

# Temporal framework of pathogen invasion elucidates contemporary host pathogen dynamics

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<b>Abstract:</b>	<p>Amphibians, the most threatened group of vertebrates, are seen as indicators of the sixth mass extinction on earth. Thousands of species are threatened with extinction and many have been affected by an emerging infectious disease, chytridiomycosis, caused by the fungal pathogen, Batrachochytrium dendrobatidis (Bd). However, amphibians exhibit different responses to the pathogen, such as survival and population persistence with infection, or mortality of individuals and complete population collapse after pathogen invasion. Multiple factors can affect host pathogen dynamics, yet few studies have provided a temporal view that encompasses both the epizootic phase (i.e. pathogen invasion and host collapse), and the transition to a more stable co-existence (i.e. recovery of infected host populations). In the Sierra Nevada mountains of California, USA, conspecific populations of frogs currently exhibit dramatically different host/ Bd-pathogen dynamics. To provide a temporal context by which present day dynamics may be better understood, we use a Bd qPCR assay to test 1165 amphibian specimens collected between 1900 and 2005. Our historical analyses reveal a pattern of pathogen invasion and eventual spread across the Sierra Nevada over the last century. Although we found a small number of Bd-infections prior to 1970, these showed no sign of spread or increase in infection prevalence over multiple decades. After the late 1970s, when mass die offs were first noted, our data show Bd as much more prevalent and more spatially spread out, suggesting epizootic spread. However, across the ~400km<sup>2</sup> area, we found no evidence of a wave-like pattern, but instead discovered multiple, nearly-simultaneous invasions within regions. We found that Bd invaded and spread in the central Sierra Nevada (Yosemite National Park area) about four decades before it invaded and spread in the southern Sierra Nevada (Sequoia and Kings Canyon National Parks area), and suggest that the temporal pattern of pathogen invasion may help explain divergent contemporary host pathogen dynamics.</p>
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Butler, H. 2017 "Sierra Nevada Retrospective Analysis" AmphibiaWeb: Amphibian Disease Portal <<https://n2t.net/ark:/21547/Ars2>>).

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# Pathogen invasion history elucidates contemporary host pathogen dynamics

Short title: Unraveling the historic invasion of a fungal pathogen

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## **ABSTRACT**

22 Amphibians, the most threatened group of vertebrates, are seen as indicators of the sixth mass extinction  
23 on earth. Thousands of species are threatened with extinction and many have been affected by an  
24 emerging infectious disease, chytridiomycosis, caused by the fungal pathogen, *Batrachochytrium*  
25 *dendrobatidis* (*Bd*). However, amphibians exhibit different responses to the pathogen, such as survival  
26 and population persistence with infection, or mortality of individuals and complete population collapse  
27 after pathogen invasion. Multiple factors can affect host pathogen dynamics, yet few studies have  
28 provided a temporal view that encompasses both the epizootic phase (i.e. pathogen invasion and host  
29 collapse), and the transition to a more stable co-existence (i.e. recovery of infected host populations). In  
30 the Sierra Nevada mountains of California, USA, conspecific populations of frogs currently exhibit  
31 dramatically different host/ *Bd*-pathogen dynamics. To provide a temporal context by which present day  
32 dynamics may be better understood, we use a *Bd* qPCR assay to test 1165 amphibian specimens  
33 collected between 1900 and 2005. Our historical analyses reveal a pattern of pathogen invasion and  
34 eventual spread across the Sierra Nevada over the last century. Although we found a small number of  
35 *Bd*-infections prior to 1970, these showed no sign of spread or increase in infection prevalence over  
36 multiple decades. After the late 1970s, when mass die offs were first noted, our data show *Bd* as much  
37 more prevalent and more spatially spread out, suggesting epizootic spread. However, across the  
38 ~400km<sup>2</sup> area, we found no evidence of a wave-like pattern, but instead discovered multiple, nearly-  
39 simultaneous invasions within regions. We found that *Bd* invaded and spread in the central Sierra  
40 Nevada (Yosemite National Park area) about four decades before it invaded and spread in the southern  
41 Sierra Nevada (Sequoia and Kings Canyon National Parks area), and suggest that the temporal pattern of  
42 pathogen invasion may help explain divergent contemporary host pathogen dynamics.

43

## 44 INTRODUCTION



45 With thousands of amphibian species experiencing population declines around the world [1],  
46 amphibians are facing a global biodiversity crisis, and many suggest this is emblematic of a global mass  
47 extinction [2]. Though multiple factors play a role in these declines, the invasion and emergence of the  
48 fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*), and the ensuing epizootics (epidemics in  
49 wildlife) are implicated as major contributing factors [3]. *Bd*, and its recently described congener  
50 *Batrachochytrium salamandrivorans* (*Bsal*), are the only chytridiomycete known to be pathogenic to  
51 vertebrates. Since the description of *Bd*, [4], it has been detected on every continent except Antarctica  
52 and is frequently associated with amphibian die-offs [5-8], but not all species are susceptible. *Bd* infects  
53 the skin of amphibians and induces a thickening of the skin (hyperkeratosis) on the host, disrupting  
54 osmotic balance which, in highly infected individuals, often results in death [4, 9-11].

