Occurrence of the Amphibian Pathogen Batrachochytrium Dendrobatidis in the Pacific Northwest

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Original Contribution

Widespread Occurrence of the Chytrid Fungus *Batrachochytrium dendrobatidis* on Oregon Spotted Frogs (*Rana pretiosa*)

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Abstract: The pathogen *Batrachochytrium dendrobatidis* (*Bd*) has been associated with amphibian declines in multiple continents, including western North America. We investigated *Bd* prevalence in Oregon spotted frog (*Rana pretiosa*), a species that has declined across its range in the Pacific Northwest. Polymerase chain reaction analysis of skin swabs indicated that *Bd* was prevalent within populations (420 of 617 juvenile and adults) and widespread among populations (36 of 36 sites) where we sampled *R. pretiosa* in Oregon and Washington. We rarely detected *Bd* in *R. pretiosa* larvae (2 of 72). Prevalence of *Bd* in postmetamorphic *R. pretiosa* was inversely related to frog size. We found support for an interactive effect of elevation and sampling date on *Bd*: prevalence of *Bd* generally increased with date, but this effect was more pronounced at lower elevations. We also found evidence that the body condition of juvenile *R. pretiosa* with *Bd* decreased after their first winter. Our data indicate that some Oregon spotted frog populations are currently persisting with relatively high *Bd* prevalence, but the risk posed by *Bd* is unknown.

Keywords: amphibian, pathogen, disease, Pacific Northwest

INTRODUCTION

Chytridiomycosis is a disease of amphibians that is caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) (Berger et al., 1998; Longcore et al., 1999). This disease has been linked with amphibian mortality or population declines on at least four continents (Lips et al., 2006; Bosch et al., 2007). In western North America, *Bd* appears to be widespread both geographically and on a variety of anuran hosts (Adams et al., 2007; Pearl et al., 2007; Muths et al., 2008). Chytridiomycosis may be linked to declines of several anurans in the western USA, including the Boreal Toad (*Bufo boreas*) in Colorado (Muths et al., 2003); Wyoming

Toad (*B. baxteri*) in Wyoming (Taylor et al., 1999); Yosemite Toad (*B. canorus*) and Mountain Yellow-Legged Frog (*Rana muscosa*) in California (Green and Kagarise Sherman, 2001; Rachowicz et al., 2006).

The Oregon spotted frog (*Rana pretiosa*) is thought to be extirpated from >70% of its historic range (Hayes, 1997), and is a candidate for listing under the Endangered Species Act (USFWS, 2007). While habitat loss and alteration are considered the leading factors in its decline (Hayes, 1997; USFWS, 2007), the highly aquatic life history of *R. pretiosa* suggests that the species could also be susceptible to an aquatic pathogen such as *Bd*. Small numbers of *R. pretiosa* have been tested at two sites in Oregon, and *Bd* was found from one site (Pearl et al., 2007). More information is needed about the distribution of this

pathogen and whether it could have played a role in declines of this species (USFWS, 2007).

We evaluated predictors of *Bd* presence on individual *R. pretiosa* in Oregon and Washington, USA where >90% of *R. pretiosa* populations occur. We also evaluated a hypothesis that presence of *Bd* decreases the body condition of overwintering juvenile *R. pretiosa*. Mortality events that may be associated with *Bd* have been reported during cold snaps and over winter, suggesting that frogs may be particularly vulnerable during these periods (Berger et al., 1998; Schlaepfer et al., 2007). We present results from other anurans sampled at extant and historic *R. pretiosa* sites to complement the limited data on *Bd* in northwestern amphibians.

METHODS

We attempted to sample 10–20 individuals from as many of the *R. pretiosa* populations as possible. Sampling was conducted between June and October of 2006 and 2007. We surveyed known *R. pretiosa* breeding sites as well as three sites that historically hosted *R. pretiosa* (Goose Lake, Crater Meadow, Clackamas Lake; Hayes, 1994). We included the sample of historic sites because other studies (e.g., La Marca et al., 2005; Lips et al., 2006) suggest that *Bd* presence could be related to extirpation in some species. At the historic and other sites, we opportunistically sampled introduced American bullfrogs (*Rana catesbeiana*) and native anurans other than *R. pretiosa*. Bullfrogs have been proposed as a vector and a reservoir for *Bd* (Daszak et al., 2004).

