

AMPHIBIAN CHYTRID FUNGUS (*BATRACHOCHYTRIUM DENDROBATIDIS*) IN COASTAL AND MONTANE CALIFORNIA, USA ANURANS

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Abstract.—We found amphibian chytrid fungus (*Bd* = *Batrachochytrium dendrobatidis*) to be widespread within a coastal watershed at Point Reyes National Seashore, California and within two high elevation watersheds at Yosemite National Park, California. *Bd* was associated with all six species that we sampled (*Bufo boreas*, *B. canorus*, *Pseudacris regilla*, *Rana draytonii*, *R. sierrae*, and *Lithobates catesbeianus*). For those species sampled at 10 or more sites within a watershed, the percentage of *Bd*-positive sites varied from a low of 20.7% for *P. regilla* at one Yosemite watershed to a high of 79.6% for *P. regilla* at the Olema watershed at Point Reyes. At Olema, the percent of *Bd*-positive water bodies declined each year of our study (2005–2007). Because *P. regilla* was the only species found in all watersheds, we used that species to evaluate habitat variables related to the sites where *P. regilla* was *Bd*-positive. At Olema, significant variables were year, length of shoreline (perimeter), percentage cover of rooted vegetation, and water depth. At the two Yosemite watersheds, water depth, water temperature, and silt/mud were the most important covariates, though the importance of these three factors differed between the two watersheds. The presence of *Bd* in species that are not declining suggests that some of the amphibians in our study were innately resistant to *Bd*, or had developed resistance after *Bd* became established.

Key Words.—Amphibian chytrid; *Batrachochytrium dendrobatidis*; *Bufo*; California; *Pseudacris regilla*; *Rana*; Sierra Nevada

INTRODUCTION

In 1998, a new infectious disease, chytridiomycosis, was described by Berger et al. (1998). The disease is caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), which was described by Longcore et al. (1999). *Bd* has now been implicated in the decline of amphibian populations in many areas around the world (Berger et al. 1998; Bosch et al. 2001; Hopkins and Channing 2003; Ron et al. 2003; Woodhams et al. 2008). However, it is unclear under what circumstances outbreaks of lethal chytridiomycosis occur. The oldest records of *Bd* come from *Andrias japonicus* (Japanese Giant Salamander) collected in 1902 in Japan (Goka et al. 2009), and from *Xenopus laevis* (African Clawed Frog) collected in 1938 in southern Africa (Weldon et al. 2004).

The first report of *Bd* in California, USA was from *Lithobates catesbeianus* (American Bullfrog) collected in 1961 in Palo Alto (Padgett-Flohr and Hopkins 2009). In the Sierra Nevada of California, *Bd* has been reported from *Bufo canorus* (Yosemite Toad) collected in 1976 (Green and Kagarise Sherman 2001), and *Rana sierrae* (Sierra Nevada Yellow-legged Frog) collected in 1993 (Fellers et al. 2001a). *Bd* has now been documented in at least 14 species of amphibians in California, nearly all

the species that have been examined carefully. However, the prevalence of *Bd* in wild populations in California is largely unknown except for *R. muscosa* (Southern Mountain Yellow-legged Frog), *R. sierrae* (formerly part of *R. muscosa*; Vredenburg et al. 2007) in the Sierra Nevada (Briggs et al. 2005; Vredenburg et al. 2010), and in all six species of local amphibians in a set of ponds in Santa Clara County (Padgett-Flohr and Hopkins 2010).

In many areas around the world, chytrid-related amphibian declines have been most dramatic at high elevations (montane tropics, Sierra Nevada, Rocky Mountains, Pyrenees Mountains), and an association between chytridiomycosis outbreaks, high altitude, and low temperatures has been proposed (Daszak et al. 2003). The highest elevation sites that support amphibian populations in the Sierra Nevada are typically at or above tree line where ponds and lakes are largely devoid of vegetation. The lack of vegetation and the related lack of microhabitat diversity might influence the presence of *Bd* or the likelihood that frogs would be exposed to the fungus, if important components for the survival of *Bd* were missing from these less complex environments. Also, the deeper portions of high elevation ponds and lakes likely favor *Bd* because they are typically quite cool. Because *Bd* does not grow or

produce live zoospores after two days at 28° C (Piotrowski et al. 2004), high elevation sites may harbor *Bd* more frequently, or in greater density compared with low elevation sites where summer water temperatures often exceed 28° C.

In North America, local declines and extirpations have been documented for frogs, and *Bd* infections have been reported for both pond-breeding and terrestrial salamanders (Muths et al. 2003; Lips et al. 2006). In the Sierra Nevada, some of the largest, most vigorous populations of *R. muscosa* and *R. sierrae* have died off within 1–2 years of the discovery of *Bd* at those sites, yet other *R. muscosa* and *R. sierrae* populations with *Bd* have persisted for many years with apparently stable populations (Briggs et al. 2005, 2010).

Because *Bd*-related declines have been reported in the Sierra Nevada, but not from any coastal areas, we examined the distribution of *Bd* in one low elevation coastal watershed at Point Reyes National Seashore (Olema) and two high elevation sierran watersheds at Yosemite National Park (Bridalveil and Dana) in California (Fig. 1). The objectives of our study were to: (1) determine the distribution of *Bd* in pond-breeding amphibians (*R. sierrae*, *R. draytonii* [California Red-legged Frog], *B. canorus*, *B. boreas* [Western Toad], *Pseudacris regilla* [Pacific Chorus Frog], and the non-native *L. catesbeianus*) in the three watersheds; (2) evaluate the geographic distribution of *Bd* within each watershed; and (3) evaluate associations between habitat variables and occurrence and prevalence of the chytrid fungus.

