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Original Contribution

Differences in Fungal Disease Dynamics in Co-occurring Terrestrial and Aquatic Amphibians

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Abstract: The fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*), has devastated biodiversity and ecosystem health and is implicated as a driver of mass amphibian extinctions. This 100-year study investigates which environmental factors contribute to *Bd* prevalence in a fully terrestrial species, and determines whether infection patterns differ between a fully terrestrial amphibian and more aquatic host species. We performed a historical survey to quantify *Bd* prevalence in 1127 *Batrachoseps gregarius* museum specimens collected from 1920 to 2000, and recent data from 16 contemporary (live-caught) *B. gregarius* populations from the southwestern slopes of the Sierra Nevada mountains in California, USA. We compared these results to *Bd* detection rates in 1395 historical and 1033 contemporary specimens from 10 species of anurans and 427 historical *Taricha* salamander specimens collected throughout the Sierra Nevada mountains. Our results indicate that *Bd* dynamics in the entirely terrestrial species, *B. gregarius*, differ from aquatic species in the same region in terms of both seasonal patterns of *Bd* abundance and in the possible timing of *Bd* epizootics.

Keywords: Chytridiomycosis, Chytrid, *Bd*, Direct-developing, Plethodontids, Salamanders

Introduction

Batrachochytrium dendrobatidis (*Bd*), a causal agent of the widespread and deadly amphibian disease chytridiomycosis, is a pathogen responsible for devastating loss of biodiversity (Skerratt et al. 2007; Fisher et al. 2009) and a likely driver of mass extinction (Wake and Vredenburg 2008). The pathogen *Bd* consists of multiple genetic lineages that vary in pathogenicity. The hypervirulent global panzootic lineage of *Bd* (*Bd*GPL) is found on every continent with amphibians (Rosenblum et al. 2013; Byrne et al. 2017) and is a major contributor to the decline or extinction of hundreds of amphibian species (Stuart et al. 2004; Skerratt et al. 2007).

With hypothesized origins in Asia, *Bd*GPL (hereafter referred to as *Bd*) is a novel pathogen across much of its current distribution (O'Hanlon et al. 2018). This pathogen was likely spread globally during the last 50 to 120 years through amphibian trade (Fisher and Garner 2007; Rosenblum et al. 2013; O'Hanlon et al. 2018; Byrne et al. 2019). Because *Bd* was identified as the cause of chytridiomycosis in 1998 (Berger et al. 1998; Longcore et al. 1999), researchers have reconstructed *Bd*'s emergence using museum specimens. Retrospective technique have helped ex-

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plain dramatic amphibian declines in Australia and Central America (Catenazzi et al. 2011; Cheng et al. 2011; Berger et al. 2016). The recent discovery in the Netherlands of a second pathogen which also causes lethal chytridiomycosis in amphibians, *Batrachochytrium salamandrivorans* (*Bsal*), underscores our need to understand the mechanisms of fungal pathogen spread in order to avoid the wider introduction of another devastating amphibian disease (Martel et al. 2013; Yap et al. 2015, 2017).

Transmission of *Bd* primarily occurs via water due to its flagellated zoospores (Berger et al. 1998; Rachowicz and Vredenburg 2004); as a result, *Bd* has been primarily studied in aquatic species, which are exposed to zoospores from both conspecifics and from re-infection (Kriger and Hero 2007; Berger et al. 2016). Though *Bd* zoospores are highly susceptible to desiccation (Piotrowski et al. 2004), they appear to survive for months in wet sand under laboratory conditions (Johnson and Speare 2005). *Bd* zoospores have also been found on environmental substrates from the forest floor to the canopy during disease outbreaks (Lips et al. 2006; McCracken et al. 2009; Kolby et al. 2015). Therefore, it is not surprising that *Bd* has been detected in a number of completely terrestrial amphibian species, including caecilians, Plethodontid salamanders, and direct-developing frogs (Cummer et al. 2005; Burrowes and Longo 2007; Weinstein 2009; Doherty-Bone et al. 2013; Gower et al. 2013; Muletz et al. 2014; Sette et al. 2015). These studies indicate that terrestrial transmission routes for *Bd* must exist, independent of aquatic-to-land vectoring driven by the emergence of metamorphosing amphibians (Kolby et al. 2015). While it is possible that infection among exclusively terrestrial amphibians occurs through contact with contaminated substrates, it is also likely to happen through skin-to-skin transmission (Rowley and Alford 2007; Kolby et al. 2015). Neither terrestrial transmission route has been experimentally demonstrated, nor few studies have explicitly studied disease dynamics of *Bd* in terrestrial hosts (Muletz et al. 2014; Kolby et al. 2015).

In this study, we use museum specimens to reconstruct historical *Bd* infection prevalence in the fully terrestrial gregarious slender salamander, *Batrachoseps gregarius* (Jockusch et al. 1998), in the inland region of California (ranging from the eastern edge of California's Central Valley to the western slope of the Sierra Nevada mountains). This species can be found in open grassland habitat in low elevations as well as high elevation coniferous forests. *B. gregarius* are fully terrestrial, direct-developing salamanders which nest communally and exhibit aggregation behaviours involving skin-to-skin contact (Jockusch et al. 1998). *B. gregarius* remains terrestrial from egg to adulthood, has extremely low vagility, and has highly gregarious nesting behaviour, all of which allows for an examination of likely terrestrial transmission of *Bd*. This species is useful for studying the historical spread of *Bd* due to its high representation in museum collections (1511 specimens listed on VertNet [http://www.vertnet.org]), and the likelihood that individuals spread the disease through direct social contact rather than via aquatic zoospores.

We performed a historical survey to quantify *Bd* infection detection in *B. gregarius* museum specimens collected from 1920 to 2000. We explored environmental factors that may contribute to historical *Bd* infection in *B. gregarius*, as well as the relevant time scales for interpreting the influence of these environmental factors. In order to determine if *Bd* infection levels have changed over time, we revisited a number of historically surveyed sites (some which revealed historical *Bd* infection) and tested these contemporary *B. gregarius* populations for *Bd*. Finally, we compared *Bd* detection rate in the fully terrestrial *B. gregarius* to historical and contemporary specimens from 10 species of aquatic anurans and three species of semi-aquatic salamanders from the Sierra Nevada mountains. Our goals were to investigate which environmental factors contribute to *Bd* infection in a fully terrestrial species, and to determine whether infection patterns differ between a fully terrestrial amphibian and host species with more aquatic lifestyles.

Methods

Historical Survey

We tested for *Bd* in all available *B. gregarius* museum specimens collected in Fresno, Madera, Mariposa, Stanislaus, and Tulare counties (California) housed permanently at two natural history museums, the California Academy of Sciences and the Museum of Vertebrate Zoology at the University of California, Berkeley (Supp. Table 1). We tested 1127 specimens available at the time (out of 1512 specimens listed across all institutions on VertNet) for *Bd*.

We collected skin swabs from museum specimens and live, field-caught salamanders using standard *Bd* skin swabbing techniques (Boyle et al. 2004; Hyatt et al. 2007; van Rooij et al. 2011): specimens were stroked 10 times each dorsally and ventrally and 5 times on each side with a

MW113 dry swab (Medical Wire and Equipment Company). Swabs were dried and stored in 1.5 ml microcentrifuge tubes at 4°C until DNA extraction (Cheng et al. 2011). Standard DNA extraction and real-time PCR quantification techniques were used: samples were run in singlicate with standard controls of 0, 0.1, 1, 10, and 100 zoospore equivalents (ZE) (Boyle et al. 2004; Hyatt et al. 2007; Cheng et al. 2011). We multiplied the qPCR genomic equivalents (GE) by the PCR dilution factor of 80 in order to quantify the number of zoospore equivalents on each swab, Z_{swab} score (Briggs et al. 2010; Vredenburg et al. 2010). Because the likelihood of false negatives when sampling in singlicate is ~ 40% (Cheng et al. 2011), we describe historical *Bd* abundance as the detection rate of positive specimens.