We focused our sampling on postmetamorphic stages of *R. pretiosa*, particularly juvenile frogs, but we also sampled larvae (defined as animals < stage 46; Gosner, 1960) at 10 sites. At roughly half our sites ($n = 17$), we sampled postmetamorphic *R. pretiosa* in both years of the study to investigate inter-year variation in *Bd*.

We used frog size and presence of limb emergence scars to assign juvenile frogs to one of two groups for which we compared condition: (1) their summer of metamorphosis (Juv Year 0), or (2) their first full summer as frogs (Juv Year 1). We sampled juvenile *R. pretiosa* from sites in the Oregon Cascade Range, soon after snows receded in 2007, to assess body condition at the beginning of their first full growing season as frogs.

We captured all animals via dip nets or gloved hands. When dip nets were used, we immediately removed frogs from nets and kept them singly in clean Ziploc bags for processing. We vigorously rinsed nets with water and air dried them between captures. We rinsed, disinfected (5%–10% dilute bleach solution), rinsed, and dried nets and footwear between sites. We used a new pair of Nitrile gloves to handle each frog. For each frog, we took 25 strokes with a cotton swab across the ventral surface and toe webbing (juveniles and adults), or mouthparts and ventral surface (larvae). After swabbing, we recorded stage (Gosner, 1960), gender, length (snout-urostyle length [SUL], measured to nearest 1-mm using rulers), and mass (to nearest 0.5 g with Pesola spring scales). We also inspected animals for signs of wounds or illness, including symptoms associated with chytridiomycosis (lethargy, excessive skin sloughing, reddish discoloration of ventral thorax that can indicate secondary bacterial infections; Berger et al., 1999). Swabs were stored in ethanol in screw top vials and submitted to Pisces Molecular Labs (Boulder, CO) for PCR (polymerase chain reaction) analysis to evaluate presence of *Bd* DNA. That analysis uses methods similar to those described by Annis et al. (2004). Throughout this article, we refer to the proportion of *Bd*-positive frogs as "prevalence."

Our hypothesized explanatory variables were derived from other recent work investigating distribution and prevalence of *Bd* (Table 1). For example, studies elsewhere indicate that elevation and time of year affect prevalence of *Bd* (Berger et al., 2004; Longcore et al., 2007). We also hypothesized that higher human use around a site could increase prevalence by acting as a stressor that might predispose frogs to infection by *Bd*. We used a "human footprint" raster data set to quantify human use (Leu et al., 2008). The Human Footprint Index represents a composite of seven models in which human presence on the landscape is assigned a code from low (1) to high (10) across the western United States in 180 m^2 cells. The models and raster scores incorporate data on human habitation, railroads, roads and highways, irrigation canals, power lines, agricultural land, campgrounds, land fills, oil and gas development, and human-induced fires (Leu et al., 2008). We used a 5-km buffer around each survey site to calculate site scores (ArcGIS 9.2). We calculated an average of all impact categories weighted by the proportion of the buffer, and used this weighted average as a human footprint score.

Analyses

We conducted two analyses. We evaluated predictors of *Bd* occurrence on juvenile and adult *R. pretiosa* ($n = 617$) with

Table 1. Predictor Variables for Two Analyses of *Bd* on Oregon Spotted Frogs (*Rana pretiosa*)[a]

Variable	Range (or sample size)	Description
Analysis 1: Factors related to prevalence of Bd in postmetamorphic R. pretiosa		
Year	2006 (*n* = 226), 2007 (*n* = 391)	2006 or 2007
Date	169 (June 18)–279 (October 6)	Ordinal date of sample
Length	19–91	Snout-urostyle length (mm)
Elevation	35–1650	Meters above mean sea level
Human footprint	1.0–7.4	Human footprint score (1–10 scale): an index of human activity (see Methods)
Date × elevation	6401–422,406	Interaction between ordinal date and elevation
Site number	1–36	Random effect
Analysis 2: Potential influence of Bd on body condition of juvenile R. pretiosa		
Bd	0 (*n* = 92), 1 (*n* = 287)	Not detected (0), detected (1)
Elevation	35–1612	Meters above MSL
JuvYr	0 (*n* = 283); 1 (*n* = 96)	Frog sampled in year of metamorphosis (0) or after first winter as frog (1)
Bd × JuvYr	0.0 (*n* = 69); 0.1 (*n* = 23); 1.0 (*n* = 214); 1.1 (*n* = 73)	Interaction between *Bd* status and JuvYr