MATERIALS AND METHODS

Study areas.—At Point Reyes National Seashore, we used the Olema Creek watershed that drains into Lagunitas Creek (UTM: 516600 E, 4212950 N, zone 10), which then empties into a bay of the Pacific Ocean, 3 km downstream. Vegetative cover of about half of the watershed was grassland that was grazed by beef cattle, and the balance was predominantly second-growth Douglas-fir (*Pseudotsuga menziesii*) forest. Several two-lane roads run through the watershed, and two small towns (2,300 total population) are located there. In Yosemite National Park, we used both the Bridalveil Creek watershed (upstream from the Pohono Trail, 268830 E, 4175130 N, zone 11), and the Dana Fork of the Tuolumne River watershed (upstream of the confluence with the Gaylor Lakes drainage, 298150 E, 4194828 N, zone 11). Yosemite is located within the Sierra Nevada, and the two watersheds were selected to represent different elevations (2,000 m for Bridalveil, and 3,000 m for Dana). The Bridalveil watershed lies 250 km east of the Pacific Ocean, and the Dana watershed lies 300 km east. Vegetative cover of the Bridalveil watershed is mostly a Lodgepole Pine (*Pinus*

contorta) forest with lesser amounts of Red Fir (*Abies magnifica*) and scattered meadows with a mixture of grasses, sedges (*Carex* spp.), and Dwarf Bilberry (*Vaccinium caespitosum*). Vegetative cover of the Dana watershed is much more open than Bridalveil, and contains primarily grasses, sedges (*Carex* spp.), Tea Leaved Willow (*Salix planifolia*), Mountain Heather (*Phyllodoce breweri*), and Lodgepole Pine. Both watersheds have a single two-lane road through them; Highway 120 runs through the Dana watershed and is heavily traveled because it is the only cross-sierran road in the park. While snow is rare in the Olema watershed, frog breeding sites in both the Yosemite watersheds are under snow and ice throughout most of the winter. Because of this, we waited at least six weeks after ice-out in Yosemite each year before beginning our sampling in each watershed. In the Olema and Bridalveil watersheds, we sampled amphibians in every pond and lake within the watershed. In meadows, only discrete ponds were sampled. Within parts of the Dana watershed, ponds were both numerous and closely spaced, so we did not sample smaller, secondary ponds within a 100 m radius of a sampled site.

Field protocol.—We attempted to capture 20 tadpoles of each species at each site. We were not able to swab 20 individuals at a few sites; however, we present the results from sites that were either positive for *Bd*, or where at least 10 tadpoles were swabbed for that species. Adults were not used because they were far less common, more difficult to capture, and would not provide a sufficient sample size.

Tadpoles were caught with a dipnet and held in stainless steel bowls, one bowl for each species. We swabbed only tadpoles between Gosner stages 30 (rear limb buds visible, length of bud is twice that of diameter) and 40 (articulated hind limbs, but forelimbs not yet protruding or visible; Rachowicz et al. 2006). Gosner stage 30 was chosen as a low cutoff to ensure that tadpoles had grown to a large enough size to be easily handled and had probably been exposed to *Bd* if it was present at the site. For each set of 20 tadpoles, we used a fresh pair of nitrile gloves (Kimberly Clarke, Roswell, Georgia; but see Cashins et al. 2008). We removed tadpoles from their holding bowl with a small aquarium net, swabbed them (Medical Wire and Equipment Co., Corsham, Wiltshire, England), and released them into a second bowl. For each individual, we swabbed the keratinized structures (beak and tooth rows) with 15 passes, rotating the swab slightly after each pass. We immediately placed swabs in individual vials, stored them in a cool place, and placed them in a freezer at the end of the day. We shipped swabs overnight with sufficient blue ice (propylene glycol) to remain frozen until received at the USGS National Wildlife Health Center in Madison, Wisconsin for DNA