We obtained historical *Bd* data (1900–2009) for aquatic-breeding anuran species in the California Sierra Nevada region from Vredenburg et al. 2019, as well as additional anuran *Bd* records from that region (data repository: AmphibiaWeb's disease portal: https://amphibiandisease.org). These include 2428 specimens (1033 of which were live-caught) of *Anaxyrus boreas:* 58 (0), *Anaxyrus canorus:* 1254 (999), *Hyliola regilla:* 355 (32), *Rana boylii:* 100 (0), *Rana cascadae:* 1 (0), *Rana catesbeiana:* 48 (0), *Rana draytonii:* 1 (0), *Rana muscosa:* 177 (2), *Rana pipiens:* 4 (0), and *Rana sierrae:* 430 (0). We also obtained 427 historical records (1900-2009) from Sierran aquatic-breeding salamanders *Taricha granulosa:* 91, *T. sierrae*: 18, and *T. torosa:* 317 originally published in Chaukulkar et al. 2018. Sampling range extents of these species were defined in Quantum GIS v.3.4 by generating convex hulls using minimum bounding geometry.

Contemporary Sampling

In addition to comparing contemporary and historical *Bd* detection rate, we also set out to compare yearly variation in contemporary *Bd* detection rate by collecting skin swab samples from field sites in 2013 and 2014. In 2013, we revisited 16 sites from the historical survey. We detected *Bd*-positive salamanders at two sites, which we revisited in 2014 for more extensive field sampling. All *B. gregarius* found in the field were swabbed for *Bd* testing, measured, weighed, and released within 5 min. We wore gloves when handling salamanders, and changed gloves between individual specimens.

Statistical Analysis

We used R (R Core Team 2014) for all statistical tests. We compared the *Bd* detection rate in historical and live-collected field samples over successive time periods using a two-tailed equal proportions test. We generated credible intervals using an exact binomial test with a confidence level of 95%. The R package MASS (Venables and Ripley 2002) was used to perform a generalized linear model (GLM); this was done to find 1) which environmental factors contribute to *Bd* prevalence, and 2) what time scale is most relevant for those same environmental factors.

We obtained daily precipitation and daily temperature values for each sample month from NOAA's Daily Summaries dataset (https://www.ncdc.noaa.gov/cdo-web/) for all weather stations in Fresno, Madera, Mariposa, Stanislaus, Tulare, and nearby Kern and Tuolumne counties. We used the data from the nearest weather station to assign daily values to each collection site. We obtained elevation data for all historical specimen collection sites using USGS's National Map bulk point query tool (https://viewer.nationalmap.gov). The nearest distance from historical collection sites to freshwater was calculated in Quantum GIS using an inland hydrologic features basemap from CalAltas at CA.gov (http://atlas.ca.gov).

Before performing the GLM, we assessed the collinearity of the environmental variables using a Pearson correlation test. We tested historical results for spatial autocorrelation, a high degree of clumping in *Bd*-positive specimens, using the R package gstat (Pebesma 2004; Gräler et al. 2016). We limited the test for autocorrelation to groups of historical sites within 10 km of one another, as amphibian dispersal events beyond this distance are rare (Smith and Green 2005).

Bd count values were zero inflated; therefore, we used a negative binomial distribution (Zuur et al. 2012). To account for variable sampling effort between sites, the natural log of total sample size per site was included as a model offset. We included the following environmental variables: average daily precipitation, average maximum daily temperature, elevation, and distance from historical collection site to the nearest fresh body of water. Model variables could not contribute to the prevalence of *Bd* prior to its arrival at any particular site; therefore, all variables were modelled in interaction with the year of sample collection. A significant interaction term indicates that the effect of an environmental variable has changed over time. We elected not to include collection site as a factor in the GLM; our

models include the assumption that every collection site/year combination is an independent data point. This results in a small degree of pseudoreplication since 10 out of 157 sites were sampled in multiple years (12 of 168 total site/year combinations). Of those, 6 sites were *Bd*-negative in all sample years.

Time scale is relevant when assessing the effect of daily precipitation and maximum daily temperature, as both may have short-term and long-term effects on *Bd* dynamics. Therefore, we calculated the average values for those two variables across time intervals ranging from 1 to 12 months long prior to the month each historical specimen was collected. When performing model selection for each of these 12 time intervals for precipitation and temperature, we began with a beyond-optimal model that consisted of the interactions between year of sample collection and each of the environmental variables. We selected the best fit model for each of the 12 models using a reverse-stepwise procedure, based on the significance of model factors and a Wald test comparing AIC values (Zuur et al. 2009). McFadden's Pseudo-R^2 was used to compare models at different time scales (Hemmert et al. 2018).

Results

Historical Survey

Bd-positive *B. gregarius* museum specimens were detected at 26 out of 157 historical specimen collection sites (Fig. 1, left panel). Sites were defined as discrete GPS coordinates in the museum database; the closest two sites were 28 m apart. We found no *Bd*-positive samples from 1920 to 1971, a time period that included 346 museum specimens (Supp. Table 1). The oldest *Bd*-positive *B. gregarius* specimens were collected in 1973 at several nearby sites in northern Mariposa and Stanislaus counties, as well as one site to the south in Tulare county (Fig. 1, right panel). *Bd* detection rate was significantly higher in the 1990s compared to any other decade (Fig. 2), but was not significantly different between any two other decades (for p-values, see Supp. Table 2). Similarly, infected specimens collected during the 1990s had an average Z_{swab} score an order of magnitude larger than the other decades (1970: 1.695, 1980: 4.270, 1990: 106.5, 2000: 11.28). Ten sites were sampled for museum collections in multiple years; four of these sites had *Bd*-positive specimens, primarily in later sampling years (4-Tulare, 5-Tulare, 13-Madera & 14-Mariposa) (Supp. Table 3).

The sampling region for *B. gregarius* historical specimens, along the western slope of the southern Sierra Nevada mountains, overlapped with approximately 18% of all Sierran aquatic-breeding anuran samples. The *Taricha* sampling region along the western slope of the Sierras overlapped approximately 18% of anuran samples and 94% of *B. gregarius* samples. Both *B. gregarius* and *Taricha* sampling regions overlapped only 5% of anuran samples. The earliest *Bd*-positive from Sierra Nevada records was from a *Rana sierrae* collected in 1939 in the northern Sierra Nevada (Vredenburg et al. 2019). The first *Bd*-positives within the range of *B. gregarius* sampling were two *Taricha sierra* collected in 1960; the first anuran positive was a *Hyliola regilla* collected in 1962; the first *Bd*-positive *B. gregarius* were collected 11 years later. Between the 1970's and 2000's, we detected *Bd* in *B. gregarius* at a lower rate compared to Sierran anurans except during the 1990's (70's: $P < 0.01$, 80's: $P < 0.01$, 90's: $P = 0.11$, 00's: $P = 0.03$) (Fig. 3). Sampling effort for *B. gregraius* was higher than for anurans during the 1970's and 1980's, yet overall *Bd* abundance was significantly lower than anurans (Fig. 3); additionally 67% of *B. gregarius Bd* positives during the 1970's originated from a single site. Detection rates of *Bd* in *Taricha* were statistically the as *B. gregarius* in all decades (60's–00's: $P > 0.05$), and were lower than Sierran anurans from the 1970's–2000's (40's–60's: $P > 0.05$, 70's–90's: $P < 0.01$, 00's: $P = 0.02$).

