

$$C_i = \sum_{j \neq i} p_j \exp(-\propto d_{ij}) A_j^b$$

where p is the estimated Bd prevalence at wetland j (see below), α is a parameter scaling the effect of distance on dispersal ($1/\alpha$ is 1000 m, a typical dispersal distance for most North American anurans; Peterson et al. 2013), d_{ij} is the Euclidean distance between target wetland i and source wetland j, A is the area of source wetland j, and b (set at 0.5) is a parameter scaling the relationship between abundance and wetland area (Schooley & Branch 2009). The estimates of p were calculated using model averaging for models from the candidate set with a $\Delta AIC_c \leq 2$ (see below).

Statistical analyses

To assess how environmental factors affect the spatial patterns of disease prevalence, I used occupancy modeling (Program PRESENCE 6.2), which typically is used for estimation of species distributions among sites despite the possibility of false negatives (MacKenzie et al. 2006). When occupancy modeling is applied to occurrence and prevalence of a disease (Adams et al. 2010; Bailey et al. 2013) the interpretation of parameters is altered because the number of independent observations is limited (i.e., finite number of host individuals). The occupancy parameter (ψ) represents the probability that Bd occurs at a site (i.e., individual wetland). The detection probability parameter (p) has two components ($p = p' \times p''$) where p' is the probability that Bd is present on an individual in a population in which Bd is present, and p'' is the probability of detecting Bd on an individual when Bd is present on the individual. If one uses an intensive swabbing procedure for Bd and assumes p'' is close to one, then it is reasonable to interpret detection probabilities (p) as being mainly due to variation in p' and representing heterogeneity in prevalence (Adams et al. 2010).

Because of problems with models not converging, I had to streamline the occupancy modeling in several ways. First, I constructed single-season models for each year instead of a multi-season model and then contrasted the results for the warm drought year (2012) with the normal year (2013). Second, because Bd was present in most of my study wetlands (naïve occupancy = 0.81 – 0.91; see Results), I did not focus on modeling covariates for occupancy. Instead, I focused my modeling on p, or Bd prevalence, which represented the probability of an individual from an infected wetland being Bd positive. Third, I did not assess complex models that included more than two covariates.

I evaluated occupancy models using four data sets: 2012 common species, 2013 common species, 2012 Spring Peepers, and 2013 Spring Peepers. The common species sets included all species sampled at ≥5 wetlands (see Tables 2 and 3). Spring Peepers were the most common species in both years and thus warranted separate analyses. All occupancy models were run using a logit-link function and had occupancy (ψ) set as intercept-only (dot model). My covariates for Bd prevalence (p) included canopy cover, July water depth, wetland area, precipitation, species, SVL, Julian date, and spatial connectivity (Table 1). Elevation was not used as a covariate because of the minimal relief for the study area. All covariates were z-transformed prior to analysis. I developed a candidate set of predictive models that included all covariates individually as well as in combinations of two (excluding collinear covariates that were correlated at $r \geq 0.5$). 'Species' was used as a covariate when modeling 2012 and 2013 common species and it identified whether or not a sample was from a Bullfrog, a species considered a Bd carrier (Daszak et al. 2004). SVL was only used for Spring Peeper models. The predictive model set also included a null model for prevalence (intercept-only). The total number of models evaluated for each of the four data set varied from 22 to 27 (see Appendix A).

I evaluated support for models using an information-theoretic approach. Models were ranked using Akaike's Information Criteria modified for small sample sizes (AIC_c; Burnham & Anderson 2002). AIC_c values were calculated using the number of wetlands in the model set as a proxy for effective sample size. Strong backing for models that included canopy cover as a covariate for Bd prevalence, and a positive relationship between the two variables, would provide support for my main hypothesis.

In a second analysis, I determined whether connectivity explained spatial variation of Bd prevalence beyond that already explained by other covariates and, if so, which measure of

connectivity was most supported. For each of the four data sets, I retained the top models from the previous analysis whose Akaike weights summed to 0.95 (a 95% confidence set). I then created additional models by separately adding each of the three measures of connectivity (Distance to nearest wetland, Distance to nearest Bd-positive wetland, IFM connectivity metric) to all models. Because the connectivity measures occurred in all models in a balanced fashion, I used summed Akaike weights across models to evaluate support for the three measures.

RESULTS

Canopy cover and environmental conditions

Canopy cover of wetlands ranged from 0.002 to 0.88 (2012: $\bar{x}$ = 0.62; 2013: $\bar{x}$ = 0.60), representing a strong gradient. Air and water temperatures at wetlands were negatively correlated with canopy cover during both 2012 and 2013. For example, the number of days during June and July that air and water temperatures exceeded Bd's maximum temperature threshold of 28°C was negatively correlated with canopy cover (Figure 1). Additional examples illustrating the relationships between wetland temperatures and canopy cover support this general pattern (see Appendix B).

During the severe drought of 2012, 18 (58%) of 31 wetlands had dried completely by July. In contrast, only 4 (12.5%) of 32 wetlands had dried by July 2013 (Fisher's exact test, p = 0.0002).

Naïve occupancy and prevalence for chytrid

During 2012, Bd was detected in 25 of the 31 wetlands (naïve occupancy = 80.7%). Of the 456 anurans of nine species captured, 91 individuals (19.96%) tested positive for Bd (Table

2). Five of the nine species captured included Bd positive individuals. Spring Peepers were the most commonly sampled species, with 236 individuals from 24 wetlands, of which 11.4% were positive. Bullfrogs had the highest naïve prevalence with 71.4% of 49 individuals testing positive.

During 2013, Bd was detected in 29 of the 32 wetlands (naïve occupancy = 90.6%). Of the 586 anurans of nine species captured, 201 individuals (34.3%) tested positive for Bd (Table 3). Positive samples included individuals from seven species. Spring Peepers were again the most commonly sampled species, with 266 individuals from 17 wetlands, of which 25.6% were Bd positive. Bullfrogs had the highest naïve prevalence again with 60% of 20 individuals testing positive.

Naïve occupancy of Bd in wetlands was similar between years (Fisher's exact test, p = 0.302). However, overall naïve prevalence of Bd increased from 2012 to 2013 (20% vs. 34%; Fisher's exact test, p = 0.0001). Likewise, naïve prevalence of Bd for Spring Peepers increased from 2012 to 2013 (11% vs. 26%; Fisher's exact test, p = 0.0001).

Predictors of Bd prevalence from occupancy models

For 2012 common species, heterogeneity in Bd prevalence (p) was best explained by species differences. All supported models contained Bullfrogs as a covariate (Table 4). Bd prevalence was higher for Bullfrogs than for other species ($\beta = 1.137$, SE = 0.184). There was secondary support for Bd prevalence being positively related to day of sampling ($\beta = 0.2439$, SE = 0.1807; Table 4). Other covariates that occurred in competitive models ($\Delta AIC \leq 2$) had little support because model fits were nearly the same as the top-ranked model containing only

Bullfrog. Based on the top model for prevalence, Bd occupancy of wetlands was estimated to be 0.89 (95% CI = 0.66 – 0.97).

For 2013 common species, one model received nearly all support and it included both 30-day precipitation and canopy cover as covariates (Table 4). Bd prevalence was positively correlated with 30-day precipitation ($\beta = 1.083$, SE = 0.138) but negatively correlated with canopy cover ($\beta = -0.718$, SE = 0.123), contrary to my hypothesis (Figure 2). Based on this top model, Bd occupancy of wetlands was estimated to be 0.97 (95% CI = 0.71 – 0.99).