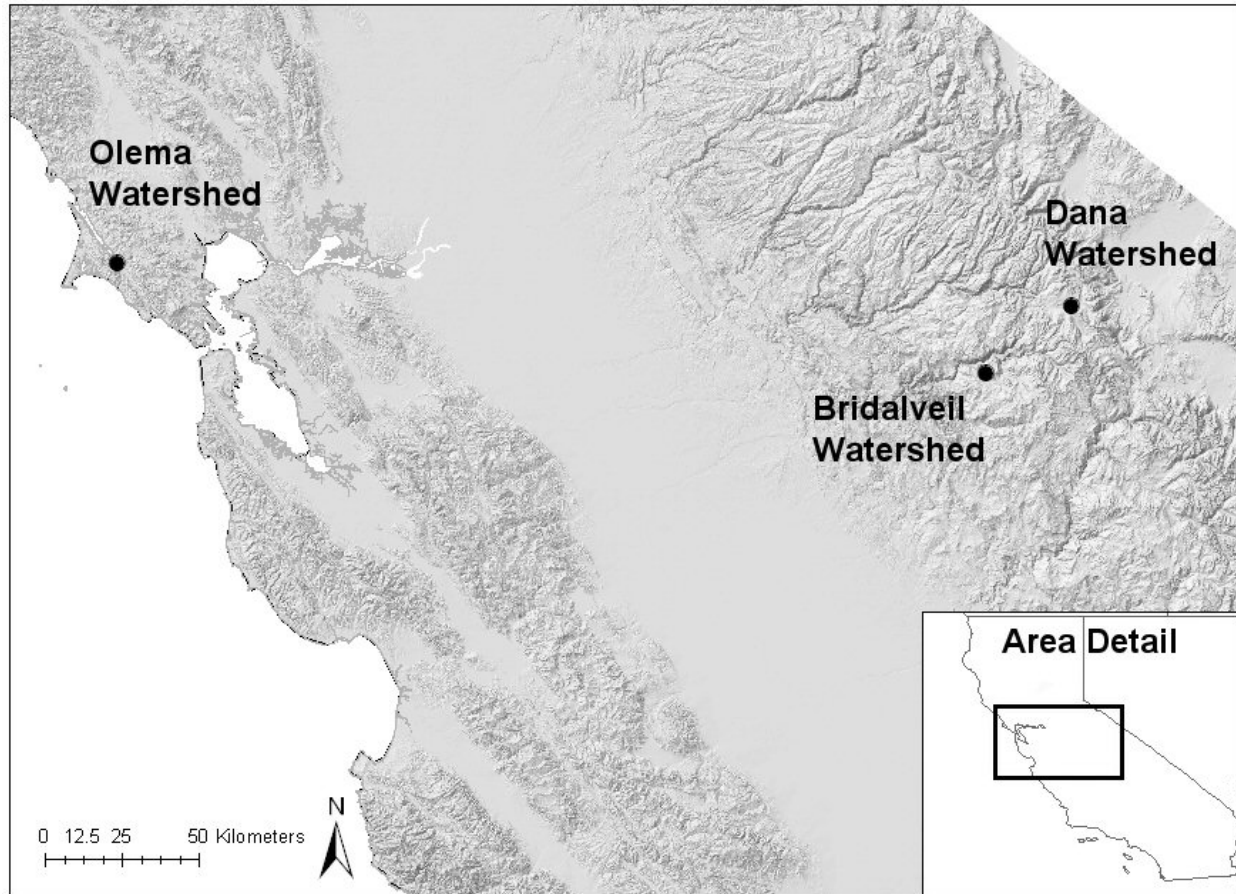


FIGURE 1. Location of Olema, Bridalveil, and Dana watersheds in central California, USA.

analysis. In a companion study, this handling procedure provided equivalent or better results for detecting *Bd* compared with swabs that were placed directly into vials with 100% ethanol (Rebecca Cole, pers. comm.). After sampling a site, we cleaned and decontaminated (30 s immersion in 10% bleach solution, then air dried) all equipment (waders, wading shoes, booties, dipnets, aquarium dip-net, thermometers, pans, etc.) that came into contact with the water.

We recorded descriptive data of each site. The site variables were: depth (average depth 3 m from shore), maximum depth (truncated at 3 m), elevation, fish presence, habitat naturalness (subjective 1–5 scale; 1 = pristine, 5 = completely unnatural such as a pond in a plowed field or a cement cistern), ordinal date, percentage cover of midday shade, permanent/temporary water body (based on 7.5' topographic maps), rooted vegetation (% cover), floating vegetation (% cover), length of shoreline (perimeter), pond in a wetland/meadow (yes/no), site length, site width, substrate predominantly silt/mud, substrate predominantly sand < 2 mm, substrate predominantly gravel 2–75 mm, turbidity (subjective 1–5 scale; 1 = clear water, 5 = turbid), UTM coordinates, water temp (15 cm deep, 0.5 m from shore; measured where tadpoles were

captured), and year. All values for percentage cover, length, and width were visual estimates. For the statistical analysis, we used site data collected on the first visit to each site (mostly from 2005).

Laboratory analyses.—We pooled swabs into groups of five and analyzed for *Bd* with Real-time TaqmanT PCR assays as described in Boyle et al. (2004). We modified the technique by using an ABI 7000 Prism system (Applied Biosystems, Foster City, California, USA) and prepared the samples by adding 60 μ l of Prepman UltraT to air-dried swabs that were stored in 1.5 ml screw-cap tubes (Fisher, San Diego, California, USA). We then incubated tubes at 100° C for 10 min in a dry heat block, cooled at room temperature for two minutes, and spun in a microfuge for three min at 14,000 RPM prior to removal of 20–25 μ l of supernatant for storage at -20° C until analysis.

Statistical analyses.—We evaluated the influence of the site variables on the presence of *Bd* in *P. regilla*, the only species present in all three watersheds, using an information theoretic approach. We used Akaike's Information Criterion (AICc values with the small-sample adjustment; Burnham and Anderson 2002) to

rank models, with those having the lowest AICc considered the most parsimonious. For each watershed, we used the following three-step process to evaluate the importance of site variables in explaining the presence of *Bd* within that watershed. First, we evaluated all 22 site variables individually and each of the resulting models was ranked using AICc values. We included site as a random factor to avoid pseudoreplication. The dataset included as many as three observations of *Bd* presence from each site (one per year), and these observations were not independent. Second, we evaluated all combinations of the four best variables (i.e., lowest individual AICc values) using logistic regressions. Finally, we tested five additional models using three to six of the individual variables that ranked within the top seven variables for that watershed. Following Burnham and Anderson (2002), we constructed these five models based on careful consideration of the covariates likely to contribute to the presence of *Bd*. We then ranked all models according to their AICc values.

Logistic regressions were run using Statistix 9 software (Analytical Software, Tallahassee, Florida, USA). The software handles collinearity as follows: if an independent variable is too highly correlated with a linear combination of other independent variables in the model, it is dropped from the model. We evaluated spatial analysis using the spatial autocorrelation tool (Moran's I) in the Spatial Statistics toolbox in ArcGIS 10 (ESRI, Redlands, California, USA). Moran's I varies from +1.0 (clustering) to -1.0 (dispersion), with values near zero indicating a random distribution. We tested the null hypothesis that there was no spatial clustering. We compared proportions with z-tests. We used $\alpha = 0.05$ to evaluate statistical significance.

