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Research

Landscape epidemiology of *Batrachochytrium dendrobatidis* in central California

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Amphibian chytridiomycosis (caused by *Batrachochytrium dendrobatis*; *Bd*) was first identified in 1998 and has since been implicated in numerous amphibian declines worldwide. Most researchers have since investigated broad-scale geographic and taxonomic occurrences of the pathogen in tropical lotic or cool montane systems. In this study, we analyzed how environmental factors, land use practices, and landscape structure may affect the dynamics of the pathogen's distribution in a landscape dominated by lentic systems within a region of Mediterranean climate. We quantified the occurrence of *Bd* testing the six resident amphibian species that occur in 54 isolated perennial and ephemeral ponds in central California between May and June annually from 2004 to 2007. The geographic distribution of *Bd* within the landscape varied markedly between years. Inter-annual variation in climate affected the pond landscape structure indicating that climate conditions indirectly influence the distribution of the pathogen. Fourteen ponds, 12 perennial and 2 ephemeral, were positive for *Bd* $\geq$3 yr of the study and were treated as *Bd* hotpots for comparative purposes. Occurrences of *Bd* within the landscape were spatially autocorrelated and ponds within $\sim$1000–1500 m of *Bd* hotspots were more likely to test positive. Local land use, (presence/absence of grazing or recreational activity and developed lands), did not influence *Bd* status of a pond, indicating that the most likely means of *Bd* transmission between ponds may be waterfowl and/or amphibians.

Nearly a dozen years have passed since the identification of amphibian chytridiomycosis, a disease associated with numerous amphibian declines worldwide (Berger et al. 1998). The discovery of the etiological agent, *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al. 1999), and subsequent investigations of the epizootiology of the pathogen have changed how scientists perceive the role of disease in amphibian populations. As recently as the 1970s, many researchers assumed that parasites and pathogens either had little impact on wildlife host populations (Hudson et al. 2004, Wobeser 2006a) or that epizootic events were not inherent to ecosystems but were merely stochastic disturbances (Keesing et al. 2008). The idea that pathogens and parasites are inconsequential (Begon 2008) has segued into a new understanding of the potential for parasites and pathogens to not only regulate host populations (McCallum and Dobson 1995, Wobeser 2006b) but to affect ecosystem structure and function as well (Ranvestel et al. 2004, Whiles et al. 2006, Eviner and Likens 2008).

Numerous studies have been conducted since 1998 as scientists have attempted to elucidate the ecology of *Bd* and epizootiology of amphibian chytridiomycosis. *Bd* and amphibian chytridiomycosis have largely been studied in tropical lotic systems (Berger et al. 1998, Lips 1999, Ron and Merino 2000, Lips et al. 2005, Woodhams and Alford 2005, Kriger and Hero 2007, Rowley and Alford 2007) and cool montane systems (Rachowicz et al. 2006, Bosch et al. 2007, Muths et al. 2008). The majority of research to date has been focused on identifying the global distribution (Hopkins and Channing 2003, Cunningham et al. 2005, Garner et al. 2005, Carnaval et al. 2006) and taxonomic (Beard and O'Neill 2005, Barrionuevo and Mangione 2006, Lampo et al. 2006, Obendorf and Dalton 2006) occurrences of *Bd*; or investigating the physiological limitations (Johnson and Speare 2003, 2005) and life history (Berger et al. 2005, Mitchell et al. 2007, Morgan et al. 2007) of *Bd*, as herpetologists have sought to obtain baseline information on the fungal pathogen.

Some studies have investigated the distribution of *Bd* across continents, countries, states and/or regions (Bradley et al. 2002, Ouellet et al. 2005, Carnaval et al. 2006, Longcore et al. 2007, Muths et al. 2008), however, we are unaware of any published studies focused on *Bd* epidemiology at the landscape level, defined as "intermediate between an organism's home range and its regional distribution" (Dunning et al. 1992). Landscape epidemiology as a discipline has its origins in the work of Pavlovsky (1966) who articulated three basic observations: diseases tend to be geographically limited; spatial variation in disease distribution is related to the variation in biotic and abiotic resources supporting a pathogen, its vectors, and reservoirs; and disease risks can be predicted if those factors can be mapped (Pavlovsky 1966, Ostfeld et al. 2005).

Pathogen transmission and persistence in a host population are functions of contact rates between susceptible and infectious individuals, which in turn are influenced by the landscape structure in which they occur (Ostfeld et al. 2005, McCallum 2008). Transmission of pathogens necessarily requires close proximity of infectious and susceptible individuals and therefore is an inherently spatial process. For example, Estrada-Peña (2005) found that abundance of *Ixodes ricinus*, a vector of Lyme disease, increases with increasing degrees of connectivity between suitable habitat patches; and Allan et al. (2003) observed an inverse relationship between abundance and proportion of infected ticks (*Ixodes scapularis*) and forest patch size. Landscape configuration (i.e. fragmentation and patch size) appears to be a key factor in pathogen transmission and disease risk (Allan et al. 2003) and thus, factors affecting suitability of habitats, patch size, and degrees of connectivity between susceptible and infectious hosts at the landscape level necessarily influence disease dynamics (Dunning et al. 1992, Ostfeld et al. 2005, Keesing et al. 2008, McCallum 2008). In this study, we examined the spatio-temporal occurrence of *Bd* in a central California landscape to investigate how landscape structure and land use practices affect disease dynamics.