55 Nearly two decades after its discovery, the dynamics and emergence of *Bd* are yet to be fully  
56 understood. Genomic studies have revealed that there are multiple lineages of *Bd*. For example, the  
57 Global Panzootic Lineage (*Bd*-GPL) is associated with *Bd*-epizootics and host population collapse [12],  
58 but other lineages are found in areas where epizootics have not been found (e.g. South Korea) [12-15]  
59 and many species survive infections. Asia is a geographic hotspot for *Bd* genetic diversity and is  
60 proposed as a possible source of the *Bd*-panzootic that began in the 20<sup>th</sup> century [16]. In the Americas  
61 (North, Central and South America), many of the reported declines of amphibians are attributed to *Bd*-  
62 GPL epizootics, yet most occurred decades before *Bd* was discovered [17, 18]. Thus, retrospective  
63 studies are needed to help create a timeline for *Bd* emergence and spread.

64 Studies of *Bd* epizootics in California were some of the first to describe host pathogen dynamics  
65 of chytridiomycosis in detail [7, 11, 19], yet, like many other areas that have suffered epizootics, the  
66 historical view of *Bd* in the region has not been fully described. The earliest evidence of *Bd*-infection in  
67 California is from an American bullfrog (*Rana catesbeiana*) specimen collected in 1928 [20]. Although

68 this widely introduced species is identified as a reservoir species for *Bd*, and thus may have facilitated  
69 *Bd* invasion [21], there is no evidence that this infection case resulted in epizootics and may represent a  
70 failed invasion. Evidence suggests that the timing of *Bd* emergence (i.e. increase in infection prevalence  
71 and geographic spread) in California is from the late 1960s to the 1980s [22-24], a time period that  
72 coincides with declines of many species in the region [25-27].

73 In the Sierra Nevada mountain range of California, population declines and local extinctions  
74 have been documented in most of the amphibians that occur there [27]. Some population declines are  
75 attributed to introduced species like non-native fishes [28], though the causes of other declines are  
76 unknown [26]. Between 1976 and 1979, a mass die-off of Yosemite toads (*Anaxyrus canorus*) was  
77 documented near Yosemite National Park [29]. A later study suggested it could have been caused by a  
78 *Bd* epizootic, but the study was ultimately inconclusive [30]. Bradford (1991) documented a mass  
79 mortality event of the southern mountain yellow-legged frog (*Rana muscosa*) in Kings Canyon National  
80 Park in 1979; the cause of mortality was not determined, but by 1989 the species was extirpated from  
81 that area of the park [25].

82 The southern mountain yellow-legged frog (*Rana muscosa*) and Sierra Nevada mountain yellow-  
83 legged frog (*Rana sierrae*) have undergone extensive population declines and local extinctions over the  
84 past 100 years [31]. Recently, population collapse and extinction in both species of frog have been  
85 shown to be caused by *Bd*-epizootics [6, 7]; however, the extent of the effect across the entire Sierra  
86 Nevada range is unknown. For example, studies of host pathogen dynamics in both species of frog have  
87 shown that some host populations co-exist with the pathogen [19], while others go extinct < 1 year after  
88 pathogen invasion [7]. In the Yosemite area (central Sierra Nevada), mass die-offs associated with *Bd*-  
89 epizootics have not been observed in either *R. sierrae* or *R. muscosa*, yet populations are all infected  
90 with *Bd*. These frog populations exhibit moderately high *Bd* infection prevalence (60-75%) and very low

*Bd* infection intensity (< 1 Zswab (zoospore equivalents of *Bd* DNA per swab) [19]). However, in the Sequoia-Kings Canyon area (southern Sierra Nevada), *Bd*- epizootics and die offs have been documented in those same species [7]. Here *Bd* infection prevalence rises rapidly to 100% soon after invasion and establishment of *Bd*. During this time, the *Bd* infection intensity rises 3-4 orders of magnitude higher than in Yosemite) (> 10,000 Zswab) [7], at which point, populations collapse. We hypothesize that *Bd* invaded and spread throughout the central Sierra Nevada (Yosemite area) long before it invaded and spread in the southern Sierra Nevada (Sequoia-Kings Canyon area) and propose that this may explain differences in present day host-pathogen dynamics. To test this, we conducted a retrospective survey using museum specimens to describe *Bd*-host dynamics in the Sierra Nevada mountain range over the past century. Because there are fewer available museum specimens from contemporary populations, we also present portions of previously published data on contemporary populations for comparison. Previous studies have hypothesized that *Bd* is an invasive pathogen in California, thus we also investigate the relationship between *Bd* infection and anthropogenic and abiotic factors (i.e., climate variables) that could help explain why *Bd* became established and spread in some areas. We also use statistical techniques to estimate when *Bd* invasion occurred in the Sierra Nevada, based on our available data dating back over a century.