Batrachoseps gregarius and *Taricha* specimen collection was primarily performed between December and April, while sampling of anuran species primarily occurred May–September (Fig. 4). *Bd* was most abundant in March, April, and June in *B. gregarius* and *Taricha*, and in June–August in anuran species (Fig. 4, Supp. Figure 1). The proportion of *Bd*-positive *B. gregarius* was significantly higher in the cool/wet season (November–April) than in the hot/dry season (May–October) (Fig. 5) ($\chi^2 = 10.50$, $df = 1$, $P < 0.01$); a similar pattern was found in *Taricha* ($\chi^2 = 10.50$, $df = 1$, $P < 0.01$); however *Bd* detection in anuran species did not differ significantly between seasons ($\chi^2 = 0.0010$, $df = 1$, p value = 0.97). For Sierran anurans, the proportion of *Bd*—positive specimens each month did not differ significantly between the samples from the entire Sierra Nevada range and those overlapping the range of *B. gregarius*, except during the month of August, when the specimens from the total range had significantly higher rates of *Bd* detection (overlapping range: 3/80, total range: 57/426; $P = 0.02$).

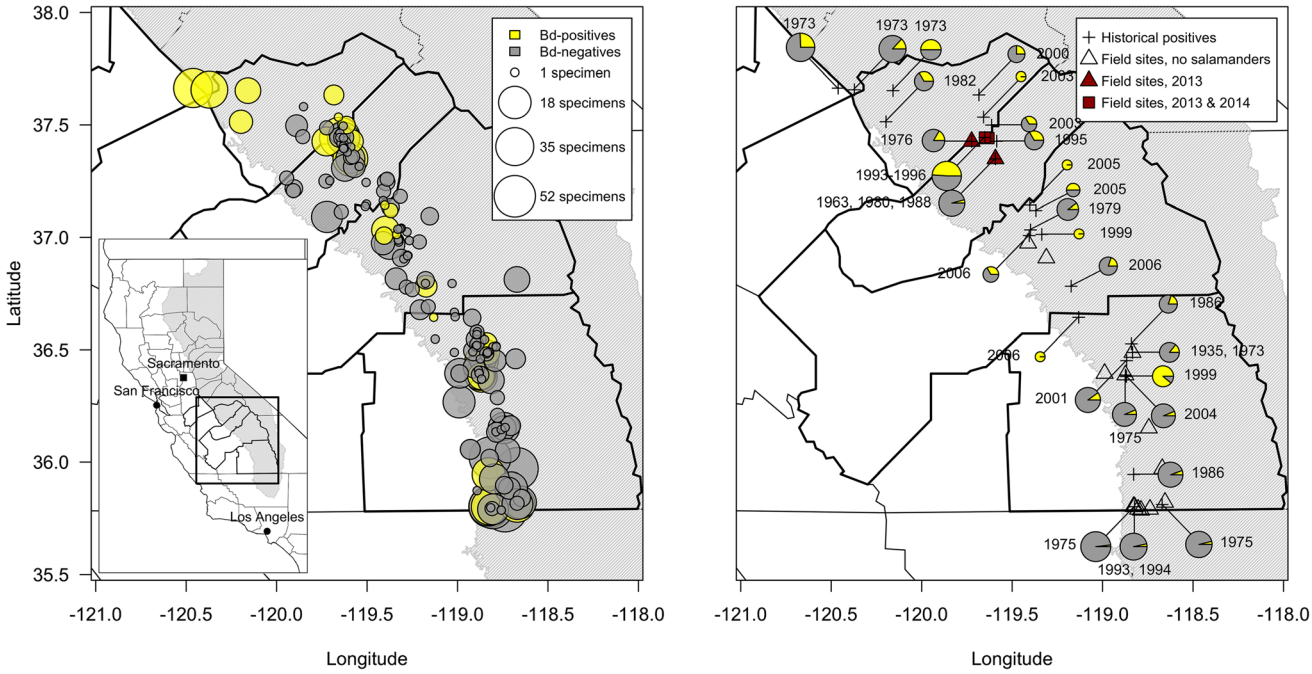

Figure 1. Left inset: Map of California showing the study area counties in bold outline and the Sierra Nevada mountains shaded grey. Left panel: *Batrachoseps gregarius* historical museum survey results. The number of samples tested for *Batrachochytrium dendrobatidis (Bd)* from each site is indicated by the size of each dot (log scale). *Bd*-negative sites are shown in grey, while *Bd*-positive sites are shown in yellow. Right panel: Pie charts indicate the proportion of *Bd*-positive *B. gregarius* specimens at each *Bd*-positive historical site (26 sites). *B. gregarius* museum localities revisited in 2013 and 2014—open triangles indicate sites where no salamanders were found during revisit, dark red triangles indicate sites visited in 2013 only, and dark red squares indicate sites visited in 2013 and 2014.

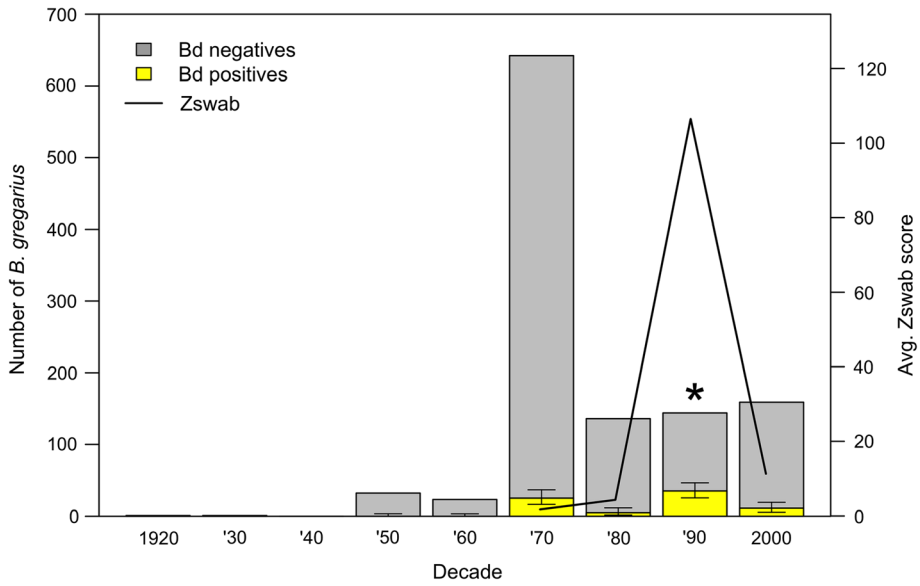

Figure 2. Proportion of *Bd*-positive *Batrachoseps gregarius* specimens and average Z_{swab} score by decade. Grey bars' height indicates sample size per decade, and yellow bars indicate proportion infected with *Bd*. The black line indicates average Zswab score per decade. Specimens collected in 1990 had a significantly higher prevalence of *Bd* than other decades (indicated with *), and higher average Z_{swab} score.