Methods

Study site

Our study site consisted of two contiguous parcels in central California (Fig. 1) that total ~6475 ha: Joseph D. Grant County Park, Santa Clara County, California (37°20.122″N, 121°42.102″W), and Blue Oak Ranch Reserve, Santa Clara County, California (37°22.567″N, 121°44.567″W). The southern boundary of Joseph D. Grant County Park (Grant County Park) is also the northern boundary of Blue Oak Ranch Reserve (Blue Oak Ranch) which was under private ownership until 2007 when it was donated to the Univ. of California, Berkeley. The study area is characterized by a Mediterranean climate with wet, temperate winters and hot, dry summers. Average annual precipitation is ~30.27 cm and is largely restricted to the period November–May. Mean monthly temperatures range from 2.6°C to 9.1°C in winter and 17.3°C to 25.9°C in summer. Elevations range between 454 and 994 m and both Grant County Park and Blue Oak Ranch are characterized by steep canyons, oak woodlands, and oak savannahs.

Lentic habitats on Blue Oak Ranch and Grant County Park consist of numerous perennial and ephemeral ponds (Fig. 1). The majority of the water bodies are man-made stock ponds historically created to collect and provide water for livestock. These ponds fill during the rainy season (November–April) via direct precipitation, and the downward overland flow of water during rainfall events, as most of the ponds are located mid- or downhill of steep canyons, valleys or swales and are ringed with man-made berms. Some of the perennial ponds are spring-fed via subterranean aquifers, but no ponds are associated with streams and thus there are no hydrologic connections between ponds. During summer the ephemeral ponds undergo extreme dessication (Keeley and Zedler 1998), drying to a hard clay-pan. During drought years, some of the perennial ponds will also dry.

Land use differs between the two sites as Grant County Park is a county park open to the public, whereas Blue Oak Ranch was a privately owned reserve until 2007. Grant County Park has equine, bovine, human, canine (i.e. domestic dogs) and vehicular traffic and all parts of the park are accessible and open to the public. Fishing, trail riding and hiking are common activities in the park. Year-round cattle grazing is the primary tool used to reduce undesirable vegetative growth; however prescribed burns are also conducted annually. Feral pigs are numerous in Grant County Park despite an active removal-trapping program and many perennial ponds contain non-native *Rana catesbeiana*, *Gambusia affinis*, *Lepomis cyanellis*, and *Micropterus salmoides*.

Blue Oak Ranch is not open to the public and has had extremely restricted human activity since 1990. During the course of this study, the sole human presence on the site was the reserve biologist and the anonymous owner. Vehicular traffic is therefore minimal, and livestock have not been present on site since before 1990. Prescribed burns are the primary management tool to reduce exotic plant species. Blue Oak Ranch has an intensive program to remove feral pigs and, at the time of this study, a single feral pig remained on the property. A fence was erected around the entire site preventing immigration of feral pigs and other large mammals onto the property. Most of the perennial ponds inhabited by non-native *R. catesbeiana* on Blue Oak Ranch have been drained and dried in recent years to eliminate introduced *R. catesbeiana*, *G. affinis*, *L. cyanellis*, and *M. salmoides* (J. Wilcox pers. comm.) and currently, a single pond remains containing these invasive species.

The amphibian community within lentic habitats differs slightly between Blue Oak Ranch and Grant County Park. Grant County Park hosts *R. draytonii*, *R. catesbeiana*, *Bufo boreas halophilus*, *Taricha torosa torosa*, *Ambystoma californiense*, and *Pseudacris regilla*. In contrast, *R. draytonii* and *T. torosa torosa* were absent on Blue Oak Ranch from 2003 to 2006. *Taricha torosa torosa* were first detected on Blue Oak Ranch in 2007 and *R. draytonii* were first detected in 2008 (Padgett-Flohr unpubl.). *Rana boylii* occurs in Smith Creek, the sole lotic habitat that also traces the eastern boundary of both sites, but lotic habitats were not a part of this study.

Of the six species that occur across the two sites, only *R. draytonii* and *R. catesbeiana* routinely overwinter, typically metamorphosing in the second summer or fall post-hatching (Wright and Wright 1949, Fellers and Wood 2004). Both species are highly aquatic and inhabit only perennial ponds. *Bufo boreas halophilus* can inhabit either perennial or ephemeral ponds, but has a quick developmental period generally metamorphosing 30–45 d post-hatching (Wright and Wright 1949). *Ambystoma californiense* as been documented to overwinter (Alvarez 2004) in perennial ponds, but this occurs rarely as this vernal pool endemic species historically bred in pools that annually dry up (Trenham et al. 2000). *Ambystoma*

