Biological Conservation 192 (2015) 20–29

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/bioc

Reconstructing historical and contemporary disease dynamics: A case study using the California slender salamander

C.M. Sette [a,*], V.T. Vredenburg [b], A.G. Zink [b]

[a] University of California, Santa Cruz, United States
[b] San Francisco State University, United States

ARTICLE INFO

Article history:
Received 10 December 2014
Received in revised form 17 August 2015
Accepted 24 August 2015
Available online xxxx

Keywords:
Disease ecology,
Chytridiomycosis,
Sociality,
Aggregation,
Amphibians,
Plethodontidae

ABSTRACT

The fungal disease chytridiomycosis, caused by the pathogen *Batrachochytrium dendrobatidis* (Bd), has been implicated in the extirpation and extinction of amphibian species throughout the world. Recent Bd epizootics (i.e. epidemics in wildlife) have driven hundreds of species to near-extinction. Several species in California have been severely affected by Bd epizootics, but most work has focused on aquatic species. Our study focused on the most abundant and widespread terrestrial amphibian species in California, the California slender salamander, *Batrachoseps attenuatus*. This species is known to be infected by Bd, but little is known of its disease dynamics. We examined the effect of disease history on contemporary disease dynamics by combining retrospective tests for Bd emergence in museum specimens with contemporary field-collected infection data from the same locations. We found that Bd rapidly emerged in *B. attenuatus* and exhibited a non-linear pattern of spread throughout Northern California, and that modern-day persistence was negatively correlated with time since first detection of infection. To understand what factors are associated with Bd emergence, we correlated standard environmental variables (e.g. temperature, precipitation) from Bd-positive sites with Bd prevalence. We also compared and contrasted the degree of sociality between *B. attenuatus* populations that were recently Bd-infected with those that had a longer history of infection. We found that Bd infection in *B. attenuatus* was positively associated with distance to nearest lentic aquatic habitat, suggesting that aquatic carriers of Bd may be important in prevalence of pathogen within terrestrial *B. attenuatus* populations. Among our 14 field sites, we also found that recently infected populations had larger group sizes, after standardizing for population density, than populations that had been infected over multiple decades. This result suggests that sociality may facilitate disease spread in terrestrial hosts and that populations with longer exposure to this pathogen may evolve away from the ancestral condition of sociality.

1. Introduction

The current global outlook for amphibians is grim (Wake and Vredenburg, 2008). Due to factors such as habitat destruction, climate change, pollution, direct exploitation, the spread of invasive species, and emergent amphibian diseases such as chytridiomycosis (Hof et al., 2011), nearly one third (32.5%) of amphibian species are threatened with extinction, and 43% of amphibian species are experiencing population declines (Stuart et al., 2004). Retrospective studies have found that chytridiomycosis was likely the cause of many of the "enigmatic" amphibian declines (Cheng et al., 2011; Olson et al., 2013) that occurred before the disease was identified in the late 1990's (Berger et al., 1998; Blaustein, 1994; Pechmann and Wilbur, 1994). Chytridiomycosis, caused by the virulent fungal pathogen *Batrachochytrium dendrobatidis* (Bd), has now been implicated in mass amphibian die-offs in western North and Central America, often in protected or remote habitats (Lips et al., 2006; Skerratt et al., 2007; Vredenburg et al., 2010), and is associated with declines and possible extinctions in Europe and Australia (Fisher and Walker, 2009; Skerratt et al., 2007).

Because chytridiomycosis was already widespread before it was described, retrospective studies utilizing museum collections are needed to fill in the timeline of disease emergence. Recently developed techniques for reconstructing the history of Bd in museum collections (Cheng et al., 2011) can, in theory, be combined with contemporary sampling of exact localities in order to address hypotheses about disease emergence and persistence. Common species with large numbers of museum specimens are ideal for this approach, because they allow for unbiased sampling across space and time in order to accurately reconstruct disease history.

The California slender salamander, *Batrachoseps attenuatus*, provides a unique opportunity to perform a retrospective survey of Bd emergence in Northern California. *Batrachoseps attenuatus* is the most abundant and widespread terrestrial species known to host Bd. This species has a large geographic range throughout coastal Northern California into Oregon and in the northern Sierra Nevada Mountains

* Corresponding author.
E-mail address: cmsette@ucsc.edu (C.M. Sette).

http://dx.doi.org/10.1016/j.biocon.2015.08.039
0006-3207/

and is estimated to attain densities of 4470 animals/acre (Anderson, 1960). Additionally, more than 23,000 specimens were collected in the last century and are deposited in permanent museum collections (HerpNet.org), allowing for random subsampling from these extensive samples.

Previous historical studies in California have found early Bd positives dating before the pathogen was first identified, however historical sampling has been both geographically and temporally haphazard. Padgett-Flohr and Hopkins (2009) performed a histological study of ranid frogs and Pacific chorus frogs (*Pseudacris regilla*), concluding that Bd experienced a slow, radial expansion from a late 1950s introduction site in the San Francisco Bay Area. However, their sampling effort was also highly concentrated in that region. Huss et al. (2014) found earlier positives in an introduced species, the American bullfrog, *Rana* (*Lithobates*) *catesbeiana*, in 1928 in Sacramento County, 1929 at Stanford University in Santa Clara County, 1931 in Butte County, and 1957 in Sonoma County (Fig. 1, Fig. S1), suggesting that Bd has been present in California for almost a century. Field observations from 1973-4 in nearby Alameda County reported *B. attenuatus* dying in the wild with clinical symptoms characteristic of chytridiomycosis (Maiorana, 1977a). This later prompted a histological study of 34 historical *B. attenuatus* specimens, which found Bd-positive specimens from as early as 1973 in Contra Costa County, CA (Weinstein, 2009). The same study showed that Bd is almost always fatal to *B. attenuatus* salamanders in captivity.

The arrival or emergence of Bd in naïve amphibian populations is characterized by a rapid increase in Bd prevalence in host populations, while infection intensities on individuals grow to over 4-5 orders of magnitude (Vredenburg et al., 2010). These epizootic events are characterized by high Bd prevalence and transmission rates, and high host mortality (Brem and Lips, 2008; Briggs et al., 2010). Epizootic events generally result in one of two alternatives: the extirpation of host populations or the establishment of more stable pathogen/host dynamics (a sustained enzootic state). Once a disease has become enzootic in a population and the number of susceptible individuals is low, lower transmission rates, disease prevalence, and infection intensities can be expected (Briggs et al., 2010). However most enzootic populations are encountered by collectors after the epizootic phase has passed; therefore a clear reconstruction of the history of disease in a population following the transition from and enzootic to epizootic state is rare.

Utilizing the large museum collections of *B. attenuatus* specimens, we conducted a randomly sampled retrospective survey to describe the spread of Bd across N. California. We evaluated the effect of disease history (based on museum specimens) on the disease presence in contemporary populations. We also evaluated historical and contemporary Bd levels with respect to several biotic and abiotic factors that may affect the ecology of Bd (e.g. temperature, precipitation, proximity to standing water). Finally, we also recorded salamander group size to examine the relationship between social behavior and disease dynamics. Given *B. attenuatus*' large range and the time elapsed since Bd's introduction to California, we predicted that a retrospective survey would reveal variation among populations in their Bd infection histories. By evaluating both historical and contemporary populations of B. attenuatus, at the same locations, we were able to reveal important insights into the temporal dynamics of Bd, as well as the ecological drivers of Bd infections.