For 2012 Spring Peepers, all competitive models included canopy cover (Table 5). During the drought year, Bd prevalence was positively related to canopy cover ($\beta = 1.334$, SE = 0.603), which supported my hypothesis (Figure 3). The top model also included June-July water depth (Table 5), which was positively correlated with estimated Bd prevalence ($\beta = 0.635$, SE = 0.320). Other covariates with support that occurred in competitive models included wetland area ($\beta = 0.658$, SE = 0.400) and 1-day precipitation ($\beta = 0.346$, SE = 0.194).

For 2013 Spring Peepers, canopy cover was not in the single competitive model (Table 5). Furthermore, the direction of the relationship between Bd prevalence and canopy cover was in contradiction to my hypothesis (Figure 3). Covariates in the top model (Table 5) were day of sampling ($\beta = 1.912$, SE = 0.375) and June-July water depth ($\beta = -0.688$, SE = 0.232).

Spatial connectivity

For 2012 common species, the summed Akaike weights indicated that none of the connectivity measures had strong support (Figure 4). Only one competitive model included connectivity (DNear; Distance to nearest wetland), but the addition of this measure did little to improve model fit (Appendix C). For 2013 common species, in contrast, there was strong

support for the DNear connectivity measure (Figure 4). The top-ranked, and only competitive model, included DNear (Appendix C). Bd prevalence was related positively to distance to the nearest wetland ($\beta = 0.308$, SE = 0.100). That is, more isolated wetlands had higher predicted Bd prevalence. For Spring Peepers in 2012 and 2013, there was no strong support for any measure of spatial connectivity (Figure 4, Appendix C).

DISCUSSION

Bd was widespread throughout the study wetlands; naïve occupancy was high during both years. During the drought year of 2012, however, naïve prevalence of Bd was substantially reduced for all anuran species combined and for Spring Peepers. As expected, air and water temperatures at closed canopy wetlands were lower than temperatures at open canopy wetlands, supporting the idea that canopy cover can create conditions favorable for Bd in my region. The influence of canopy cover on Bd was complex, however, and I found limited support for my main hypothesis. Estimated Bd prevalence was related positively with canopy cover only for Spring Peepers during the 2012 drought. Effects of other environmental covariates also differed between 2012 and 2013 as well and were probably influenced by the different weather conditions in the two years. Lastly, connectivity was generally not an important predictor of spatial heterogeneity in Bd prevalence. Connectivity was only significant for common species in 2013, but with an unexpected outcome (i.e., higher prevalence in more isolated wetlands).

The springs of 2012 and 2013 when sampling was conducted had drastically different weather conditions. The precipitation total for spring 2012 was the lowest recorded in the past 30 years. Moreover, temperatures during spring 2012 were much higher than the 30-year average. In contrast, spring 2013 had precipitation levels and temperatures near the 30-year

average. Summer water depth also reflected differences in weather conditions; 18 wetlands dried completely between spring sampling and summer 2012, but only four dried by summer 2013. This sharp contrast in weather conditions provided insights into how the chytrid fungus could respond to climate change. The severe drought of 2012 was associated with reduced prevalence of Bd on anurans. This outcome supports the argument by Kriger (2009) that unusually dry weather should favor amphibians by reducing the prevalence and spread of chytridiomycosis. Similarly, drought has also been shown to be beneficial to Crawfish Frogs by reducing Bd infection intensity and mortality (Terrell et al. 2014). Weather conditions also appear to interact with other environmental factors, including canopy cover, in influencing Bd prevalence.

Bd prevalence for spring peepers during 2012 was related positively to canopy cover, as I hypothesized. In contrast, Bd prevalence of Spring Peepers was not strongly affected by canopy cover in 2013. With cooler temperatures during 2013 compared to 2012, the role of canopy cover in preventing large fluctuations in wetland temperature may have been reduced. Therefore, the anticipated effect of canopy cover on Bd prevalence for Spring Peepers was only expressed during the unusually warm and dry conditions of 2012. For common species combined, the effect of canopy cover also differed between years. Canopy cover was not a predictor of Bd prevalence in 2012, but Bd prevalence was related negatively to canopy cover in 2013. The negative relationship may have been driven by the fact that when temperatures are low enough that canopy cover does not have a large impact on wetland temperature, it instead is influencing other factors, such as anuran densities. Wood Frogs and Spring Peepers prefer to breed in open canopy wetlands because their tadpoles develop more rapidly in them (Halverson et al. 2003). Therefore, the density of anurans in open canopy wetlands may be higher than in closed canopy wetlands, and greater density allows for more Bd transmission between

individuals, leading open canopy wetlands to have higher Bd prevalence than closed canopy. When temperatures were warmer during 2012, open canopy wetland anurans had lower Bd prevalence perhaps because temperatures were exceeding Bd's maximum critical temperature threshold more often. Becker et al. (2012) reported that Green Frog populations in New York from forested wetlands had higher Bd prevalence than those in open wetlands. I would have reached the same conclusion had I only sampled one species (Spring Peepers) during one year (2012). Instead, by additionally sampling during a year with normal weather conditions, and sampling multiple amphibian species, I found the effect of canopy cover on Bd prevalence is complex and contingent on weather conditions. Other studies examining the influence of temperature profiles on Bd infections have recommended the clearing of canopy to raise the temperature of wetlands in an effort to reduce the impacts of Bd on amphibian populations (Woodhams et al. 2011; Heard et al. 2014). Based on my results, such a manipulation could be effective for particular species under certain weather conditions, but not under different conditions in other years.

Of the other covariates that I examined, Bd prevalence for common species in 2012 and Spring Peepers in 2013 were related positively to day of sampling. I expected this positive relationship because spring temperatures start low and gradually increase throughout the season until they occur more often within Bd's optimal temperature range. During 2013, 30-day precipitation had a strong positive influence on Bd prevalence for the common species. Because Bd requires water for survival and reproduction, more precipitation resulting in higher Bd prevalence is expected. During the drought of 2012, the range of 30-day precipitation amounts between wetlands was minimal compared to 2013, so the amount of variation the covariate could explain was minimal. Other studies also found a positive correlation between Bd prevalence and

30-day precipitation amounts (Kriger et al. 2007; Riley et al. 2013). For Spring Peepers, the most influential covariates during the drought of 2012, in addition to canopy cover, were June-July water depth, wetland area, and 1-day precipitation amounts. Bd prevalence was positively related to all three covariates. Water depth was supported for 2013 Spring Peepers as well, but during the average weather year, Bd prevalence was negatively related to water depth. The difference in weather conditions between years not only influenced which covariates had the greatest influence on Bd prevalence, but the direction of influences as well.

Bullfrogs had an obvious impact on Bd. During both 2012 and 2013, Bullfrogs had higher naïve prevalence than any other species, a result consistent with other studies (Sanchez et al. 2008; Schloegel et al. 2010; Spitzen-Van Der Sluijs et al. 2014). Occupancy models for 2012 common species reflected the influence of Bullfrogs as all supported models contained 'species' as a covariate (Bullfrog or not), and estimated Bd prevalence was greater for Bullfrogs than for other species. During 2013, fewer Bullfrogs were captured so the lack of Bullfrogs as a supported covariate could be attributed to a smaller sample size. Overall, my results are consistent with the notion that Bullfrogs are particularly important carriers of Bd (Daszak et al. 2004). While all species I captured can technically be defined as Bd carriers, having Bd infections without exhibiting visual signs of chytridiomycosis, Bullfrogs have the additional distinction of having very high Bd prevalence compared to other sampled species. This means that a higher percentage of Bullfrog individuals can introduce Bd to new areas compared with species with lower Bd prevalence.