[a]JuvYr, juvenile year.

the first analysis. We used the second analysis to assess whether body condition of juveniles was related to presence of *Bd* (*n* = 379). We limited the second analysis to juvenile frogs for two reasons: (1) juvenile and metamorphic stages in a variety of anurans appear to be the life stages that are most vulnerable to *Bd* (Rachowicz and Vredenburg, 2004; Kriger and Hero, 2007b), and (2) demographic modeling suggests that factors that affect survival of juvenile and adult life stages (such as condition and size; Chelgren et al., 2008) have the biggest per capita effect on population growth rate (Biek et al., 2002; Vonesh and de la Cruz, 2002). We report *Bd* prevalence from *R. pretiosa* larvae and non-*R. pretiosa* samples, but did not include these data in our analyses.

Analysis 1: Factors Related to Prevalence of *Bd* in Postmetamorphic *R. pretiosa*

To evaluate predictors of *Bd* in *R. pretiosa*, we used a generalized linear mixed model approach in a Bayesian framework (Gilks et al., 1994). Modeling was conducted using program WinBugs 1.4 (Spiegelhalter et al., 1994). This approach allows inclusion of fixed and random effects, as well as site and individual predictor data, and relaxes assumptions of independence among samples within sites

(Zeger and Karim, 1991). This latter feature is appropriate for studying infectious diseases like *Bd* that can be spread by zoospores among individuals in a pond (Rachowicz and Vredenburg, 2004; Rachowicz and Briggs, 2007). The disease status of *R. pretiosa* captured in the same wetland is likely to be more correlated than frogs captured in different wetlands.

Our response variable was *Bd* status (Positive or Negative) on individual juvenile and adult *R. pretiosa*. We fit a model that included the main effects of Human Footprint and other potential predictors of *Bd* that had support from other studies of *Bd*: Elevation, Length (SUL), Ordinal Date, and Year. We also included an interaction term for Date × Elevation, since both are associated with *Bd* in other studies and an interaction is likely (Berger et al., 2004; Muths et al., 2008). We did not include latitude, which is related to *Bd* prevalence in other studies (e.g., Kriger et al., 2007). Latitude is strongly correlated with elevation for *R. pretiosa* extant and historical sites, with those in the species' northern range (western Washington and southwestern British Columbia) at markedly lower elevations than those in the Cascades of central and southern Oregon (Hayes, 1997). We included a random effect of Site. We did not include invasive bullfrogs as a predictor of *Bd* because we only had two *R. pretiosa* sites in

our sample pool where bullfrogs breed. We standardized continuous variables to 1 SD.

We used noninformative prior distributions: priors for the regression coefficients and intercept were Normal (mean = 0, SD = 100); the prior for the random effect standard deviation was Uniform (range, 0–5). We report 95% credibility intervals for Bayesian parameter estimates. As the indicator of support for each predictor variable, we provide the posterior probability (Pr) that each β coefficient was greater than 0. A variable can be considered strongly supported if Pr values are <0.05 or >0.95 (Gelman et al., 1995).

We used a Bayesian P-value to test model fit. This value is based on a Freeman-Tukey goodness-of-fit test comparing observed and expected (simulated) numbers of positive swabs at each site. The P-value is the percentage of the time that a Freeman-Tukey statistic based on real field collected data exceeds a Freeman-Tukey statistic computed with the simulated data. A perfect-fitting model should have a Bayesian P-value of 0.5; poor fit would be indicated by P-values <0.05 or >0.95 (Gelman et al., 1995).