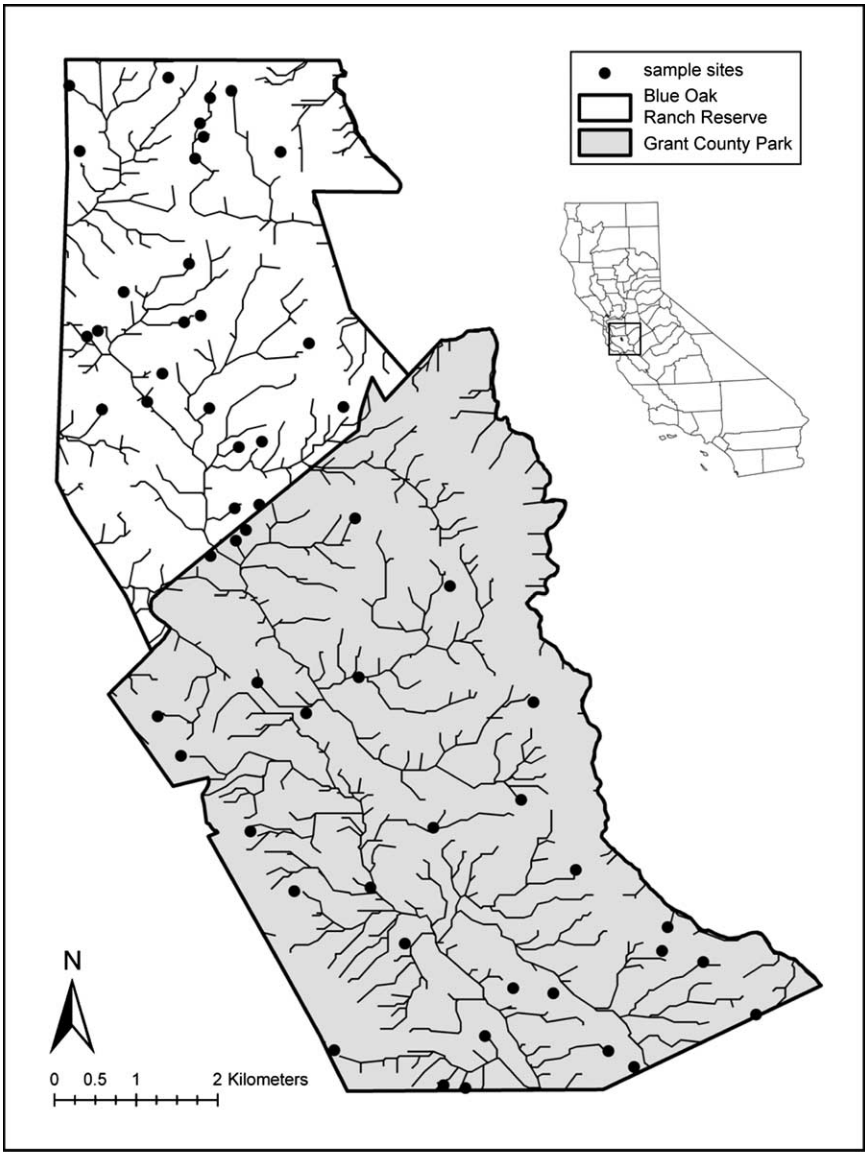

Figure 1. Location of contiguous study sites in California and ponds present on Blue Oak Ranch Reserve (white background) and Joseph D. Grant County Park (gray background). Dendritic networks show the pattern of drainage in the canyons that occasionally form intermittent streams during extreme rainfall events. The dendritic network is included on the graphic to aid in visualizing the extreme topography of the study sites. The appearance of a pond in an intermittent stream drainage, is an artifact of the scale of the graphic; all ponds are isolated and there are no connections to any above-ground water sources.

californiense larvae metamorphose as the ponds dry, which usually occurs starting in May but can occur as late as August in very wet years. *Pseudacris regilla* and *T. torosa torosa* breed in either perennial or ephemeral ponds, typically metamorphosing within 60–74 d (Nussbaum et al. 1983) and 75–92 d (Kaplan 1985) respectively; however rare occurrences of *T. torosa torosa* larvae overwintering in perennial ponds has been documented (Storer 1925).

Sample collection

We sampled all amphibian species present in all extant ponds (i.e. those holding water) on Blue Oak Ranch and Grant County Park annually from 2004 to 2007. We visited 54 ponds each year, but not all ponds had water at the time of sampling, which generally occurred within the last two weeks of May and first two weeks of June in any given year. *Bd* infects only the keratinized mouthparts of anuran larvae and swabbing of mouthparts is minimally successful (Hyatt et al. 2007), therefore sacrificing of anuran specimens is necessary to test for *Bd* infection. Up to 30 larvae (per species) of *R. draytonii*, *R. catesbeiana*, *B. boreas halophilus*, *T. torosa torosa*, and *P. regilla* were collected from each pond each year, using hand-held dip nets or two-pole seines. Labial toothrow formulae were used to identify larvae to species (Stebbins 1985). Larvae were euthanized in MS-222, preserved in 70% ethanol and stored in individual containers.

Ambystoma californiense is a federally protected species and larvae of this caudate species can be infected throughout the epidermis (Padgett-Flohr and Longcore 2005); thus we did not have to sacrifice salamander larvae for *Bd* testing. We obtained swab samples from up to 30 *A. californiense* larvae per pond by rubbing a cotton swab across the ventral surfaces of the abdomen and feet at least 25 times. Swabs were stored in individual vials and preserved in 70% ethanol. Strict decontamination protocols were followed for all collection procedures (<www.ccadc.us/docs/DeconForProfessionals.pdf>) and all personnel and equipment were decontaminated prior to arriving at a new pond.