Connectivity was generally not an important predictor of spatial patterns of Bd prevalence. Only for 2013 common species was connectivity a supported covariate in prevalence models. Connectivity may not have had a noticeable influence on Bd prevalence for

most data sets because of the relatively fine scale of my study. Many of the wetlands were in close proximity (median nearest-neighbor distance of 80 m), which should be within the dispersal distances for focal amphibian species (Werner et al. 2009; Peterson et al. 2013).

For 2013 common species, the distance to nearest wetland (DNear) was clearly more supported than the other connectivity metrics (Figure 4). This result is consistent with other studies that found simple connectivity metrics can perform as well or better than more complex measures (Prugh 2009). What is most surprising, however, is that for 2013 common species, estimated Bd prevalence was higher at more isolated wetlands (larger DNear). This result contrasts with a study that found Bd prevalence was higher at more connected wetlands (smaller DPos; Padgett-Flohr & Hopkins 2010), and with the general idea that greater connectivity should increase immigration (Werner et al. 2009) and disease transmission (Ostfeld et al. 2005). One hypothesis is that if species concentrate at more isolated wetlands when alternative breeding wetlands are scarce in an area, density could be greater at isolated wetlands (Veysey et al. 2011), fostering a favorable environment for Bd transmission. Increased host density is known to increase Bd transmission in Mountain Yellow-Legged Frogs (Rachowicz & Briggs 2007).

My results demonstrate that Bd prevalence for anurans was heterogeneous across the landscape and influenced by many environmental factors in ways that may be unpredictable. Effects of forest canopy cover on Bd prevalence rates are embedded in this web of interacting factors. Weather conditions had a large impact on Bd prevalence patterns, which makes predicting pathogen dynamics difficult. In the Midwest region, climate change models predict more frequent droughts, but also more frequent extreme rainfall events (Pryor et al. 2014). More frequent droughts may generally inhibit Bd (and degrade amphibian habitat) while also altering the role that canopy cover plays in Bd prevalence. In contrast, more frequent extreme rainfall

events and flooding may promote Bd proliferation. Fluctuating weather conditions may also inhibit amphibian immune systems, making them more susceptible to Bd infection (Raffel et al. 2012). Overall, my study supports the complex nature of climate change and disease prevalence (Lafferty 2009a, 2009b; Ostfeld 2009; Altizer et al. 2013) and the need for long-term studies assessing the synergistic effects of climate and habitat factors on Bd spatial dynamics so that the effects of the pathogen on amphibian populations can be mitigated.

TABLES AND FIGURES

Table 1. Covariates of Bd prevalence (*p*) used in occupancy modeling.

Covariate	Range	Description
Design		
Day	66 to 150	Day of the year that anuran was swabbed
Local		
Canopy	0.002 to 0.88	Proportion of wetland obstructed by tree canopy
JDepth	0 to 75	Wetland depth recorded in June or July (cm).
Landscape		
Ci	4.1 to 75.2	Wetland connectivity weighted by wetland area and Bd prevalence
DNear	15.0 to 712.5	Distance to nearest wetland (m)
DPos	15.0 to 712.5	Distance to nearest wetland where Bd was detected (m)
Area	46.8 to 3616.4	Wetland area (m^2)
Precipitation		
1DPrec	0 to 3.6	Precipitation on day of survey (cm)
30DPrec	0.6 to 19	Precipitation during 30 days prior to survey (cm)
Taxonomic		
Lca	1 or -1	1 if sample was Bullfrog (*Lithobates catesbeianus*)
SVL	9.2 to 34.2	Snout to vent length (mm)

Table 2. Naïve prevalence of Bd (percent of individuals testing Bd positive) in anurans sampled in 2012 by species. N = number of individuals captured during the sampling season. Bolded species were sampled from ≥ 5 wetlands and were included in the 2012 common species data set.

Species	N	Naïve prevalence (%)
Spring Peeper (*Pseudacris crucifer*)	**236**	**11.4**
Blanchard's Cricket Frog (*Acris blanchardi*)	**84**	**22.6**
American Bullfrog (*Lithobates catesbeianus*)	**49**	**71.4**
Gray treefrog complex (*Hyla versicolor-chrysoscelis*)	22	0
Green Frog (*Lithobates clamitans*)	**20**	**30.0**
Boreal Chorus Frog (*Pseudacris maculata*)	**20**	**20.0**
Wood Frog (*Lithobates sylvaticus*)	18	0
American Toad (*Anaxyrus americanus*)	4	0
Fowler's Toad (*Anaxyrus fowleri*)	3	0

Table 3. Naïve prevalence of Bd (percent of individuals testing Bd positive) in anurans sampled in 2013 by species. N = number of individuals captured during the sampling season. Bolded species were sampled from ≥ 5 wetlands and were included in the 2013 common species data set.

Species	N	Naïve prevalence (%)
Spring Peeper (*Pseudacris crucifer*)	**266**	**25.2**
Blanchard's Cricket Frog (*Acris blanchardi*)	**140**	**49.3**
Boreal Chorus Frog (*Pseudacris maculata*)	**75**	**42.7**
Green Frog (*Lithobates clamitans*)	**49**	**36.7**
American Bullfrog (*Lithobates catesbeianus*)	**20**	**60.0**
Gray treefrog complex (*Hyla versicolor-chrysoscelis*)	**17**	**11.8**
Wood Frog (*Lithobates sylvaticus*)	17	0
Plains Leopard Frog (*Lithobates blairi*)	1	100
American Toad (*Anaxyrus americanus*)	1	0

Table 4. Ranking of models evaluating Bd prevalence (*p*) for 2012 common species and 2013 common species based on Akaike's Information Criterion (AIC_c). Occupancy (ψ) was included as an intercept-only model. $\Delta AIC_c = AIC_c$ for a given model minus AIC_c for the best model, w_i = Akaike model weight, and K = number of model parameters. Models with $\Delta AIC_c \leq 4$ are presented.

Model	ΔAIC_c	w_i	K	-2LogLikelihood
2012				
Psi(.), *p*(Lca)	0.00	0.3021	3	363.21
Psi(.), *p*(Lca, Day)	0.81	0.2015	4	361.37
Psi(.), *p*(Lca, JDepth)	1.80	0.1228	4	362.36
Psi(.), *p*(Lca, 1DPrec)	2.09	0.1062	4	362.65
Psi(.), *p*(Lca, Canopy)	2.11	0.1052	4	362.67
Psi(.), *p*(Lca, Area)	2.61	0.0819	4	363.17
2013				
Psi(.), *p*(30DPrec, Canopy)	0.00	0.9975	4	642.27

Notes: Lca = Bullfrog, Day = Julian date, JDepth = June-July water depth (cm), 1DPrec = 1 day precipitation (cm), Canopy = canopy cover, Area = wetland area (m^2), and 30DPrec = 30 day precipitation total (cm).

Table 5. Ranking of models evaluating Bd prevalence (*p*) for 2012 and 2013 Spring Peepers based on Akaike's Information Criterion (AIC_c). $\Delta AIC_c = AIC_c$ for a given model minus AIC_c for the best model, w_i = Akaike model weight, and K = number of model parameters. Models with $\Delta AIC_c \leq 4$ are presented.

