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Short Communication

Invasion of the Fungal Pathogen *Batrachochytrium dendrobatidis* on California Islands

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Abstract: *Batrachochytrium dendrobatidis* (*Bd*), an amphibian fungal pathogen, has infected >500 species and caused extinctions or declines in >200 species worldwide. Despite over a decade of research, little is known about its invasion biology. To better understand this, we conducted a museum specimen survey (1910–1997) of *Bd* in amphibians on 11 California islands and found a pattern consistent with the emergence of *Bd* epizootics on the mainland, suggesting that geographic isolation did not prevent *Bd* invasion. We propose that suitable habitat, host diversity, and human visitation overcome isolation from the mainland and play a role in *Bd* invasion.

Keywords: amphibian pathogen, *Batrachochytrium dendrobatidis*, chytridiomycosis, emerging infectious disease, California islands

Emerging infectious diseases are increasingly affecting wildlife and are a major threat to global biodiversity. Fungal infections have caused dramatic declines across many taxa worldwide, including bats (e.g., white-nose syndrome), soft corals, bees, oak trees, and frogs (Fisher et al. 2012). *Batrachochytrium dendrobatidis* (*Bd*), a fungal pathogen that causes chytridiomycosis in amphibians, is implicated in a recent pandemic (Wake and Vredenburg 2008), infecting over 500 species (Olson et al. 2013) and causing declines and extinctions in over 200 species globally (Fisher et al. 2009; Skerratt et al. 2007).

Studies investigating the emergence of *Bd* in California are restricted to the mainland. The earliest reported case was from an invasive *Rana catesbeiana* in 1928 (Huss et al. 2013) that apparently did not result in epizootic spread and may represent pathogen invasion followed by a fade. However, *Bd* appears to have spread widely throughout the state beginning in the late 1960s through the 1970s, a time period which coincides with declines and extirpations of amphibians in California (Bradford 1991; Padgett-Flohr and Hopkins 2009; Wake and Vredenburg 2008). To date, there are no published reports on *Bd* from California islands. Describing the historical to present day distribution of *Bd* on the islands may help understand the invasion biology of this pathogen. Here we report the first records of

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Bd on California islands, discover a new host species for *Bd*, and discuss the presence of suitable *Bd* habitat, host diversity, and human visitation as possible factors that could influence the invasion and sustained presence of *Bd* on these islands and elsewhere.

We sampled 656 preserved museum specimens, with collection dates ranging from 1910 to 1997, from five of the Channel Islands in southern California (San Miguel, Santa Cruz, Santa Rosa, Anacapa, and Santa Catalina) and six islands in northern California (Año Nuevo, the Farallones, Alcatraz, Angel, Brooks, and Yerba Buena) (Fig. 1). We did not include three Channel Islands (Santa Barbara, San Clemente, and San Nicolas) because amphibians do not occur there. The amphibians sampled included *Aneides lugubris*, *Batrachoseps attenuatus*, *Batrachoseps major*, *Batrachoseps nigriventris*, *Batrachoseps pacificus*, and *Pseudacris regilla* (Table 1). We sampled specimens using a non-destructive skin swab technique (Cheng et al. 2011). In cases where more than 30 individuals were collected within a single year, species, and island, 30 specimens were randomly selected for inclusion in the study (Gray et al. 2015). All specimens were formalin-fixed, stored in 70 % ethanol, and archived in the permanent collection of the Museum of Vertebrate Zoology (Berkeley, California, USA).