2. Methods

2.1. Historical survey with museum specimens

We generated a list of 15,007 available *B. attenuatus* museum specimens housed at the Museum of Vertebrate Zoology in Berkeley and the California Academy of Sciences (using http://www.herpnet2.org). The 12 California counties with the highest museum representation spanning seven decades, 1940-2009, were chosen to be included in this study (Alameda, Butte, Contra Costa, Del Norte, Humboldt,

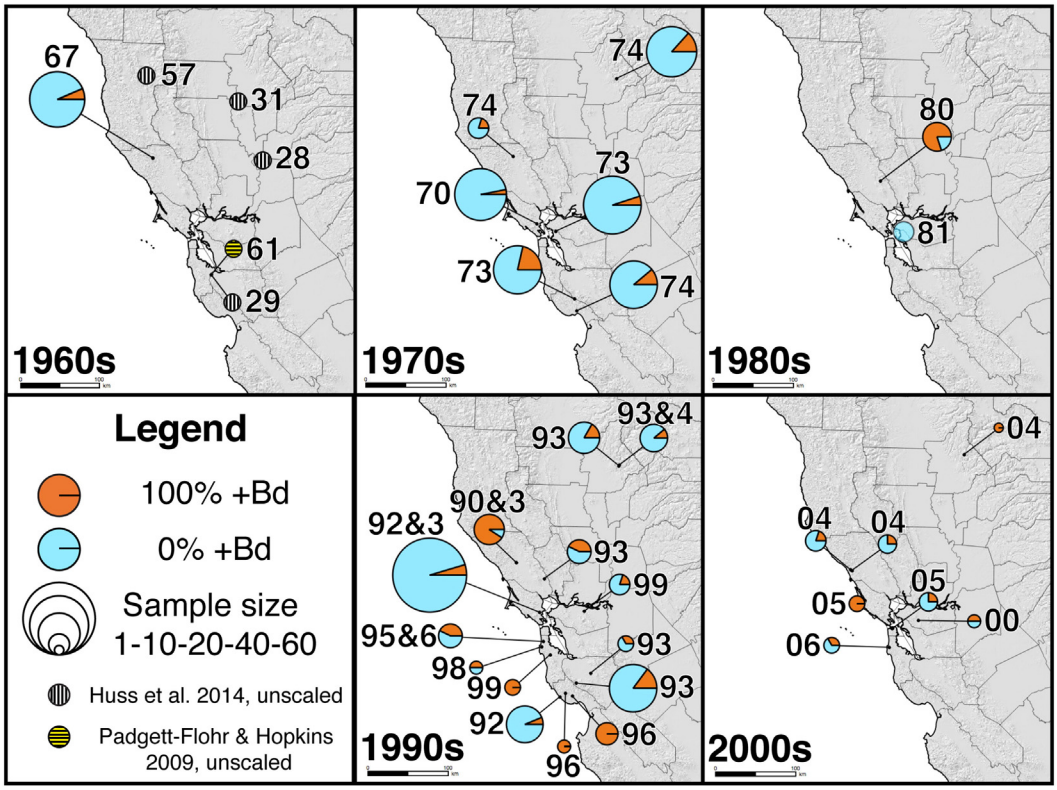

Fig. 1. Map of historical Bd-positive sites by decade. Charts are proportional to the number of specimens from each site with the proportion of Bd-negative specimens in blue and proportion of Bd-positive specimens in orange. The numbers outside the circles indicate the year(s) of sampling. The early positives from Huss et al., 2014 are in white and the 1961 positive from Padgett-Flohr and Hopkins (2009) is in yellow, not scaled.

Marin, Mendocino, San Francisco, San Mateo, Santa Clara, Santa Cruz, Sonoma; Fig. S1). Up to twenty specimens from each of these 12 counties were randomly selected from all available for each of the 7 decades (20/county/decade). Specimens were tested from 458 separate localities, based on the museum record. Due to collector bias, occasionally the randomly-selected 20 samples included multiple samples from the same locality.

The selection process generated a list of 1266 museum specimens (Fig. S2) which were then sampled for the presence of Bd using standard skin swabbing techniques (Boyle et al., 2004; Hyatt et al., 2007). Specimens were stroked 30 times with a MW113 dry swab (Medical Wire and Equipment Company) – ten times dorsally and ventrally, and five times on each laterum, spanning the majority of individual's body length. To decrease the chance of cross contamination between specimens, each specimen was rinsed in 70% EtOH before swabbing and gloves were changed between handling every specimen. Swabs were stored in 1.5-mL microcentrifuge tubes at 4°C until DNA extraction and qPCR (Cheng et al., 2011).

We used standard Bd DNA extraction and real-time quantitative PCR methods (Boyle et al., 2004; Hyatt et al., 2007). These methods have been validated both in live specimens and formalin-fixed museum specimens stored in 70% ethanol (Cheng et al., 2011; Richards-Hrdlicka, 2012). In museum specimens, qPCR has nearly the same Bd detection rate as histology when run in triplicate; in singlicate, qPCR has about 60% the detection rate of histology (Cheng et al., 2011). Due to cost considerations, samples were run in singlicate rather than in triplicate. Genomic equivalent results were multiplied by 80, the dilution factor in qPCR sample preparation, to estimate the number of zoospores on the entire swab (Z_{swab}). Bd-positive samples were defined as having a Z_{swab} score > zero. DNA extractions from positive Bd samples were re-run in triplicate and the Z_{swab} scores were then averaged across the three runs.

2.2. Field sampling in contemporary populations and historical population sampling

Of the 27 Bd-positive localities derived from the historical survey, spanning all decades, five localities per decade were randomly selected and numbered sequentially from the earliest to the most recent historical positive. We revisited these 17 sites (the 1960s and 1980s had only 1 positive site) from February – May of 2013 (Fig. S3). No salamanders were found at three sites in Butte County, leaving a total of 14 field sites. Every effort was made to sample as near to the geo-referenced collection site as possible, however unavoidable geographic error estimation in older sampling sites combined with modernization and habitat alteration (e.g. land development) made it impossible to sample at the exact same historical locations at four sites (site numbers 3, 5, 7, 12). In these four cases, we sampled from the closest possible available habitat. Localities sampled ranged from 36.08 to 1355.01 m (distances averaged across all cover items where salamanders were found) from the historical locality.

Up to 30 *B. attenuatus* were swabbed and released at each of the 14 sites. Snout-vent length (SVL) and tail length (TL) were measured for every salamander caught following Hendrickson (1954). Handling time was minimal (i.e. < 5 min). When other salamander species were encountered, they were also swabbed. Swabs were air-dried and stored in 1.5-mL microcentrifuge tubes at 4°C until DNA extraction and PCR. GPS locations were recorded using a Garmin GPSMAP 62stc Handheld Navigator GPS. We also recorded the area of the cover object where salamanders were found. For example, the length and diameter of a log were multiplied together to get total area. When sampling under leaf litter, approximately a 1 m^2 area was cleared. This allowed us to count the number of individuals per group and calculate density of individuals within the group (relative to the cover item defining that group or cleared leaf litter area). We swabbed 389 *B. attenuatus* across all of the 14 field sites where salamanders were found (Table 1). Of these, 34 tested positive for Bd.

2.3. Second round of historical sampling at Bd-positive sites

A second round of historical sampling tested all additional museum specimens that were collected between 1940-2009 at Bd-positive sites identified by our first round of historical sampling. This was to insure that our analyses included the first detectable positive at each locality. In order for historical sampling to encompass areas comparable to the areas sampled in the field in 2013, additional historical specimens were sampled based on the comparable field areas. Due to land use changes and irregular scattering of suitable cover objects for salamanders, the areas searched in order to find a maximum of 30 individuals in 2013 differed widely between field sites (e.g. two field sites were over 1000 m from the historical locality). Distances between salamanders sampled in the field (2013) and the exact historical localities were calculated in Quantum GIS (http://qgis.osgeo.org). The 3rd quartile distance of all field sampling sites from their historical positives, 538.20 m, was used because the distribution of these distances had a long positive-skewed tail of distribution. Quantum GIS was used to generate a list of additional HerpNet museum specimen search results within a radius of 538.20 m around each historical Bd-positive locality. For the four field sites further than 538.20 m from the historical locality, the distance between historical and field sites was used instead of 538.20 m. All additional historical specimens within selected radii were sampled as before: resulting in 311 additional specimens across all 17 sites for a total of 1577 historical samples.

Table 1
Results of 2013 field re-survey of historically positive localities by site. "Historical positive" refers to the oldest historical positive at that site.