Model	ΔAIC_c	w_i	K	-2LogLikelihood
2012				
Psi(.), *p*(Canopy, JDepth)	0.00	0.1842	4	154.13
Psi(.), *p*(Canopy, Area)	0.72	0.1285	4	154.85
Psi(.), *p*(Canopy)	0.75	0.1266	3	157.90
Psi(.), *p*(Canopy, 1DPrec)	0.77	0.1253	4	154.90
Psi(.), *p*(.)	2.36	0.0566	2	162.21
Psi(.), *p*(1DPrec)	2.74	0.0468	3	159.89
Psi(.), *p*(JDepth)	3.14	0.0383	3	160.29
Psi(.), *p*(Canopy, SVL)	3.37	0.0342	4	157.50
Psi(.), *p*(Canopy, Day)	3.59	0.0306	4	157.72
Psi(.), *p*(Canopy, 30DPrec)	3.69	0.0291	4	157.82
2013				
Psi(.), *p*(Day, JDepth)	0.00	0.6594	4	232.57
Psi(.), *p*(Day,1DPrec)	3.02	0.1457	4	235.59
Psi(.), *p*(Day, Canopy)	3.42	0.1193	4	235.99

Notes: Canopy = canopy cover, JDepth = June-July water depth (cm), Area = wetland area (m^2), 1DPrec = 1 day precipitation (cm), SVL = snout to vent length, Day = Julian date, and 30DPrec = 30 day precipitation total (cm).

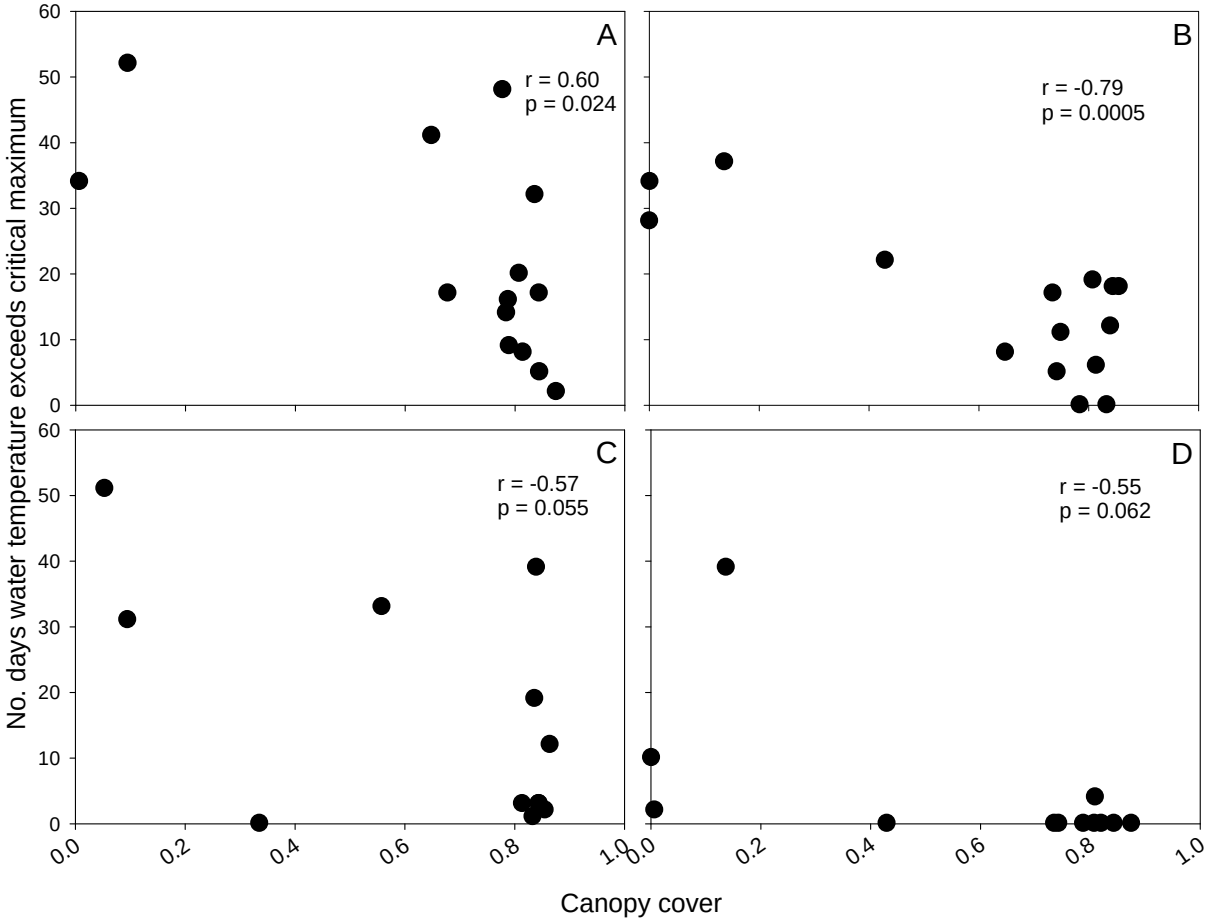

Figure 1. Effect of canopy cover on air and water temperatures of wetlands from 3 May to 30 June, 2012 and 2013. Shown is the number of days on which temperature was ≥28°C and thus exceeded the critical maximum temperature for Bd. A) 2012 air temperature, B) 2013 air temperature, C) 2012 water temperature, and D) 2013 water temperature.

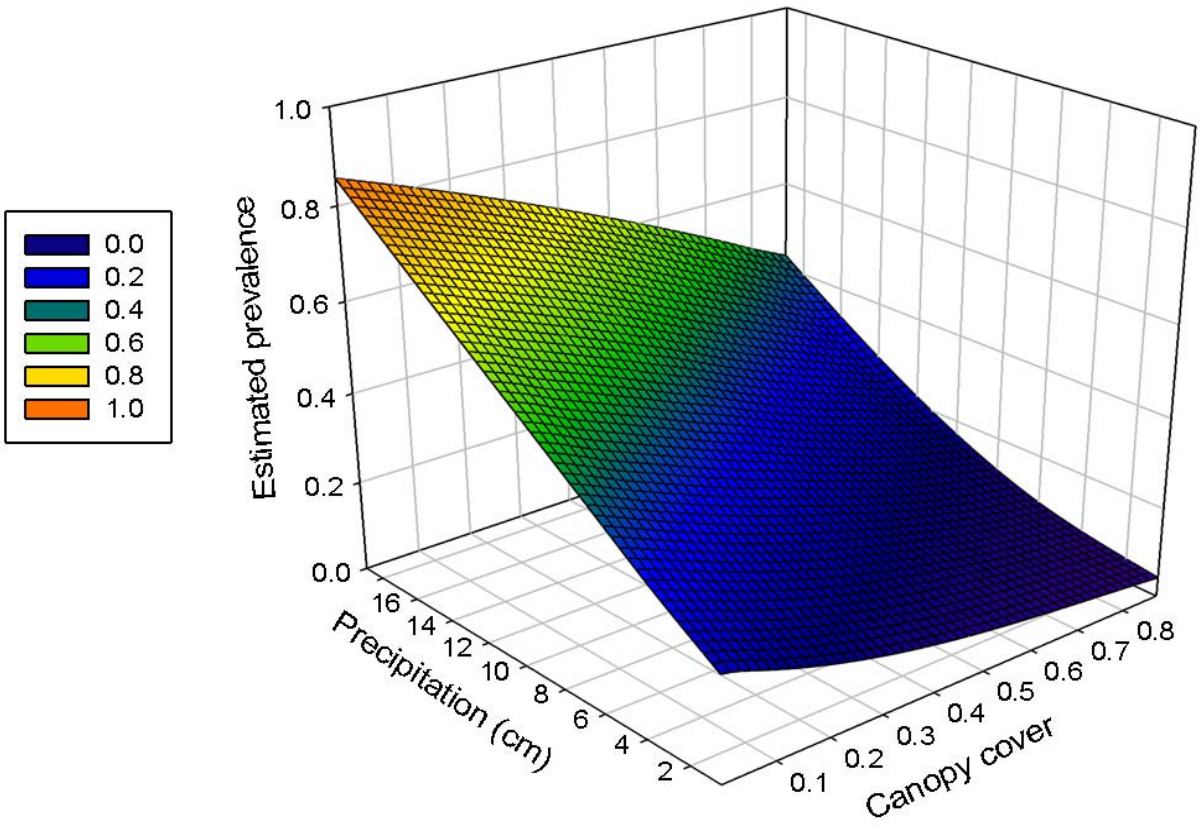

Figure 2. Effect of precipitation (total within 30 days of sampling) and canopy cover on estimated Bd prevalence for common anuran species in 2013. Prevalence estimates are from the top-ranked occupancy model (see Table 4).