RESULTS

From 2005–2007, we collected *Bd* swab samples from tadpoles at 53, 30, and 58 sites in Olema, Bridalveil, and Dana watersheds, respectively (Table 1). We attempted

to sample each site each year, but a few sites in each watershed were dry in some years. We swabbed five species of native anurans, *B. boreas*, *B. canorus*, *R. draytonii*, *R. sierrae*, *P. regilla*, and the non-native *L. catesbeianus*. We found *Pseudacris* in all watersheds; we found *B. boreas*, *R. draytonii*, and *L. catesbeianus* only at Point Reyes NS (Olema), and *R. sierrae* and *B. canorus* only at the two Yosemite watersheds (Bridalveil and Dana). For Bridalveil, we captured tadpoles an average of 60.5 days after ice-out (range 43–75 d), while at Dana we sampled 57.5 days after ice-out (range 44–77 d).

When summed over all years and all species, we detected *Bd* at 53.2% of the sites, but the percentage of sites with at least one infected frog differed between Olema and the two Yosemite watersheds. Olema, the coastal watershed, had 83.0% positive sites, whereas in the Sierra Nevada, Bridalveil had 33.3 % positive sites and Dana had 36.2% (Table 1). For species with at least five sites sampled within a watershed, the species that was *Bd*-positive at the highest percentage of sites was *P. regilla* in the Olema watershed; 79.6% of the sites had at least one *Bd*-positive individual during the three years of our study. The second highest rate was 65.5% for *R. draytonii* in the Olema watershed. For species with at least 10 sites sampled, the lowest percentage of infected sites during all years was 20.7% for *P. regilla* at Bridalveil (Table 1).

Though there was a trend for the higher elevation Dana watershed (3,000 m) to have a higher proportion of *Bd*-positive sites than Bridalveil (2,000 m), this was not significant when comparing only *P. regilla* ($Z = 0.90$, $P = 0.37$), or all species ($Z = 0.27$, $P = 0.79$; Table 1). However, *P. regilla* at Olema were *Bd*-positive at a significantly higher proportion of sites than *P. regilla* at the combined Yosemite watersheds ($Z = 5.84$, $P < 0.001$).

Bd by species and watershed.—In the Olema watershed, we were only able to swab *B. boreas* at two sites, and *L. catesbeianus* at four sites; both species were

TABLE 1. Summary of number of sites positive for *Batrachochytrium dendrobatidis* (*Bd*) over total number of sites sampled (defined as sites that were either positive for *Bd*, or where at least 10 tadpoles were swabbed for that species). Percent of sites positive at any time during 2005–2007 are indicated in parentheses. Dashes indicate the lack of that species within the watershed. The total number of sites sampled for all species is not a sum of that column because multiple species occurred at many sites.

	Olema	Bridalveil	Dana	All Sites
<i>Bufo boreas</i>	1/2 (50.0)	-	-	1/2 (50.0)
<i>Bufo canorus</i>	-	0/8 (0.0)	8/27 (29.6)	8/35 (22.9)
<i>Pseudacris regilla</i>	39/49 (79.6)	6/29 (20.7)	15/50 (30.0)	60/128 (46.9)
<i>Lithobates catesbeianus</i>	2/4 (50.0)	-	-	2/4 (50.0)
<i>Rana draytonii</i>	19/29 (65.5)	-	-	19/29 (65.5)
<i>Rana sierrae</i>	-	5/10 (50.0)	2/2 (100.0)	7/12 (58.3)
All species	44/53 (83.0)	10/30 (33.3)	21/58 (36.2)	75/141 (53.2)

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TABLE 2. Summary of sites sampled, defined as sites that were either positive for *Batrachochytrium dendrobatidis* (*Bd*) or where at least 10 tadpoles were swabbed for that species, and the annual percentage that were positive for *Bd* in the three watersheds. Dashes indicate the absence of the species within the watershed. The total number of sites sampled is not a sum of the column because multiple species occurred at many sites.

	Olema		Bridalveil		Dana	
	Sites (n)	% Positive	Sites (n)	% Positive	Sites (n)	% Positive
2005						
<i>Bufo boreas</i>	2	50.0	-	-	-	-
<i>Bufo canorus</i>	-	-	1	0.0	4	50.0
<i>Pseudacris regilla</i>	44	84.1	27	22.2	36	41.7
<i>Lithobates catesbeianus</i>	4	50.0	-	-	-	-
<i>Rana draytonii</i>	14	85.7	-	-	-	-
<i>Rana sierrae</i>	-	-	3	100.0	2	100.0
2006						
<i>Bufo boreas</i>	2	0.0	-	-	-	-
<i>Bufo canorus</i>	-	-	1	0.0	3	0.0
<i>Pseudacris regilla</i>	39	82.1	26	0.0	37	0.0
<i>Lithobates catesbeianus</i>	0	-	-	-	-	-
<i>Rana draytonii</i>	9	55.6	-	-	-	-
<i>Rana sierrae</i>	-	-	3	100.0	2	100.0
2007						
<i>Bufo boreas</i>	1	100.0	-	-	-	-
<i>Bufo canorus</i>	-	-	0	-	2	50.0
<i>Pseudacris regilla</i>	33	51.5	19	15.8	28	39.3
<i>Lithobates catesbeianus</i>	0	-	-	-	-	-
<i>Rana draytonii</i>	9	77.8	-	-	-	-
<i>Rana sierrae</i>	-	-	2	100.0	0	-
Total	49		26		37	

positive on at least one occasion (Table 2). *Rana draytonii* were more common, and we were able to sample tadpoles at 9–14 sites each year. We detected *Bd* at 55.6–85.7% of these sites (Table 2). We sampled *P. regilla* at 33–43 sites in the Olema watershed each year. The percentage of sites positive for *Bd* declined through the three years of the study, with values decreasing from 84.1% to 82.1% to 51.5% ($X^2 = 12.36$, $P = 0.002$; Table 2).