To minimize chances of cross contamination among specimens stored in the same preservation jars, individuals were rinsed with 70 % ethanol before swabbing, gloves were changed between animals, and specimens were swabbed in ascending chronological order of collection date. Specimens were stroked 30 times on the ventral side (abdomen, sides, and tail [in the case of salamanders], and plantar side of the forelimbs and hindlimbs) with sterile, synthetic cotton swabs (Cheng et al. 2011; Van Rooij et al. 2011, Richards-Hrdlicka 2012). Swab samples were dried, placed in 1.5 mL tubes, and stored at 4°C. DNA was extracted using Prepman Ultra (Cheng et al. 2011) and analyzed using quantitative polymerase chain reaction (qPCR) assays (Boyle et al. 2004) using an Applied BioSystems 7300 Real-Time PCR system. Standards of known zoospore concentrations and negative controls were included in each assay. Samples were processed in singlicate and considered positive if both a sigmoidal amplification occurred and the qPCR score was greater than zero. To calculate infection intensity, we multiplied the qPCR score by 80 to account

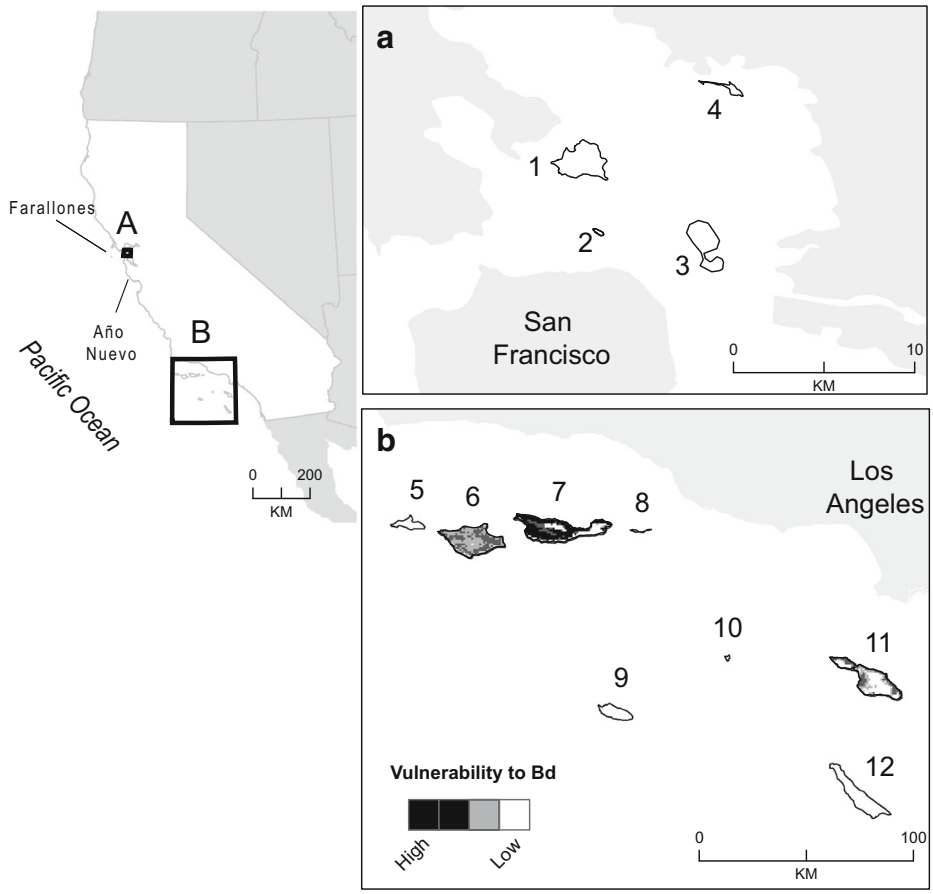

Figure 1. Map of the California Islands. **a** San Francisco Bay Islands: *1* Angel Island; *2* Alcatraz; *3* Yerba Buena and Treasure Island; *4* Brooks Island, where only Alcatraz had *Bd*-positive specimens. **b** The Channel Islands and their vulnerability to *Bd* invasion: *5* San Miguel; *6* Santa Rosa; *7* Santa Cruz; *8* Anacapa; *9* San Nicolas; *10* Santa Barbara; *11* Santa Catalina; *12* San Clemente. *Bd*-positive specimens were recorded at Santa Catalina, Santa Cruz, and Santa Rosa islands, where vulnerability to *Bd* was predicted to be highest.