Site	County	Historical positive	*B. attenuatus*	Field + Bd	Field % Bd	95% Credible Interval	Field ZE avg.
1	**Sonoma**	**1967**	**28**	0	0.00%	0 – 11.94%	0
2	**Alameda**	**1973**	**30**	4	**13.33%**	5.45 – 29.83%	**0.8832**
3	**Santa Cruz**	**1973**	**29**	0	0.00%	0 – 11.57%	0
4	**Butte**	**1974**	0		--		--
5	**Santa Cruz**	**1974**	**22**	0	0.00%	0 – 14.82%	0
6	**Sonoma**	**1974**	**31**	0	0.00%	0 – 10.89%	0
7	**Sonoma**	**1980**	**11**	0	0.00%	0 – 26.46%	0
8	**Santa Cruz**	**1992**	**9**	0	0.00%	0 – 30.85%	0
9	**Butte**	**1993**	0		--		--
10	**San Mateo**	**1997**	**35**	4	**11.43%**	4.67 – 26.06%	**1.261**
11	**San Mateo**	**1998**	**24**	0	0.00%	0 – 13.72%	0
12	**San Mateo**	**1999**	**32**	6	**21.88%**	11.09 – 38.91%	**220.4**
13	**Contra Costa**	**2000**	**36**	2	**5.56%**	1.70 – 18.19%	**6.749**
14	**Butte**	**2004**	0		--		--
15	**Sonoma**	**2004**	**32**	3	**9.38%**	3.40 – 24.33%	**3.237**
16	**San Francisco**	**2005**	**33**	4	**12.12%**	4.95 – 27.45%	**2.350**
17	**San Francisco**	**2005**	**37**	10	**27.03%**	15.42 – 43.10%	**36.77**

2.4. Climate and terrain data correlations for historically positive Bd sites

Generalized linear mixed modeling (GLMM) was used to examine biotic and abiotic environmental factors that correlate with Bd occurrence in both historical (museum) and contemporary (field) populations. We included monthly mean maximum temperature because Bd zoospore growth is strongly influenced by temperature. Bd growth is enhanced in culture by increased temperatures up to 30°C, above which zoospores experience rapid mortality (Piotrowski et al., 2004). However, *in vivo* host defenses are more effective at curbing Bd growth at higher temperatures (Raffel et al., 2012). During the months that *B. attenuatus* are active aboveground, temperatures rarely reach Bd-lethal levels, yet temperature likely contributes to both Bd growth and host defenses. The majority of historical specimens with collection dates available were collected during October-April, the times of year with the most precipitation and generally lower temperatures, therefore we did not explore seasonal variation in Bd prevalence. We explored elevation as a factor because greater amphibian loss due to Bd has been observed at higher elevations in tropical and subtropical regions (Wake, 2012; Woodhams and Alford, 2005), although this pattern has not yet been observed in temperate regions (Knapp et al., 2011; Piovia-Scott et al., 2011). We used mean monthly precipitation in the model because *B. attenuatus* are more active during rainy months (Hendrickson, 1954) and are potentially exposed to Bd at a higher frequency during this time. Distance to permanent water bodies was included in the model as Bd is primarily an aquatic disease; we hypothesize local Bd emergence in terrestrial salamanders (e.g. *B. attenuatus*) is in part due to pathogen dispersal from permanent water sources. Distance to the nearest highway (both US and state) was used as a proxy for distance to human disturbance, as land use and development have changed over the historically relevant time period. The effect of the year specimens were collected on Bd prevalence was also explored.

Climate data were obtained from PRISM Climate Group, Oregon State University (http://www.prism.oregonstate.edu) for field and Bd-positive historical sites within every year of specimen collection. Quantum GIS was used to extract values from monthly mean maximum temperature and total monthly precipitation map raster layers for field sample localities. Yearly averages were calculated for the entire year (Jan-Dec) encompassing collection date of historical (museum) samples, or 12 months preceding the collection date of all field samples. Elevation data for both historical and field specimens were obtained from the U.S. Geological Survey, Department of the Interior (http://viewer.nationalmap.gov).

An inland hydrologic features basemap was downloaded from Cal-Altas at CA.gov (http://atlas.ca.gov/). Water feature types categorized as "reservoirs, lake, all bays, swamp, pond" were included in the *permanent still freshwater* (lentic waters) map layer. Additionally, the features "slough, coves, cuts, lagoons, bypasses, bends of water, channels, deep water channels" and "lakes, bays, swamps, sloughs, and ponds" were included in the *permanent freshwater* map layer (lentic and lotic waters). Brackish water, including sloughs and bays, were excluded from the data. GIS basemap for state highways (2000) and US highways (2000) were downloaded from Cal-Altas at CA.gov (http://atlas.ca.gov/). Quantum GIS was used to generate distances between sample locations and water layers and historical positives and highway map layers.

2.5. Statistical analysis

All statistical analyses were performed using the R statistical environment (R Development Core Team, 2010). The "geoR" package for R (Ribeiro and Diggle, 2007) was used to test for spatial autocorrelation, non-independence of samples based on spatial structure of the data, among historical positives. Spatial analysis was performed using the "akima" package (Gebhardt, 2013) to interpolate the spatio-temporal progression of Bd using historical positives from this study, as well as positives from other historical studies performed in the same geographic region. Because Butte County is geographically discontinuous from the other counties sampled in this study (Fig. S1), we excluded Butte County positives from the analysis of spatial spread of Bd. *Batrachoseps attenuatus* are largely absent from California's Central Valley (the area between Butte County and the remaining geographic region we sampled, i.e. the greater Bay Area), therefore museum records from this region are rare (Jockusch et al., 2007). The spatial analysis also included *B. attenuatus* positives from Weinstein, 2009, the 1957 *Rana catesbeiana* positive from Sonoma County from Huss et al., 2014, and the 1961 positive from Padgett-Flohr and Hopkins, 2009.

For the historical population survey, GLMM with zero-inflation and a binomial distribution was performed using the "glmmADMB" package (Skaug et al., 2014) which utilizes AD Model Builder (Fournier et al., 2012). Our response variable was the presence or absence of Bd in each of the 424 museum samples collected from Bd-positive sites. Non-independence of samples and sampling regime, respectively, were accounted for by using Site and County as random factors in a mixed-effects model. The environmental factors explored were: year of specimen collection (Year), elevation of collection site (Elev), mean monthly maximum temperature at site in the year sample was collected (MaxTemp), mean monthly precipitation at site in the year sample was collected (Precip), distance to any permanent freshwater (WaterDist), and distance to the nearest California or US highway (HwyDist) (Fig. S4). All factors except Year were centered and scaled following Gelman and Hill (2007). Because environmental factors could not have affected the presence or absence of Bd in individuals before Bd originally arrived at a given site, all of the model factors were explored as interactions with Year. We additionally included the three-way interaction between Year, precipitation (Precip), and temperature (MaxTemp) to test whether temperature and precipitation work in concert to drive Bd infection. Finally, a reverse stepwise procedure was used to build a multivariable model which only contained statistically significant factors associated with Bd presence. Wald's test was used to compare AIC values to select the best model. It is important to note that we interpret the main effects as informative only when they do not appear in significant interactions. This is due to our understanding that main effects that are also involved in significant interactions cannot be considered independent. Therefore interpreting the significance and direction of these main effects independently from their interactions can mask any non-linear trends within one main effect at different levels of the interaction term (Underwood, 1997).

For field samples (2013 only), a hurdle GLMM was performed using "glmmADMB" using a reverse stepwise procedure. The response variable was the number of Bd-positive salamanders per cover item across 17 field sites. The log of the total number of salamanders per cover item was used as an offset due to differences in sample number between cover items. Site and County were included as random effects. A hurdle model was chosen in order to compare the factors which contribute to Bd presence/absence and those which contribute to increased abundance under particular cover objects at contemporary sites. In the first part of the hurdle model, a binomial distribution was used to model the probability of presence or absence of Bd. In the second part of the model, a Poisson distribution was used to model count data, the number of Bd-positive salamanders per cover (Bolker et al., 2009). Furthermore, Bd prevalence counts were zero-inflated, and zero values could result either from the true absence of Bd-positive specimens, or unsampled Bd-positive specimens (i.e. burrowing at the time of sampling) (Martin et al., 2005; Zuur et al., 2009).