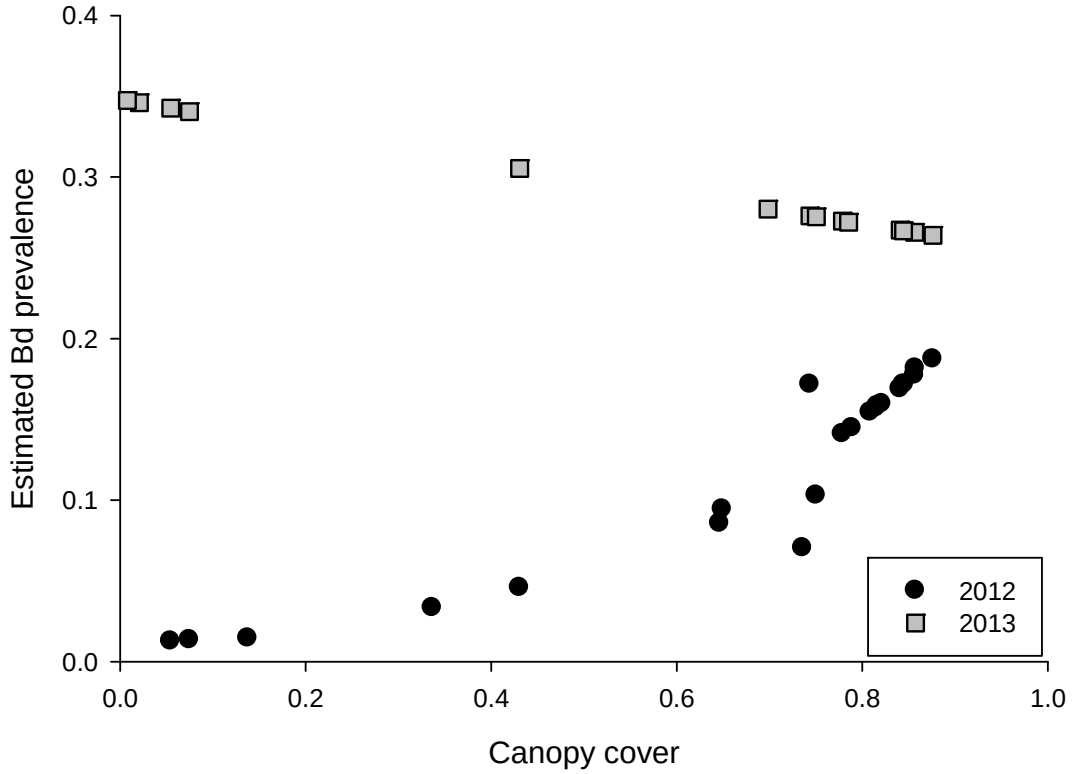

Figure 3. Relationship between Bd prevalence and canopy cover for Spring Peepers in 2012 and 2013. Bd prevalence was estimated from the *psi*(.), *p*(Canopy) model for each year.

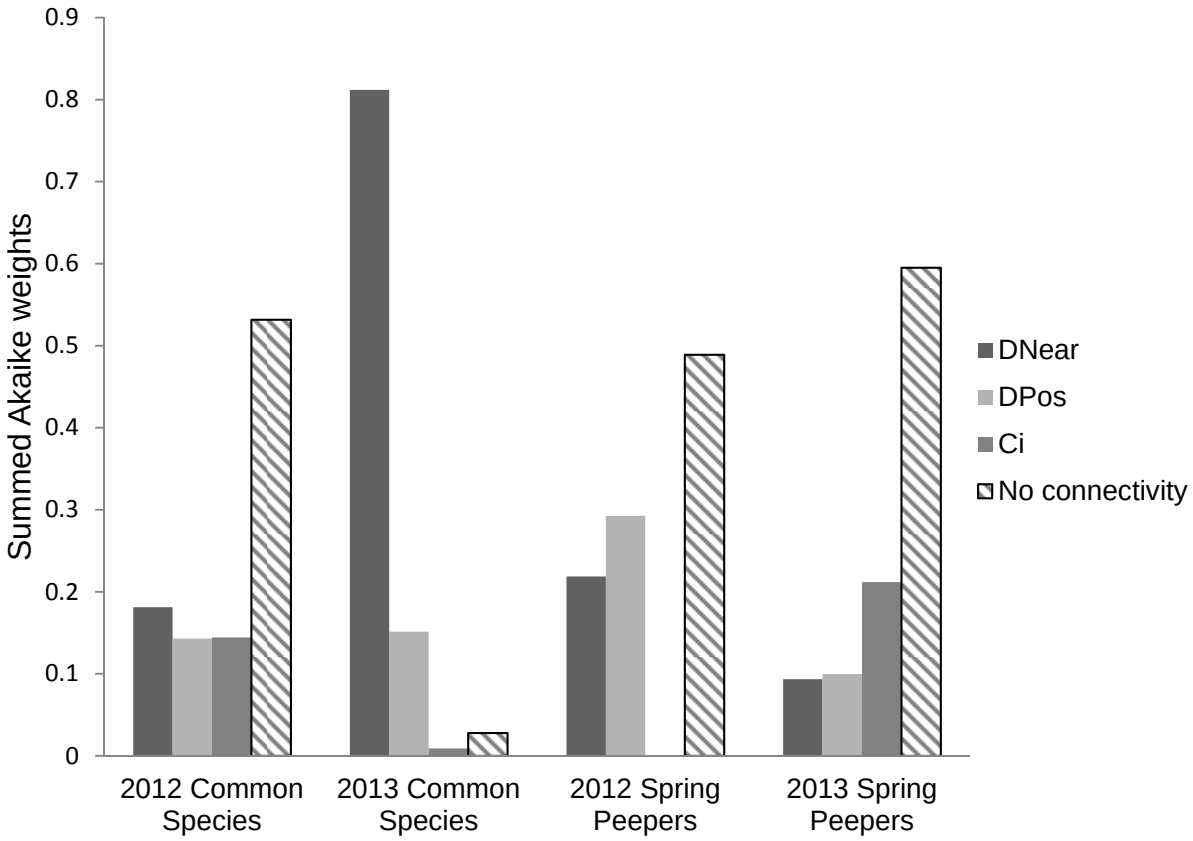

Figure 4. Summed Akaike weights for models of Bd prevalence comparing support for three measure of spatial connectivity: DNear (distance to nearest wetland), DPos (Distance to nearest Bd positive wetland), and C_i (IFM connectivity metric). "No connectivity" represents models without any measure of connectivity. For 2013 common species, only one model was in the 95% confidence set (see Table 4), so Akaike weights are not summed across models. For 2012 Spring Peepers, C_i was not evaluated due to problems with models not converging.

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APPENDIX A: CANDIDATE SET OF OCCUPANCY MODELS

Table A1. Candidate model set for occupancy modeling.