Assay for infection status

We amputated the entire oral disc intact from each anuran larva, including the underlying soft tissue, and then divided the oral disc into left and right halves. We flame-sterilized all instruments between each procedure. We preserved the left halves in 70% ethanol in individually numbered vials and cross-referenced them to the right halves which were batched in 70% ethanol by species for each pond and year. We removed one hind foot from each *T. torosa torosa* larva and batched each by pond and year. Batched samples of amphibian tissues and batched swab samples from *A. californiense* preserved in 70% ethanol were tested for *Bd* infections by PCR. Swabs were batched in groups of five swabs per vial (Hyatt et al. 2007), and tissues were batched in vials with 5 ml of tissue per vial (J. Wood pers. comm.).

Batched samples were homogenized and centrifuged to coagulate DNA and the pelleted DNA was subjected to PCR assay. PCR was carried out at Pisces Molecular Laboratory, Boulder, Colorado, according to the procedure outlined in Annis et al. (2004) with the following modifications: PCR cycles were increased from 35 to 45, annealing temperature was increased from 60°F to 65°F and Mg^{2+} concentration was also increased from 1.5 mM to 3.5 mM (J. Wood pers. comm.). Each PCR run included controls of positive *Bd* DNA, no DNA (amplification reaction mix with distilled water added instead of a DNA sample), and contamination detection (sterile water). The *Bd* PCR assay test is highly specific for the *Bd* ribosomal RNA Internal Transcribed Spacer and the test is very sensitive as it will detect the presence of <10 *Bd* zoospores in a 2 μl sample (Annis et al. 2004).

Climate data

We obtained daily weather data for November 2003–October 2007 via the National Oceanic and Atmospheric Association measured and recorded at the Mt. Hamilton weather station (37°20′49″N, 121°37′48″W, elevation 1282 m) that is ~6 km from Grant County Park and Blue Oak Ranch. From these data we calculated monthly means for maximum and minimum temperature and precipitation (cm) for each of the four years of our study.

Data analysis

We conducted repeated measures logistic regression analysis using Generalized Estimating Equations (GEE), to test whether *Bd* status of a pond was independent of year, site (Grant Count Park versus Blue Oak Ranch), pond type (ephemeral versus perennial), or species. All analyses were conducted using SAS 9.1 (SAS Inst., Cary, NC, USA).

We geolocated each pond using a global positioning system (±10 m) and mapped all ponds using ArcGIS 9.3 (Fig. 1). We created maps showing the *Bd* status of each pond and year to assess spatio-temporal patterns of *Bd* occurrence. To investigate whether the spatial configuration of ponds influenced the dynamics of *Bd* occurrences, we calculated four nearest-neighbor distances for each pond: 1) distance to nearest *Bd* hotspot, 2) distance to nearest *Bd*-positive pond, and 3) distance to nearest *Bd*-positive perennial pond. For comparative purposes, we defined *Bd* hotspots as ponds that tested *Bd*-positive ≥3 yr. We compared each distance measurement (1–4 above) between ephemeral and perennial ponds. Distance measurements showing some associative pattern with *Bd* occurrences were further investigated for statistical significance with matrix randomization. *Bd* statuses of ponds were compared using a similarity matrix coded as follows: $-1=\text{pond}_x$ *Bd*-positive and pond_y *Bd*-negative, $0=$same *Bd* status in each pond and, $1=\text{pond}_x$ *Bd*-negative and pond_y *Bd*-positive.

Results

Statistical analysis

We sampled an average of 50 ponds (Table 1) and 2478 larval amphibians (Supplementary material Table S1) per year, and found that *Bd* regularly occurs at both Blue Oak Ranch and Grant County Park, although the total number of ponds infected varied by year, indicating that climatic variables indirectly influence the distribution of *Bd* (Table 2). Combining years, we collected an average of 55 larval samples (range = 6–130 larvae) per *Bd*-positive pond and 45.3 larval samples for *Bd*-negative ponds (range = 2–159 larvae).

Bd distribution did not differ between sites or pond types (Table 2). Species composition differed among ponds, with two ponds on Grant County Park hosting all six species and we detected a mean of 1.88 ± 0.56 species per pond at Blue Oak Ranch and 2.71 ± 1.11 species per pond

Table 1. *Batrachochytrium dendrobatidis* (*Bd*) sampling effort and results by pond type (ephemeral versus perennial) at Blue Oak Ranch Reserve (BOR) and Joseph D. Grant County Park (GCP), Santa Clara County, California, 2004–2007.

Ponds sampled	2004	2005	2006	2007
BOR ephemeral	9	9	9	2
No. BOR ephemeral *Bd* positive	5	4	3	0
% BOR ephemeral *Bd* positive	55.5	44.4	33.3	0
BOR perennial	14	13	15	15
No. BOR perennial *Bd* positive	11	12	5	3
% BOR perennial *Bd* positive	78.6	92.3	33.3	20
GCP ephemeral	12	11	12	3
No. GCP ephemeral *Bd* positive	6	6	4	0
% GCP ephemeral *Bd* positive	50.0	54.6	33.3	0
GCP perennial	19	19	18	18
No. GCP perennial *Bd* positive	11	14	5	7
% GCP perennial *Bd* positive	57.9	73.7	27.8	38.9
No. total ponds *Bd* positive	33	36	17	10
% total ponds *Bd* positive	61.1	69.2	31.5	26.3

Table 2. Results of the multivariable analysis using Generalized Estimating Equations to investigate potential associations between *Batrachochytrium dendrobatidis* presence and year, site (Blue Oak Ranch Reserve versus Joseph D. Grant County Park), pond type (ephemeral versus perennial), and species.