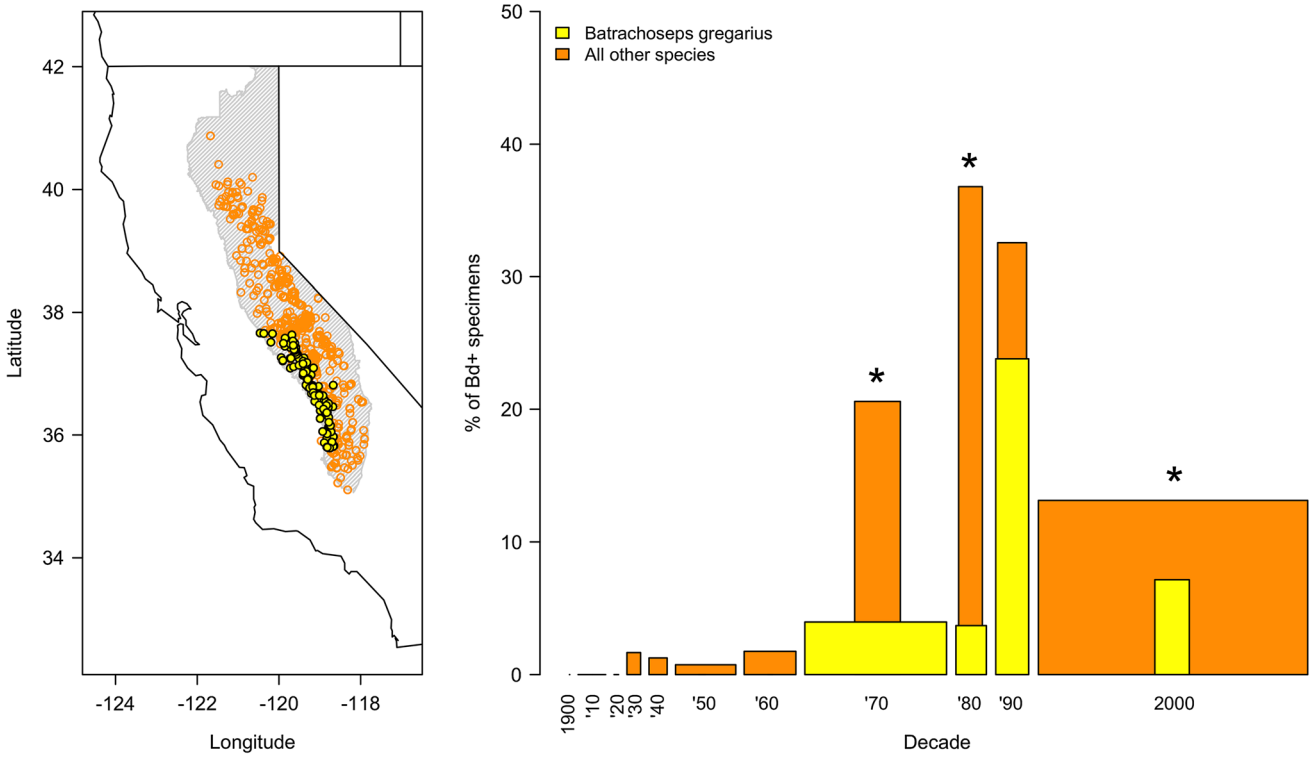

Figure 3. Left panel: Map of *Bd* sampling in the Sierra Nevada mountains including data from Vredenburg et al. 2019. Stippled grey fill shows the extent of the Sierra Nevada mountains. *Batrachoseps gregarius* specimens are shown in yellow, all anuran species are shown in orange. Right panel: Bar chart showing the proportion of *Bd*-positive specimens detected in the Sierra Nevada mountains by decade. Yellow bars show *Bd* prevalence for *B. gregarius*. Orange bars show *Bd* prevalence for all anuran species. Bar width is proportional to sample size. Stars indicate decades in which *Bd* prevalence in *B. gregarius* differs significantly from anuran Sierra Nevada host species.

The proportion of *Bd* positives was not significantly different between *Taricha* specimens collected during their aquatic or terrestrial life stages ($P = 0.59$).

Historical GLM

The average distance from each historical *B. gregarius* collection site to the nearest weather station with data available during the specimen collection year was 13.24 ± 7.16 km. Home range size for *Batrachoseps* salamanders likely varies by species, but recorded dispersal distances are typically small, ranging from 1.7 to 18.3 m (Hendrickson 1954; Cunningham 1960). Spatial autocorrelation tests show that *Bd* presence is independent across sites above 38 m apart (Supp. Figure 2); only one pair of sites in our historical samples was below this distance apart (28 m), and both of these sites tested negative for *Bd*. Therefore, the geographic location of our historical sites relative to one another is unlikely to have an effect on the historical GLM, particularly with respect to the multi-decade sampling timeframe.

The reverse-stepwise procedure for beyond-optimal models that began with environmental variables of 12-, 11-, 10-, 9-, 4-, 3-, 2-, and 1-month time intervals prior to sample date resulted in best models that contained only the interaction between elevation and year of sample collection (AIC: 203.65.17, 2 × log-lik: -189.652) (Table 1). For 8-, 7-, 6-, and 5-month time intervals, the best models contained elevation and average maximum daily temperature in interaction with the year of sample collection. However for the 8-month model, year and the intercept had P values > 0.05. We used McFadden's Pseudo-R^2 to compare these models to the fitted data (8-month R^2: 0.28, 7-month R^2: 0.30, 6-month R^2: 0.31, 5-month R^2: 0.28). We report the parameters for the model with the best fit, the 6-month model (AIC: 203.65, 2 × log-lik: − 189.652) (Table 1, Supp. Figures 2 & 3).

Contemporary Sampling

In 2013, we visited 16 sites where *B. gregarius* specimens were collected during historical surveys. We detected *Bd* in

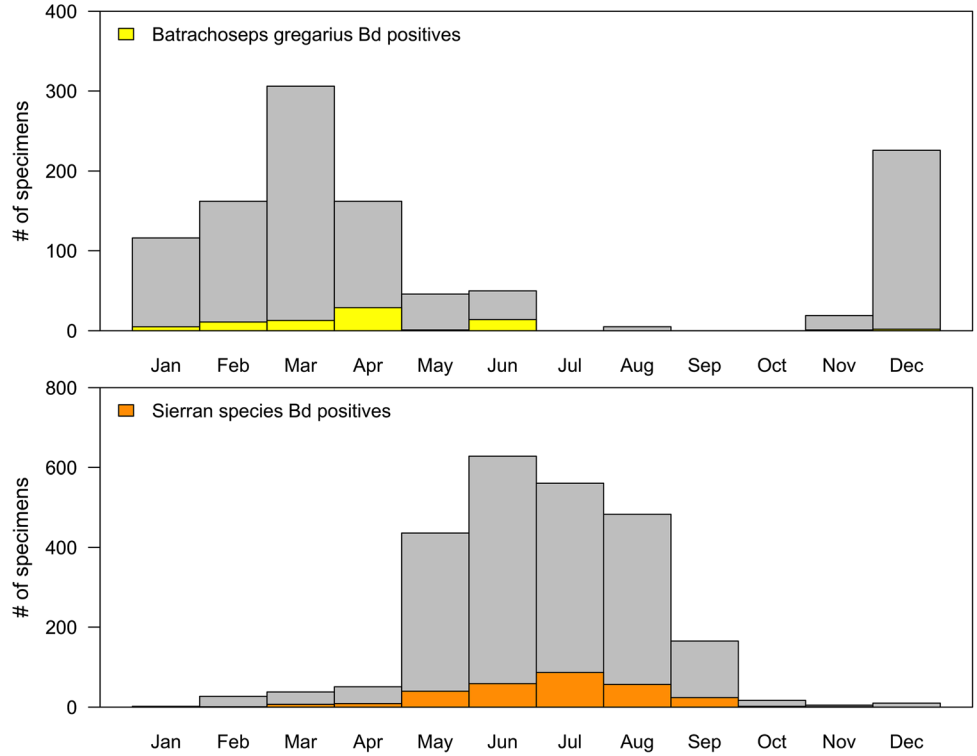

Figure 4. Historical sampling effort of *Batrachoseps gregarius* (top panel) and Sierra Nevada anuran species (bottom panel) by month. Grey bars show total sampling effort (1127 *B. gregarius*, 2423 Sierra Nevada anurans); coloured bars show the proportion of *Bd* positives per month collected.