## **METHODS**

### **Sampling of Museum Specimens**

We collected skin swabs from 1165 formalin-fixed, ethanol-preserved, post-metamorphic anurans in museum archives from 1900 to 2005. Using the VertNet.org database, we identified and sampled specimens from permanent collections housed at the California Academy of Sciences, Museum of

113 Vertebrate Zoology, Natural History Museum of Los Angeles County, the Slater Museum of Natural  
114 History, and the Carnegie Museum of Natural History. To maximize the probability of detecting *Bd*, we  
115 selected all available *R. muscosa* and *R. sierrae* museum specimens, because these species are known to  
116 have undergone dramatic *Bd*-related population declines [7]. In decades where there were <100 *R.*  
117 *muscosa* and *R. sierrae* specimens available, we randomly sampled other sympatric anuran species  
118 (*Anaxyrus canorus*, *Anaxyrus boreas*, and *Hyla regilla*) within the Sierra Nevada range until we  
119 reached the 100-sample size for those decades. All of the data we collected and analyzed are freely  
120 accessible on the AmphibiaWeb amphibian disease portal online database (Butler, H. 2017 "Sierra  
121 Nevada Retrospective Analysis" AmphibiaWeb: Amphibian Disease Portal  
122 <<https://n2t.net/ark:/21547/Ars2>>).

123 We followed the museum swabbing technique described in Cheng et al. (2011) [32]. Each specimen  
124 was swabbed a total of 30 strokes with a sterile rayon-tipped swabs (MW113, Medical Wire and  
125 Equipment, Corsham, UK): 10 strokes on each side the ventral surface (running from the abdomen  
126 towards the pelvis and inner thighs), and 5 strokes on the toes and webbing of each hind foot. To reduce  
127 the possibility of cross contamination from multiple specimens kept in the same jar, each specimen was  
128 rinsed with 70% ethanol prior to swabbing, held in a unique plastic bag to prevent glove contamination,  
129 and gloves were changed between specimens. Swabs were stored in 1.5 mL microcentrifuge tubes and  
130 refrigerated at 4°C until extraction. Prior to extraction, swab vials were placed in a SpinVac (Savant  
131 Instruments, Farmingdale, NY, USA) for 15-20 min to evaporate any ethanol which could inhibit PCR.  
132 DNA extraction from the swabs was done using 40µL of Prepman Ultra (Applied Biosystems, Carlsbad,  
133 CA, USA) and diluted 1:10 with 0.25×TE Buffer. Presence of *Bd* was assayed by real-time qPCR,  
134 following the method described in Boyle et al. (2004) [33]. Samples were run in duplicate along with  
135 negative controls (H<sub>2</sub>O, TE Buffer) and positive controls at dilutions of 100, 10, 1, and 0.1 Zoospore

136 Equivalents. Raw qPCR output was multiplied by a factor of 80 to account for the dilution factor (1:80),  
137 giving a relative measure in terms of zoospore equivalents (Zswab) on the specimen. A sample was  
138 considered *Bd* positive if the amplification curve was sigmoidal with a Zswab value greater than zero.

139 Specimen collection localities were plotted in Quantum GIS 3.0 Girona ([www.qgis.org](http://www.qgis.org)). Because  
140 not all museum databases or collection labels have been updated to reflect the 2007 taxonomic split of *R.*  
141 *muscosa* (*sensu lato*) into allotypic northern *R. sierrae* and a southern *R. muscosa* (*sensu stricto*) [31],  
142 all specimens of “*Rana muscosa*” were checked against range information and were assigned to a  
143 species given morphology (*i.e.* *R. muscosa* having a longer leg length to body size ration compared to *R.*  
144 *sierrae*) and locality data [31].

145

## 146 **Statistical Analyses**

147 All statistical analyses were performed using the statistical software R (version 3.5.0). For each decade  
148 we calculated *Bd* prevalence with 95% binomial confidence intervals (CI). We also calculated the  
149 probability of not detecting *Bd* in each decade based on our sample size, assuming a binomial  
150 distribution.

151 We performed a stepwise binomial logistic regression using *Bd* infection status as the response  
152 (dependent) variable on the historical survey data, as individuals are either infected ( $Z_{swab} > 0$ ) or not  
153 infected ( $Z_{swab} = 0$ ). The data were analyzed in two categories: 1) all decades and 2) only the decades  
154 before emergence, which we defined as the decades prior to the decade with the greatest change in *Bd*  
155 prevalence. We did not perform a regression for post-emergence decades since our question was focused  
156 on understanding factors associated with the *Bd* invasion. We used the following explanatory variables  
157 in our model: annual mean temperature, annual minimum temperature, annual maximum temperature,  
158 elevation, annual precipitation, human footprint, distance to the closest water body, category of the