At Bridalveil, *B. canorus* and *R. sierrae* were both uncommon; for *B. canorus*, the number of sites we sampled ranged from zero to one, while *R. sierrae* ranged from two to three. We never detected *Bd* in *B. canorus* at the limited number of sites (eight) where they were present in the Bridalveil watershed, but 100% of the *R. sierrae* sites were positive. *Pseudacris regilla* were present at many more sites (19–27 sites, depending on year), but the number of sites where this species tested positive was low, ranging from 0.0–22.2% in the three years of sampling (Table 2).

The Dana watershed also supported three species of anurans. *Bufo canorus* was somewhat more common at Dana, compared with Bridalveil, but the number of sites

sampled still ranged from only two to four. We detected *Bd* in *B. canorus* at 0–50.0% of sites. *Rana sierrae* were also uncommon, being found at zero to two sites over the three years. However, at those sites where we swabbed at least 10 *R. sierrae*, we always detected *Bd*. *Pseudacris regilla* were present at 28–37 sites, but during the three years, 0–41.7% of sites were *Bd* positive (Table 2). Overall, *P. regilla* was infected at a smaller proportion of sites in Yosemite compared with Point Reyes ($Z = 5.84$, $P < 0.001$).

Comparing *P. regilla* with the sympatric species of *Rana*, there was no difference in the proportion of sites with *Bd*-positive *P. regilla* and *R. draytonii* at Olema ($Z = 1.38$, $P = 0.170$), but there was a significant difference between *P. regilla* and *R. sierrae* at the Yosemite watersheds ($Z = 2.22$, $P = 0.026$). The distribution of *Bd*-positive sites was remarkably uniform; sites that tested positive were not spatially clustered, but randomly distributed among the sites within each of the watersheds (Moran's Index, Olema, $Z = 0.29$, $P = 0.77$; Bridalveil, $Z = 0.18$, $P = 0.86$; Dana, $Z = -0.41$, $P = 0.68$).

TABLE 3. Comparison of the number of sites where two species were sampled at the same site, in the same year. Pos = at least one individual of that species was positive for *Bd* at that site over the three years of the study. Neg = *Bd* was not detected in any individual of that species at that site (minimum sample size = 10 individuals). The top Pos or Neg refers to the first species in the species pair and the lower Pos or Neg refers to the second species in the pair. Note that the *Rana* comparison includes two species of *Rana*, *R. draytonii* at Olema, and *R. sierrae* at the Bridalveil and Dana watersheds.

Species Pair / Site	Pos Pos	Neg Neg	Pos Neg	Neg Pos
<i>Pseudacris</i> / <i>Rana</i>				
Olema	17	2	6	3
Bridalveil	1	0	0	4
Dana	1	0	0	3
Totals	19	2	6	10
<i>Pseudacris</i> / <i>Bufo</i>				
Olema	-	-	-	-
Bridalveil	0	1	1	0
Dana	1	2	0	3
Totals	1	3	1	3

Bd non-congruence.—At some sites, we sampled multiple species for *Bd*, and the results were not always congruent at sites where we were able to sample two species in the same year. In the Olema watershed, we sampled both *P. regilla* and *R. draytonii* at 28 sites. Of these, six sites were *Bd* positive for *P. regilla*, but not for *R. draytonii*, and three sites were the reverse (Table 3). In the combined Bridalveil and Dana watersheds, seven sites were positive for *R. sierrae*, but not for *P. regilla*, but none had the reverse situation. While this was a small sample, our data suggest that *R. sierrae* are more susceptible to *Bd* than *P. regilla* ($Z = 2.22$, $P = 0.026$). At Olema, no sites supported both *Pseudacris* and *Bufo*; in Yosemite, *P. regilla* and *B. canorus* occurred together at eight sites, but *Bd* was not more prevalent in either species (Table 3).

Covariate analysis for *Bd* presence in *Pseudacris regilla*.—The most parsimonious model (lowest AICc value) for the occurrence of *Bd* in *P. regilla* varied among watersheds (Table 4). At Olema, both year and the length of shoreline were included in all six of the best models, and both covariates were negatively related to *Bd*-presence in *P. regilla* (Table 5). Water depth (3 m from shore) and the percentage cover of rooted vegetation were only slightly less important, with summed AICc weights of 0.87 (Table 5). *Bd* was more likely to occur at sites with more rooted vegetation, and less likely to occur in deep ponds with a long shoreline. The best model included all four of these covariates (Table 4). Water temperature (mean = $18.0 \pm 3.5^\circ\text{C}$) ranked 14th of 22 variables ranked by AIC values at

TABLE 4. The six best, null, and global models for the seven best covariates for the presence of amphibian chytrid (*Bd*) in each of three watersheds. Models are ranked by increasing AICc value. Within a model, variables are listed in order of increasing AICc values for each individual variable. Site variables are defined as: 2_75 - substrate predominantly gravel 2–75 mm, scored as 0 or 1; depth - average depth 3 m from shore; veg_float - percentage cover of floating vegetation; m_depth - maximum water depth, truncated at 3 m; ordinal - ordinal date; perm - permanent body of water, scored as 0 or 1; veg_root - percentage cover of rooted vegetation; shade - percentage of site shaded at midday; shore - length of shoreline (perimeter); silt/mud - substrate predominantly silt/mud, scored as 0 or 1; temp - water temperature measured 15 cm deep, 0.5 m from shore; turbidity - water turbidity, scored as 0–5; year - calendar year.