The following centered and scaled factors were explored in the GLMM: age of the historical positive (YHist), distance of cover item from the earliest historical positive (DHist), area of cover object (Cover), ratio of *B. attenuatus*' tail length to snout-vent length (Condition), elevation (Elev), mean monthly maximum temperature (MaxTemp), mean monthly precipitation (Precip), distance to permanent still freshwater (LakeDist), distance to any permanent freshwater

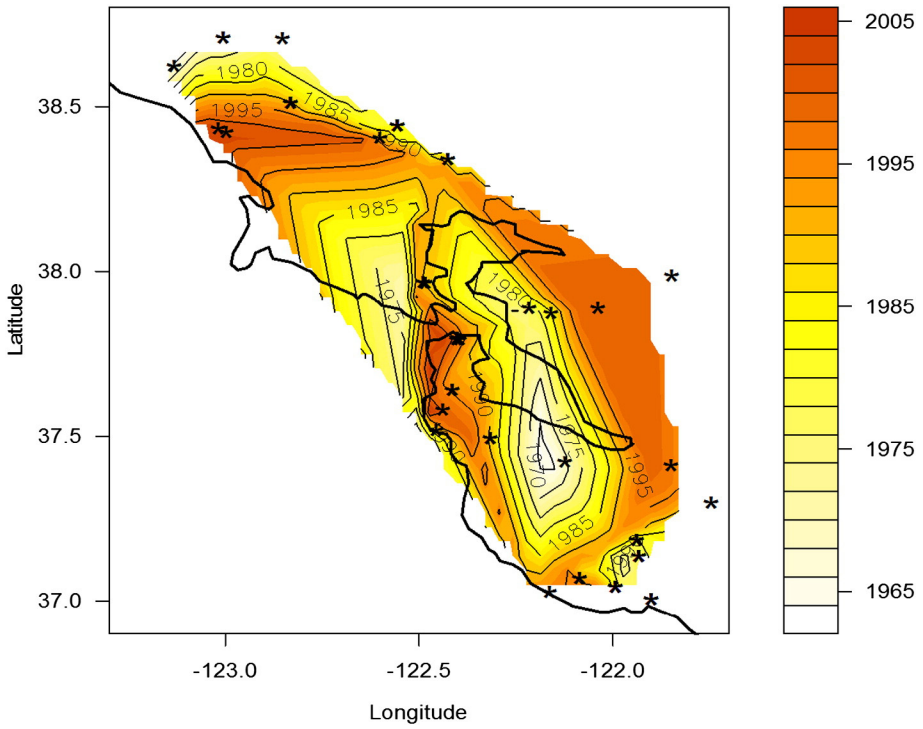

Fig. 2. Modeled spatio-temporal spread of Bd in the greater Bay Area of California based on historical positives. Temporal spread of Bd has been interpolated using the localities and years of Bd-positive *B. attenuatus* from this historical survey, excluding those in Butte County, and including additional historical *B. attenuatus* positives (Weinstein, 2009), a 1957 *Rana catesbeiana* positive in Sonoma County (Huss et al., 2014), and the 1961 positive *Rana catesbeiana* in Santa Clara County (Padgett-Flohr and Hopkins, 2009).

(WaterDist), and distance to the nearest California or US highway (HwyDist) (Fig. S4).

95% credible intervals for historical and field Z_{swab} scores were calculated using the *credint* function in the R package "emdbook" (Bolker, 2013). The *prop.test* function in R was used to compare the equality of proportions to compare prevalence between decades. A two-tailed Welch t-test was used to compare Z_{swab} scores between decades. Additionally, because snout-vent length is not proportional to age once sexual maturity has been reached (Wake and Castanet, 1995), condition, the ratio of *B. attenuatus*' tail length to snout-vent length, was compared between infected and uninfected field specimens within juvenile and adult size groups respectively using the Welch t-test function in R. Linear regression was used to explore the relationships between the time since first historical detection of Bd at a site and proportion of infected *B. attenuatus*, *B. attenuatus* density, and *B. attenuatus* aggregation size.

3. Results

3.1. Linking historical and contemporary spread of Bd

Of the 1266 specimens included in the retrospective survey, 40 specimens from 27 distinct localities tested positive for Bd. The earliest positive detected was a 1967 specimen collected in Sonoma County. By the early 1970s, Bd was present in Sonoma, Santa Cruz, and Butte Counties (Fig. 1). Bd was not detected in the northernmost coastal counties Mendocino, Humboldt, and Del Norte in any decade. The second round of historical sampling at Bd-positive sites added 311 specimens at historically positive sites, allowing for comparison between historically positive populations with varying prevalence of Bd. It is important to note that several sites had been sampled in multiple years but that expanded (second round) sampling sometimes changed year of first positive for a given site. In one case, the increased second-round sampling at a Marin County site revealed that Bd arrived two decades earlier than was detected in the initial survey.

We modeled the spatio-temporal spread of Bd in the greater Bay Area of California (Fig. 2), based on georeferenced historical positives from this study and other studies performed within the same California region. The map shows a complex pattern of Bd spread through the region. Bd prevalence increased from its appearance in the late 1960s and levels off after the 1990s (Fig. 3). The equal proportions test shows that Bd increased significantly between the 1960s and 1970's (p-value = 0.0342), experienced no significant change between the 1970's and 1980's (p-value = 0.7090), and increased significantly between the 1980's and 1990's (p-value < 0.01). Neither the drop in prevalence between the 1990s and 2000s (p-value = 0.2537) nor the drop in infection intensity (t-test: t = 0.7321, df = 10.4, p-value = 0.4803) was statistically significant. The highest Z_{swab} scores for the years 2000-09

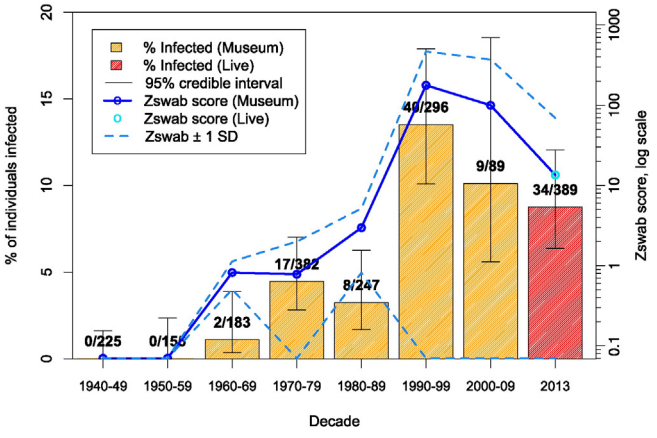

Fig. 3. Graph of historical and field sampling results by decade. Orange bars (left axis) show proportion of Bd-positive museum specimens per decade. 2013 Field results from live animals are in red. Whiskers show Bayesian 95% credible intervals. Solid blue line (right axis) shows average Z_{swab} scores per decade. Dotted lines show ± 1 SD, The highest Z_{swab} scores for 2000-09 and 2013 were excluded, as these were outliers. Labels are number of positives over sample size per decade.

and 2013 (2000-09: 1653, 2013: 4518) were excluded from Fig. 3 and subsequent analyses as these were extreme outliers compared to other infection intensities in those decades (2000-09 Z_{swab}: 0.1251-769.6, 2013 Z_{swab}: 0.0029-322.1). Bd prevalence at contemporary field sites was significantly lower than prevalence in the 1990s (p-value = 0. 0308), but not significantly lower than the 2000s (p-value = 0.4197). The same is true for Z_{swab} values (1990s vs. 2013 t-test: t = 3.5318, df = 43.629, p-value = < 0.01; 2000s vs. 2013 t-test: t = 0.8958, df = 7.144, p-value = 0.3995).

Many of the sites with early historical positives were Bd-free in 2013 (Fig. 4). There was a significant negative relationship between the proportion of individuals infected at a site and the number of years since first historical detection (p-value = 0.024). The average Z_{swab} score for the 1990's was 177.2 (SD 290.3) and the global average Z_{swab} score of *B. attenuatus* sampled in 2013 was 145.9 (SD 774.5), however this was dramatically raised by two individuals from sites 12 and 17. Excluding the highest score from these two 2013 sites reduced the average Z_{swab} score to 99.67 (SD 270.8), and removing both scores gave an average Z_{swab} score of 3.818 (SD 5.829), indicating low infection intensities in the majority of contemporary specimens.

3.2. Environmental Correlates of Bd presence in historical and contemporary specimens

At this large (regional) scale of Nothern California, we found no spatial autocorrelation among Bd-positive localities in the historical analysis. Bd prevalence across all museum specimens sampled from historically positive sites was affected by the three-way interaction between the year that the sample was collected (Year), mean monthly precipitation in that year (Precip), mean monthly maximum temperature in that year (MaxTemp), as well as the interaction between Year and distance to permanent freshwater (WaterDist) (df:12, AIC: 318.156) (Table 2).