159 closest water body, croplands, built environment, population density, roads, railways, and pastures.  
160 Using the scale function in R's base package, we scaled the covariates using their mean and standard  
161 deviation to enable a better comparison of coefficients [34]. Elevation and topographic information for  
162 the distance to the closest water body were downloaded from the US Geological Survey National  
163 Hydrography Dataset (nationalmap.gov, <https://nhd.usgs.gov/data.html>) using the package FedData  
164 (version 2.4.6) and distance to the closest water body was calculated using the gdistance package  
165 (version 1.2-1). Annual temperature and precipitation information were downloaded from PRISM  
166 (PRISM Climate Group, Oregon State University, <http://prism.oregonstate.edu>, created 10 Feb 2018),  
167 and human footprint and land use information were downloaded from Venter et al (2016) [35] using the  
168 package raster (2.5-8). We performed a Pearson correlation test to determine if any of the explanatory  
169 variables were highly correlated with each other ( $r > 0.9$  or  $r < -0.9$ ) and eliminated the variables that  
170 were highly correlated to reduce multicollinearity. The eliminated highly correlated variables were:  
171 minimum temperature, maximum temperature, and elevation – all of which are highly correlated with  
172 mean temperature. We validated the models using  $k$ -fold cross validation.

173 We conducted Bayesian hierarchical modeling using Markov Chain Monte Carlo (MCMC) to  
174 estimate the arrival year of *Bd* in the Sierra Nevada with the R package rjags (version 4-6). In this  
175 model, *Bd* arrival is described using a threshold model where *Bd* switches from absent to present in the  
176 population with some mean prevalence. The number of infected individuals in each year was treated as a  
177 draw from a binomial distribution with a sample size equal to the number of individuals sampled that  
178 year [36, 37].

179 As a baseline for *Bd* infection prevalence comparison, we used a conservative probability of 0.05.  
180 This probability is based on a previous study that used the same qPCR technique on museum specimens

and showed a mean of 11% infection prevalence in a population where *Bd* was determined to be endemic (Illinois, USA) for specimens collected over a 100-year timespan [38].

## Results

### Museum Sampling

A total of 132 out of 1164 archived specimens sampled across the Sierra Nevada were *Bd*-positive (Fig 1); six were collected before 1970 and the remaining 126 positives were collected after 1970 (Table 1; Fig 2). The probability of not detecting *Bd*, given our sample size, was low ( $p < 0.01$ ) for each time period (Table 1). The pre-1970 *Bd*-positive specimens were collected in 1939, 1942, 1955, 1959, 1962, and 1965, at isolated and widely distributed sites across the mountain range (Fig 3). The overall *Bd* infection prevalence by decade ranged from 0.8% in the 1950s to 34.7% in the 1980s, with the greatest change occurring in the 1970s (Fig 2). When we included previously published data collected from live animals in the field ( $n=3492$ ) [7], the prevalence in the 2000s was over 40% (Fig 2). Based on the museum data, the Bayesian hierarchical modeling iterations predicted that *Bd* would have likely arrived between 1932-1939 (95% of iterations;  $\bar{x} = 1936$ ) in the Sierra Nevada, though this arrival time may also signal a failed invasion attempt.

**Fig 1:** Spatial distribution of 1165 amphibian museum specimens collected between 1900-2005 and tested for *Bd* infection (red circles *Bd*-positive; grey circles *Bd*-negative) in the Sierra Nevada mountains.

**Table 1:** Sample size per decade and the probability of detecting no *Bd* based on a 5% conservative probability of detection (5% conservative *Bd* prevalence). Credible intervals (CI) were calculated using binomial confidence intervals.

Time Period	Negatives	Positives	n	Lower CI	Upper CI	Pr
1900-1949	235	2	237	0.1	3.01	< 0.01
1950-1959	234	2	236	0.1	3.03	< 0.01
1960-1969	182	2	184	0.13	3.87	< 0.01
1970-1979	118	32	150	15.07	28.76	< 0.01
1980-1989	66	35	101	25.46	44.77	< 0.01
1990-1999	84	32	116	19.69	36.65	< 0.01
2000-2009	114	27	141	13.01	26.62	< 0.01

205

**Fig 2:** *Bd* infection prevalence in anurans of the Sierra Nevada from 1900-2009. Bar graphs denote sample size from each time period. Dark gray bars denote samples from museum specimens, and light gray bars denote samples collected from live animals in the field (live animal data from [7]). Blue line denotes *Bd* infection prevalence calculated from museum specimens only, and dotted blue line denotes *Bd* infection prevalence including both museum specimens and live animals in the field (i.e. museum specimens and data from [7]).

212

**Fig 3:** The incidence (number of new positives in each time period) of *Bd*-positive amphibians in the Sierra Nevada mountain range (light grey) in California from 1900-2009. The earliest *Bd* positives (12/26) were detected in Yosemite National Park were in the 1970s (mass die offs documented there in 1978 [Sherman and Morton 1993]). The first *Bd* positives (1586/3492) detected in Sequoia- Kings Canyon National Parks were in the 2000s (mass die offs documented there beginning in 2004; [7]).