Parameter	DF	Estimate	95% confidence limit (lower)	95% confidence limit (upper)	Chi-square	p-value
2004	1	2.17	1.06	3.28	14.57	0.0001
2005	1	2.70	1.50	3.85	21.17	<0.0001
2006	1	0.76	−0.35	1.86	1.81	0.18
Ephemeral	1	−0.40	−1.25	0.45	0.83	0.36
Blue Oak Ranch Reserve	1	0.60	−0.34	1.54	1.57	0.21
Ambystoma californiense	1	0.04	−0.75	0.83	0.01	0.92
Pseudacris regilla	1	1.17	−0.13	2.48	3.09	0.078
Rana catesbeiana	1	2.02	0.80	3.23	10.54	0.001
Rana draytonii	1	−1.1569	−2.17	−0.14	−2.23	0.026
Taricha torosa	1	0.81	−0.20	1.81	1.58	0.11
Bufo boreas	1	0.05	−0.82	0.92	0.11	0.91

at Grant County Park in any given year. At least one individual from all six species tested positive for *Bd* during the course of our study (Supplementary material Table S2), however we never found >3 species positive in a single pond in any year (Table 3). GEE analysis showed that *R. catesbeiana* was infected when the species occurred, whereas *R. draytonii* was uninfected and found to be associated with uninfected ponds (Table 2). Both species had low occurrences relative to the other four species, as *R. catesbeiana* inhabited between 6 and 10 ponds and *R. draytonii* was found in only 5–6 ponds annually. We detected a mean of 1.37 ± 0.58 species infected per pond at Blue Oak Ranch and 1.41 ± 0.69 species infected per pond at Grant County Park in any given year. *Pseudacris regilla* were the most commonly occurring and most commonly infected species in all ponds and years (Supplementary material Table S1, S2). *Pseudacris regilla* was the strongest indicator of *Bd* presence, as 81 out of 96 positive findings in the ponds from 2003 to 2007, were positive due to the presence of infected *P. regilla* (Table 3). Eight of the 96 positive findings from 2003 to 2007 were in ponds in which *P. regilla* did not occur, whereas in the remaining seven of the 96 positive findings *P. regilla* were present but negative for *Bd*.

Weather data

Visual inspection of the monthly mean maximum temperatures for each year of our study (Supplementary material Table S3) showed that temperatures reached the optimal range (17–25°C) for *Bd* growth (Piotrowski et al. 2004) only during the months of June–September. The remainder of the year (i.e. October–May), mean maximum temperatures are below the optimal growth range for *Bd* (Table 4).

Precipitation was largely restricted to November–April, peaking in December and February. In 2004–2005 unusual sustained rainfall from December 2004 through March 2005 averaged 14.67 ± 1.43 cm per month. Conversely, precipitation in 2006–2007 was significantly less than any other sample year, and in combination with warmer temperatures conditions were insufficient to fill ephemeral ponds. Particularly notable, was that some perennial ponds that historically had never dried (D. Clark and J. Wilcox pers. comm.) had actually dried prior to the field season.

Spatial analysis

Patterns of *Bd* occurrences varied markedly over the study period. *Bd* distribution expanded slightly, early in the study, with a peak in number and proportions of infected ponds in 2005 followed by a sharp decrease in 2006 and further reduction in 2007 (Fig. 2). We identified 14 of 56 ponds (25%) as *Bd* hotspots (Fig. 3). These hotspots included seven of 10 ponds that were *Bd*-positive in 2007 when *Bd* distribution was most spatially constricted.

Bd status in ephemeral ponds showed no consistent relationship with any nearest-neighbor measure. *Bd* status in perennial ponds also responded inconsistently to all nearest-neighbor measurements except that it was negatively related to distance from nearest *Bd* hotspot (Fig. 4; $r = -0.23$, $p < 0.0001$).

Discussion

Climatic conditions and the spatial proximity of ponds influenced the landscape patterns of *Bd* occurrence in our

Table 3. Comparison of the number of ponds positive for *Batrachochytrium dendrobatidis* relative to *Pseudacris regilla* by year at Blue Oak Ranch and Joseph D. Grant County Park, California, 2004–2007.

Year	No. positive ponds	1 species positive (*P. regilla*)	1 species positive (not *P. regilla*)	2 species positive (*P. regilla*)	2 species positive (not *P. regilla*)	3 species positive (*P. regilla*)	3 species positive (not *P. regilla*)
2004	33/54	19	3	6	0	5	0
2005	36/52	18	3	13	0	2	0
2006	17/52	13	3	0	0	1	0
2007	10/38	3	4	1	2	0	0

Table 4. Comparison of climate data by month for Blue Oak Ranch Reserve and Joseph D. Grant County Park for the combined study period (November 2003–October 2007).