Site / Covariates	n	Δ AICc	w
Olema, Point Reyes NS			
depth, year, veg_root, shore	113	0.00	0.41
depth, year, veg_root, shore, m_depth	113	1.96	0.24
year, veg_root, shore	115	2.29	0.13
depth, year, shore	113	2.33	0.13
depth, year, veg_root, shore, m_depth, perm	113	3.33	0.07
depth, year, veg_root, shore, m_depth, perm, 2_75	113	5.41	0.03
null model	116	26.71	0.00
global model	112	35.49	0.00
Bridalveil, Yosemite NP			
depth, temp, silt/mud	60	0.00	0.40
depth, temp, silt/mud, turbidity	59	0.69	0.28
depth, temp	60	2.30	0.13
depth, temp, silt/mud, turbidity, perm	59	2.39	0.12
depth, silt/mud	65	3.71	0.06
depth, temp, silt/mud, turbidity, perm, ordinal, s_2_75	59	7.30	0.01
null model	70	57.89	0.00
global model	59	44.35	0.00
Dana, Yosemite NP			
temp, silt/mud, depth	83	0.00	0.25
temp, silt/mud	84	0.25	0.22
temp	84	0.55	0.19
temp, depth	83	0.69	0.17
temp, silt/mud, turbidity	83	1.01	0.15
temp, veg_float, turbidity, 2_75, shade, silt/mud, depth	82	4.83	0.02
null model	101	22.54	0.00
global	82	26.53	0.00

Olema, and was not included in any of the best models. However, mean water temperature for all sites with *P. regilla* in the Olema watershed was significantly lower than at the two Yosemite watersheds ($18.0 \pm 3.5^\circ\text{C}$ versus

TABLE 5. Summed AICc weights for the best seven covariates for the three watersheds. Arrows denote those covariates that were negatively related to the presence of amphibian *Bd*.

Olema	Sum w	Bridalveil	Sum w	Dana	Sum w
year ↓	1.00	depth	1.00	temp	1.00
shore ↓	1.00	temp	0.94	silt/mud	0.64
depth ↓	0.87	silt/mud ↓	0.87	depth	0.44
veg_root	0.87	turbidity ↓	0.41	turbidity	0.17
m_depth ↓	0.34	perm	0.13	veg_float	0.02
perm ↓	0.10	ordinal ↓	0.01	2_75 ↓	0.02
2_75 ↓	0.03	s_2_75	0.01	shade ↓	0.02

$19.7 \pm 4.2^{\circ}\text{C}$; $t = 3.55$, $df = 315$, $P = 0.005$).

At Bridalveil, the best model included three covariates: water depth, water temperature, and a substrate that was predominantly silt/mud (Table 4). Across models, these covariates had the highest summed AICc weights, 1.0, 0.94, and 0.87, respectively (Table 5). Silt/mud was the only one of the top three covariates with a negative relationship with *Bd* presence at a site. Turbidity was the next most important covariate, but its summed AICc weight was less than half of the next best covariate. None of the last three covariates listed in Table 5 played an important role in the best models. Hence, sites where *P. regilla* were *Bd*-positive tended to have deeper water, higher water temperature, and less silt/mud and turbidity.

At Dana, water temperature, silt/mud, and water depth were the covariates in the best model (Table 4), and also the three covariates with the highest summed AICc weights (Table 5). These were the same three covariates that were most important in the Bridalveil watershed, but the covariates ranked in a different order. The presence of substantial amounts of silt/mud was positively related to sites where *P. regilla* were *Bd*-positive, opposite of Bridalveil. Turbidity was the fourth best covariate at Dana, but its summed AICc weight was low (0.17). In the Dana watershed, sites with *Bd*-positive *P. regilla* had warmer water, a substrate that was predominantly silt/mud, and greater water depth.

DISCUSSION

Patterns of *Bd* occurrence.—*Bd* was geographically and taxonomically widespread; it was present in all six species within our three watersheds, even though sample sizes were small for some of the taxa. Furthermore, the percentage of sites containing *Bd*-positive amphibians was high within the coastal Olema watershed at Point Reyes NS; 83.0% of the 53 sites had at least one individual that tested positive. The percentage of sites with *Bd* at the sierran Bridalveil (33.3%) and Dana (36.2%) watersheds in Yosemite National Park was much lower than at Olema. Although *Bd* became

established in central California at least by 1961 (Padgett-Flohr and Hopkins 2009), we do not know when it arrived at any of our three study areas. The Olema watershed has had a long history of disturbance, including clear-cut logging and beef cattle ranching. Some of these activities may have contributed to the spread of *Bd*. In all the watersheds, *Bd* might also have spread with the assistance of birds, amphibians, livestock (horses, mules, and burros), hikers, fishermen, and biologists.

