



High fungal pathogen loads and prevalence in Baja California amphibian communities: The importance of species, elevation, and historical context

Andrea J. Adams^{a,b,*}, Anny Peralta-García^c, Carlos A. Flores-López^d,
Jorge H. Valdez-Villavicencio^c, Cheryl J. Briggs^b

^a Earth Research Institute, University of California, Santa Barbara, CA 93106, USA

^b Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA 93106, USA

^c Conservación de Fauna del Noroeste, A.C., Ensenada, Baja California 22800, Mexico

^d Facultad de Ciencias, Universidad Autónoma de Baja California, Km. 103 Carretera Tijuana – Ensenada, Pedregal Playitas, 22860 Ensenada, Baja California, Mexico

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ABSTRACT

Amphibians are declining globally, and the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), which causes the disease chytridiomycosis, has been the culprit in many of these declines. In the Mediterranean region of Baja California, Mexico—a biodiversity hotspot—native amphibians are also in decline, and Bd is present. To determine which factors best predict Bd infection prevalence and intensity in Baja California anurans, we conducted Bd sampling at three disparate sites at varying elevations. We observed an overall Bd prevalence of 68%, and found that species was an important predictor of pathogen prevalence and burden (load) both across and within sites. Pathogen prevalence and load increased positively with elevation. The highest Bd load we detected was 139,188 zoospore equivalents (ZE) in a western toad (*Anaxyrus boreas*)—more than 10 times the pathogen burden at which chytridiomycosis is lethal in some species. We observed high Bd prevalence (84–99%) and Bd loads (> 44,000 ZE) in the California red-legged frog (*Rana draytonii*)—a cause for concern in this threatened anuran, especially at La Grulla—the highest elevation (2050 m) site for this species across its range. We sampled museum specimens to determine how long Bd has been present in the region, and detected the pathogen as early as 1932. Contemporary Bd prevalence and load in Baja California are considerably higher than would be expected given similar habitats and the same community composition as in southern California, USA. We therefore highlight the importance of historical context and comparative analyses in examining contemporary Bd dynamics, as well as adhering to strict biosecurity measures when implementing reintroductions and translocations.

1. Introduction

Chytridiomycosis, the disease caused by the fungal pathogens *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium*

* Correspondence to: Earth Research Institute, University of California, 6832 Ellison Hall, Santa Barbara, CA 93106, USA.

E-mail addresses: andrea_adams@ucsb.edu (A.J. Adams), annyperaltagarcia@yahoo.com (A. Peralta-García), cflores2@uabc.edu.mx (C.A. Flores-López), j_h_valdez@yahoo.com.mx (J.H. Valdez-Villavicencio), briggs@lifesci.ucsb.edu (C.J. Briggs).

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salamandrivorans, is unequivocally implicated in global amphibian declines (Longcore et al., 1999; Stuart et al., 2004; Martel et al., 2013, 2014; Scheele et al., 2019). *Batrachochytrium dendrobatidis* can infect multiple hosts, persisting in competent host species while simultaneously affecting more susceptible species (Catenazzi, 2015). Therefore, assessing community-wide Bd infection is an important task when enigmatic amphibian declines have occurred and species of conservation concern are extant or in reintroduction planning stages.

Retrospective examinations of novel pathogen distribution and emergence can also inform contemporary research and management decisions, including reintroduction programs (Jules et al., 2002; Sainsbury and Vaughan-Higgins, 2012). The role of historical emergence and distribution of Bd in shaping amphibian communities is increasingly supported by a growing literature of museum specimen research (Lips, 2011; Zhu et al., 2014; De León et al., 2016; Adams et al., 2017b; Karwacki et al., 2021).

The complex dynamics of Bd infection are frequently dependent upon environmental factors, with temperature and elevation among the most important (Lips, 2016; Adams et al., 2017a). Moist, temperate climates have been identified as some of the highest disease risk areas (Berger et al., 1998; Puschendorf et al., 2011; Becker et al., 2012), and higher elevations (>900 m) are also associated with increased risk of Bd infection (Lips et al., 2003, 2004; Catenazzi et al., 2010). High elevation, lotic environments may also be