Month	Maximum (°C) 2003–2007	Mean maximum (°C) 2003–2007	Minimum (°C) 2003–2007	Mean minimum (°C) 2003–2007	Mean precipitation (cm) 2003–2007
January	19.44	7.74	−5.56	1.91	7.56
February	21.11	9.56	−4.44	3.19	21.23
March	23.89	10.83	−5.00	4.34	9.80
April	25.56	12.86	−3.33	4.72	6.35
May	27.22	16.33	−0.56	8.56	1.63
June	31.67	21.01	1.11	12.92	0.50
July	35.00	25.20	8.33	18.28	0.02
August	31.67	23.78	7.78	17.10	0.00
September	30.00	22.58	2.22	14.73	0.38
October	26.67	15.69	0.56	8.57	4.63
November	22.22	11.64	−3.33	5.34	5.28
December	21.11	8.73	−5.00	2.65	15.02

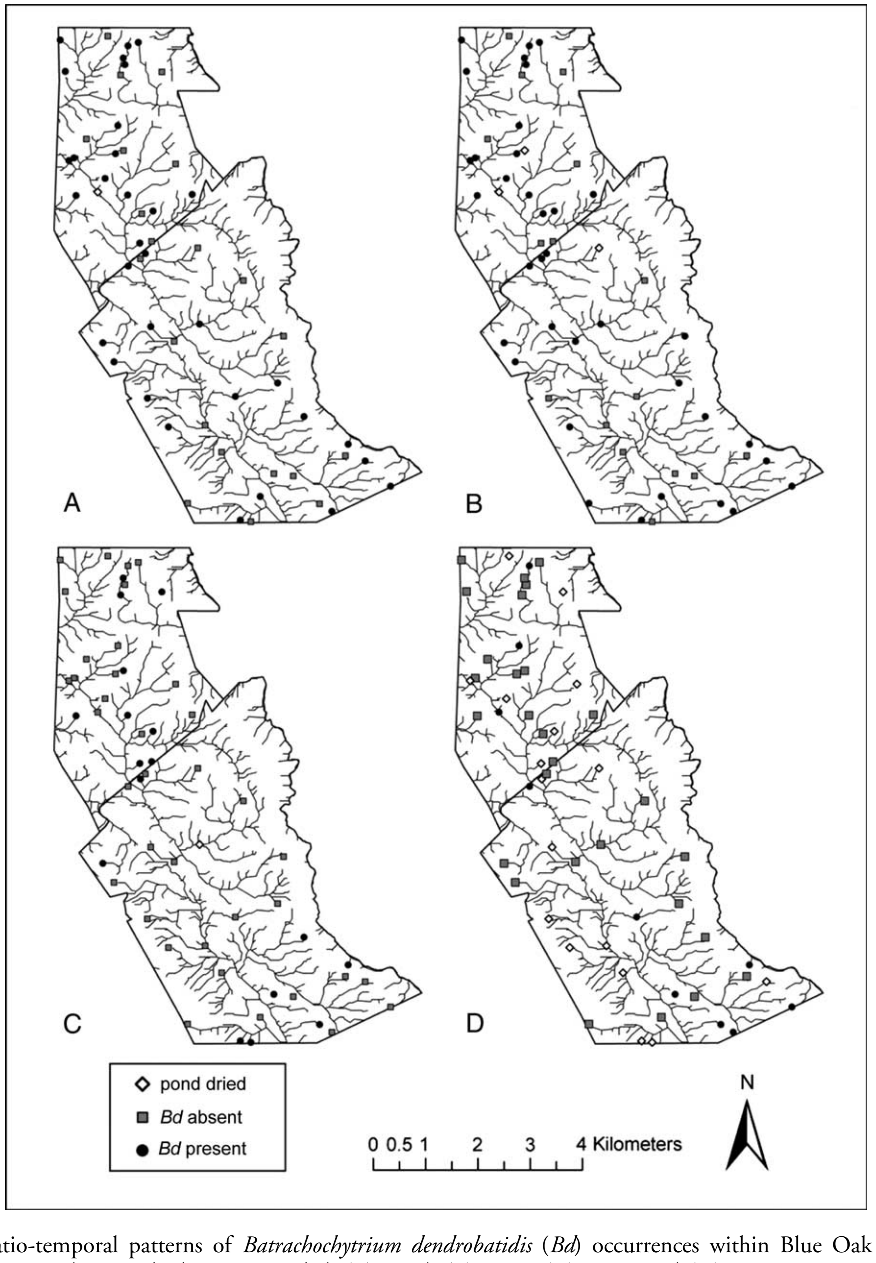

Figure 2. Annual spatio-temporal patterns of *Batrachochytrium dendrobatidis* (*Bd*) occurrences within Blue Oak Ranch Reserve and Joseph D. Grant County Park. Periods shown are coded: (A) 2004, (B) 2005, (C) 2006, and (D) 2007.