historical specimens collected at six of those 16 sites. We found salamanders at only four of the 16 field sites (Fig. 1, right panel). Three of those four sites had historical *Bd*-positives, and three of those four sites were historically sampled in multiple years: site 0-Madera (1963: 0/11 samples; 1993: 0/6), site 8-Mariposa (1976: 2/12), site 13-Madera (1963: 0/12, 1980: 0/5, 1988: 1/7), site 14-Mariposa (1993: 0/1, 1994: 9/17, 1994: 13/26, 1996: 1/3).

We sampled salamaders for contemporary *Bd* at four sites In 2013, and detected *Bd* at two sites: 0-Madera and 14-Mariposa (Table 2). We revisited these sites in 2014 for more extensive sampling. In 2013, the proportion of *Bd*-positive salamanders was the same among all four field sites sampled (i.e. the two negative sites did not have enough samples to rule out the presence of *Bd*), but in 2014, the proportion of *Bd*-positive salamanders was significantly higher in 14-Mariposa than 0-Madera (2013: P value = 0.81; 2014: P value = 0.03). The proportion of *Bd*-positive salamanders held steady from 2013 to 2014 at 0-Madera but increased in 2014 at 14-Mariposa (0-Madera: P value = 0.32; 14-Mariposa: P value = 0.04) (Table 2).

Discussion

Bd was present in California as early as 1915 (Adams et al. 2017) and in the Sierra Nevada mountains as early as 1939 (Vredenburg et al. 2019), but apparently it did not spread rapidly. In multiple retrospective studies of *Bd* in California, detection rates of *Bd* began to increase during the 1960s and 1970s, indicative of an epizootic spread that peaked in the 1980s or 1990s, though many of these studies have focused on coastal California (Padgett-Flohr and Hopkins 2009; Huss et al. 2013; Sette et al. 2015; de León et al. 2017; Chaukulkar et al. 2018; Vredenburg et al. 2019). Concurrent high pathogen prevalence and high zoospore levels support the hypothesis that *Bd* reached epizootic levels across parts of California during those two decades.

It is important to note that *Bd*-positive specimens collected in California prior to 1960 were scattered in both time and space and were apparently not followed by epizootic spread and outbreaks. These may represent failed invasions—disease introductions which disappeared from host populations (Anderson and May 1979; Briggs et al. 2010). Early positives and subsequent fadeout is apparent in pre-1960 Sierran anuran samples (Vredenburg et al.

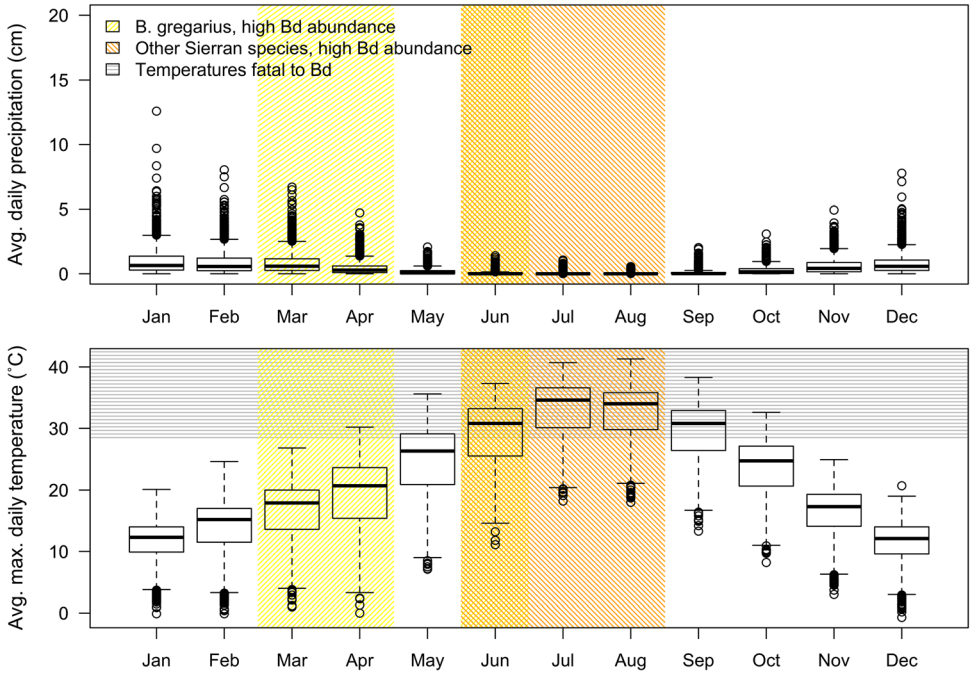

Figure 5. Top panel: Average daily precipitation (cm) by month for 1963–2009 for Fresno, Madera, Mariposa, Stanislaus, and Tulare counties, California. Bottom panel: Average maximum daily temperature (°C) by month for Fresno, Madera, Mariposa, Stanislaus, and Tulare counties. Yellow (downward hash) area shows the 3 months with highest *Bd* detection rate in *Batrachoseps gregarius*, and the orange area (upward hash) shows the 3 months with highest *Bd* prevalence in other Sierra Nevada anuran *Bd* hosts. Grey box (horizontal hash) shows lethal temperature for *Bd*.

Table 1. Historical GLM Explanatory Variables' Estimated Value and Significance for the Best Model for: 12-, 11-, 10-, 9-, 4-, 3-, 2-, and 1-Month Time Periods, and the 6-Month Time Period.

12-, 11-, 10-, 9-, 4-, 3-, 2-, 1-month model: Bd prevalence ~ Year		
(Intercept)	21.36	0.72
Year	− 0.0121	0.03*
Elevation (m)	− 0.1500	0.69
Elevation × Year	0.0001	0.03*
6-month model: Bd prevalence ~ Elevation + AvgTmax, 6mo) × Year		
(Intercept)	458.1	0.02*
Year	− 0.2297	< 0.01*
Elevation (m)	− 0.2230	0.02*
Avg. daily maximum temperature, 6 mo. (°C)	− 22.33	0.02*
Elevation × Year	0.0001	< 0.01*
Temperature × Year	0.0111	0.02*

2019), but not in *B. gregarius* where *Bd* steadily increased in prevalence after its first emergence in the 1970s. While *Bd* detection rates for Sierran anurans peaked in the 1980s and remained high through the 1990s, the *Bd* peak in *B. gregarius* was offset by a decade, reaching epizootic levels in the 1990s. *Taricha* positives appear earlier than *B. gregarius*, despite lower sampling effort, but also peak in the 1990s. Difference in timing are likely explained by *Bd's* mode of transmission switching between density-dependent transmission in aquatic environments to slower frequency-dependent transmission in terrestrial environments.