Analysis 2: Potential Influence of Bd on Body Condition of Juvenile R. pretiosa

This analysis investigates the potential for Bd to act as a stressor rather than as the response variable. We used body condition (a function of mass and length) since it is likely to be more responsive to stressors than length, and can be linked to a variety of fitness traits such as likelihood, timing, and duration of breeding (Lüddecke, 1997; Reading, 2007; Waelti and Reyer, 2007). Our response variable for this analysis was Fulton's K, an index of body condition commonly used with fishes which has also been used for frogs (Murphy and Willis, 1996; Lüddecke, 1997):

$$K_F = \left(\text{Mass}/\text{SUL}^3\right) \times 10,000.$$

We used generalized linear modeling (S-Plus, 2003) and Akaike's Information Criterion (AICc for small sample size) to rank regression models (Burnham and Anderson, 2002). We considered seven models (including the Null model) that we hypothesized could explain condition. These models included a subset of combinations of the predictor variables Bd, Elevation, Juvenile Year, and the Bd × Juvenile Year. The interaction term expresses a hypothesis that the first winter for a Bd-infected new juvenile could be particularly stressful and result in diminished mass per length, which would be evident early

in the following summer. This is consistent with reports of Bd-mortality and reduced frog condition during winter. The term "Juvenile Year" compares the condition of two age groups of juveniles at one point in time, rather than comparing the condition of juveniles to their own previous condition.

Results

We sampled a total of 617 postmetamorphic R. pretiosa for Bd at 36 sites (average frogs per site 17.1 ± 9.6 SD; range, 2–41) (Figure 1). This site total represents the vast majority of known R. pretiosa populations. We detected Bd in 2 of 72 larvae (2.8%), 287 of 379 juveniles (75.7%), and 133 of 238 (55.9%) adults (Figure 2). Prevalence of Bd in postmetamorphic R. pretiosa by site averaged 63.8 % (±24.6 SD; range, 16.7%–100%). At least one postmetamorphic R. pretiosa tested positive for Bd at all sites. We did not observe any evidence of die-offs during our sampling, nor did we detect any of the outward symptoms of chytridiomycosis that have been reported from Bd infected frogs of other species.

We collected swab samples from amphibians other than R. pretiosa (Figure 2): B. boreas from three sites (range, 1–8 postmetamorphs per site); R. aurora from one site (10 postmetamorphs); R. cascadae from five sites (range, 1–7 postmetamorphs); R. catesbeiana from five sites (range, 0–7 larvae, 2–34 postmetamorphs per site). All R. aurora, one B. boreas, and eight R. cascadae came from two historic R. pretiosa sites where the species is no longer found (Goose Lake, Little Crater Meadow); the rest of the non-R. pretiosa samples came from sites where R. pretiosa are extant. We found roughly similar overall rates of Bd prevalence in postmetamorphic R. cascadae (69.2%) and R. catesbeiana (72.6%) as with post metamorphic R. pretiosa, but the sample sizes were too small for statistical comparison. Two of 10 R. aurora and 0 of 10 B. boreas tested positive for Bd.

Analysis 1: Factors Related to Prevalence of Bd in Postmetamorphic R. pretiosa

Our regression analysis (Table 2) indicated support for the following predictors of Bd status on postmetamorphic R. pretiosa: Length (Pr = 0.001), Elevation (Pr = 0.998), Date (Pr = 0.998), and Date × Elevation (Pr = 0.004). Back-transforming the respective coefficients yields the following
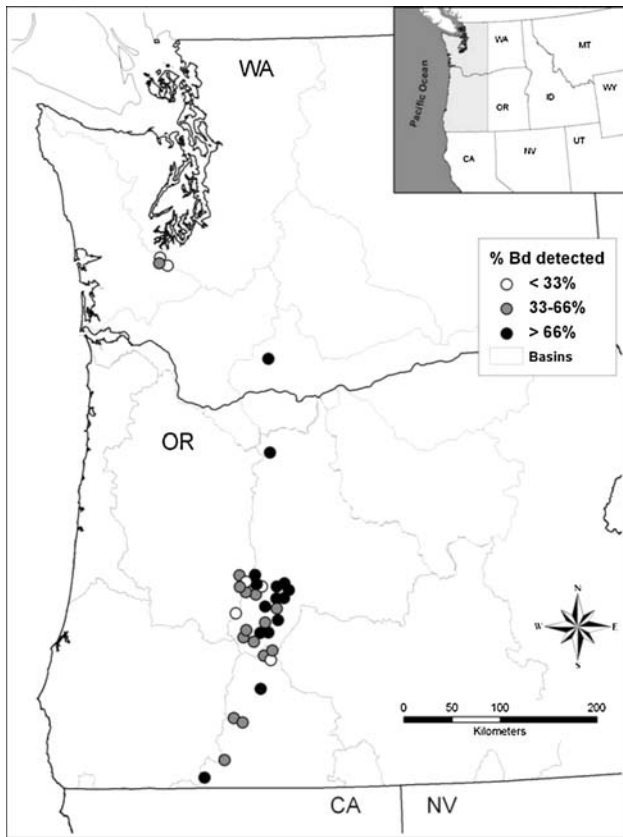
the odds of *Bd* increased by a factor of 1.54 (1.15–2.09) each week during our study period (mid-June to early October). At an elevation of 1000 m, the odds of *Bd* increased by a factor of 1.14 (1.03–1.29) each week. At 1500 m elevation, there was little temporal effect; the odds of *Bd* changed by a factor of 0.98 (0.89–1.09) per week (Figure 3).