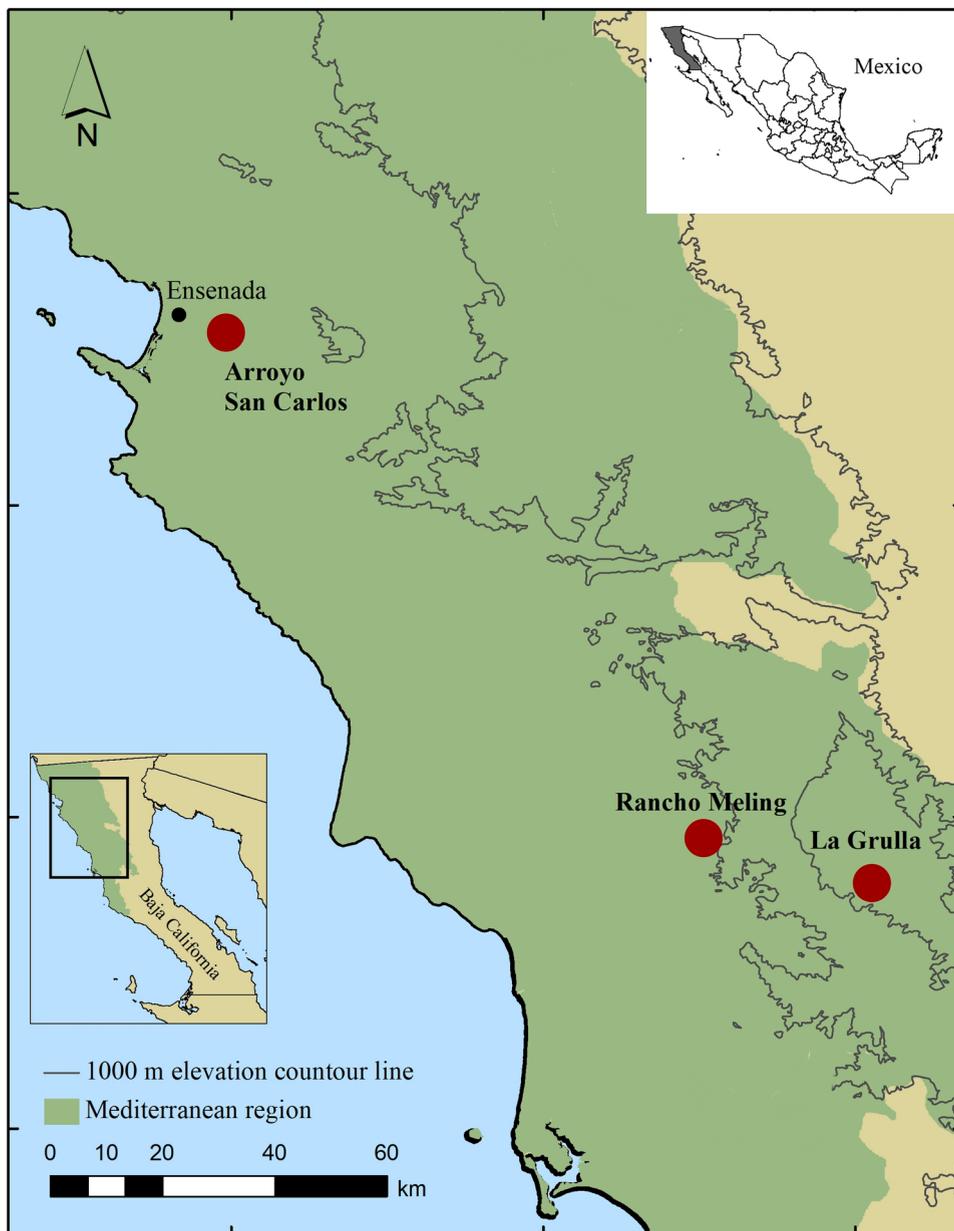


Fig. 1. Location of the study area and sampling sites in the Mediterranean Region of Baja California, Mexico.

susceptible to Bd-driven epizootic declines, because ambient temperatures at these sites frequently match the optimal conditions for Bd growth *ex situ* (Piotrowski et al., 2004).

Chytridiomycosis driven by Bd has been the direct cause of decline for at least 43 amphibian species in Latin America (Lips et al., 2006), and Bd has been detected in at least 78 species of amphibians in Mexico alone (Bolom-Huet et al., 2019). In the high-elevation Trans-Mexican Volcanic Belt in Michoacán, high Bd prevalence has been observed in amphibian populations in areas with relatively high temperature (>10 °C) and increased precipitation (>100 mm) (Nava-González et al., 2020). Pathogen detection at sites below 100 m in Baja California has expanded the range of altitudes in Mexico where Bd has been confirmed (Luja et al., 2012; Peralta-García et al., 2018). Species distribution models of Bd predict high suitability for the pathogen in northwestern Baja California based on environmental factors (Bolom-Huet et al., 2019). Infection burdens greater than 10,000 zoospore equivalents (ZE; a measure of infection intensity) are associated with chytridiomycosis mortality in Bd-susceptible North American anurans (Briggs et al., 2010; Kinney et al., 2011; Adams et al., 2017a, 2020), and infections > 10,000 ZE have been observed in Baja California frogs (Peralta-García et al., 2018), prompting a need for further investigation of Bd in this region.

The northwestern Mediterranean region of Baja California is a biodiversity hotspot, with many endemic species threatened by habitat loss (Bauder and McMillan, 1998; Vanderplank et al., 2014). This region hosts 11 amphibian species, including California red-legged frogs (*Rana draytonii*), which are threatened throughout their range (Peralta-García et al., 2016), and western toads (*Anaxyrus boreas*), which exhibit high susceptibility to Bd infection (Muths et al., 2008). In Baja California, Bd has been detected in all species sampled, except *Xenopus laevis*, the non-native African clawed frog (Peralta-García et al., 2018). The presence of non-native American bullfrogs (*Lithobates catesbeianus*), a competent Bd vector and reservoir host (Greenspan et al., 2012; Schloegel et al., 2012; Adams et al., 2017a), has been associated with higher Bd prevalence in native anurans in Baja California (Luja et al., 2012). Increased Bd prevalence in Baja California has also been observed at higher elevations, and with greater remoteness from urban areas and agricultural land (Peralta-García et al., 2018).

In this study, we present the results of one year of Bd monitoring across four seasons in three Baja California amphibian communities. We aimed to assess the biotic and abiotic factors that influence Bd prevalence and infection intensity, and hypothesized that water temperature, season, elevation, lifestage, species, and American bullfrog presence could be evident in the Bd status of amphibian populations at our study sites. We also present the results of museum specimen sampling to examine the historical emergence of the pathogen in Baja California. A better understanding of Bd dynamics in this region in the past and present can assist in conservation management decisions, especially for the threatened *R. draytonii*, for which conservation interventions such as reintroductions are currently being evaluated.

2. Materials and methods

We conducted contemporary Bd sampling in the field at three sites in the Mediterranean region of Baja California (Fig. 1), and sampled museum specimens that had been collected from across Baja California.

2.1. Study sites

Arroyo San Carlos, Campo Delicias (100 m elev), is located ~20 km south of Ensenada. Arroyo San Carlos is a ~40 km perennial stream with a series of natural and modified pools that are frequently used for recreation. Of all our sampling sites, this site is the closest to an urban area, and hot springs are located along the stream's length. Pool depth is variable, but never exceeds 2 m. Groundwater pumping prevents the stream from reaching the Pacific Ocean, and dries ~13 km inland. Arroyo San Carlos is heavily affected by introduced species, including American bullfrogs, crayfish (*Procambarus clarkii*), sunfish (*Lepomis cyanellus*), mosquitofish (*Gambusia affinis*), and introduced gastropods. Campo Delicias is one of several recreational sites along the stream. It was once occupied by *R. draytonii* and southwestern pond turtles (*Actinemys pallida*), but these species have since been extirpated. Native amphibian species are uncommon relative to other less anthropogenically-impacted sites, but include *A. boreas* and Baja California tree frogs (*Hylliola hypochondriaca*). The habitat is dominated by riparian vegetation, California live oak (*Quercus agrifolia*), western sycamore (*Platanus racemosa*), and arroyo willow (*Salix lasiolepis*), and several ponds host native cattail (*Typha domingensis*).