Table 2. Contemporary Sampling Data in 2013 and 2014 by Locality.

Latitude	Longitude	Site		2013							2014					
				Total *B. gregarius*	# Bd-positive	% Bd-positive	95% Confidence		Avg. Zswab	Avg. group size	Total *B. gregarius*	# Bd-positive	% Bd-positive	95% Confidence		Avg. Zswab
							Lower	Upper						Lower	Upper	
37.441025	− 119.63054	0	Madera	52	1	1.92	0.05	10.26	0.3072	1.61 ± 1.22	95	5	5.26	1.73	11.86	112.3
37.426322	− 119.72394	8	Mariposa	17	0	0.00	0	19.51	–	1.35 ± 0.49	–	–	–	–	–	–
37.348779	− 119.59227	13	Madera	23	0	0.00	0	14.82	–	1.09 ± 0.29	–	–	–	–	–	–
37.444067	− 119.65064	14	Mariposa	39	1	2.56	0.06	13.48	2.288	1.19 ± 0.40	102	15	14.71	8.47	23.09	19.25

Batrachoseps gregarius and aquatic Sierran anurans showed clear seasonal differences in *Bd* abundance, providing evidence of a unique terrestrial transmission mode. Specifically, *Bd* detection in *B. gregarius* was significantly lower during the hot/dry season (May–October) compared to the cool/wet season (November–April); there was no such seasonal trend for Sierran anurans. One explanation for this result is that aquatic amphibians can potentially be exposed across seasons, as *Bd* zoospores persist in freshwater bodies (Chestnut et al. 2014). Because the mechanisms of *Bd* exposure in a fully terrestrial host are likely limited to skin-to-skin transmission or contact with contaminated substrate (Berger et al. 2016), exposure is unlikely during hot/dry summer months when *Batrachoseps* salamanders burrow deep underground to escape desiccation (Hendrickson 1954; Cunningham 1960). *Taricha* shows a seasonal pattern of *Bd* more that is similar to *B. gregarius* than stream-breeding anurans. However, *Taricha's* aquatic breeding occurs predominantly January-May (Stebbins 2003), which may account for the earlier seasonal peak. However, these results may also reflect elevational differences in *Bd* seasonality, since some of the range for aquatic Sierran anurans is under snow during winter, affecting capture rates. Ultimately, additional sampling is needed to confirm these seasonal patterns among terrestrial salamanders, aquatic-breeding salamanders, and aquatic-breeding anurans.

The delay between the onset of the cool/wet season in November and the April peak in *Bd* detection rate in *B. gregarius* matches the historical GLM results, which indicate a six-month time interval is best for assessing the impact of climate factors (temperature) on *Bd* levels in this species. Because the majority of *Bd*-positive *B. gregarius* were collected in February-June, this six-month time frame begins in August–December and extends into the cool, rainy winter. This interval may reflect the duration of the exposure period for salamanders, beginning when the rains start, and *Batrachoseps* become more socially active at the surface.

The GLM includes significant interactions between year and average daily maximum temperature, as well as year and elevation. Both interactions have saddle-shaped curves, with earlier (1970s) positives occurring at lower temperatures and lower elevations compared to later (2000s) positives (Supp. Figure 2). The change in the effect of temperature and elevation over time may reflect differences between the introduction of *Bd* and post-epizootic dynamics. In addition, the pattern of early *B. gregarius* positives at lower elevations with *Bd* appearing at higher elevations in later years is consistent with human activity driving *Bd* emergence (Chestnut et al. 2014). The residuals of both GLMs are large, particularly at higher levels of *Bd* (Supp. Figure 3). This is likely due to the fact that our model explores landscape-level factors affecting *Bd*, ignoring factors that might influence local disease dynamics.

At the local population level, pathogen prevalence is driven by the transmission rate among hosts in combination with host susceptibility (May and Anderson 1979). Unfortunately, since our contemporary sampling occurred during the 2012–2014 California drought, the most severe drought in the last 1200 years (Griffin and Anchukaitis 2014), we were unable to explore factors such as host aggregation size that may contribute to *Bd* detection rate at the local level. Salamanders were absent from the majority of sites visited in 2013, though they were likely to have been present underground. The four sites where we did find salamanders in 2013 were located near creeks, and we even found communal nests at three sites. 2014 was the worst drought year in California (Griffin and Anchukaitis 2014), and we only found salamanders at the two sites. Future research on the social *B. gregraius* should prioritize a focus on within-site patterns of infection.

With the recent discovery of second chytridiomycosis-causing pathogen, *Batrachochtrium salamandrivorans* (*Bsal*) (Martel et al. 2014), it is important to understand how these pathogens become established as they enter new geographic regions and how they spread through a terrestrial and aquatic habitats. Climate suitability models predict that within 100 years of introduction, *Bsal* has the potential to spread across the range of nearly every North America salamander species (Yap et al. 2017). Historical *Bd* studies are crucial because they increase our understanding of how these pathogens spread in both time and space across the backdrop of species with different life histories and behaviours. Though aquatic species have been the focus of most *Bd* research, direct-developing host species (i.e. Plethodontid salamanders) are also extremely sensitive to *Bd* (Mesquita et al. 2017). Many species of direct-developing tropical frogs have been devastated by *Bd* (Longo and Burrowes 2010; Catenazzi et al. 2011). Our results reinforce previous studies that suggest that declines in amphibian species are highly influenced by host species' terrestrial lifestyle (Lips et al. 2003).

Salamanders are the most abundant terrestrial vertebrates in North American forests (e.g. *Batrachoseps* in California) and facilitate important carbon cycle functions

such as leaf litter retention and carbon capture (Best and Welsh 2014); therefore, their susceptibility to *Bd* could have important implications for biodiversity and ecosystem function. The impact of chytridiomycosis on terrestrial amphibians in North America may not only threaten biodiversity directly, it may also have major impacts on carbon cycling and storage, compounding the threat of *Bd* in amphibians throughout the world. Our data indicate that *Bd* dynamics in this entirely terrestrial species of amphibian differs from aquatic species in the same region with both seasonal patterns of *Bd* detection rate and in the possible timing of *Bd* epizootics.