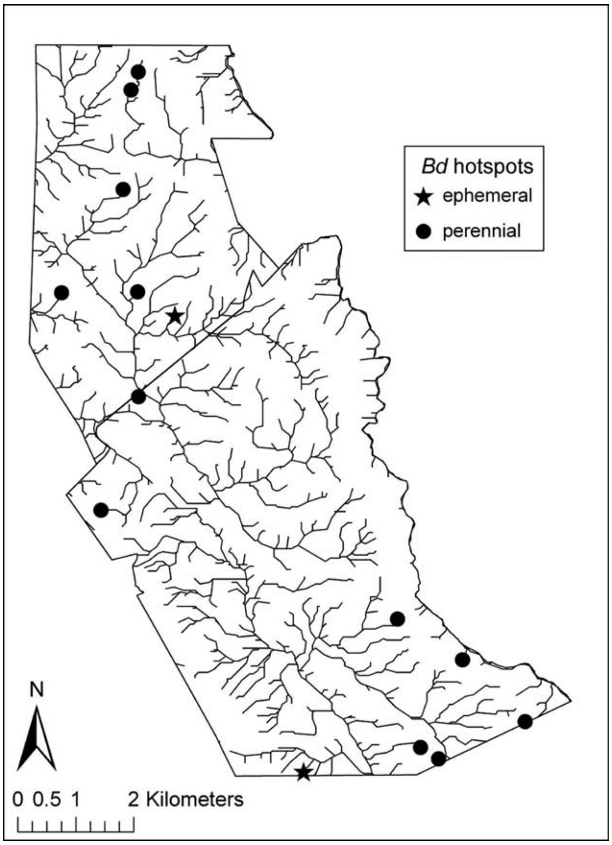

Figure 3. Location of ponds identified as *Bd* hotspots on Blue Oak Ranch Reserve and Joseph D. Grant County Park. Pond types (perennial versus ephemeral) are indicated.

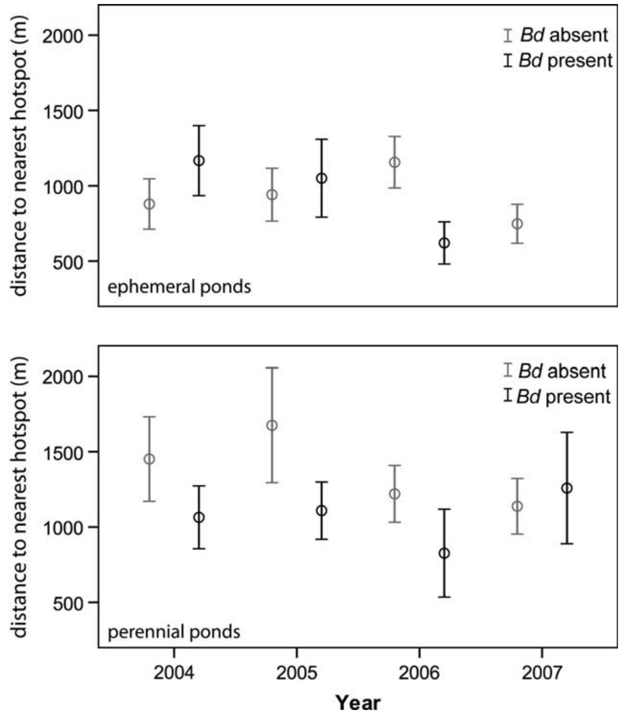

Figure 4. Distance to nearest *Bd* hotspot for each pond sampled, excluding hotspots. Observed values ± 1 SE are shown. Results are segregated by pond type, year, and *Bd* status. No ephemeral ponds were positive for *Bd* in 2007.

study area. Despite contrasting land management strategies, we detected no difference between Blue Oak Ranch and Grant County Park in occurrences of *Bd*-infected ponds. This suggests that local land use, as defined by the presence/absence of grazing or recreational activity and developed lands, does not influence *Bd* status of a pond. Our results corroborate those of St-Amour et al. (2008) who found no association between *Bd* occurrence and recreational or agricultural activity.

Although anthropogenic activities remain the most likely means of global dissemination of *Bd* (Daszak and Cunningham 2003), our study suggests that local transmission of the pathogen is independent of anthropogenic activities such as fishing, hiking and trail riding. Grant County Park is a public park with numerous recreational activities and associated use and human presence; whereas Blue Oak Ranch was a restricted reserve that had only two to five people present annually and hosted no recreational activities. Despite the extreme differences in human presence and uses, the two sites were found to have no differences in distribution of *Bd* and across the four years, the proportions of infected ponds were similar for both sites.

Kriger and Hero (2007) studying a tropical system, found a single occurrence of *Bd* in ephemeral aquatic habitat, and speculated that *Bd* grows better in streams than in ponds due to the increased shade canopy and cooler water inputs from upstream. In the Mediterranean climate, we found that *Bd* was widely present in both ephemeral and perennial ponds, although the spatial distribution varied annually. The differences in the results of these two studies provide further evidence that findings applicable to one region or climate may not be universally applicable.

Bd appears to survive well in the temperate climate observed in central California, despite our finding that *Bd* optimal growth range temperatures are only reached on average, four of the 12 months. Muths et al. (2008) suggest that *Bd* across the region of the Rocky Mountains is constrained by temperature as they found that *Bd* was more prevalent at low elevation sites which had higher mean maximum temperatures than their high elevation sites. Based on our finding that year, and thus, climate conditions, significantly affect the distribution of *Bd* within ponds, we hypothesize that in central California, precipitation may be a key factor in the successful transmission and distribution of the pathogen. The majority of the year temperatures within our study site are below the range considered optimal for *Bd* success, yet we consistently detected *Bd* in all years.

If temperature is a key factor to *Bd* success, global warming could increase the frequency and distribution of *Bd*. Currently the pathogen's distribution may be partially limited by mean minimum temperatures observed October–May as *Bd* does not grow or grows slowly below 10°C (Piotrowski et al. 2004). Conversely, global warming could decrease the distribution of *Bd* as the pathogen does not grow well above 25°C (Piotrowski et al. 2004) and increased mean maximum temperatures could also impact the timing and duration of filling and drying of ponds.