The presence of *Bd* within a species varied widely. *Pseudacris regilla* was *Bd*-positive at 79.6% of the sites in the Olema watershed, compared with 20.7% and 30.0% at the two Yosemite National Park watersheds. It may be that *P. regilla* is not particularly susceptible to chytridiomycosis, but serves as a host for the pathogen, similar to *L. catesbeianus* (Daszak et al. 2002) and *Ambystoma tigrinum* (Davidson et al. 2003). Why would *P. regilla* have significantly lower infection rates in Yosemite compared with Point Reyes? There might be different *Bd* strains in the two areas that differ in virulence (Morgan et al. 2007). Water temperature could be an important factor because it can influence *Bd* survival and reproduction (Piotrowski et al. 2004), or there might be differences in life history or phenology of either the host or pathogen. Though water temperature for Olema sites with *P. regilla* was lower (mean = 18.0°C when swabs were collected) than at the two Yosemite watersheds (20.3°C and 19.0°C), all three watersheds fall within a range that should allow for good growth and reproduction of *Bd* (Piotrowski et al. 2004). However, the Yosemite watersheds are in the Sierra Nevada and have snow cover for 6–8 months of the year, while the Olema watershed has a mild coastal climate with annual air temperatures closer to the optimum range for *Bd* (17 – 25°C ; Piotrowski et al. 2004). Only twice did we record water temperature in any of the watersheds that exceeded 28°C (29°C and 32°C , both at Bridalveil); these temperatures are high enough that they might reduce the intensity of infection in a host.

It is not clear why the number of *Bd*-positive sites in Olema *P. regilla* declined through the study. Water temperature is known to be a key aspect of *Bd* growth and survival, but there was no correlation between average water temperature on the day we collected swabs and the percentage of sites where *P. regilla* tested positive. Differences in *Bd* infection rates have been reported among anurans in both Australian and Central American (Berger et al. 1998; Retallick et al. 2004; Kriger and Hero 2006). Longcore et al. (2007) noted that species with lower incidences of *Bd* tended to be less aquatic. Our data suggest that *B. canorus* and *P. regilla* were equally likely to be infected in Yosemite, and the same was true for *R. draytonii* and *P. regilla* in the Olema watershed. However, there was a significant difference in *Bd* occurrence between *R. sierrae* (58.6%) and *P.*

regilla (26.6%) in Yosemite. This might be expected because *R. sierrae* have a notably long developmental period, with tadpoles metamorphosing after the end of the third summer (Vredenburg et al. 2005). By comparison, *P. regilla* tadpoles metamorphose 2–3 months after egg laying (Rorabaugh and Lannoo 2005). Within the Olema watershed, *P. regilla* and *R. draytonii* have similar life histories with most individuals spending much of the year away from aquatic breeding sites, and tadpoles taking about the same length of time to metamorphose (Gary Fellers, pers. obs.); they also have roughly similar infection rates, 79.6% and 65.5%, respectively.

Site covariates.—Site covariates that were closely associated with the detection of *Bd* in *P. regilla* differed considerably between Olema and the two Yosemite watersheds. At Olema, year, length of shoreline, water depth (3 m from shore), were all negatively related to *Bd* presence, whereas the percentage cover of rooted vegetation was positively related. The relationship with shoreline length and water depth was not expected because there is no known dry-tolerant *Bd* life-history stage, and sites with short shorelines or shallow water would be more likely to become dry in the summer or fall. At Olema, water temperature was not an important factor, ranking 14th of 22 variables.

The two Yosemite watersheds had the same three highest-ranking covariates; water temperature was the highest-ranking covariate at Dana, and it was a close second at Bridalveil. We detected *Bd* most often at sites that were closer to the optimum temperature for the survival and reproduction of *Bd* (17–25 °C; Piotrowski et al. 2004). Temperatures were rarely high enough to inhibit the growth of *Bd* ($\geq 28^\circ\text{C}$; Piotrowski et al. 2004), and never high enough to allow amphibians to clear *Bd* from their systems (37° C; Woodhams et al. 2003).

Water depth was positively related to the detection of *Bd* in Yosemite. This might suggest that *Bd* was present at sites that were deeper and tended to be permanent, but whether a site was permanent or not was only weakly associated with *Bd* at Bridalveil, and not at all at Dana. Deeper water is typically colder, and that might influence development or survival of *Bd*; however, nearly all temperatures we measured at the surface (0.5 m out from the shore) were well within the preferred temperature range of *Bd* (17–25° C; Piotrowski et al. 2004).

A substrate that was predominantly silt/mud was an important variable at both Bridalveil and Dana watersheds. At Bridalveil, *Bd* was found at sites with less silt/mud, while at Dana, the opposite was true. It is unclear why silt/mud should have any effect. Drew et al. (2006) reported that *Bd* occurred more often at higher elevations (> 500 m) in Australia, and Young et al. (2001)

reported the same for Latin America, where high elevation was defined as > 500 m for Central America and > 1,000 m for the Andes. We found *Bd* at a higher proportion of higher elevation sites (Dana watershed at 3,000 m compared with Bridalveil at 2,000 m), but neither of the comparisons was statistically significant. It could be that Bridalveil is already at a sufficiently high elevation that any elevational influences were similar for the two watersheds. Clearly the concept of high elevation is relative; the highest point in Australia (2,228 m) is within the range of elevations in the Bridalveil watershed.

Amphibian die-offs.—*Bd* has been closely associated with amphibian declines in many parts of the world (Stuart et al. 2004; IUCN, Conservation International, and NatureServe 2008). Presumably the widespread occurrence of *Bd* in our study has had an impact on local anurans, especially *Rana*, which have been involved in most of the declines in California (e.g., Fellers and Drost 1993; Drost and Fellers 1996; Vredenburg et al. 2007). We have been conducting research on pond breeding amphibians in the Olema watershed since 1993 (Fellers et al. 2001b; Shaffer et al. 2004; Fellers and Kleeman 2006, 2007), in part to evaluate population trends of amphibians, especially *R. draytonii*. This work has included > 1,000 site visits within the Olema watershed since 1993. Even though *R. draytonii* was *Bd*-positive at 65.5% of the sites where it occurred, we have not observed any declines unrelated to habitat loss (Gary Fellers, unpubl. data). Similarly, *P. regilla* and *B. boreas* have not obviously declined, though *B. boreas* is rare at Point Reyes (Gary Fellers, unpubl. data). If *Bd* did cause declines, species in the Olema Valley seem to have recovered, at least partially. Perhaps anurans in the Olema watershed have developed a resistance to the local strain of *Bd*, as suggested by Longcore et al. (2007) for anurans in the northeastern U.S. Also, there may have been subtle, sublethal effects from the presence of *Bd* (Venesky et al. 2010) that we have not detected.