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References

Adams AJ, Pessier AP, Briggs CJ (2017) Rapid extirpation of a North American frog coincides with an increase in fungal pathogen prevalence: Historical analysis and implications for reintroduction. *Ecology and Evolution* 7:10216–10232. https://doi.org/10.1002/ece3.3468

Anderson RM, May RM (1979) Population biology of infectious diseases: Part I. *Nature* 280:361–367. https://doi.org/10.1038/280361a0

Berger L, Roberts AA, Voyles J, Longcore JE, Murray KA, Skerratt LF (2016) History and recent progress on chytridiomycosis in amphibians. *Fungal Ecology* 19:89–99. https://doi.org/10.1016/j.funeco.2015.09.007

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America* 95:9031–9036. https://doi.org/10.1073/pnas.95.15.9031

Best ML, Welsh HH (2014) The trophic role of a forest salamander: Impacts on invertebrates, leaf litter retention, and the humification process. *Ecosphere* . https://doi.org/10.1890/ES13-00302.1

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD (2004) Rapid quantitative detection of chytridiomycosis (Batrachochytrium dendrobatidis) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 60:141–148. https://doi.org/10.3354/dao060141

Briggs CJ, Knapp RA, Vredenburg VT (2010) Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 107:9695–9700. https://doi.org/10.1073/pnas.0912886107

Burrowes PA, Longo AV (2007) Potential fitness cost of batrachochytrium dendrobatidis in eleutherodactylus coqui, and comments on environment-related risk of infection. *Herpetotropicos* 4:51–57

Byrne AQ, Rothstein AP, Poorten TJ, Erens J, Settles ML, Rosenblum EB (2017) Unlocking the story in the swab: A new genotyping assay for the amphibian chytrid fungus Batrachochytrium dendrobatidis. *Molecular Ecology Resources* 17:1283–1292. https://doi.org/10.1111/1755-0998.12675

Byrne AQ, Vredenburg VT, Martel A, Pasmans F, Bell RC, Blackburn DC, Bletz MC, Bosch J, Briggs CJ, Brown RM, Catenazzi A, López MF, Figueroa-Valenzuela R, Ghose SL, Jaeger JR, Jani AJ, Jirku M, Knapp RA, Muñoz A, Portik DM, Richards-Zawacki CL, Rockney H, Rovito SM, Stark T, Sulaeman H, Tao NT, Voyles J, Waddle AW, Yuan Z, Rosenblum EB (2019) Cryptic diversity of a widespread global pathogen reveals expanded threats to amphibian conservation. *Proceedings of the National Academy of Sciences of the United States of America* 116:20382–20387. https://doi.org/10.1073/pnas.1908289116

Catenazzi A, Lehr E, Rodriguez LO, Vredenburg VT (2011) Batrachochytrium dendrobatidis and the collapse of anuran species richness and abundance in the upper Manu National Park, southeastern Peru. *Conservation Biology* 25:382–391. https://doi.org/10.1111/j.1523-1739.2010.01604.x

Chaukulkar S, Sulaeman H, Zink AG, Vredenburg VT (2018) Pathogen invasion and non-epizootic dynamics in Pacific newts in California over the last century. *PLoS ONE* 13:1–10. https://doi.org/10.1371/journal.pone.0197710

Cheng TL, Rovito SM, Wake DB, Vredenburg VT (2011) Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen Batrachochytrium dendrobatidis. *Proceedings of the National Academy of Sciences of the United States of America* 108:9502–9507. https://doi.org/10.1073/pnas.1105538108

Chestnut T, Anderson C, Popa R, Blaustein AR, Voytek M, Olson DH, Kirshtein J (2014) Heterogeneous occupancy and density estimates of the pathogenic fungus Batrachochytrium dendrobatidis in waters of North America. *PLoS ONE* . https://doi.org/10.1371/journal.pone.0106790

Cummer MR, Green DE, O'Neill EM (2005) Aquatic chytrid pathogen detected in terrestrial Plethodontid. *Herpetological Review* 36:248–249

Cunningham JD (1960) Aspects of the ecology of the Pacific slender salamander. *Batrachoseps pacificus, in Southern California* 41:88–99

de León ME, Vredenburg VT, Piovia-Scott J (2017) Recent emergence of a chytrid fungal pathogen in California cascades frogs (Rana cascadae). *EcoHealth* 14:155–161. https://doi.org/10.1007/s10393-016-1201-1

Doherty-Bone TM, Gonwouo NL, Hirschfeld M, Ohst T, Weldon C, Perkins M, Kouete MT, Browne RK, Loader SP, Gower DJ, Wilkinson MW, Rödel MO, Penner J, Barej MF, Schmitz A, Plötner J, Cunningham AA (2013) Batrachochytrium dendrobatidis in amphibians of Cameroon, including first records for caecilians. *Diseases of Aquatic Organisms* 102:187–194. https://doi.org/10.3354/dao02557

Fisher MC, Garner TWJ (2007) The relationship between the emergence of Batrachochytrium dendrobatidis, the international trade in amphibians and introduced amphibian species. *Fungal Biology Reviews* 21:2–9. https://doi.org/10.1016/j.fbr.2007.02.002

Fisher MC, Garner TWJ, Walker SF (2009) Global emergence of Batrachochytrium dendrobatidis and amphibian chytridiomycosis in space, time, and host. *Annual Review of Microbiology* 63:291–310. https://doi.org/10.1146/annurev.micro.091208.073435

Gower DJ, Doherty-Bone T, Loader SP, Wilkinson M, Kouete MT, Tapley B, Orton F, Daniel OZ, Wynne F, Flach E, Müller H, Menegon M, Stephen I, Browne RK, Fisher MC, Cunningham AA, Garner TWJ (2013) Batrachochytrium dendrobatidis Infection and lethal chytridiomycosis in caecilian amphibians (Gymnophiona). *EcoHealth* 10:173–183. https://doi.org/10.1007/s10393-013-0831-9

Gräler B, Pebesma E, Heuvelink G (2016) Spatio-temporal interpolation using gstat. R Journal 8:204–218. https://doi.org/10.32614/rj-2016-014

Griffin D, Anchukaitis KJ (2014) How unusual is the 2012-2014 California drought? *Geophysical Research Letters* 41:9017–9023. https://doi.org/10.1002/2014GL062433

Hemmert GAJ, Schons LM, Wieseke J, Schimmelpfennig H (2018) Log-likelihood-based Pseudo-R 2 in Logistic Regression: Deriving Sample-sensitive Benchmarks. *Sociological Methods & Research* 47:507–531. https://doi.org/10.1177/0049124116638107

Hendrickson JR (1954) Ecology and Systematics of Salamanders of the genus Batrachoseps. *University of California Publications in Zoology* 54:1–46

Huss M, Huntley L, Vredenburg V, Johns J, Green S (2013) Prevalence of Batrachochytrium dendrobatidis in 120 archived specimens of Lithobates catesbeianus (American bullfrog) collected in California, 1924-2007. *EcoHealth* 10:339–343. https://doi.org/10.1007/s10393-013-0895-6

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Hero M, Hines H, Phillott R, Campbell R, Marantelli G, Gleason F, Colling A (2007) Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. *Diseases of Aquatic Organisms* 73:175–192. https://doi.org/10.3354/dao073175

Jockusch EL, Wake DB, Yanev KP (1998) New species of slender salamanders, Batrachoseps (Amphibia: Plethodontidae), from the Sierra Nevada of California. Contributions in Science 1–17

Johnson ML, Speare R (2005) Possible modes of dissemination of the amphibian chytrid Batrachochytrium dendrobatidis in the environment. *Diseases of Aquatic Organisms* 65:181–186. https://doi.org/10.3354/dao065181

Kolby JE, Ramirez SD, Berger L, Richards-Hrdlicka KL, Jocque M, Skerratt LF (2015) Terrestrial dispersal and potential environmental transmission of the amphibian chytrid fungus (Batrachochytrium dendrobatidis). *PLoS ONE* 10:1–13. https://doi.org/10.1371/journal.pone.0125386

Kriger KM, Hero JM (2007) The chytrid fungus Batrachochytrium dendrobatidis is non-randomly distributed across amphibian breeding habitats. *Diversity and Distributions* 13:781–788. https://doi.org/10.1111/j.1472-4642.2007.00394.x

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier AP, Collins JP (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America* 103:3165–3170. https://doi.org/10.1073/pnas.0506889103

Lips KR, Reeve JD, Witters LR (2003) Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* 17:1078–1088. https://doi.org/10.1046/j.1523-1739.2003.01623.x