218

We found that *Bd*-positive specimens appeared earlier and in higher numbers in the central and northern part of the Sierra Nevada range (e.g. the vicinity of Yosemite National Park and northwards), compared to the southern areas (e.g. the vicinity of Sequoia-Kings Canyon National Parks; Fig 3), but few samples were collected in the southern areas between the late 1970s and 1990s. In the Yosemite National Park area, the first positive was detected in 1972, and the first mass declines noted in 1978. In the Sequoia-Kings National Parks area the first detection of *Bd* was in 1998, with the first evidence of a

224



225 rapid increase in *Bd* prevalence being in 2004 (Fig 4). We also detected several early positives from  
 226 animals collected in 1976 in southwest Inyo County in the most extreme southern reaches of the Sierra  
 227 Nevada, far south of the Sequoia-Kings National Parks area (Fig 3).

228  
 229 **Fig 4.** Chronology of *Bd* infected amphibians collected in and around Yosemite National Park (central  
 230 Sierra Nevada) and Sequoia-Kings Canyon National Parks (southern Sierra Nevada).

231  
 232 The best model for the stepwise binomial logistic regressions, using *Bd* presence as a response  
 233 variable, (Table 2) shows differences in which scaled coefficients were significant at predicting *Bd*-  
 234 positive individuals between pre-1970's and all time periods. The best model for all decades shows both  
 235 human factors (human footprint, croplands, built environment, human population density, roads, and  
 236 railways), and climatic variables (precipitation and mean temperature) as significant predictors of *Bd*  
 237 infections (Table 3a). For the pre-1970s time period (before *Bd* emergence), anthropogenic factors (built  
 238 environment, human footprint, and railways) are significant predictors of *Bd* infection (Table 3b).  
 239 Models that included amphibian species as a factor did not suggest that that factor was significant in the  
 240 context of this study. Our *k*-fold cross validation for all time periods and pre-1970s models show a  
 241 cross-validation estimation of accuracy of 88.7% and 75.5%; respectively.

242  
 243 **Table 2:** Lowest AIC models for stepwise binomial logistic regression

<i>Predictors</i>	<b>All Time Periods</b>		<b>Pre-1970s</b>	
	<i>Odds Ratios</i>	<i>p</i>	<i>Odds Ratios</i>	<i>p</i>
(Intercept)	0.11	<b>&lt;0.001</b>	0.31	<b>&lt;0.001</b>
ppt	1.63	<b>&lt;0.001</b>	1.15	0.111
tmean	1.57	<b>&lt;0.001</b>		

HFP 2009	5.57	<b>0.001</b>	3.43	<b>0.003</b>
croplands 2005	0.81	0.086	0.82	0.134
Built 2009	0.26	<b>&lt;0.001</b>	0.37	<b>0.011</b>
Popdensity 2010	0.62	<b>0.009</b>		
Roads	0.48	<b>0.016</b>	0.66	0.087
Railways	0.71	<b>0.034</b>	0.76	<b>0.041</b>
distwater			1.19	0.108
Pasture 2009			0.84	0.135
AIC	783.788		564.465	

244

245 **Table 3a:** Selection for the lowest AIC model for all time periods sampled

Predictors	Model 1	Model 2	Model 3	Model 4
Precipitation	X	X	X	X
Mean Temperature	X	X	X	X
Human Footprint Index	X	X	X	X
Croplands	X	X	X	X
Built Environment	X	X	X	X
Population Density	X	X	X	X
Roads	X	X	X	X
Railways	X	X	X	X
Category of Closest Water Body				X
Distance to Closest Water Body		X	X	X
Pasturelands			X	X
AIC	783.79	785.02	786.70	792.79
Resid. Dev	765.79	765.02	764.70	756.79
Resid. Df	1155.00	1154.00	1153.00	1146.00
Deviance	0.77	0.31	7.91	NA

246

247 **Table 3b:** Selection for the lowest AIC model for pre-1970s

Predictors	Model 1	Model 2	Model 3	Model 4
Precipitation	X	X	X	X

Mean Temperature			X	X
Human Footprint Index	X	X	X	X
Croplands	X	X	X	X
Built Environment	X	X	X	X
Population Density		X	X	X
Roads	X	X	X	X
Railways	X	X	X	X
Category of Closest Water Body				X
Distance to Closest Water Body	X	X	X	X
Pasturelands	X	X	X	X
AIC	564.47	566.08	568.06	575.02
Resid. Dev	546.47	546.08	546.06	541.02
Resid. Df	498.00	497.00	496.00	490.00
Deviance	0.39	0.02	5.04	NA

## Discussion

The global collapse of amphibian species caused, in part, by the *Bd* panzootic, stands as an example that we have entered a sixth mass extinction [2]. Because the pathogen was discovered and described after causing epizootics in Central America and Australia [4, 9], retrospective studies are vital to improve our understanding of *Bd*'s pathogen invasion and spread. Despite sampling biases of museum specimens that were collected for reasons unrelated to disease ecology, retrospective studies, like this one, can provide insight regarding pathogen invasion history and disease dynamics.