Rancho Meling (625 m elev) is located in the western foothills of the Sierra San Pedro Mártir, a mountain range in northern Baja California and a continuation of the Transverse and Peninsular ranges in southern California, USA (Rebman et al., 2012). A perennial stream, Arroyo San José (~12 km in length), crosses through the ranch, a popular stop for tourists entering Sierra de San Pedro Mártir National Park. The low-gradient stream has few natural shallow ponds; however, several ponds have been created for cattle watering and irrigation. These constructed ponds are used by native species, including *R. draytonii*, *A. boreas*, arroyo toads (*Anaxyrus californicus*), western spadefoot toads (*Spea hammondi*), *H. hypochondriaca*, and California tree frogs (*Hylliola cadaverina*). Non-native aquatic taxa have not been observed at Rancho Meling. Vegetation is characterized by western sycamore, Fremont cottonwood (*Populus fremontii*), and arroyo willow. Small-scale cattle grazing and agricultural activities are present.

La Grulla (2050 m elev) is a mountain meadow located in Sierra de San Pedro Mártir National Park. The habitat is dominated by coniferous forest vegetation, mainly Jeffrey pine (*Pinus jeffreyi*) and scrub such as manzanita (*Arctostaphylos* spp.), peninsular oak (*Quercus peninsularis*), and rose sage (*Salvia pachyphylla*) (Rebman et al., 2012). Cattle have grazed the area for over 100 years. La Grulla is located at the headwaters of the Santo Domingo watershed, which is the largest watershed in Baja California. Natural ponds with depths of 15–200 cm (Ruiz-Campos, 1993) support native amphibian species, including *R. draytonii*, *A. boreas*, *A. californicus*, *H. cadaverina*, and *H. hypochondriaca*. No non-native aquatic species are present at the La Grulla ponds; however, Baja California rainbow trout (*Oncorhynchus mykiss nelsoni*) have been translocated to the stream from Arroyo San Antonio Murillos, a lower-elevation

tributary of this watershed ~20 km downstream, where the species is native but was otherwise prevented from migrating to La Grulla by natural barriers.

2.2. Sampling methods

We sampled all amphibian species encountered from November 2015 to October 2016, surveying both nocturnally and diurnally. We sampled each of the three study sites once per season, for a total of 12 surveys. Search effort was consistent, and methods were standardized, across sampling sites. We walked along the banks, waded in the channel, and captured juvenile and adult amphibians with gloved hands. Upon capture, we swabbed for Bd using sterile, rayon-tipped swabs following a standardized protocol developed specifically for Bd sampling and detection (Hyatt et al., 2007). Post-metamorphic individuals were swabbed five times each on the bottoms of the feet, on the ventral thighs, and both sides of the lower abdomen. Gloves were changed between each capture to prevent cross-contamination. Swabs were individually placed in sterile screw-cap vials with 96% molecular-grade ethanol. Water temperature was measured with HOBO data loggers (Onset Computer Corp., Pocasset, MA) placed at a depth of 10 cm in the vicinity of survey locations at all three sites. The loggers were placed in flowing streams at Rancho Meling and La Grulla and in a pool at San Carlos, where the stream does not run year-round.

Historical samples were collected from specimens stored at the San Diego Natural History Museum. We sampled all available post-metamorphic anuran specimens in the collection from across Baja California. Museum specimens were sampled by first rinsing specimens individually with fresh ethanol and then swabbing 30 times in each body area mentioned above; these extra strokes are required to obtain an adequate sample of skin cells from formalin-fixed tissue (Adams et al., 2015). Fresh ethanol rinses have been shown to effectively eliminate the risk of cross-contamination from communal specimen storage in ethanol (Adams et al., 2015). Field and specimen samples were air-dried under a laminar flow fume hood in the laboratory prior to DNA extraction to remove all residual ethanol.

To characterize Bd dynamics in an infected population, we use the proportion of infected individuals (Bd prevalence) and infection intensity (Bd load) as separate metrics (Briggs et al., 2010). We used a real-time PCR assay (qPCR) to test for the quantity of Bd in each sample (Bd load). Using qPCR analysis, infection intensity is determined in terms of zoospore equivalents (ZE), the number of zoospores on the swab sample, as compared to a standard curve of serial dilutions of standard Bd DNA (Boyle et al., 2004). Swabs collected from the field were DNA extracted using 40 μ L of PrepMan Ultra (Applied Biosystems) using standardized protocols (Boyle et al., 2004; Hyatt et al., 2007). Swabs collected from museum specimens were DNA extracted using Macherey-Nagel DNA FFPE, a method shown to be 40% more effective at detecting Bd DNA from museum specimens than swabs extracted using the PrepMan extraction method (Adams et al., 2015). For all samples, qPCR analysis followed Boyle et al. (2004), and samples were run on a StepOnePlus real-time PCR system (Applied Biosystems). Positive controls in quantities of 0.1, 1, 10, and 100 ZE were run in addition to negative controls (PCR water only), with one set of standards per qPCR plate. Negative controls indicated that there was no false positive amplification on any of the qPCR plates. Museum specimen samples were run in triplicate, and when one or more replicates amplified in qPCR, they were considered positive for the study. Single-replicate positives are not expected to result from cross-contamination, because a large number of positives in museum specimen Bd studies show up as positive only in a single qPCR run.

2.3. Analyses and hypothesis evaluation

Based on the literature, we expected Bd prevalence and load to vary according to different biotic and abiotic factors (Table 1). The rate of Bd growth is temperature-dependent and can be mediated by anthropogenic, behavioral, or climatic shifts (Piotrowski et al., 2004; Puschendorf et al., 2011; Adams et al., 2017a); therefore, we tested for effects of water temperature and season on Bd status. The

Table 1

Variables used in mixed effects models to determine the best predictors of Bd load and prevalence. The order of predictor variables in the table indicates the order in which they were tested in candidate models.

| Predictor | Type | Range or levels | Description |
|----------------------------|-------------------------------|---|--|
| Water Temperature (mean) | Environmental/ Temporal | 6.1–21.6 C | Temperature of water body where sampling occurred |
| Water Temperature (median) | Environmental/ Temporal | 5.8–20.9 C | Temperature of water body where sampling occurred |
| Season | Environmental/ Temporal | Wet/Cool, Dry/Warm | Wet/Cool season: 1 December to 31 May; Dry/Warm season: 1 June to 30 November. |
| Elevation ^a | Environmental | 110–2050 m | Elevation of site where sampling occurred |
| Bullfrogs ^a | Biological | Present/Absent | Whether American bullfrogs (<i>Lithobates catesbeianus</i>) were present at the sampling site |
| Site ^a | Environmental & Biological | Arroyo San Carlos, Rancho Meling, La Grulla | Site that sampling occurred (Fig. 1) |
| Life stage | Biological | Adult, Juvenile | Life stage of individual sampled |
| Species | Biological | ANBO; HYHY; LICA; RADR | ANBO = <i>Anaxyrus boreas</i> (Western toad); HYHY = <i>Hyla hachisontana</i> (Baja California treefrog); LICA = <i>Lithobates catesbeianus</i> (American bullfrog); RADR = <i>Rana draytonii</i> (California red-legged frog) |