Longcore JE, Pessier AP, Nichols DK (1999) Batrachochytrium dendrobatidis gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91:219–227. https://doi.org/10.1080/00275514.1999.12061011

Longo AV, Burrowes PA (2010) Persistence with chytridiomycosis does not assure survival of direct-developing frogs. *EcoHealth* 7:185–195. https://doi.org/10.1007/s10393-010-0327-9

Martel A, Blooi M, Adriaensen C, van Rooij P, Beukema W, Fisher MC, Farrer RA, Schmidt BR, Tobler U, Goka K, Lips KR, Muletz C, Zamudio KR, Bosch J, Lötters S, Wombwell E, Garner TWJ, Cunningham AA, Spitzen-Van Der Sluijs A, Salvidio S, Ducatelle R, Nishikawa K, Nguyen TT, Kolby JE, van Bocxlaer I, Bossuyt F, Pasmans F (2014) Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science* 346:630–631. https://doi.org/10.1126/science.1258268

Martel A, Spitzen-Van Der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC, Woeltjes A, Bosman W, Chiers K, Bossuyt F, Pasmans F (2013) Batrachochytrium salamandrivorans sp. nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 110:15325–15329. https://doi.org/10.1073/pnas.1307356110

May RM, Anderson RM (1979) Population biology of infectious diseases: Part II. *Nature* 280:455–461. https://doi.org/10.1038/280455a0

McCracken S, Gaertner JP, Forstner MRJ, Hahn D (2009) Detection of Batrachochytrium dendrobatidis in amphibians from the forest floor to the upper canopy of an Ecuadorian Amazon lowland rainforest. *Herpetological Review* 40:190–195

Mesquita AFC, Lambertini C, Lyra M, Malagoli LR, James TY, Toledo LF, Haddad CFB, Becker CG (2017) Low resistance to chytridiomycosis in direct-developing amphibians. *Scientific Reports* 7:1–7. https://doi.org/10.1038/s41598-017-16425-y

Muletz C, Caruso NM, Fleischer RC, McDiarmid RW, Lips KR (2014) Unexpected rarity of the pathogen Batrachochytrium dendrobatidis in Appalachian Plethodon salamanders: 1957-2011. *PLoS ONE* . https://doi.org/10.1371/journal.pone.0103728

O'Hanlon SJ, Rieux A, Farrer RA, Rosa GM, Waldman B, Bataille A, Kosch TA, Murray KA, Brankovics B, Fumagalli M, Martin MD, Wales N, Alvarado-Rybak M, Bates KA, Berger L, Böll S, Brookes L, Clare F, Courtois EA, Cunningham AA, Doherty-Bone TM, Ghosh P, Gower DJ, Hintz WE, Höglund J, Jenkinson TS, Lin CF, Laurila A, Loyau A, Martel A, Meurling S, Miaud C, Minting P, Pasmans F, Schmeller DS, Schmidt BR, Shelton JMG, Skerratt LF, Smith F, Soto-Azat C, Spagnoletti M, Tessa G, Toledo LF, Valenzuela-Sánchez A, Verster R, Vörös J, Webb RJ, Wierzbicki C, Wombwell E, Zamudio KR, Aanensen DM, James TY, Thomas M, Weldon C, Bosch J, Balloux F, Garner TWJ, Fisher MC (2018) Recent Asian origin of chytrid fungi causing

global amphibian declines. *Science* 360:621–627. https://doi.org/10.1126/science.aar1965

Padgett-Flohr GE, Hopkins RL (2009) Batrachochytrium dendrobatidis, a novel pathogen approaching endemism in central California. *Diseases of Aquatic Organisms* 83:1–9. https://doi.org/10.3354/dao02003

Pebesma EJ (2004) Multivariable geostatistics in S: The gstat package. *Computers and Geosciences* 30:683–691. https://doi.org/10.1016/j.cageo.2004.03.012

Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of Batrachochytrium dendrobatidis, a chytrid pathogen of amphibians. *Mycologia* 96:9–15. https://doi.org/10.1080/15572536.2005.11832990

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rachowicz LJ, Vredenburg VT (2004) Transmission of Batrachochytrium dendrobatidis within and between amphibian life stages. *Diseases of Aquatic Organisms* 61:75–83. https://doi.org/10.3354/dao061075

Rosenblum EB, James TY, Zamudio KR, Poorten TJ, Ilut D, Rodriguez D, Eastman JM, Richards-Hrdlicka K, Joneson S, Jenkinson TS, Longcore JE, Olea GP, Toledo LF, Arellano ML, Medina EM, Restrepo S, Flechas SV, Berger L, Briggs CJ, Stajich JE (2013) Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. *Proceedings of the National Academy of Sciences of the United States of America* 110:9385–9390. https://doi.org/10.1073/pnas.1300130110

Rowley JJL, Alford RA (2007) Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. *Diseases of Aquatic Organisms* 77:1–9. https://doi.org/10.3354/dao01830

Sette CM, Vredenburg VT, Zink AG (2015) Reconstructing historical and contemporary disease dynamics: A case study using the California slender salamander. *Biological Conservation* 192:20–29. https://doi.org/10.1016/j.biocon.2015.08.039

Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134. https://doi.org/10.1007/s10393-007-0093-5

Smith AM, Green DM (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: Are all amphibian populations metapopulations? *Ecography* 28:110–128. https://doi.org/10.1111/j.0906-7590.2005.04042.x

Stebbins RC (2003) A Field Guide to Western Reptiles and Amphibians (Peterson Field Guides)

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786. https://doi.org/10.1126/science.1103538

van Rooij P, Martel A, Nerz J, Voitel S, van Immerseel F, Haesebrouck F, Pasmans F (2011) Detection of Batrachochytrium dendrobatidis in Mexican bolitoglossine salamanders using an optimal sampling protocol. *EcoHealth* 8:237–243. https://doi.org/10.1007/s10393-011-0704-z

Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Springer

Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ (2010) Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proceedings of the National Academy of Sciences of the United States of America* 107:9689–9694. https://doi.org/10.1073/pnas.0914111107

Vredenburg VT, McNally SVG, Sulaeman H, Butler HM, Yap T, Koo MS, Schmeller DS, Dodge C, Cheng T, Lau G, Briggs CJ (2019) Pathogen invasion history elucidates contemporary host pathogen dynamics. *PLoS ONE* 14:1–14. https://doi.org/10.1371/journal.pone.0219981

Wake DB, Vredenburg VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians *PNAS* 105:11466–11473

Weinstein SB (2009) An aquatic disease on a terrestrial salamander: Individual and population level effects of the amphibian chytrid fungus, Batrachochytrium dendrobatidis, on Batrachoseps attenuatus (Plethodontidae). *Copeia* 2009:653–660. https://doi.org/10.1643/ch-08-180

Yap TA, Koo MS, Ambrose RF, Wake DB, Vredenburg VT (2015) Averting a North American biodiversity crisis. Science 349:481–482. https://doi.org/10.1111/dech.12325

Yap TA, Nguyen NT, Serr M, Shepack A, Vredenburg VT (2017) Batrachochytrium salamandrivorans and the risk of a second amphibian pandemic. *EcoHealth* 14:851–864. https://doi.org/10.1007/s10393-017-1278-1

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed Effects Models and Extensions in Ecology with R. Springer

Zuur AF, Savaliev AA, Ieno EN (2012) Zero Inflated Models & Generalized Linear Mixed Models with R. Highland Statistics, Ltd.