Our survey of museum specimens collected over a 100-year period in the Sierra Nevada found no evidence of *Bd* before 1939. For almost the next four decades, we detected only a few *Bd*-infected frogs and no sign of spread or an increase in infection prevalence until the late 1970's. These results are consistent with a growing body of evidence showing that *Bd* may have invaded and spread in California and the west coast of North America approximately a decade before mass die offs were discovered [21,

262 22, 23, 36]. Unfortunately, those studies did not provide disease data from the Sierra Nevada, where  
263 studies first described pathogen host dynamics during epizootics that resulted in local host extinctions  
264 [7, 19]. However, one previous retrospective study did discover a *Bd*-positive Yosemite toad (*Anaxyrus*  
265 *canorus*) collected at Tioga Pass Meadow in 1978, the year before a mass population die-off at the site  
266 (Green & Sherman 2001). We also detected *Bd*-infected *R. sierrae* in the vicinity of Tioga Pass before  
267 the population collapse, suggesting that a *Bd* epizootic may have swept through the Yosemite area in the  
268 mid to late 1970s.

269 We found evidence of *Bd* invasion and spread that varies geographically and temporally across  
270 the Sierra Nevada mountain range. Unlike previous historical studies that occurred over very large  
271 (continental) or limited (1-2 Km) geographic scales [5, 7], we did not detect a directional wave-like  
272 spread of *Bd* across the entire mountain range. Instead, we found *Bd* invaded host populations  
273 asynchronously in separate regions across the 400 km<sup>2</sup> area, but specimens were collected for purposes  
274 not related to this study, and this limited our ability to detect a wave. In the central Sierra Nevada  
275 (Yosemite area), *Bd* was first detected in the 1970s (Fig 3), and epizootics are suggested to have  
276 occurred soon thereafter (Sherman and Morton 1993). In the southern Sierra Nevada (Sequoia-Kings  
277 Canyon area), *Bd* invaded more recently in the 2000s (Fig 3), and epizootics have been documented in  
278 2004-2008 [7]. Interestingly, these areas currently have different *Bd*-host dynamics, with frog  
279 populations in the central Sierra Nevada persisting in an enzootic state with *Bd* [19, 40] while frog  
280 populations in the southern Sierra Nevada are now experiencing epizootics and collapse [7]. Although  
281 the invasion patterns could be an artifact of the biased spatial-temporal spread of the museum  
282 specimens, our results indicate that *Bd* may have invaded these separate geographical areas at different  
283 times, and that difference in timing could account for the varying present-day *Bd*-host dynamics at these  
284 locations.

285           Infected frogs found prior to the 1970s may represent failed invasions, a pathogen invasion that  
286 took decades to establish, or may be evidence of the presence of non-virulent, endemic lineages of *Bd*.  
287 Currently, the only known lineage of *Bd* in the Sierra Nevada is the Global Pandemic Lineage (*Bd*-GPL)  
288 [12,14], but in other areas (*e.g.* Brazil) virulent and non-virulent lineages of *Bd* have been found in the  
289 same populations of hosts [41]. More studies are needed to determine which *Bd* lineages are present in  
290 the Sierra Nevada.

291           There could be other explanations for the differing present-day dynamics of these frog  
292 populations. It could be a result of differences in the pathogen, differences in the host or host  
293 community, and/or differences in habitat (abiotic factors). The virulence hypothesis states that  
294 pathogens gradually lose their highly virulent invasive qualities in order to maintain themselves in host  
295 populations [42, 43]. It could be that after the initial invasion, *Bd* has become less virulent, allowing  
296 remaining frog populations to persist. Another possibility is that instead of *Bd* varying in virulence, it  
297 may be that differences in varying host population genetics at the geographically separate sites could  
298 result in differences in *Bd* susceptibility [44]. In addition, abiotic factors like temperature can limit *Bd*  
299 growth [45]. However, a recent study in the Sierra Nevada found that neither differences in *Bd* strains  
300 from Yosemite and Sequoia-Kings Canyon nor abiotic factors explained differences in host  
301 susceptibility [40]. Instead, differences in host-pathogen dynamics were explained by the geographic  
302 location of the frog populations; frogs from the Yosemite area were less susceptible to *Bd* than frogs  
303 from the Sequoia-Kings area [40]. This further supports the hypothesis that the timing of pathogen  
304 invasion may explain present day differences in *Bd* host dynamics in the contemporary populations in  
305 these areas.

306           At the larger spatial scale of the Sierra Nevada range, our regression analyses show that  
307 anthropogenic factors likely played a role in the arrival and spread of *Bd*, which is similar to previous

308 studies in California [21-24]. We found that, human footprint index in general, and railways, and built  
309 environment specifically, were significant predictors of frog specimen being a *Bd* positive (Table 2).  
310 These results support the idea that *Bd* invaded the high country (areas above 2500m) of the Sierra  
311 Nevada earliest in areas with the most human influence, such as Tioga Pass road in Yosemite National  
312 Park. The last area of the Sierra Nevada to become infected by *Bd*, almost four decades later, appears to  
313 be the Sequoia-Kings Canyon National Parks area, where the largest wilderness areas are located and  
314 access by humans is limited.