Model	*K*
Psi(.),*p*(.)	2
Psi(.),*p*(Day)	3
Psi(.),*p*(Canopy)	3
Psi(.),*p*(JDepth)	3
Psi(.),*p*(Area)	3
Psi(.),*p*(1DPrec)	3
Psi(.),*p*(30DPrec)[6]	3
Psi(.),*p*(Lca)[1,4]	3
Psi(.),*p*(SVL)[2]	3
Psi(.),*p*(Day, Canopy)	4
Psi(.),*p*(Day, JDepth)	4
Psi(.),*p*(Day, Area)	4
Psi(.),*p*(Day, 1DPrec)	4
Psi(.),*p*(Day, 30DPrec)[3,5]	4
Psi(.),*p*(Canopy, JDepth)	4
Psi(.),*p*(Canopy, Area)	4
Psi(.),*p*(Canopy, 1DPrec)	4
Psi(.),*p*(Canopy, 30DPrec)[6]	4
Psi(.),*p*(JDepth, 1DPrec)	4
Psi(.),*p*(JDepth, 30DPrec)[6]	4

Table A1. (cont.)

Psi(.),*p*(Area, 30DPrec)[6]	4
Psi(.),*p*(1DPrec, 30DPrec)[6]	4
Psi(.),*p*(Day, Lca)[1]	4
Psi(.),*p*(Canopy, Lca)[1]	4
Psi(.),*p*(JDepth, Lca)[1]	4
Psi(.),*p*(Area, Lca)[1]	4
Psi(.),*p*(1DPrec, Lca)[1]	4
Psi(.),*p*(30DPrec, Lca)[1]	4
Psi(.),*p*(Day, SVL)[2]	4
Psi(.),*p*(Canopy, SVL)[2]	4
Psi(.),*p*(JDepth, SVL)[2]	4
Psi(.),*p*(Area, SVL)[2]	4
Psi(.),*p*(1DPrec, SVL)[2]	4
Psi(.),*p*(30DPrec, SVL)[2,6]	4

Notes: [1] Models only run with 2012 and 2013 common species sets. [2] Models only run with 2012 and 2013 Spring Peeper sets. [3] Covariates correlated in 2012. [4]Model did not run with 2013 common species. [5]Model did not run with 2012 Spring Peepers. [6]Model did not run with 2013 Spring Peepers.

APPENDIX B: SUPPLEMENTAL MATERIAL FOR CANOPY-TEMPERATURE RELATIONSHIP

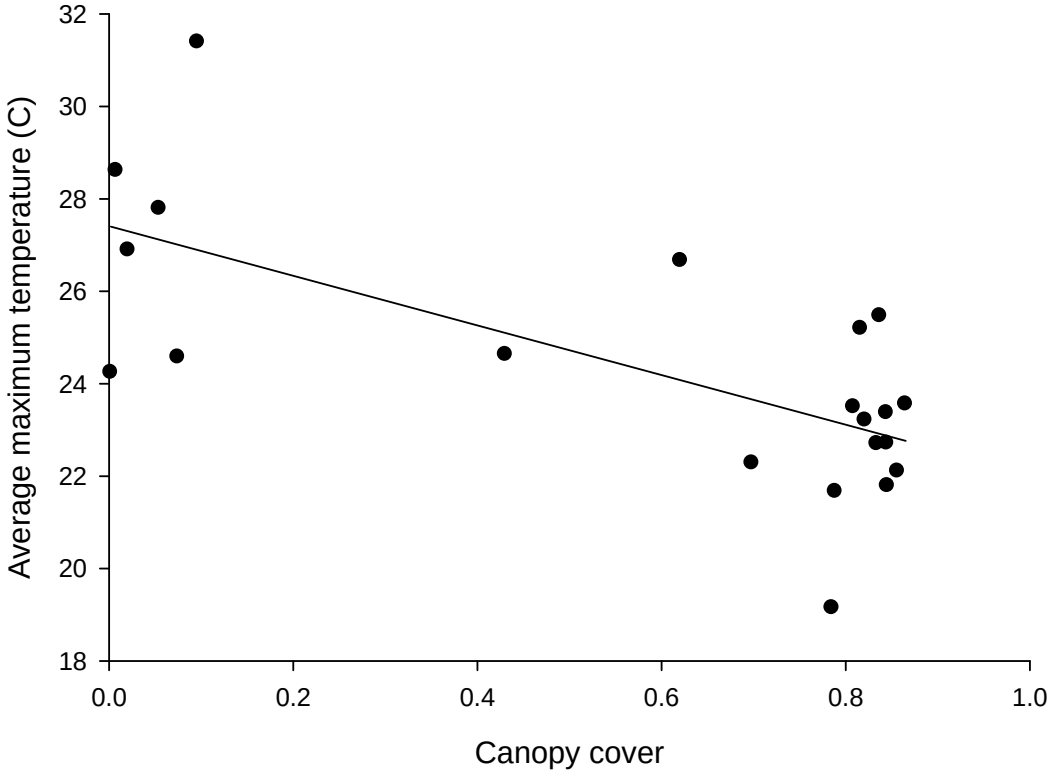

Figure B1. Effect of canopy cover on daily maximum air temperatures between 19 July 2012 and 19 November 2012.

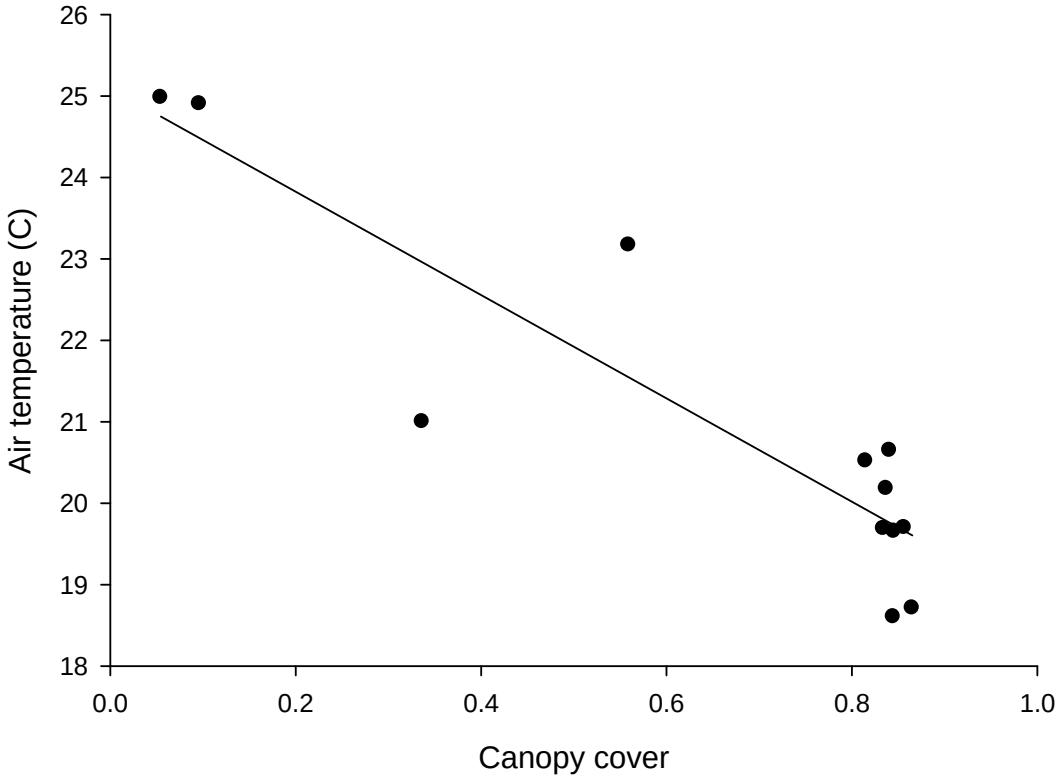

Figure B2. Effect of canopy cover on average water temperatures between 2 May 2012 and 2 July 2012.