^a Predictors used only in models that included all sites.

literature also suggests that elevation, life stage, and species can influence Bd prevalence and load (Garner et al., 2009; Johnson et al., 2011; Searle et al., 2011); therefore, we tested these as predictor variables. Finally, bullfrogs are a Bd vector and reservoir host that can drive Bd dynamics in native amphibians (Peterson and McKenzie, 2014; Adams et al., 2017a); therefore, we used bullfrog presence as a predictor variable. We expected Bd prevalence in museum specimens to vary spatiotemporally (De León et al., 2016; Adams et al., 2017b), and so tested year, decade, distance from the earliest positive, latitude, longitude, and 20-year time interval as predictor variables in the models of Bd prevalence in museum specimens.

We used generalized linear mixed effects models (GLMMs) and an information-theoretic approach to test various hypotheses for which factors best predict Bd prevalence and load. Using mixed effects models enabled us to account for non-independence of samples that were collected at the same locality during the same time period—in every model we included “survey event”, a variable created to group animals sampled on the same visit (within the same 4-day time period) and at the same site, as a random effect. We developed a set of candidate models to determine the best predictors of Bd prevalence and load across sites and within sites (8 candidate model sets total), because amphibian community composition and local environmental factors varied between sites.

Using a stepwise forward selection procedure with the GLMMs, we determined the predictor variables that were the best fit to the data, according to the aforementioned hypotheses. We tested the predictor variables in the order presented in Table 1 and computed likelihood ratio tests for nested models. We log-transformed ZI values for the model with Bd load as a response variable and z-transformed all continuous predictor variables so that effect sizes of different predictors were comparable. We included only Bd-positive samples in the models of Bd load. We included interactions in the models whenever biologically appropriate and they did not prevent model convergence. We ranked candidate models according to Akaike’s information criterion corrected for small sample sizes (AIC_c) to determine the relative importance of predictor variables within each model set. The models with the lowest AIC_c were considered the best-supported models by the data. To ensure that none of the fixed effects in the best-fit models were collinear, we used candidate predictors in the same model only if their variance inflation factors (VIFs) taken together had values less than 3 (Zuur et al., 2010). We conducted all analyses in R (R Core Team, 2019). The GLMMs were fit using the “glmer” (for Bd presence/absence models) and “lme” (for the Bd load models) functions in the “lme4” and “nlme” packages, respectively (Pinheiro et al., 2019; Bates, 2020). The GLMM for Bd presence/absence included a logit-link function and a binomial error structure. Similarly, the error was normally distributed for the log-transformed Bd loads. For the best-fit model in each model set, statistics were obtained using the “summary” and “intervals” commands for the binomial and linear GLMMs, respectively. The “summary” command calculated P-values for glmer models using asymptotic Wald tests. To avoid pseudoreplication in the museum specimen analysis, we included only one sample per series (i.e., individuals collected on the same date at the same location); if there were any positive samples in a series, we used the positive sample in the analysis (Adams et al., 2017b).

3. Results

3.1. Field sampling

3.1.1. Bd prevalence

We detected Bd in 361 of 533 post-metamorphic individuals sampled, an overall Bd prevalence of 68% (95% CI 0.63–0.72; Table S1). Elevation, lifestage, and species were included in the best-fit model of Bd prevalence at all sites (Table 2). *Lithobates catesbeianus* and *R. draytonii* had significantly higher prevalence than *A. boreas* and *H. hypochondriaca* ($p < 0.0001$). Prevalence increased significantly with elevation ($p < 0.0001$). Lifestage (whether juvenile or adult) was also an important predictor of Bd prevalence, though the difference between juveniles and adults was not significant ($p = 0.66$). Pathogen prevalence at La Grulla was 84% (177/

Table 2

Parameter estimates for best-fit models (Tables S2–S5) used to determine the best predictors of Bd prevalence in post-metamorphic anurans. ANBO = *Anaxyrus boreas* / Western toad; HYHY = *Hyliola hypochondriaca* / Baja California tree frog; LICA = *Lithobates catesbeianus* / American bullfrog; RADR = *Rana draytonii* / California red-legged frog. * $p < 0.05$.

| Model | Parameter | Estimate | SE | z | P |
|-------------------|-----------------------------------|----------|------|-------|------------|
| All sites | Intercept (ANBO adults) | -0.14 | 0.24 | -0.16 | 0.81 |
| | Stage (juvenile) | -0.14 | 0.32 | -0.45 | 0.66 |
| | Elevation | 1.85 | 0.47 | 3.91 | < 0.0001* |
| | Species (HYHY) | 0.14 | 0.42 | -0.33 | 0.74 |
| | Species (LICA) | 3.44 | 0.81 | 4.27 | < 0.0001* |
| | Species (RADR) | 2.18 | 0.47 | 4.62 | < 0.00001* |
| La Grulla | Intercept (ANBO) | 1.70 | 1.01 | 1.69 | 0.09 |
| | Species (HYHY) | -0.21 | 0.47 | -0.44 | 0.66 |
| | Species (RADR) | 3.87 | 1.09 | 3.53 | < 0.001* |
| Rancho Meling | Intercept (HYHY adults) | -0.66 | 0.51 | 0.60 | 0.55 |
| | Stage (juvenile) | -0.37 | 0.60 | -0.62 | 0.54 |
| | Species (RADR) | 2.57 | 0.75 | 3.43 | < 0.001* |
| | Stage (juvenile) x Species (RADR) | -1.92 | 0.89 | -2.14 | 0.03* |
| Arroyo San Carlos | Intercept (HYHY adults) | -3.34 | 1.22 | -2.75 | 0.006* |
| | Stage (juvenile) | 1.77 | 0.71 | 2.50 | 0.01* |
| | Species (LICA) | 4.55 | 0.91 | 4.99 | < 0.0001* |

211 samples positive; 95% CI 0.78 – 0.89; Table S1), the highest of all sites sampled in this study. Prevalence of Bd in *R. draytonii* at La Grulla was 99%. Whether a frog at La Grulla was infected with Bd was best predicted by species, with *R. draytonii* having significantly higher prevalence than *H. hypochondriaca* or *A. boreas* (Table 2 and Fig. 2; S3).