315         Archived amphibian museum specimens provide important historical insights that help increase  
316 our understanding of the host-pathogen dynamics of *Bd* at a local, regional, and global scale. For  
317 example, a study in Central America showed *Bd* invasion coincident with collapse of amphibian  
318 communities [32], and this helped explain the timing and losses of amphibian species that had been  
319 proposed as *Bd*-epizootics [5]. Other studies have found a 100-year history of amphibians co-occurring  
320 with *Bd* and no evidence of *Bd* invasion in areas with no known history of *Bd* epizootics [38, 46]. Here  
321 we show an increase in *Bd* prevalence before collapse and disappearance of many amphibians in two  
322 geographically separate locations within the Sierra Nevada four decades apart. We found that *Bd*  
323 became established and began to increase in prevalence in the late 1970's in the central Sierra Nevada  
324 (Yosemite area), which is coincident with declines in those areas [26, 29], and is consistent with the  
325 hypothesis that chytridiomycosis was associated with declines recorded in several Sierra Nevada  
326 amphibian species [2, 26, 29]. We also found *Bd* invasion prior to documented epizootics in the 2000s in  
327 the southern Sierra Nevada [7]. Last, we detected early presence (before the 1970s) of *Bd* at low  
328 prevalence in spatially spread out locations that apparently did not lead to epizootics, which may be  
329 indicative of either an endemic but less virulent lineage of *Bd* or failed historic invasions. Further

330 retrospective studies in conjunction with current field and lab studies are needed to improve our  
331 understanding of pathogen invasion history and host-pathogen dynamics.