Unlike the Olema watershed, amphibian declines have been well documented in Yosemite National Park. Drost and Fellers (1996) conducted surveys in 1992 and reported that five of the seven species within the study area had undergone significant declines compared with the 1915–1919 surveys of Grinnell and Storer (1924). Vredenburg et al. (2007) reported a 93.3% loss of *R. sierrae* populations in the Sierra Nevada, and some populations have crashed simultaneously with the appearance of *Bd* (Vredenburg et al. 2010). By contrast, no dramatic die-offs of *P. regilla* have been observed within the park, or elsewhere in the Sierra Nevada. While *P. regilla* are not as common or widespread as they once were (Gary Fellers, pers. obs.), vigorous populations remain throughout their historic range (Gary Fellers, unpubl. data).

Kagarise Sherman and Morton (1993) studied *B.*

canorus at Tioga Pass Meadow (TPM), just north of the Dana watershed, and documented steep declines in the toad population, primarily from 1979 to 1982. They never found toads in the Dana watershed during their most intensive field work in 1971–1976, or during less frequent visits through 1990. They also reported that Karlstrom detected no toads in a 1991 visit to the Dana watershed where he studied toads from 1954–1958. Kagarise Sherman and Morton (1993) attributed the toad decline to a combination of drought and disease. Green and Kagarise Sherman (2001) confirmed the presence of *Bd* in toads collected during the 1977–79 die-offs. Though we were able to swab only a limited number of *B. canorus* in the Dana watershed, we detected *Bd* in all three years of our study; hence, the pathogen has been present at TPM or the immediately adjacent Dana watershed in four years over a 23-year period, and presumably those years in between.

We have surveyed for *B. canorus* at TPM every year from 1992–2010, and in the Dana watershed all but four of those years (1992, '94, '97, and '98) and found toads in all but two years (Gary Fellers, unpubl. data). In 1997, we only visited one TPM pond late in the season when the pond was almost dry (Gary Fellers, unpubl. data). In 1998, we spent 45 min surveying that same pond when conditions were good, but found no toads (Gary Fellers, unpubl. data). However, from 1993–2010 we found *B. canorus* at 47 sites in the Dana watershed, and located 40 egg masses at one site in 1996 and 25 masses at that site in 1999 (Gary Fellers, unpubl. data). Because we do not normally conduct surveys right at snow melt when the toads are breeding, our counts do not reflect actual population size. Regardless, it is clear that toads are more common at both TPM and Dana than in 1990, and that *B. canorus* is persisting in the presence of *Bd*.

The situation at the Bridalveil watershed appears to be similar. *Bd* was detected in Summit Meadow in 1998 (Fellers et al. 2001a), 2001 (D. Earl Green, unpubl. data), 2004 (Cheryl Briggs, unpubl. data), and in each year of the current study (2005–2007). No one has studied *B. canorus* at the meadow, but we have visited the meadow every year from 1992–2010. Most of our work focused on *R. sierrae*, and we did not always search the smaller ephemeral pools where *B. canorus* breed. Nonetheless, we recorded toads in 11 of 19 years, including eight of the last nine (Gary Fellers, unpubl. data.). Clearly *B. canorus* has persisted in the presence of *Bd*, though perhaps at a reduced level. Unfortunately, there are no population data from Summit Meadow that predate the occurrence of *Bd*.

The situation with *R. sierrae* is similar to that of *B. canorus*. While significant declines of both *R. sierrae* and the closely related *R. muscosa* have been documented throughout the Sierra Nevada, populations in at least some areas have persisted in the presence of

Bd (Briggs et al. 2010). For example, two of us (Gary Fellers and Patrick Kleeman) have been conducting a mark-recapture study of *R. sierrae* at Summit Meadow since 2003. During that time, the *R. sierrae* population has fluctuated in size, but not discernibly declined, even though *R. sierrae* from the meadow tested positive for *Bd* in 1998, and 2004–2007 (this study, Gary Fellers, unpubl. data). It is possible that both the *R. sierrae* and *B. canorus* populations declined after initially being infected with *Bd*, subsequently recovered, and are now coexisting with the pathogen, as has been reported for other anurans (e.g., Retallick et al. 2004; Daszak et al. 2005; Puschendorf et al. 2006; Rachowicz et al. 2006). The third amphibian that occurs at Summit Meadow, *P. regilla*, tested negative for *Bd* in all three years of our study. That raises interesting questions about the susceptibility of *P. regilla* in Yosemite to *Bd*, and what the history of infection at that site might be. Those are questions that probably cannot be answered at this time.

As pointed out by Daszak et al. (2003), the relationship between host and pathogen is complex and influenced by the environment. In trying to understand the interaction, it is often necessary to have information on the duration of coexistence, changes in virulence and resistance, changes in populations of both host and pathogen, immune capabilities and anti-fungal fauna of the host, and changes to the environment. Much of this information is missing from studies on *Bd*, including ours. However, we have provided insight into patterns of *Bd*-infected sites in parts of California and some of the variables related to *Bd* occurrence for several amphibian communities.

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