At Rancho Meling, Bd prevalence was 70% (124/176 samples positive; 95% CI 0.63 – 0.77; Table S1). The best-fit models indicated that a model including a species-lifestage interaction was the best predictor of Bd prevalence at Rancho Meling (Table 2 and S4), with higher Bd prevalence in *R. draytonii* adults than *R. draytonii* juveniles (Table 2). We removed *H. cadaverina* samples from the data used in the GLMMs because the sample size for this species was small (N = 5); therefore, we included samples from *A. boreas*, *R. draytonii*, *H. hypochondriaca*, and *L. catesbeianus* in our models. Pathogen prevalence at Arroyo San Carlos was 42% (59/141 samples positive; 95% CI 0.34 – 0.51; Table S1), and in the best-fit model, species and lifestage were important predictors of Bd prevalence (Table 2 and S5). *Lithobates catesbeianus* had significantly higher Bd prevalence at this site than *H. hypochondriaca* (Fig. 2).

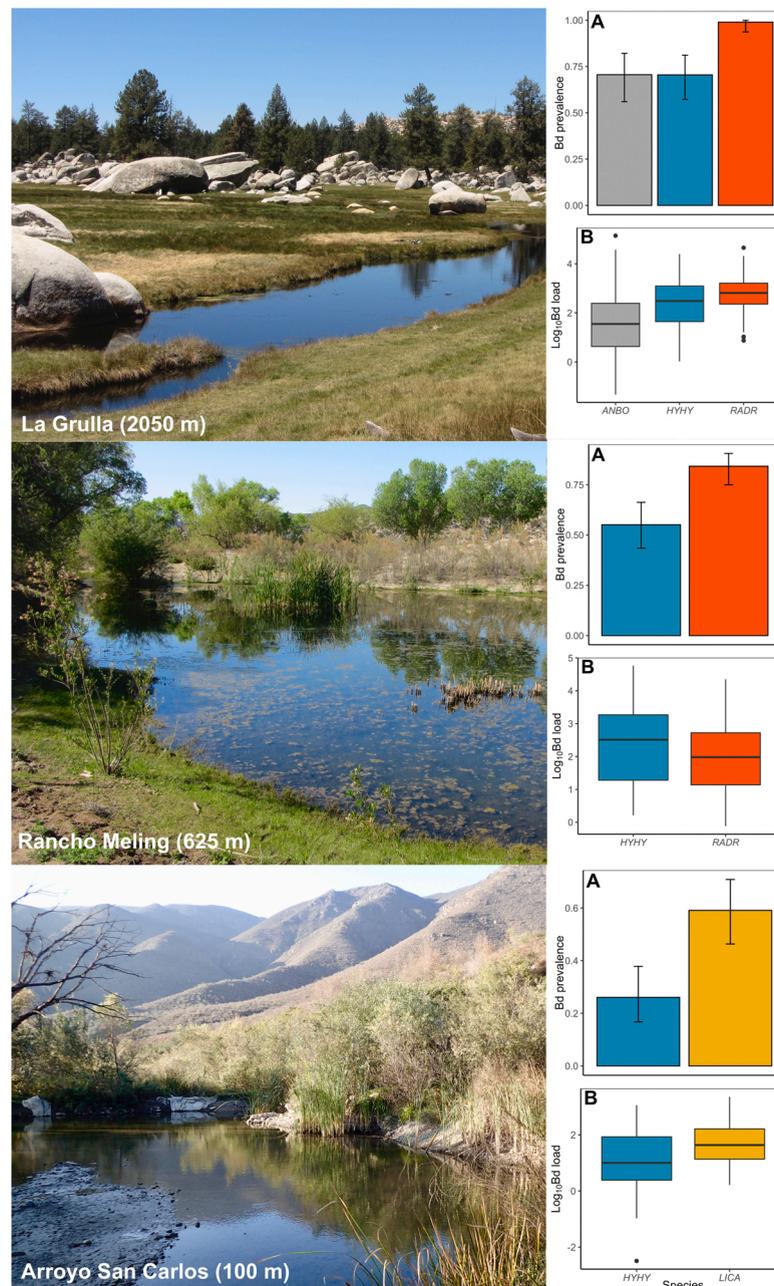


Fig. 2. Bd prevalence (A) and load (B) from the anuran communities of each of the three sites sampled. ANBO = *Anaxyrus boreas* / Western toad; HYHY = *Hyliola hypochondriaca* / Baja California tree frog; LICA = *Lithobates catesbeianus* / American bullfrog; RADR = *Rana draytonii* / California red-legged frog. Colors correspond to individual species. Bd load is presented on a log₁₀ scale. Images represent the habitat at each site indicated.

3.1.2. *Bd* load

The mean *Bd* load from all sites (*Bd*-positive samples only) was 2053 ZE \pm 9409 SD, and the highest *Bd* load detected was > 139,000 ZE in *A. boreas* (Table S1). The best-fit model of *Bd* load from all sites included species, and load increased positively with elevation (Table 3 and S6). Mean *Bd* load at La Grulla was 2733 ZE \pm 11,632 SD. The best-fit model of *Bd* load at La Grulla included species (Table 3 and S7). Mean *Bd* load at Rancho Meling was 2352 ZE \pm 7973 SD. The best-fit model of *Bd* load at Rancho Meling included season, though this effect was not significant (Table 3). Rancho Meling models that included species and lifestage were equivalent ($<2 \Delta AIC_c$) to the best-fit model (Table S8). At Arroyo San Carlos, mean *Bd* load was 218 ZE \pm 461 SD. The best-fit model of *Bd* load at this site included species (Table 3 and S9).

3.2. Museum specimens

We sampled 145 specimens of six species from four anuran families (Table S10). Overall historical *Bd* prevalence was 21% (95% CI 0.14 – 0.28). None of the candidate models outperformed the null model, indicating that none of the covariates we tested were good predictors of *Bd* infection in historical samples (Table S11). We detected *Bd* in museum specimens as early as 1932 in *A. boreas* (SDNHM 8859) from San José (an historical name for Rancho Meling), Baja California (Fig. 3).