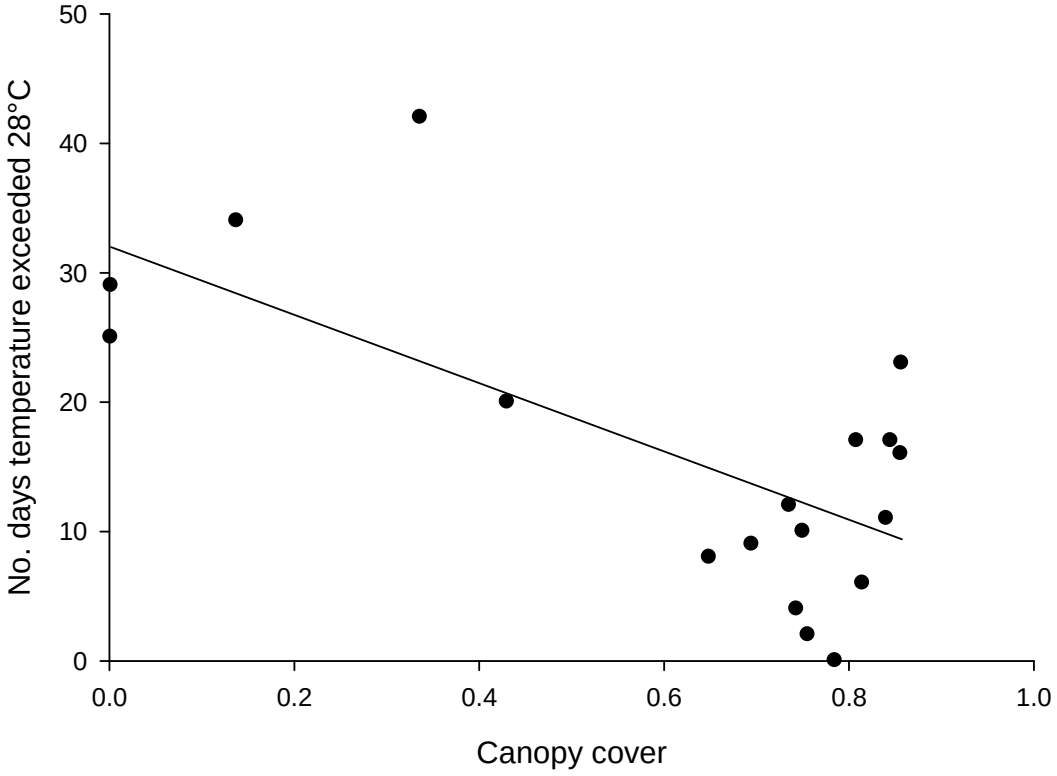

Figure B3. Number of days where air temperature was ≥28˚C between 14 May 2013 and 1 July 2013 compared to canopy.

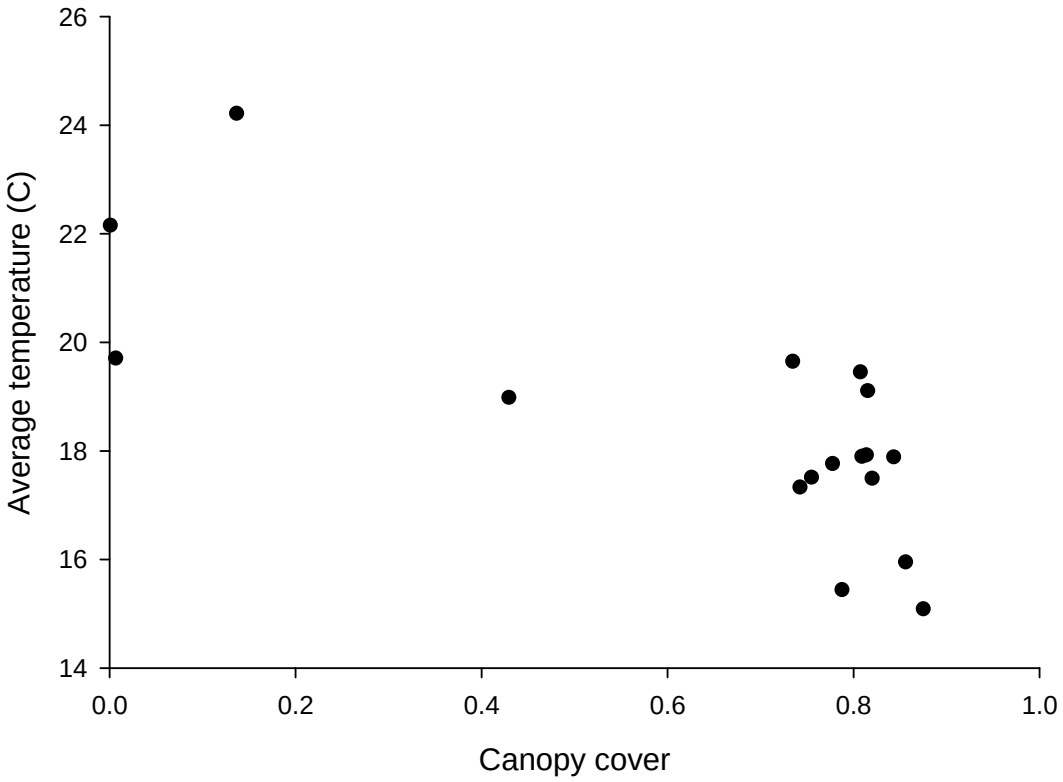

Figure B4. Effect of canopy cover on average water temperatures between 18 May 2013 and 19 June 2013.

APPENDIX C: SUPPLEMENTAL MATERIAL FOR MODEL PERFORMANCE OF CONNECTIVITY COVARIATES

Table C1. 2012 common species connectivity measures assessed with top models

Model	$\triangle AIC_c$	w_i	K	-2LogLikelihood
Psi(.), *p*(Lca)	0.00	0.1747	3	363.21
Psi(.), *p*(Lca, Day)	0.81	0.1165	4	361.37
Psi(.), *p*(Lca, JDepth)	1.80	0.0710	4	362.36
Psi(.), *p*(Lca, DNear)	1.81	0.0707	4	362.37
Psi(.), *p*(Lca,1DPrec)	2.09	0.0614	4	362.65
Psi(.), *p*(Lca, Canopy)	2.11	0.0608	4	362.67
Psi(.), *p*(Lca, Ci)	2.38	0.0532	4	362.94
Psi(.), *p*(Lca, DPos)	2.42	0.0521	4	362.98
Psi(.), *p*(Lca, Area)	2.61	0.0474	4	363.17
Psi(.), *p*(Day, Lca, DNear)	3.58	0.0292	5	361.28
Psi(.), *p*(Day, Lca, DPos)	3.64	0.0283	5	361.34
Psi(.), *p*(Day, Lca, Ci)	3.67	0.0279	5	361.37
Psi(.), *p*(JDepth, Lca, DNear)	4.01	0.0235	5	361.71
Psi(.), *p*(Canopy, Lca, Ci)	4.23	0.0211	5	361.93
Psi(.), *p*(Canopy, Lca, DNear)	4.25	0.0209	5	361.95
Psi(.), *p*(JDepth, Lca, Ci)	4.28	0.0206	5	361.98
Psi(.), *p*(1DPrec,Lca,DNear)	4.35	0.0198	5	362.05
Psi(.), *p*(JDepth, Lca, DPos)	4.49	0.0185	5	362.19
Psi(.), *p*(Area, Lca, DNear)	4.66	0.0170	5	362.36
Psi(.), *p*(Canopy, Lca, DPos)	4.79	0.0159	5	362.49
Psi(.), *p*(1DPrec,Lca,DPos)	4.86	0.0154	5	362.56

Table C1. (cont.)