We found little support for effects of Year or Human Footprint on the odds of *Bd* for individuals. The Bayesian *P*-value for the simultaneous modeling of our six predictors was 0.62, indicating that the model fit the data well.

Analysis 2: Potential Influence of *Bd* on Body Condition of Juvenile *R. pretiosa*

We measured body condition for 379 juvenile *R. pretiosa* from 31 breeding sites (mean = 12 per site; range, 1–28 frogs). Of these, 287 tested positive for *Bd*. We found similar proportions of *Bd*-positive juveniles in their summer of metamorphosis (75.6% [214/283]) as in their first full summer as frogs (76.0% [73/96]). Condition of juvenile frogs that tested positive for *Bd* averaged 1.002 ± 0.010 SE; range, 0.501–1.650); *Bd*-negative juveniles averaged 1.020 ± 0.019 SE; range, 0.702–1.707).

The best supported model suggested an effect of *Bd* × Juvenile Year on condition: the diminished condition after overwintering was greater for individuals that were positive for *Bd* than for individuals that were negative (Tables 3, 4; Figure 4). Our best-supported model (model weight = 0.466) for juvenile *R. pretiosa* condition included *Bd*, juvenile year, and the interaction between them. The second and third ranked models were within ΔAICc of 1.95 and 1.99 (weights 0.175, 0.172), respectively, so the best model did not receive overwhelming support among those we considered. Each of the top three models included *Bd* and Juvenile Year, and the top two models both contained the *Bd* × Juvenile Year interaction. All of these models had low explanatory power (multiple r^2, 7.3%–8.3%).

DISCUSSION

Chytridiomycosis has received much attention worldwide in recent years, mainly due to associations with declines in tropical and subtropical anurans (Berger et al., 1998; Pounds et al., 2006; Lips et al., 2006). The distribution and effects of *Bd* in temperate species are less well defined. Our data add to others (Adams et al., 2007; Pearl et al., 2007) that suggest that *Bd* is widespread in the Pacific

Figure 1. Sample sites and *Bd* prevalence in Oregon spotted frogs (*Rana pretiosa*) in northwestern USA, 2006–2007. Prevalence categories are <33%, 33%–66%, >66% of postmetamorphic *R. pretiosa* at each site.
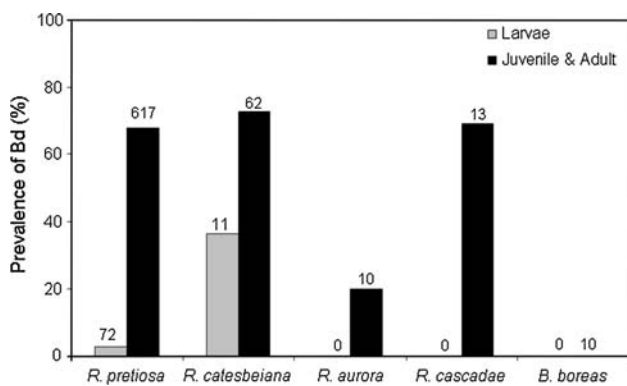

Figure 2. Prevalence of *Bd* in *R. pretiosa* and other northwestern anurans. Numbers above bars represent sample size for each species and life stage; we did not sample larvae of *B. boreas, R. aurora,* or *R. cascadae.*

effects on the odds that an individual postmetamorphic *R. pretiosa* had *Bd*: 1) for each 15.1 mm increase in length, the odds of *Bd* decreased by a factor of 0.71 (95% CI: 0.56–0.89); 2) the effect of date depended on elevation. At 50 m elevation,