4. Discussion

4.1. *Bd* susceptibility in focal species

The Mediterranean region of Baja California represents a largely unexplored area of western North America with regard to *Bd*'s distribution and driving factors. Much can be learned about the spatiotemporal distribution and dynamics of *Bd* by comparing Baja California *Bd* results to other areas of western North America. Susceptibility to *Bd* is extensively variable among amphibian species (Bancroft et al., 2011; Searle et al., 2011; Blaustein et al., 2018; Olson et al., 2021), and our results show that species is an important predictor of *Bd* prevalence and load both within and among our study sites in Baja California. We found that elevation is an important predictor of *Bd* load and prevalence in Baja California, consistent with work suggesting that higher elevations in Mexico, and Baja California in particular, have higher environmental suitability for *Bd* growth (Bolom-Huet et al., 2019; Nava-González et al., 2020).

Loads > 10,000 ZE are associated with lethal chytridiomycosis in some susceptible *Rana* species (Briggs et al., 2010; Kinney et al., 2011; Adams et al., 2017a), and we observed relatively high *Bd* loads (>10,000 ZE) in multiple species in this study (*A. boreas*, *R. draytonii*, and *H. hypochondriaca*; Fig. 2). High loads in Baja California tree frogs (*H. hypochondriaca*) might be expected, as a closely related congener, the Sierran chorus frog (*H. sierra*), is a suitable *Bd* reservoir host, able to tolerate high loads without succumbing to disease (Reeder et al., 2012).

The role individual species play in the host-pathogen system—including whether they act as vector or reservoir hosts—is a key factor to consider when examining community effects on *Bd* outcome (Venesky et al., 2014). In the present study, the non-native American bullfrog—a *Bd* vector and reservoir host (Miaud et al., 2016)—was present at only one site (Arroyo San Carlos), where the species had higher *Bd* prevalence and load than the sympatric *H. hypochondriaca* (Tables 2 and 3). The presence of bullfrogs has been associated with increased *Bd* prevalence and load in the threatened foothill yellow-legged frog (*Rana boylei*) in California (Adams et al., 2017a).

The highest *Bd* load we detected in this study was > 130,000 ZE in a single *A. boreas* individual. *Anaxyrus boreas* is highly susceptible to chytridiomycosis in the Rocky Mountains, USA, where it has experienced significant declines at higher (2200–3400 m) elevations (Muths et al., 2003; Pilliod et al., 2010). In Baja California, *Bd* prevalence in *A. boreas* has previously been reported at 40%, though the sample size ($N = 5$) was small (Peralta-García et al., 2018). In the present study, *Bd* prevalence in *A. boreas* was 69%, based on 55 samples. By contrast, at lower elevations (<1000 m) in the Mediterranean region of southern California, *A. boreas* exhibited the lowest *Bd* prevalence (1%) and load (1 ZE) in the anuran community (Adams et al., 2017b). This pattern across three disparate

Table 3

Parameter estimates for best-fit models (Tables S6–S9) used to determine the best predictors of *Bd* infection intensity (load, log-transformed) in post-metamorphic anurans. ANBO = *Anaxyrus boreas*; HYHY = *Hyliola hypochondriaca*; LICA = *Lithobates catesbeianus*; RADR = *Rana draytonii*. *Parameter estimates with 95% CI that do not overlap zero.

| Model | Parameter | Estimate | 95% CI (lower, upper) |
|-------------------|----------------------|----------|-----------------------|
| All sites | Intercept (ANBO) | 3.12 | 2.17, 4.05* |
| | Species (HYHY) | 2.17 | 1.23, 3.11* |
| | Species (LICA) | 3.52 | 2.02, 5.02* |
| | Species (RADR) | 2.03 | 1.13, 2.93* |
| | Elevation | 1.41 | 0.78, 2.05* |
| La Grulla | Intercept (ANBO) | 4.30 | 3.08, 5.53* |
| | Species (HYHY) | 1.67 | 0.69, 2.64* |
| | Species (RADR) | 2.27 | 1.36, 3.18* |
| Rancho Meling | Intercept (dry/warm) | 4.56 | 4.03, 5.09* |
| | Season (wet/cool) | 1.17 | -1.25, 3.59 |
| Arroyo San Carlos | Intercept (HYHY) | 2.51 | 1.48, 3.53* |
| | Species (LICA) | 1.42 | 0.14, 2.69* |