Table 1. *Bd* Prevalence and Infection Intensities of Species Sampled.

Island group	Island	Decades sampled	Year of first *Bd*-positive record	Species	Number of positives	Number sampled	Prevalence (95 % C I)	Median infection intensity ZE (min, max)
Channel Islands	Anacapa	1950s, 1970s, 1980s, 1990s	–	*Batrachoseps pacificus*	0	25	0 (0–7.3)	–
	San Miguel	1970s	–	*Batrachoseps pacificus*	0	38	0 (0–4.9)	–
	Santa Catalina	1910s, 1940s, 1970s, 1990s	1979	*Batrachoseps major*	1	96	1 (0.1–4.7)	1.35
				Pseudacris regilla	0	6	0 (0–26.4)	–
	Santa Cruz	1920s, 1940s, 1950s, 1970s, 1990s	1973	*Batrachoseps nigriventris*	16	88	18.2 (11.2–27.2)	3.05 (0.07, 1495)
				Batrachoseps pacificus	1	143	0.6 (0.07–3.2)	0.89
				Pseudacris regilla	0	96	0 (0–1.9)	–
	Santa Rosa	1920s, 1950s, 1970s, 1980s	1978	*Batrachoseps pacificus*	3	75	4 (1.1–10.2)	1.46 (1.41, 1.66)
				Pseudacris regilla	0	25	0 (0–7.3)	–
Northern California San Francisco Bay Islands	Alcatraz	1980s	1987	*Batrachoseps attenuatus*	1	3	33.3 (3.8–82.3)	20.16
	Angel	1930s, 1950s, 1970s	–	*Aneides lugubris*	0	2	0 (0–56.9)	–
				Batrachoseps attenuatus	0	27	0 (0–6.8)	–
	Brooks	1950s	–	*Aneides lugubris*	0	4	0 (0–36.2)	–
	Yerba Buena	1920s	–	*Batrachoseps attenuatus*	0	12	0 (0–14.5)	–
Northern California other Islands	Año Nuevo	1960s, 1970s	–	*Aneides lugubris*	0	8	0 (0–20.7)	–
	Farallones	1970s	–	*Aneides lugubris*	0	8	0 (0–20.7)	–

for subsampling and dilution that occurred during the DNA extraction, resulting in a zoospore equivalents (ZE) estimate on each specimen (Briggs et al. 2010; Vredenburg et al. 2010).

To determine if the islands exhibited an endemic prevalence pattern consistent with areas of known *Bd* endemism, such as Brazil (Rodriguez et al. 2014) and Illinois (Talley et al. 2015), we calculated the power to detect a 20 % prevalence (the approximate *Bd* prevalence these studies found over a 100-year period in museum specimens) given our sample size. We divided our samples into pre-1960 and post-1960 time periods to increase power and because a previous study on the California mainland estimated that *Bd* epizootics first occurred after 1960 (Padgett-Flohr and Hopkins 2009). We calculated prevalence and 95 % Bayesian confidence intervals (CIs) for these time periods. We used R version 3.1.2 (R Development Core Team 2008; 'pwr' package (Champely 2015), 'binom' package (Dorai-Raj 2014)) for statistical calculations.

We estimated the vulnerability of the Channel Islands to *Bd* invasion by combining host availability with a climate-driven, presence-only habitat suitability model (HSM) for *Bd* with maximum entropy modeling software, Maxent version 3.3.3 k (Phillips et al. 2004), following previously described methodologies (Rödder et al. 2009; Yap et al. 2015). Training data for the HSM included 197 *Bd*-positive sites within the California mainland (Vredenburg et al. unpublished) and six bioclimatic variables from the Worldclim database (Hijmans et al. 2005): annual mean temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, annual precipitation, precipitation of the wettest month, and precipitation of the driest month. We ran 100 model simulations using cross-validation, which were then averaged by Maxent to produce a probabilistic density function of suitable *Bd* habitat on the Channel Islands. We weighted the HSM with amphibian host diversity from each island to produce a predictive model of the probability of *Bd* establishment on these islands (Fig. 1b). We used ArcGIS 10.2.2 (ESRI) to produce all GIS layers. We only used the Channel Islands (excluding Santa Barbara Island) for the model because climate data were incomplete for the other small California islands.