Woodhams et al. (2003) found that *Litoria chloris* cleared infections when exposed to 37°C for 16 h and

Johnson et al. (2003) showed that cultured *Bd* on agar plates is exterminated in 4 h at 37°C. Although increased temperatures can directly affect pathogen survival, maximum temperatures further restrict the spatial distribution of the pathogen as the hosts that can carry *Bd* (i.e. amphibians) move to those ponds that continue to hold water when ephemeral ponds desiccate in the heat of summer. In the years 2003–2006, there was sufficient rainfall to fill most of the ponds; however in 2007, rainfall was insufficient and approximately one-fourth of the ponds did not fill. Further, higher temperatures combined with lack of rainfall in that year also resulted in desiccation of some perennial ponds; thus the spatial distribution of *Bd* was markedly constricted as compared to previous years. Whether global warming may increase or decrease the impact of *Bd* within the landscape of our study site is a question for future research.

Ostfeld et al. (2005) suggest that spatial proximity is important in determining likelihood of disease transmission. We found that proximity to a *Bd* hotspot was correlated with the presence of *Bd* in perennial ponds, suggesting a clustering pattern of *Bd* dynamics at the landscape level. We speculate that the lack of clustering relative to ephemeral ponds is likely related to the ephemeral nature of the ponds and the restricted amphibian assemblage that inhabits and breeds in them. Ephemeral ponds can only be sampled when water and amphibians are present; thus clustering patterns in these ponds may become apparent if adequate rainfall renders the ponds full for a number of consecutive years. Alternatively, the lack of a pattern may be due to the annual sampling that occurred in May–June. Some of the ephemeral ponds are dry by May or June and thus, an earlier sampling conducted when all ponds are full, could show patterns associated with ephemeral ponds.

Summer drying of ephemeral ponds in central California is likely lethal to *Bd* as the pathogen does not survive desiccation (Johnson et al. 2003) and the soils in that region dry to a hard clay pan (Keeley and Zedler 1998); therefore detection of *Bd* in ephemeral ponds in consecutive years indicates that *Bd* was most likely re-introduced each year. Johnson and Speare (2005) speculated that *Bd* may be vectored between aquatic habitats by waterfowl, as they found that *Bd* survived on moist bird feathers for 1–3 h. Our study seems to provide some support for that hypothesis, as waterfowl could move from a *Bd* hotspot to a nearby pond, thus transporting the pathogen and rendering a clustering pattern such as we detected. However, the distance at which we detected some level of association to *Bd* hotspots was ~1000–1500 m, which is also the observed range of movement for *P. regilla* (Smith and Green 2005) and *A. californiense* (Trenham et al. 2001). Both species are resistant carriers of *Bd* (Garcia et al. 2006, Padgett-Flohr 2008), and are the two most widely distributed and abundant amphibians occurring on Blue Oak Ranch and Grant County Park. In addition, these two species were the most commonly infected of the six species that occur across the study site and we therefore hypothesize that these two species may be re-introducing *Bd* into ponds.

Padgett-Flohr (2008) demonstrated that *A. californiense* does not lose *Bd* infection and can survive infected for at least three years in the lab. Thus, infected *A. californiense* appear capable of introducing or re-introducing the pathogen to breeding ponds over several years. *Pseudacris regilla*, likewise, have wide home ranges that encompass upland foraging sites, aestivation sites, multiple breeding ponds and movement corridors between ponds (Schaub and Larsen 1978). *Pseudacris regilla* can breed multiple times in a year and thus have multiple opportunities to disperse the pathogen within and among aquatic habitats. Recent retrospective analysis of museum specimens has also shown that historic spatio-temporal patterns of *Bd* occurrences in central California are associated with the presence of infected *P. regilla* (Padgett-Flohr and Hopkins 2009). We speculate that many ephemeral ponds consistently test positive for *Bd* because the primary species that occur in them are *A. californiense* and *P. regilla*. The amphibian communities present in most of the ponds on Blue Oak Ranch, for example, were composed solely of those two species. Therefore, it seems that further studies are needed to investigate the potential role of amphibians and waterfowl in the transport of *Bd* between ponds.

Some perennial ponds that tested negative for *Bd* may actually have harbored the pathogen as pathogen detectability is based on sample size. Assuming a 100% diagnostic sensitivity, the ability to detect a pathogen at a 3% prevalence level with 95% confidence in an infinite population, requires a minimum of 59 samples (DiGiacomo and Koepsell 1986) and in our study 43 of 115 *Bd*-negative findings were based on sample sizes averaging >59 samples. The remaining 72 *Bd*-negative ponds had an average sample size of 29.35 which would allow us to detect 7.5% prevalence with 95% confidence, but does not provide reliable information regarding prevalence below that level.

In summary, we consistently detected *Bd* on our site in both ephemeral and perennial ponds and the pathogen's distribution was indirectly influenced by climatic variables. Detectable geographic patterns of *Bd* occurrences that were clustered within ~1000–1500 m of *Bd* hotspots supports the suggested importance of spatial proximity in the transmission of pathogens. Presence/absence of grazing or recreational activity and developed lands did not influence *Bd* status of a pond indicating that livestock and humans via recreational activity are not transporting *Bd* between ponds. It is likely that waterfowl and/or the amphibian hosts are the primary transport mechanisms of the pathogen between ponds and further studies are needed to investigate their potential roles.

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