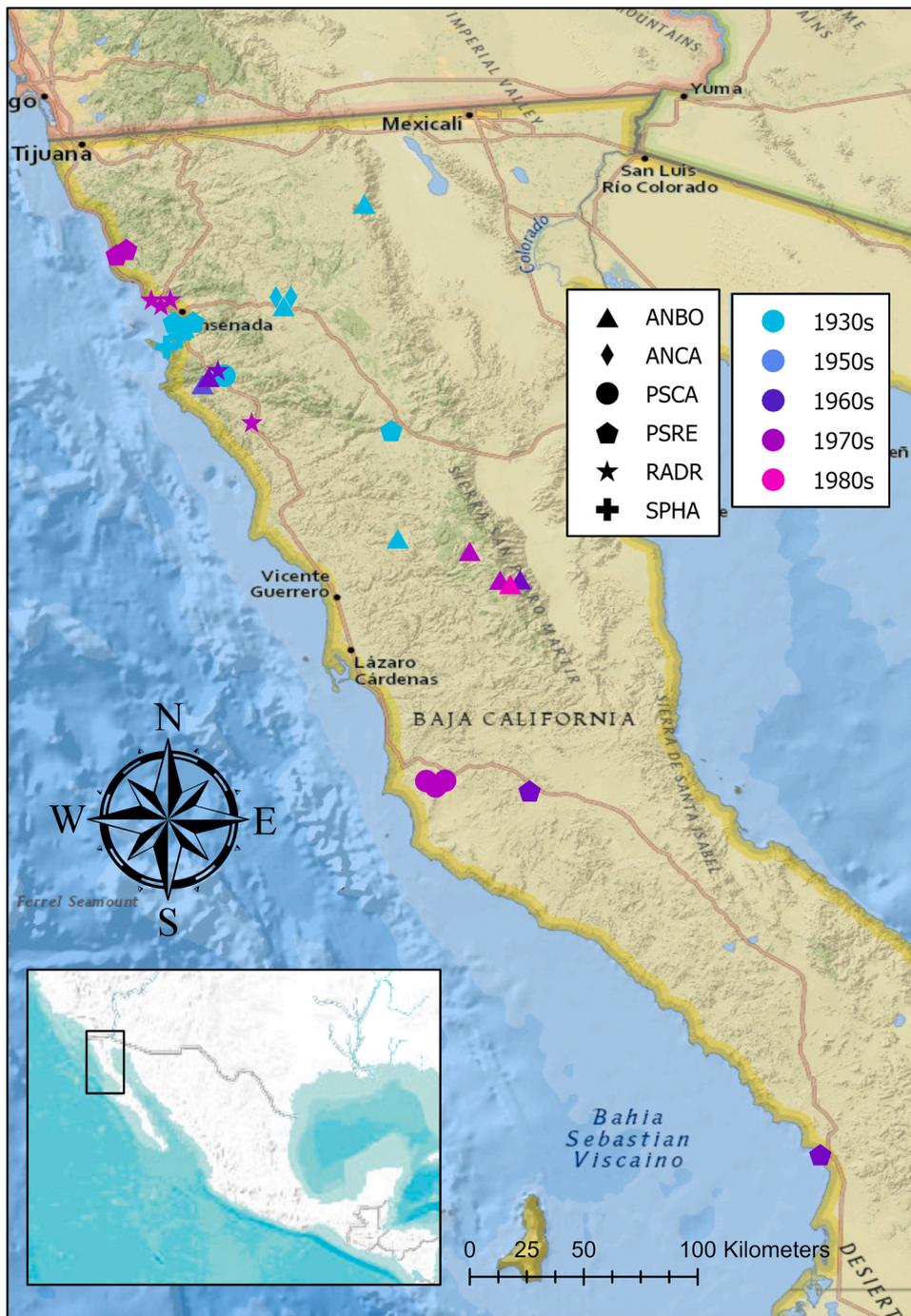


Fig. 3. Map of *Batrachochytrium dendrobatidis* (Bd)-positive museum specimens in Baja California by species and decade. ANBO = *Anaxyrus boreas*; ANCA = *Anaxyrus californicus*; HYCA = *Hylliola cadaverina*; HYHY = *Hylliola hypochondriaca*; RADR = *Rana draytonii*; SPHA = *Spea hammondi*.

geographic regions suggests that elevation may be an important factor in Bd outcome for *A. boreas*.

Within-species disease susceptibility across the entire range of individual wildlife pathogens is a phenomenon deserving of further examination. For example, little brown bats (*Myotis lucifugus*) in eastern North America are highly susceptible to *Pseudogymnoascus destructans* (Pd), the fungal pathogen that causes white nose syndrome. Since the discovery of Pd in western North American bats in 2016, the same catastrophic declines have not yet been observed in *M. lucifugus* (Rodhouse et al., 2019; Hoyt et al., 2021). Mirroring these longitudinal differences, amphibians in western North America have experienced declines on a latitudinal gradient: *R. boylei* was rapidly extirpated from southern California coincident with the arrival of Bd in the region, yet populations are persisting in the

northern part of their extant range despite presence of the pathogen (Adams et al., 2017b; Kupferberg et al., 2021). *Rana draytonii* have also experienced a range contraction from southern California northward, a pattern consistent with a deadly disease (Richmond et al., 2013).

We found a positive effect of elevation on Bd load, with higher Bd loads at higher-elevation sites, which is consistent with previous work in Baja California (Peralta-García et al., 2018) and elsewhere (Catenazzi et al., 2010; Johnson et al., 2011; Nava-González et al., 2020). Chytridiomycosis has affected montane anuran populations in the tropics (La Marca et al., 2005; Lips et al., 2005), Peruvian Andes (Catenazzi et al., 2010), Europe (Bosch et al., 2001, 2007), and the western USA (Muths et al., 2003; Briggs et al., 2010; Vredenburg et al., 2010). The causes of this observed pattern are as yet unknown, but may be related to immunosuppression resulting from lower ambient temperatures or smaller amounts of genetic variance at more isolated high-elevation sites (Carey et al., 1999; Lips et al., 2003).

The patterns in Bd prevalence and load that we observed in *R. draytonii* are consistent with this elevational trend. We observed 99% Bd prevalence in *R. draytonii* at La Grulla (2050 m elev) and 84% at Rancho Meling (625 m elev; Fig. 2). The highest Bd load we detected in *R. draytonii* was > 44,000 ZE at La Grulla, our highest-elevation site. High Bd loads and prevalence are a concern for this threatened species. Lethal chytridiomycosis has been recorded in *R. draytonii* (Adams et al., 2020), and lower survivorship is associated with populations with higher Bd prevalence (Russell et al., 2019). At a chytridiomycosis mortality event in *R. draytonii* in the Sierra Nevada of California (975 m elevation), a moribund frog had a Bd load > 297,000 ZE (Adams et al., 2020). This highlights the need for continuing to monitor the La Grulla *R. draytonii* population, which is the highest-elevation site in the species' entire range. With high Bd prevalence combined with low genetic diversity (Peralta-García, 2017), the population at this site is at a high risk of extirpation.

4.2. High Bd prevalence in Baja California

The overall Bd prevalence we observed (68%) is higher than that observed (47%) in the most comprehensive examination of Bd in Baja California prior to this study (Peralta-García et al., 2018). In that study, elevation was a significant predictor of Bd prevalence, but not Bd load; by contrast, we found that elevation was an important predictor of Bd prevalence and load. This difference may be a result of sampling design: Peralta-García et al. (2018) sampled at 33 localities across Baja California, whereas we conducted repeated sampling at three designated sites. While the mixed effects modeling employed for the statistical analyses in both studies accounted for repeated sampling, there were still differences in the sites and species sampled between the two studies.

The Bd prevalence observed in the present study is also markedly higher than the prevalence (15%) observed in a 4-year Bd sampling effort with identical community composition in the Mediterranean region of southern California (Adams et al., 2017b). One explanation for this difference could be that the epizootic following Bd's arrival in the Mediterranean region of Baja California is more recent, and therefore in an earlier stage than the enzootic state of the Mediterranean region of southern California.