332

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345

## 346 **Competing interests**

347 The authors have declared that no competing interests exist.

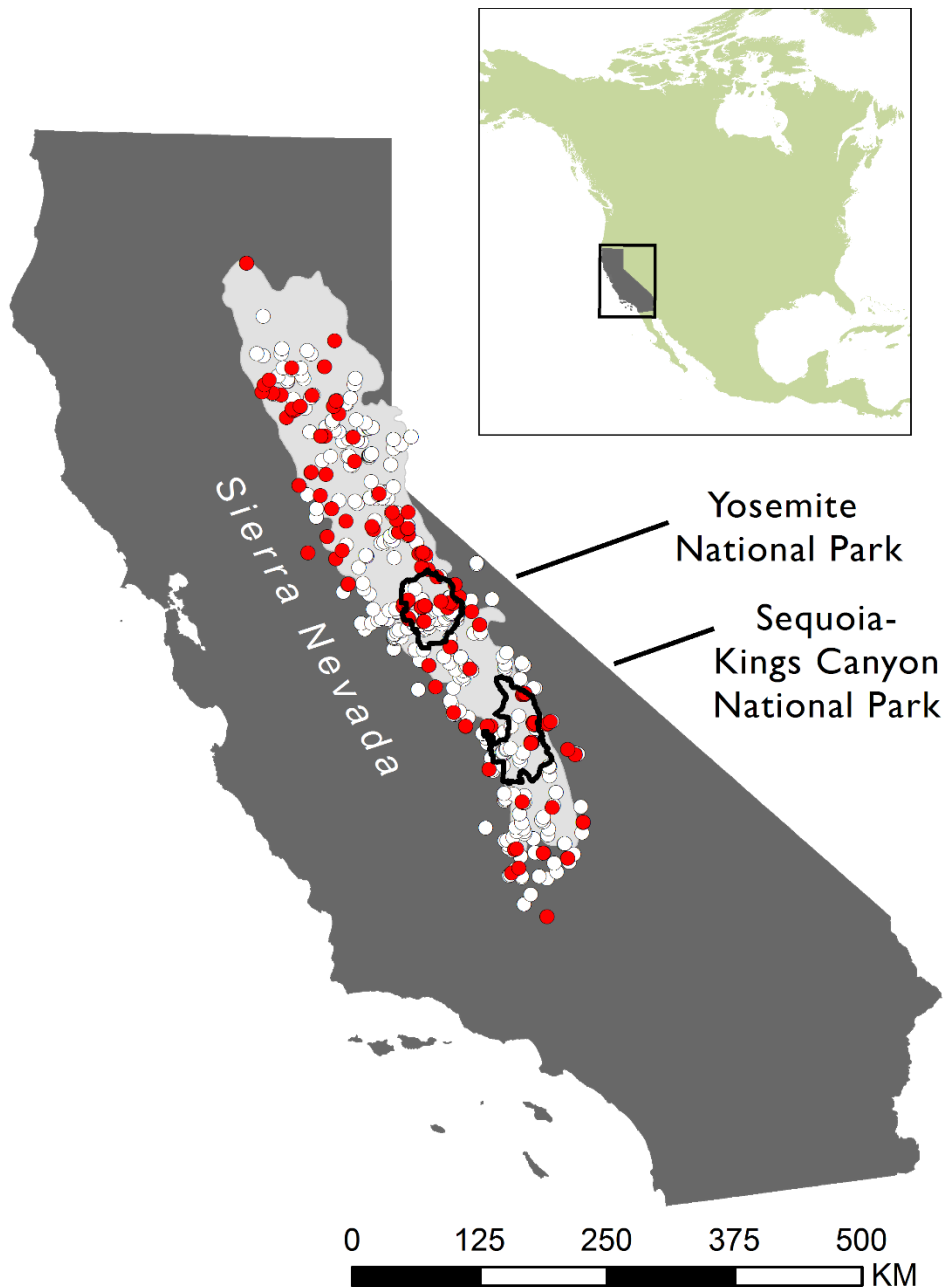
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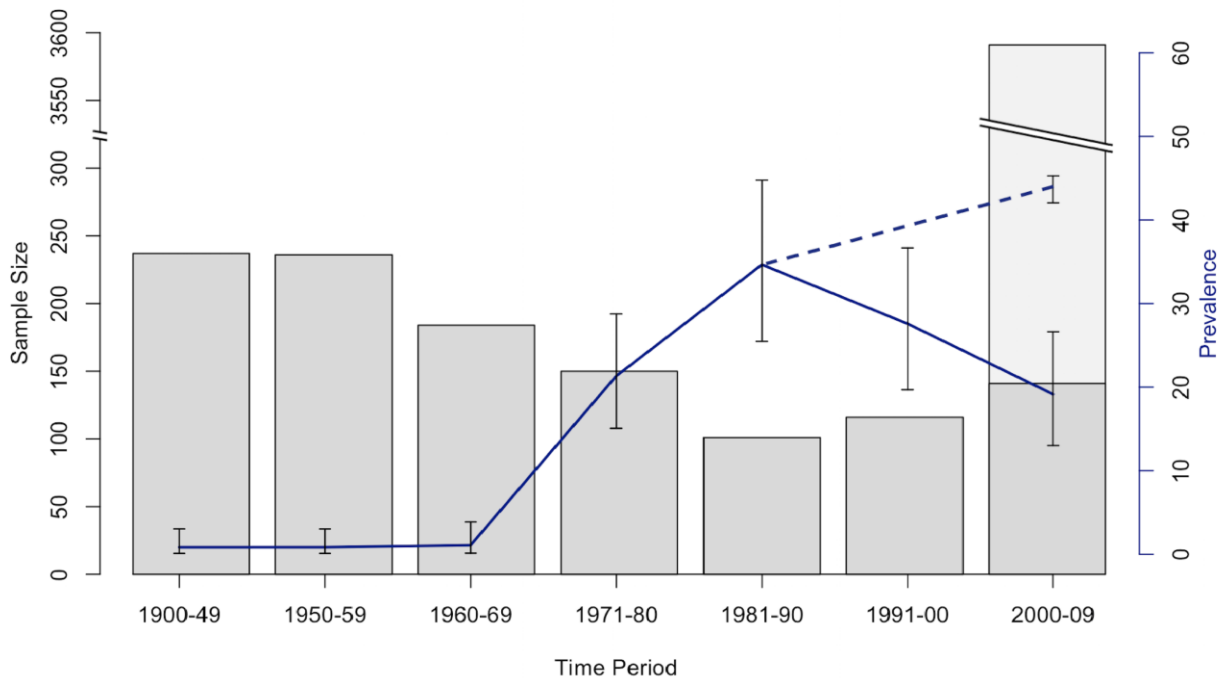
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**Figure 1:** Spatial distribution of 1165 amphibian museum specimens collected between 1900-2005 and tested for *Bd* infection (red circles *Bd*-positive; grey circles *Bd*-negative) in the Sierra Nevada mountains.



**Figure 2:** *Bd* infection prevalence in anurans of the Sierra Nevada from 1900-2009. Bar graphs denote sample size from each time period. Dark gray bars denote samples from museum specimens, and light gray bars denote samples collected from live animals in the field (live animal data from Vredenburg et al. 2010). Blue line denotes *Bd* infection prevalence calculated from museum specimens only, and dotted blue line denotes *Bd* infection prevalence including both museum specimens and live animals in the field (i.e. museum specimens and data from Vredenburg et al. 2010).



**Figure 3:** The incidence (number of new positives in each time period) of *Bd*-positive amphibians in the Sierra Nevada mountain range (light grey) in California from 1900-2009. The earliest *Bd* positives (12/26) detected in Yosemite National Park were in the 1970s (mass die offs documented there in 1978 [Sherman and Morton 1993]). The first *Bd* positives (1586/3492) detected in Sequoia- Kings Canyon National Parks were in the 2000s (mass die offs documented there beginning in 2004; Vredenburg et al. 2010).