In the hurdle GLMM, different factors were included in the binomial and Poisson portions of the model (Table 3). In the binomial GLMM, age of the historical positive (YHist) had a negative relationship with the probability of Bd positives at a site, indicating that more recently infected sites were more likely to have Bd. Also, sites further from permanent still freshwater (LakeDist) were less likely to have Bd-positive salamanders. Only the intercept was included in the final model in the Poisson portion of the hurdle model.

Table 2
Explanatory variables included in historical GLMM. Factors are Year, mean monthly precipitation (Precip), mean monthly maximum temperature (MaxTemp), and distance to permanent freshwater (WaterDist). All factors except Year are centered and scaled.

	Estimate	z Value	Pr (>|z|)
(Intercept)	-73.14	-1.36	0.1739
Year	0.0360	1.33	0.1831
Precip	240.7	2.27	0.0234
MaxTemp	-201.1	-1.64	0.1004
WaterDist	333.9	2.59	<0.01
Year x Precip	-0.1211	-2.27	0.0233
Year x MaxTemp	0.1025	1.66	0.0966
Year x WaterDist	-0.1678	-2.58	<0.01
Precip x MaxTemp	-832.80	-2.73	<0.01
Year x Precip x MaxTemp	0.4207	2.75	<0.01

3.3. Linking group size and density with historical and contemporary Bd

Linear regression showed no trend in *B. attenuatus* population density (which factors in the area of cover objects) by years since detectable infection per site ($F_{1, 214} = 1.10$, Pr(>|t|) = 0.30) (Fig. 5a), however, there was a significantly negative relationship between the total number of *B. attenuatus* under a cover object and the years since detectable infection ($F_{1, 214} = 17.91$, Pr(>|t|) < 0.01) (Fig. 5b). Group size did not correlate with the area of cover objects ($F_{1, 214} = 1.753$, Pr(>|t|) = 0.1870) suggesting that cover items are not limiting and groups form through social aggregations. In the populations surveyed in 2013, larger groups were more likely than smaller groups to have Bd-infected individuals ($F_{1, 214} = 42.14$, Pr(>|t|) < 0.01). Additionally, the proportion of Bd-positive *B. attenuatus* under a given cover item had a strong positive relationship with group size both across all cover items ($F_{1, 214} = 10.21$, Pr(>|t|) < 0.01) and Bd-positive cover items alone ($F_{1, 20} = 8.579$, Pr(>|t|) < 0.01).

We compared salamander condition between Bd-positive and Bd-negative groups across field sites. Adults and juvenile size classes had significantly different condition scores (t-test: t = 3.5461, df = 266.538, p-value < 0.01), consistent with expectation that juveniles have smaller resource stores (Maiorana, 1977b). Within age classes,

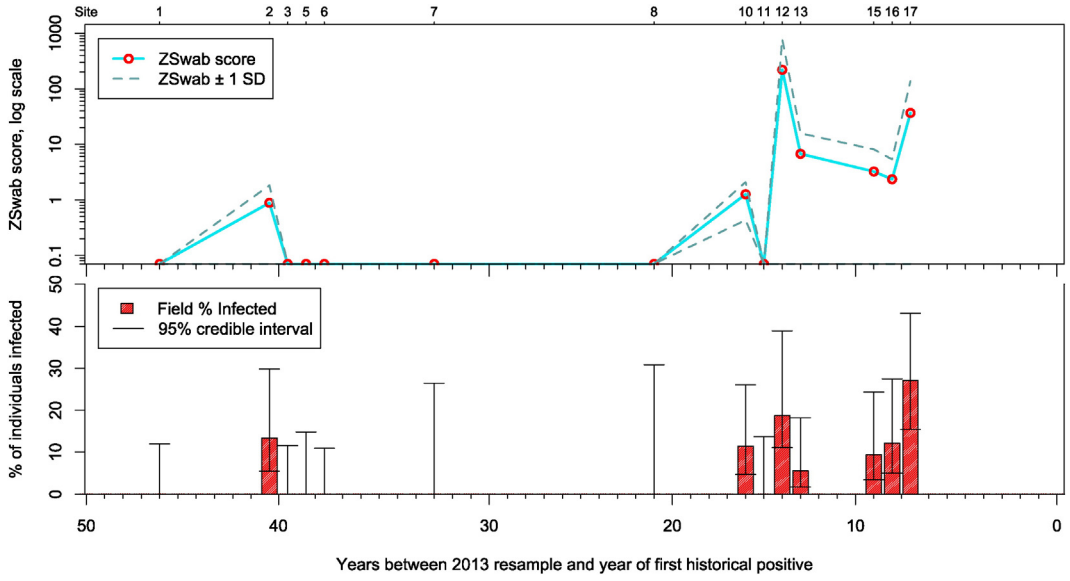

Fig. 4. Contemporary field sampling results by site. Sites are labeled (1-17) above and arranged by the number of years between the field re-sampling (2013) and earliest detected historical positive at that site. The solid red circles (top pane) show average Z_{swab} scores per site, with dotted lines for ± 1 SD. Red bars (bottom pane) show the proportion of Bd-positive individuals per site. Whiskers show Bayesian 95% credible intervals.

Table 3
Explanatory variables included in field hurdle GLMM. Factors are age of the historical positive (YHist) and distance to permanent still freshwater (LakeDist). All factors are centered and scaled.

Binomial distribution	Estimate	z Value	Pr (>|z|)
(Intercept)	-2.83	-5.56	<0.01
YHist	-2.52	-2.68	<0.01
LakeDist	-1.48	-1.68	0.0939
Poisson distribution	Estimate	z Value	Pr (>|z|)
(Intercept)	-0.88	-5.13	<0.01

Bd-positive and negative individuals did not have significantly different condition scores – for adults (t-test: t = 0.5582, df = 31.383, p-value = 0.5806) and juveniles (t-test: t = 0.365, df = 14.455, p-value = 0.7204).

4. Discussion

The emergence of Bd in the 1960's in our samples and subsequent proliferation corresponds with known amphibian die-offs in California beginning in the 1970's and continuing through the present day (Maiorana, 1977a; Bradford, 1991; Weinstein, 2009; Vredenburg et al., 2010). On the other hand, we found no positive Bd individuals from the 1940's and 50's. Our retrospective survey of over 1500 samples suggests that Bd likely experienced saltatory spread through California, given the distances between the earliest known Bd-positive sites. Previously discovered 1950's and 60's positives in Sonoma County and Santa Clara County (Padgett-Flohr and Hopkins, 2009; Huss et al., 2014) are also consistent with the timing and pattern of Bd emergence and geographic spread in *B. attenuatus*.

Disease ecology theory predicts that a pathogen will fade out when its host population is driven below a minimum threshold density (De Castro and Bolker, 2004; May and Anderson, 1979). It is possible that early positives from the 1920s and 1930s in California (Huss et al., 2014) were Bd strains with lower pathogenicity or failed invasions. While we systematically sampled California counties with the most consistent historical records, we did not sample all counties available. Additional sampling to increase geographic coverage or likelihood of finding early Bd positives present at low frequencies in the host population may improve the accuracy of our spatio-temporal spread models. Unfortunately, there was insufficient DNA to sequence Bd DNA to determine the Bd strain, either in this study, Huss et al. (2014), or the Padgett-Flor study (2009).

The rapid geographic spread of Bd revealed in this study was likely due to a combination of factors including multiple introduction points, anthropogenic spread (Daszak et al., 2003; McKenzie and Peterson, 2012), and other host vectors with larger dispersal distances. Though *B. attenuatus'* range is continuous, it nevertheless contains reproductively isolated and deeply genetically differentiated populations (Jockusch et al., 2007). This extreme lack of gene flow across *B. attenuatus*' range, in combination with behavioral observations of short dispersal distances (Hendrickson, 1954) and a lack of spatial autocorrelation among Bd-positive historical sites (this study) strongly imply that *B. attenuatus* hosts are unlikely to spread disease across large distances. However, *B. attenuatus'* high population density presents a potential carpet of Bd hosts, making it an important backdrop to document spread across a region. Further historical sampling in outlying areas and the inclusion of historical results from other species may further resolve patterns of Bd emergence in California.