4.3. Historical Bd presence in Baja California

In the museum specimens we sampled, we found Bd in Baja California as early as 1932. The earliest record of Bd detection from the state of Baja California is 1926 (Basanta et al., 2021). The same study found the earliest Bd detection in Mexico from 1894 in the state of Baja California Sur, suggesting that Bd could have arrived in Baja California from the north or the south, and possibly earlier than in southern California. Comparatively, the earliest record of chytridiomycosis in California is from Los Angeles County in 1915 (Adams et al., 2017b), and in eastern North America, the earliest record is from 1888 in the state of Illinois (Talley et al., 2015). A number of amphibian declines in California and Mexico have been attributed to the arrival of chytridiomycosis in the 1960s and 1970s (Padgett-Flohr and Hopkins, 2009; Cheng et al., 2011; Sette et al., 2015; Adams et al., 2017b); however, depending on how "regions" are defined, the earliest Bd detections can pre-date evidence of epizootics by decades (Adams et al., 2017b; De León et al., 2019). There are several possible reasons for this, one of which is that there is an endemic Bd variant that has been present for much longer than the one that is causing the declines observed in more recent decades.

We observed low Bd prevalence in the museum specimens we sampled (21%); however, Bd prevalence estimates from museum specimens are likely to be underestimated, because the damage caused to Bd DNA by the sample preservation process makes qPCR detection extremely difficult (Adams et al., 2015). Therefore, relatively low Bd prevalence in museum specimens compared to the field data does not indicate that prevalence has increased through time. Nevertheless, spikes in historical prevalence have been linked to Bd outbreaks: significant increases in Bd prevalence have been associated with amphibian declines in Illinois (Talley et al., 2015), California (Adams et al., 2017b), and Brazil (Carvalho et al., 2017). In Mexico, high historical prevalence was observed between 1930 and 1949 (Basanta et al., 2021), which coincides with the earliest Bd positive specimen we detected (1932).

Anthropogenic movements facilitate the spread of Bd intercontinentally, largely through the global amphibian trade (Fisher and Garner, 2007; Schloegel et al., 2009, 2012). When looking for potential culprits prior to widespread globalization, it can be useful to look at changes in historical land use for probable causes of Bd introduction and spread (Adams et al., 2017b). Two of these potential anthropogenic activities common in Baja California throughout the early twentieth century were cattle ranching and recreational hunting.

When cattle are moved between grazing areas and across landscapes, they may transport Bd (Derlet et al., 2010). As many as 20,000 cattle were grown in the main mountain ranges of Baja California (Sierra Juárez and Sierra San Pedro Mártir) in 1911, and subsequently transported to the US-Mexico border to be slaughtered in Mexico and imported as meat to the USA (Acosta Montoya, 2009). In southern California, an increase in recreational activities coincided with increased Bd prevalence and the decline of *R. boylei* from that region (Adams et al., 2017b). In Baja California, recreational hunting and exploration began with the early establishment of several

gun clubs (in Ensenada, Uruapan, Sierra Juárez, Ojos Negros, and San Quintín); these activities significantly grew during the 1920s and 1930s (Tenderfoot, 1924; Salisbury and Mc Lean, 1925). Thus, we suspect cattle ranching and recreational activities, such as hunting and exploration, could have facilitated Bd's spread throughout Baja California in the early twentieth century.

Genetic variance may also play a role in the high Bd prevalence we observed in Baja California, as Bd virulence can vary both within and among genotypes (Berger et al., 2005; Fisher et al., 2009; Farrer et al., 2011). The Bd-GPL (Global Panzootic Lineage)-1 genotype, which predominates in North America and Europe and has been associated with die-offs and declines in the Sierra Nevada of California (Rachowicz et al., 2006; Vredenburg et al., 2010; James et al., 2015), is also widespread across Mexico, including Baja California (Basanta et al., 2021). In addition, samples from Mexico are genetically similar to isolate CJB4 from the Sierra Nevada of California (Rosenblum et al., 2013; Basanta et al., 2021). A second genotype, Bd-GPL-2, which has been associated with mortality events in Central America, was isolated from one *A. boreas* individual in Baja California (Basanta et al., 2021). The co-occurrence of Bd-GPL-1 and Bd-GPL-2 in Baja California creates the potential for coinfection with multiple lineages, which can affect pathogen diversity, and therefore potentially influence disease outcome (Jenkinson et al., 2018). We did not genotype the Bd strains infecting the anurans in our study; thus, the role of genotype in virulence in these Baja California anuran communities compared to southern California remains unknown, and is an avenue for further research.

5. Conclusion and conservation recommendations

Our work suggests that Bd is prevalent in the Mediterranean region of Baja California, that Bd loads capable of causing morbidity and mortality affect anurans in this area, and that increased infection burdens occur at higher elevations. Amphibians are in decline in this biodiverse region, and continued, regular Bd monitoring should be conducted to determine when these populations shift from an epizootic to an enzootic phase, which should be signaled by lower Bd prevalence and load. No chytridiomycosis mortalities have yet been recorded in Baja California, and we did not observe clinical signs of chytridiomycosis infection in this study. However, such observations are unusual because frogs succumb rapidly once morbidity is present (Savage et al., 2011; Piovio-Scott et al., 2015), and scavengers, the environment, and natural processes facilitate rapid decay.

Reintroductions and habitat restoration (i.e., creating additional breeding sites) may improve population persistence for the threatened *R. draytonii* experiencing high Bd prevalence and load here. We caution, however, that with the diversity of Bd lineages present in the region, strict biosecurity measures (including decontamination of field equipment) should be adhered to when conducting monitoring and when translocating anurans between sites. Any translocated individuals should be confirmed Bd-negative or cleared of Bd in an *ex situ* facility prior to translocation. Preferably—and more economically—egg masses, which are not keratinized and therefore cannot be infected with Bd, should be used for translocations rather than tadpoles and post-metamorphic individuals.

More broadly, our work adds to a growing body of evidence that Bd arrived in western North America earlier than was previously presumed, and that the history of Bd invasion globally is highly complex and nuanced. High Bd prevalence and load in Baja California compared to ecologically similar areas further north suggest there may be more factors influencing Bd outcome than have yet been measured. Further work in Bd's genotypic variation in the region may advance scientific understanding of enigmatic differences in Bd virulence in anuran communities of Baja California and nearby regions.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.gecco.2021.e01968](https://doi.org/10.1016/j.gecco.2021.e01968).

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