Psi(.), *p*(1DPrec,Lca, Ci)	4.89	0.0152	5	362.59
Psi(.), *p*(Area, Lca, Ci)	6.66	0.0063	5	364.36
Psi(.), *p*(.)	49.44	0.0000	2	415.11

Table C2. 2013 common species connectivity measures assessed with top models

Model	$\triangle AIC_c$	w_i	K	-2LogLikelihood
Psi(.), *p*(30DPrec, Canopy, DNear)	0.00	0.8118	5	632.67
Psi(.), *p*(30DPrec, Canopy, DPos)	3.36	0.1513	5	636.03
Psi(.), *p*(30DPrec, Canopy)	6.74	0.0279	4	642.27
Psi(.), *p*(30DPrec, Canopy, Ci)	9.02	0.0089	5	641.69
Psi(.), *p*(.)	75.33	0.0000	2	715.97

Table C3. 2012 Spring Peeper connectivity measures assessed with top models

Model	$\triangle AIC_c$	w_i	K	-2LogLikelihood
Psi(.), *p*(Canopy, JDepth)	0	0.0878	4	154.13
Psi(.), *p*(Canopy, Area, Ci)	0.31	0.0752	5	151.04
Psi(.), *p*(Canopy, Area)	0.72	0.0612	4	154.85
Psi(.), *p*(Canopy)	0.75	0.0603	3	157.9
Psi(.), *p*(Canopy, 1DPrec)	0.77	0.0597	4	154.9
Psi(.), *p*(Canopy, Area, DPos)	0.89	0.0562	5	151.62
Psi(.), *p*(Canopy, JDepth, DPos)	1.1	0.0506	5	151.83
Psi(.), *p*(Canopy, Area, DNear)	1.32	0.0454	5	152.05
Psi(.), *p*(Canopy, DPos)	1.85	0.0348	4	155.98
Psi(.), *p*(Canopy, JDepth, DNear)	2.1	0.0307	5	152.83
Psi(.), *p*(Canopy, DNear)	2.34	0.0272	4	156.47
Psi(.), *p*(.)	2.36	0.027	2	162.21
Psi(.), *p*(1DPrec)	2.74	0.0223	3	159.89
Psi(.), *p*(DPos)	2.78	0.0219	3	159.93
Psi(.), *p*(JDepth)	3.14	0.0183	3	160.29
Psi(.), *p*(Canopy, 1DPrec, DPos)	3.18	0.0179	5	153.91
Psi(.), *p*(DNear)	3.36	0.0164	3	160.51
Psi(.), *p*(Canopy, SVL)	3.37	0.0163	4	157.5
Psi(.), *p*(Canopy, 1DPrec, DNear)	3.5	0.0153	5	154.23
Psi(.), *p*(Canopy, Day)	3.59	0.0146	4	157.72
Psi(.), *p*(Canopy, 30DPrec)	3.69	0.0139	4	157.82
Psi(.), *p*(1DPrec, 30DPrec)	4.04	0.0116	4	158.17
Psi(.), *p*(JDepth, DPos)	4.14	0.0111	4	158.27

Table C3. (cont.)

Psi(.), *p*(30DPrec, DPos)	4.17	0.0109	4	158.3
Psi(.), *p*(30DPrec)	4.19	0.0108	3	161.34
Psi(.), *p*(1DPrec, DPos)	4.41	0.0097	4	158.54
Psi(.), *p*(Canopy, SVL, DPos)	4.59	0.0088	5	155.32
Psi(.), *p*(30DPrec, DNear)	4.78	0.008	4	158.91
Psi(.), *p*(1DPrec, DNear)	4.81	0.0079	4	158.94
Psi(.), *p*(1DPrec, JDepth)	4.83	0.0078	4	158.96
Psi(.), *p*(Area)	4.87	0.0077	3	162.02
Psi(.), *p*(Canopy, 30DPrec, DPos)	4.89	0.0076	5	155.62
Psi(.), *p*(SVL	4.94	0.0074	3	162.09
Psi(.), *p*(JDepth, DNear)	4.99	0.0072	4	159.12
Psi(.), *p*(Day)	5	0.0072	3	162.15
Psi(.), *p*(Canopy, SVL, DNear)	5.09	0.0069	5	155.82
Psi(.), *p*(Canopy, Day, DPos)	5.24	0.0064	5	155.97
Psi(.), *p*(1DPrec, Day)	5.28	0.0063	4	159.41
Psi(.), *p*(1DPrec, Area)	5.37	0.006	4	159.5
Psi(.), *p*(JDepth, 30DPrec)	5.37	0.006	4	159.5
Psi(.), *p*(1DPrec, 30DPrec, DPos)	5.4	0.0059	5	156.13
Psi(.), *p*(Area, DPos)	5.5	0.0056	4	159.63
Psi(.), *p*(Day, DPos)	5.53	0.0055	4	159.66
Psi(.), *p*(SVL, DPos)	5.58	0.0054	4	159.71
Psi(.), *p*(Canopy, Day, DNear)	5.73	0.005	5	156.46
Psi(.), *p*(1DPrec, 30DPrec, DNear)	5.82	0.0048	5	156.55
Psi(.), *p*(JDepth, 30DPrec, DPos)	5.83	0.0048	5	156.56

Table C3. (cont.)

Psi(.), *p*(Day, DNear)	6.1	0.0042	4	160.23
Psi(.), *p*(Area, DNear)	6.1	0.0042	4	160.23
Psi(.), *p*(SVL, DNear)	6.16	0.004	4	160.29
Psi(.), *p*(JDepth, 30DPrec, DNear)	6.71	0.0031	5	157.44
Psi(.), *p*(1DPrec, JDepth, DPos)	6.87	0.0028	5	157.6
Psi(.), *p*(1DPrec, Day, DPos)	7.1	0.025	5	157.83
Psi(.), *p*(1DPrec, Area, DPos)	7.4	0.0022	5	158.13
Psi(.), *p*(1DPrec, Day, DNear)	7.46	0.0021	5	158.19
Psi(.), *p*(1DPrec, JDepth, DNear)	7.46	0.0021	5	158.19
Psi(.), *p*(1DPrec, Area, DNear)	7.8	0.0018	5	158.53

Table C4. 2013 Spring Peeper connectivity measures assessed with top models

Model	ΔAIC_c	w_i	*K*	-2LogLikelihood
Psi(.), *p*(Day, JDepth)	0.00	0.4246	4	232.57
Psi(.), *p*(Day, JDepth, Ci)	1.74	0.1779	5	229.25
Psi(.), *p*(Day, 1DPrec)	3.02	0.0938	4	235.59
Psi(.), *p*(Day, JDepth, DPos)	3.22	0.0849	5	230.73
Psi(.), *p*(Day, Canopy)	3.42	0.0768	4	235.99
Psi(.), *p*(Day, JDepth, DNear)	3.45	0.0756	5	230.96
Psi(.), *p*(Canopy, Day, Ci)	5.74	0.0241	5	233.25
Psi(.), *p*(Day, 1DPrec, DNear)	7.17	0.0118	5	234.68
Psi(.), *p*(Day, 1DPrec, Ci)	7.53	0.0098	5	235.04
Psi(.), *p*(Day, 1DPrec, DPos)	7.81	0.0086	5	235.32
Psi(.), *p*(Canopy, Day, DPos)	8.47	0.0061	5	235.98
Psi(.), *p*(Canopy, Day, DNear)	8.48	0.0061	5	235.99
Psi(.), *p*(.)	41.60	0.0000	2	281.52