In completely terrestrial species such as *B. attenuatus*, the effect of social behavior on Bd transmission may be stronger than in aquatic hosts. In aquatic systems, zoospores can swim to other hosts, but because Bd is susceptible to desiccation on land (Piotrowski et al., 2004), zoospore transmission is likely to depend on host behaviors such as sociality. Our historical GLMM results show that distance to any permanent (lentic or lotic) freshwater source interacts with year to influence Bd prevalence. The negative interaction between year and distance to fresh water suggests that distance to water played a larger role in Bd presence earlier in the historical record for *B. attenuatus* specimens. Though we did not explicitly test which species are the most likely potential hosts for between-species transmission of Bd, based on published ranges, aquatic amphibian species with larger home ranges are likely candidates. Potential carriers of Bd in California are the invasive African clawed frog, *Xenopus laevis* (Vredenburg et al., 2013), the invasive American bullfrog, *Rana catesbeiana* (Huss et al., 2014), and the native Pacific treefrog, *Pseudacris regilla* (Pessier and Vredenburg, 2012). All three species are highly tolerant of Bd infection (Daszak et al., 2003; Parker et al., 2002; Reeder et al., 2012) and are found throughout *B. attenuatus'* range.

We propose that across the *B. attenuatus* populations we surveyed, there was a potential switch from epizootic to enzootic disease dynamics, characteristic of novel disease emergence. First, there has been a stabilization of Bd prevalence since the 1990s. The lower availability of

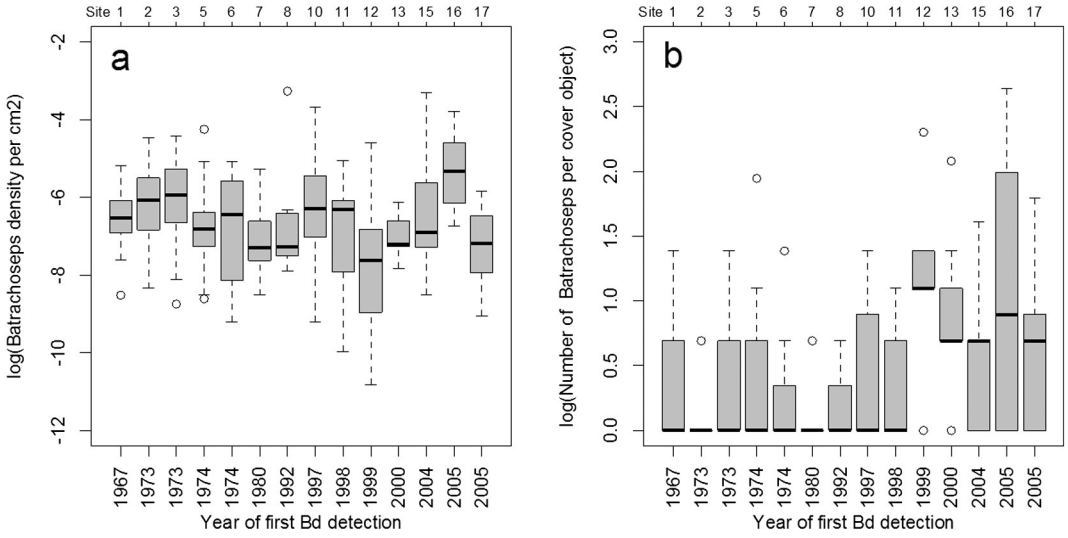

Fig. 5. The density of *B. attenuatus* did not differ across sites that differed in years since first detection of Bd (6a Boxplot of log(*B. attenuatus* density per cm^2) and Year of first Bd detection at site); however, the number of *B. attenuatus* found under a cover object was significantly higher for populations with more recent detection of Bd infection (b Boxplot of log(the total number of B. attenuatus per cover object) and Year of first Bd detection at site).

historical specimens collected in the 2000s could account for the difficulty in resolving statistical significance between 1990s and 2000s prevalence. However, 2013 field samples support the existence of a stabilization Bd infection prevalence in *B. attenuatus*, reinforced by very low infection intensities in all but two contemporary specimens. Additionally, Bd presence at contemporary sites negatively correlates with time since historical exposure. We also found no significant drop in body condition of Bd-infected *B. attenuatus* in populations with prior exposure to the pathogen. While our measures of body condition may not correlate directly with the effects of Bd, a lack of difference could be explained by the evolution of host resistance to Bd (immunological or behavioral). Alternative explanations for low prevalence at contemporary sites are simple density-dependent host parasite dynamics, or stochasticity – the random loss of infection over time due to low infection prevalence. Taken together, though, these results suggest a shift from epizootics upon first arrival of Bd to an eventual enzootic or more stable state.

The binomial portion of the hurdle GLMM shows that *B. attenuatus* individuals have a higher probability of having Bd when their populations are closer to lentic standing waters. The three potential carriers of Bd inhabit lentic waters. Likewise, the earliest positives in California were found in *R. catesbeiana* museum specimens (Huss et al., 2014), and one juvenile specimen was collected near Lake Sonoma in 1964 indicating a breeding population was present at the lake shortly before the local *B. attenuatus* population tested positive for Bd.

The three-way interaction between year, precipitation, and mean monthly maximum temperature in the historical GLMM indicates that Bd prevalence varies non-linearly with different combinations of temperature and precipitation over time. By plotting these variables against one another (fig. S6), we can see trends in Bd levels across these variables. Historical Bd-positives become more abundant over time, but positives occur more frequent both at higher temperatures and at higher precipitation, indicating that warm, wet years may increasingly contribute to Bd prevalence in *B. attenuatus*, particularly as Bd becomes more widespread. Warmer temperatures enhance Bd growth, however temperatures above 28°C are lethal to Bd (Piotrowski et al., 2004). In the regions in question, temperatures do reach those levels during summer months. However, during hot and dry periods, *B. attenuatus* retreat underground to cool temperatures. This behavior may decrease social interactions (exposure to conspecifics) and disease transmission. Conversely, *B. attenuatus* are more active during the rainy season, which may increase transmission, particularly at warmer temperatures.

We measured sociality simply as the number of individuals found in close proximity to each other under one cover item as these animals are found under cover objects (mostly downed wood, logs, etc.) and can easily move among cover items. Given that in all surveyed populations, the majority of cover objects were not occupied, pairings were by active choice rather than a byproduct of habitat saturation. Salamander group size did not correlate with the size of cover objects; in populations with more recently detected infection, salamanders formed larger aggregations under larger cover objects, while at sites with earlier first date of infection, *B. attenuatus* formed smaller group sizes even though population densities were no different than more recently infected populations. We suggest that populations with a longer history of Bd (i.e. the earliest infected populations) may have evolved reduced sociality to avoid exposure to the pathogen, however, these results arise from a small number of sites (i.e. only 14 contemporary sites) and there may be other hypotheses that could explain this pattern. For example, more recently exposed populations may increase aggregation behavior to spread Bd-fighting microbes. Furthermore, this number of field sites was insufficient to resolve what environmental factors might contribute to prevalence of Bd among contemporary Bd-positive sites and what factors might be causing changes in aggregation behavior.

The introduction of a novel pathogen into a gregarious host system provides the opportunity to explore this relationship between disease and sociality. Further experimental work can be done in captivity to test our observation that populations with longer exposure to Bd have evolved reduced sociality. Both *B. attenuatus'* aggregative behavior and social nesting increase rate of contact of potential hosts, and increased host group size is generally correlated with increased parasite prevalence (Côté and Poulinb, 1995). Pathogens are expected to disrupt the evolution of sociality if their spread is facilitated by social activity (Altizer et al., 2003). This has been observed in the two gregariously roosting species of bats since the fungal pathogen responsible for white-nose syndrome has emerged in those populations, the number of bats roosting singly has increased significantly (Langwig et al., 2012). Alternately, gregariousness in *B. attenuatus* may facilitate the spread of mutualistic bacteria, such as *Janthinobacterium lividum* (Brucker et al., 2008; Harris et al., 2009), counteracting to some degree the cost of sociality that Bd imposes. Thus, if parasites are transmitted socially, models predict that populations will evolve away from sociality, both to decrease personal cost of receiving parasites and inclusive fitness costs of passing parasites to kin; alternatively kin selection could increase sociality by increasing rates of mutualist transmission (Zink, 2015). While numerous studies have analyzed the effects of environmental variables on Bd dynamics, few studies have tested the effects of host aggregation size on Bd disease ecology (Alford, 2007; Han et al., 2008; Venesky et al., 2011).