We detected *Bd* in 22 of the 656 specimens and on four (Alcatraz Island, Santa Catalina Island, Santa Cruz Island, and Santa Rosa Island) of 11 California islands (Table 1; Fig. 1). Infected hosts included *B. major*, *B. nigriventris*, and *B. pacificus*; this is the first report of *Bd* in *B. pacificus* (Table 1). The earliest *Bd* positives for each island were detected in 1973 (Santa Cruz Island), 1978 (Santa Rosa Island), 1979 (Santa Catalina Island), and 1987 (Alcatraz Island) (Table 1). Prevalence and *Bd* infection intensities for the species and islands sampled were low. *Bd* prevalence was 0 % (0–0.7 CI, $N = 254$, power to detect 20 % *Bd* prevalence = 0.73) in samples collected before 1960 and 5.4 % (3.5–8.0 CI, $N = 402$, power to detect 20 % *Bd* prevalence = 0.88) in samples collected after 1960. *Bd* infection intensities ranged from 0.07 ZE to 1495 ZE (Table 1). Our *Bd* vulnerability model shows that despite the Channel Islands having generally low *Bd* habitat suitability (<0.42, scale of 0–1), the islands with the highest relative model predictions are the same islands where we detected *Bd*-infected specimens (Fig. 1b).

Our discovery of *Bd* on four of 11 California islands may be a conservative estimate. There is a chance we did not detect all *Bd*-positive specimens through our singlicate qPCR assays (Cheng et al. 2011). In addition, the museum samples were not collected for this study's purpose; thus, specimens were not equally available across time and space. However, collectively, the samples from 1910 to 1960 provide ample support that *Bd* was likely not present on the islands prior to 1960. This indicates that *Bd* is not endemic to the islands and likely invaded after 1960, which coincides with the hypothesized emergence of *Bd* epizootics on the mainland (Huss et al. 2013; Padgett-Flohr and Hopkins 2009; Vredenburg et al. 2013, 2010).

Several studies propose that humans play a role in the worldwide spread of *Bd* (Fisher and Garner 2007; Picco and Collins 2008; Une et al. 2008; Schloegel et al. 2012). California's islands provide insight in the extent of human influence on the spread of *Bd* because the islands vary dramatically in human visitation rate. For example, the Farallon Islands are closed to the public, while Alcatraz Island is a major tourist attraction that receives $\sim$1.4 million visitors per year (National Parks Conservation Association 2010), and Santa Catalina Island has $\sim$4000 year-round residents (U.S. Census Bureau 2010) and $>$600,000 visitors per year (Catalina Island and Chamber of Commerce Visitors Bureau 2012). The remaining islands are National Parks where human visitation is restricted. Our results suggest that human visitation may affect *Bd* invasion because the two most visited islands, Alcatraz and Santa Catalina Island, had *Bd*-infected animals (Fig. 1; Table 1), though this factor is not the only factor associated with *Bd* invasion. Based on our results, we propose the presence of suitable, even non-optimal *Bd*

habitat, host diversity, and human visitation all play a role in the invasion and establishment of *Bd* on these islands. Other factors may also influence the spread of *Bd* to isolated islands, such as bird or rainwater dispersal (Johnson and Speare 2003; Kolby et al. 2015). In addition, salinity affects *Bd* dynamics, and these are habitats that are exposed to high levels of salinity (Stockwell et al. 2015a, b). We suggest that island systems present a unique framework that could be used to elucidate the invasion biology of *Bd* as well as other emerging infectious pathogens.

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