Both Bd prevalence and Z_{swab} scores were significantly lower for field samples than for museum specimens collected during the 1990's. Because the rate of DNA degradation in historical specimens is not always consistent with the infection intensities of actual populations (Cheng et al., 2011), when comparisons are made between historical and contemporary infection levels the potential for false historical negatives should be considered (Miller et al., 2012). In particular, it would be good to know if the probability of false negatives increases with older museum samples. Additionally, there is a possibility of false negatives in singlicate qPCR. For this reason, we limited our historical GLMM to the factors that contributed to the relative abundance of Bd in historically positive sites alone. Conversely, in this case, we can be confident that the *relative* values in the 1990's and contemporary populations accurately reflect disease dynamics in these populations. Moreover, the drop in both Bd prevalence and Z_{swab} scores in contemporary samples compared to preserved historical specimens highlights the robustness of PCR recovery of Bd DNA. Historical Bd positives as early as 1894 have been detected in anuran museum specimens using the same singlicate qPCR technique (Rodriguez et al., 2014; Talley et al., 2015).

Our approach, which links historical sampling for Bd with contemporary sampling at the same locations, is a novel method that can lend great insight into the temporal dynamics of Bd in populations of amphibians that are well-represented in museums (and have not yet gone extinct in contemporary populations). Because these museum specimens were collected for systematic studies and evolutionary relationships, rather than for ecological patterns, the sampling regime was not initially random with respect to geography and time but rather a reflection of sampling effort. Though the specimens in museum collections were not collected randomly, the museum collections of *B. attenuatus* are so extensive both in number of specimens and geographic coverage that we propose it is possible to obtain unbiased results of disease spread by utilizing a random sample of these collections. Future studies can expand this approach to account for intrinsic sampling bias within museum collections, balancing specimens available with more even spatial representation for historically exposed amphibians. Dynamics of currently abundant populations, such as *B. attenuatus*, can lend insight into more vulnerable species in which sample size is insufficient due to lower numbers.

Acknowledgements

We are grateful to the numerous graduate students at San Francisco State University who assisted with salamander swabbing in the field: Angel Conde, Celeste Dodge, Silas Ellison, Raul Figueroa, Andrea

Manzano, Audrey Salinas, Jon Young. Also thanks to the undergrads who assisted in swabbing museum collections and field specimens: Jason Anders, Cheyenne Anderson, Allen Atallah, Eleonora Bondareva, Michelle Davila, Alex Harencar, Pearl Tam, Susi Ngoi. Thanks to Dr. Ed Connor and Celeste Dodge for invaluable advice on the statistical methods used. We are grateful to the California Academy of Sciences and Museum of Vertebrate Zoology at Berkeley for providing access to their collections and to Jens Vindum and Carol Spencer, particularly, for their help and patience. Financial support was provided by NSF grant IOS - 1258133 awarded to Andrew G. Zink and Vance T. Vredenburg and ORSP small research grant awarded to Andrew G. Zink. Carla Sette was supported by an NIH-RISE grant #5425-GM059298-12 awarded to Frank Bayliss.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.biocon.2015.08.039.

References

Alford, R.A., 2007. Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. Dis. Aquat. Org. 77, 1–9. http://dx.doi.org/10.3354/dao01830.

Altizer, S., Nunn, C.L., Thrall, P.H., Gittleman, J.L., Antonovics, J., Cunningham, A.A., Dobson, A.P., Ezenwa, V.O., Jones, K.E., Pedersen, A.B., Poss, M., Pulliam, J.R.C., 2003. Social organization and parasite risk in mammals: integrating theory and empirical studies. Annu. Rev. Ecol. Evol. Syst. 34, 517–547. http://dx.doi.org/10.1146/annurev.ecolsys.34.030102.151725.

Anderson, P.K., 1960. Ecology and evolution in island populations of salamanders in the San Francisco Bay region. Ecol. Monogr. 30, 359–385.

Berger, L., Spears, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. PNAS USA 95, 9031–9036.

Blaustein, A.R., 1994. Chicken little or Nero's fiddle? A perspective on declining amphibian populations. Herpetologica 50, 85–97.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135. http://dx.doi.org/10.1016/j.tree.2008.10.008.

Bolker, B., 2013. emdbook: Ecological Models and Data in R; R package version 1.3.4-03. http://CRAN.R-project.org/package=emdbook.

Boyle, D.G., Boyle, D.B., Olsen, V., Hyatt, A.D., 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis. Aquat. Org. 60, 141–148.

Bradford, D.F., 1991. Mass mortality and extinction in a high-elevation population of *Rana muscosa*. J. Herpetol. 174–177.

Brem, F.M.R., Lips, K.R., 2008. Batrachochytrium dendrobatidis infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. Dis. Aquat. Org. 81, 189–202. http://dx.doi.org/10.3354/dao01960.

Briggs, C.J., Knapp, R.A., Vredenburg, V.T., 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. Proc. Natl. Acad. Sci. 107, 9695–9700. http://dx.doi.org/10.1073/pnas.0912886107</p>.

Brucker, R.M., Harris, R.N., Schwantes, C.R., Gallaher, T.N., Flaherty, D.C., Lam, B.A., Minbiole, K.P.C., 2008. Amphibian chemical defense: antifungal metabolites of the microsymbiont *Janthinobacterium lividum* on the salamander *Plethodon cinereus*. J. Chem. Ecol. 34, 1422–1429. http://dx.doi.org/10.1007/s10886-008-9555-7.

Cheng, T.L., Rovito, S.M., Wake, D.B., Vredenburg, V.T., 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. PNAS 108, 9502–9507. http://dx.doi.org/10.1073/pnas.1105538108.

Côté, I.M., Poulinb, R., 1995. Parasitism and group size in social animals: a meta-analysis. Behav. Ecol. 6, 159–165.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2003. Infectious disease and amphibian population declines. Divers. Distrib. 9, 141–150.

De Castro, F., Bolker, B.M., 2004. Mechanisms of disease-induced extinction. Ecol. Lett. 8, 117–126. http://dx.doi.org/10.1111/j.1461-0248.2004.00693.x (Mechanisms of disease-induced extinction).

Fisher, M.C., Walker, S.F., 2009. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. Annu. Rev. Microbiol. 63, 291–310. http://dx.doi.org/10.1146/annurev.micro.091208.073435.

Fournier, D.A., Skaug, H.J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M.N., Nielsen, A., Sibert, J., 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optim. Method Softw. 27, 233–249. http://dx.doi.org/10.1080/10556788.2011.597854.

Gebhardt, A., 2013. akima: Interpolation of irregularly spaced data R package version 0.5-11 http://CRAN.R-project.org/package=akima.

Gelman, A., Hill, J., 2007. Data Analysis Using Regression and Multilevel/Hierarchical Models. Cambridge University Press, Cambridge.

Han, B.A., Bradley, P.W., Blaustein, A.R., 2008. Ancient behaviors of larval amphibians in response to an emerging fungal pathogen, *Batrachochytrium dendrobatidis*. Behav. Ecol. Sociobiol. 63, 241–250. http://dx.doi.org/10.1007/s00265-008-0655-8.

Harris, R.N., Brucker, R.M., Walke, J.B., Becker, M.H., Schwantes, C.R., Flaherty, D.C., Lam, B.A., Woodhams, D.C., Briggs, C.J., Vredenburg, V.T., Minbiole, K.P.C., 2009. Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. ISME J. 3, 818–824. http://dx.doi.org/10.1038/ismej.2009.27.

Hendrickson, J.R., 1954. Ecology and systematics of salamanders of the genus *Batrachoseps*. UC Pub. Zool. 54.

Hof, C., Araújo, M.B., Jetz, W., Rahbek, C., 2011. Additive threats from pathogens, climate and land-use change for global amphibian diversity. Nature 480, 516–519. http://dx.doi.org/10.1038/nature10650.

Huss, M., Huntley, L., Vredenburg, V., Johns, J., Green, S., 2014. Prevalence of *Batrachochytrium dendrobatidis* in 120 archived specimens of *Lithobates catesbeianus* (American Bullfrog) collected in California, 1924–2007. EcoHealth 10, 339–343. http://dx.doi.org/10.1007/s10393-013-0895-6.

Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., Berger, L., Obendorf, D., Dalton, A., Kriger, K.M., Heros, M., Hines, H.B., Phillott, R., Campbell, R., Marantelli, G., Gleason, F., Coiling, A., 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis. Aquat. Org. 73, 175–192. http://dx.doi.org/10.3354/dao073175.

Jockusch, E.L., Wake, D.B., Martínez-Solano, I., 2007. Extreme population subdivision throughout a continuous range: Phylogeography of *Batrachoseps attenuatus* (Caudata: Plethodontidae) in western North America. Mol. Ecol. 16, 4335–4355. http://dx.doi.org/10.1111/j.1365-294X.2007.03527.x.

Knapp, R.A., Briggs, C.J., Smith, T.C., Maurer, J.R., 2011. Nowhere to hide: impact of a temperature-sensitive amphibian pathogen along an elevation gradient in the temperate zone. Ecosphere 2 (online).

Langwig, K.E., Frick, W.F., Bried, J.T., Hicks, A.C., Kunz, T.H., Kilpatrick, A.M., 2012. Sociality, densdy-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. Ecol. Lett. 15, 1050–1057. http://dx.doi.org/10.1111/j.1461-0248.2012.01829.x.

Lips, K.R., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Livo, L., Pessier, A.P., Collins, J.P., 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. PNAS USA 103, 3165.

Maiorana, V.C., 1977a. Observations of salamanders (Amphibia, Urodela, Plethodontidae) dying in the field. J. Herpetol. 1-5.

Maiorana, V.C., 1977b. Tail autotomy, functional conflicts and their resolution by a salamander. Nature 265, 533–535.

Martin, T.G., Wintle, B.A., Rhodes, J.R., Kuhnert, P.M., Field, S.A., Low-Choy, S.J., Tyre, A.J., Possingham, H.P., 2005. Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. Ecol. Lett. 8, 1235–1246. http://dx.doi.org/10.1111/j.1461-0248.2005.00826.x (Modelling excess zeros in ecology).

May, R.M., Anderson, R.M., 1979. Population biology of infectious diseases: Part II. Nature.

McKenzie, V.J., Peterson, A.C., 2012. Pathogen pollution and the emergence of a deadly amphibian pathogen. Mol. Ecol. 21, 5151–5154. http://dx.doi.org/10.1111/mec.12013.

Miller, D.A.W., Talley, B.L., Lips, K.R., Campbell Grant, E.H., 2012. Estimating patterns and drivers of infection prevalence and intensity when detection is imperfect and sampling error occurs. Methods Ecol. Evol. 3, 850–859. http://dx.doi.org/10.1111/j.2041-210X.2012.00216.x (Detection in epidemiological studies).

Olson, D.H., Aanensen, D.M., Ronnenberg, K.L., Powell, C.I., 2013. Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. PLoS ONE http://dx.doi.org/10.1371/journal.pone.0056802.

Padgett-Flohr, G.E., Hopkins, R.L., 2009. *Batrachochytrium dendrobatidis*, a novel pathogen approaching endemism in Central California. Dis. Aquat. Org. 83, 1–9. http://dx.doi.org/10.3354/dao02003.

Parker, J.M., Mikaelian, I., Hahn, N., Diggs, H.E., 2002. Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). Comp. Med. 52, 265–268.

Pechmann, J.H., Wilbur, H.M., 1994. Putting declining amphibian populations in perspective: natural fluctuations and human impacts. Herpetologica 65-84.

Piotrowski, J.S., Annis, S.L., Longcore, J.E., 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96, 9–15.

Piovia-Scott, J., Pope, K.L., Lawler, S.P., Cole, E.M., Foley, J.E., 2011. Factors related to the distribution and prevalence of the fungal pathogen *Batrachochytrium dendrobatidis* in *Rana cascadae* and other amphibians in the Klamath Mountains. Biol. Conserv. 144, 2913–2921. http://dx.doi.org/10.1016/j.biocon.2011.08.008.

Raffel, T.R., Romansic, J.M., Halstead, N.T., McMahon, T.A., Venesky, M.D., Rohr, J.R., 2012. Disease and thermal acclimation in a more variable and unpredictable climate. Nat. Clim. Chang. 2, 1–6. http://dx.doi.org/10.1038/nclimate1659.

Reeder, N.M.M., Pessier, A.P., Vredenburg, V.T., 2012. A reservoir species for the emerging amphibian pathogen *Batrachochytrium dendrobatidis* thrives in a landscape decimated by disease. PLoS ONE 7, e33567 http://dx.doi.org/10.1371/journal.pone.0033567.g005.

Ribeiro Jr., P.J., Diggle, P.J., 2007. The geoR package. R News.

Richards-Hrdlicka, K.L., 2012. Extracting the amphibian chytrid fungus from formalin-fixed specimens. Methods Ecol. Evol. 3, 842–849. http://dx.doi.org/10.1111/j.2041-210X.2012.00228.x (Extracting chytrid DNA from fixed amphibians).

Rodriguez, D., Becker, C.G., Pupin, N.C., Haddad, C.F.B., Zamudio, K.R., 2014. Long-term endemism of two highly divergent lineages of the amphibian-killing fungus in the Atlantic Forest of Brazil. Mol. Ecol. 23, 774–787. http://dx.doi.org/10.1111/mec.12615.

Skaug, H.J., Fournier, D.A., Bolker, B.M., 2014. Generalized Linear Mixed Models using AD Model Builder (R package version 0.8.0).

Skerratt, L.F., Berger, L., Spears, R., Cashins, S.D., McDonald, K.R., Phillott, A.D., Hines, H.B., Kenyon, N., 2007. Spread of cytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4, 125–134. http://dx.doi.org/10.1007/s10393-007-0093-5.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306, 1783–1786. http://dx.doi.org/10.1126/science.1103538.

Talley, B.L., Muletz, C.R., Vredenburg, V.T., Fleischer, R.C., Lips, K.R., 2015. A century of *Batrachochytrium dendrobatidis* in Illinois amphibians (1888–1989). Biol. Conserv. 182, 254–261.

Underwood, A.J., 1997. Experiments in Ecology. Cambridge University Press.

Venesky, M.D., Kerby, J.L., Storfer, A., Parris, M.J., 2011. Can differences in host behavior drive patterns of disease prevalence in tadpoles? PLoS ONE 6, e24991. http://dx.doi.org/10.1371/journal.pone.0024991.t001.

Vredenburg, V.T., Felt, S.A., Morgan, E.C., McNally, S.V.G., Wilson, S., Green, S.L., 2013. Prevalence of *Batrachochytrium dendrobatidis* in *Xenopus* collected in Africa (1871–2000) and in California (2001–2010). PLoS ONE 8, e63791. http://dx.doi.org/10.1371/journal.pone.0063791.t003.

Vredenburg, V.T., Knapp, R.A., Tunstall, T.S., Briggs, C.J., 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. PNAS 107, 9689–9694. http://dx.doi.org/10.1073/pnas.0914111107.

Wake, D.B., 2012. Facing extinction in real time. Science 335, 1052–1053. http://dx.doi.org/10.1126/science.1218364.

Wake, D.B., Castanet, J., 1995. A skeletochronological study of growth and age in relation to adult size in *Batrachoseps attenuatus*. J. Herpetol. 29, 60–65.

Wake, D.B., Vredenburg, V.T., 2008. Colloquium paper: are we in the midst of the sixth mass extinction? A view from the world of amphibians. PNAS 105 (Suppl. 1), 11466–11473. http://dx.doi.org/10.1073/pnas.0801921105.

Weinstein, S.B., 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. http://dx.doi.org/10.1643/CH-08-180.

Woodhams, D.C., Alford, R.A., 2005. Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. Conserv. Biol. 19, 1449–1459.

Zink, A.G., 2015. Kin selection and the evolution of mutualism between species. Ethology.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R, Springer New York. Springer New York, New York, NY http://dx.doi.org/10.1007/978-0-387-87458-6.