Table 2.   Posterior Distributions of Beta Parameters (and 95% Credibility Intervals [LCI, UCI]) for Predictors of *Bd* on Postmeta-morphic Oregon Spotted Frogs (*Rana pretiosa*) (*n* = 617) in Northwestern USA, 2006–2007[a]

Beta	Median	LCI	UCI	Pr	expMedian	expLCI	expUCI
Intercept	0.634	0.032	1.231	0.981	1.885	1.032	3.425
Length	−0.348	−0.571	−0.121	0.001	0.707	0.565	0.886
Elevation	3.878	1.258	6.560	0.998	48.328	3.518	706.289
Year	0.420	−0.273	1.148	0.885	1.522	0.761	3.152
Date	2.189	0.693	3.760	0.998	8.926	2.000	42.950
Human footprint	0.357	−0.230	0.945	0.889	1.429	0.794	2.574
Elevation × date	−4.415	−7.756	−1.143	0.004	0.012	0.000	0.319

[a]Pr is the probability; the value of the parameter is >0. expMedian is the multiplicative effect of 1 SD change in the predictor on the odds that an individual will be *Bd* positive.

Table 3.   Results of GLM Analysis of Factors Associated with Body Condition for Juvenile Oregon Spotted Frogs (*Rana pretiosa*) (*n* = 379) in Northwestern USA, 2006–2007[a]

Model	No. parameters	AICc	ΔAICc	Model likelihood	ω Weight
Bd + JuvYr + *Bd* × JuvYr	4	−1337.44	0.000	1.000	0.466
Elev + *Bd* + JuvYr + *Bd* × JuvYr	5	−1335.48	1.957	0.376	0.175
Bd + JuvYr	3	−1335.45	1.987	0.370	0.172
Elev + JuvYr	3	−1334.78	2.660	0.264	0.123
Elev + *Bd* + JuvYr	4	−1333.46	3.980	0.137	0.064
Null	1	−1310.87	26.568	0.000	0.000
Elev + *Bd*	3	−1309.03	28.403	0.000	0.000

[a]Elev, elevation; JuvYr, juvenile year.
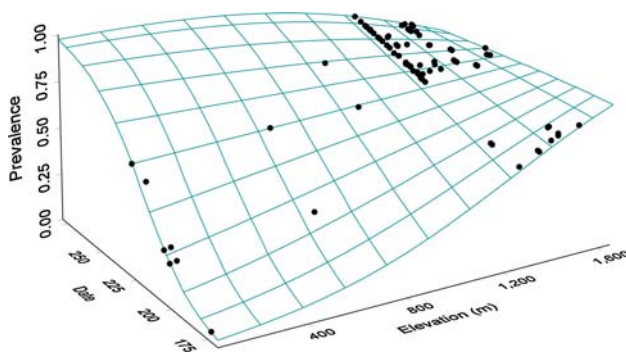
Figure 3. Interactive effects of elevation and sampling date on prevalence of *Bd* in postmetamorphic *R. pretiosa* (*n* = 617). Points are predicted values computed for the ranges of ordinal date and elevation in the data.

Northwest. Laboratory and field surveys confirm marked variation in *Bd* prevalence and responses to infection among amphibian species (Garcia et al., 2006; Woodhams et al., 2006; Retallick et al., 2004; McDonald et al., 2005). Our study of *R. pretiosa* found one of the highest rates of *Bd* prevalence (68.1% of juveniles and adults) and occupancy (100% of surveyed sites) that we have seen reported. We found very low prevalence of *Bd* in larvae (2.8%). In contrast, larvae showed markedly higher *Bd* prevalence than adults in some species inhabiting Australian streams (Woodhams and Alford, 2005). This pattern led to a hypothesis that larvae in those systems might provide the local reservoir for the disease. Low *Bd* prevalence in larval *R. pretiosa*, along with the relatively short duration of that stage (usually 3–4 months with no evidence of overwintering) suggests they are unlikely to serve as a disease reservoir. High prevalence and heavy use of aquatic habitats by postmetamorphic *R. pretiosa* in all seasons may suggest they provide a local reservoir for *Bd*. Low prevalence in larvae could be related to resistance of those *R. pretiosa* life stages to infection by *Bd,* or problems detecting *Bd* with swabs of those life stages (e.g., Retallick et al., 2006). Regardless, sampling *Bd* in larvae does not appear to be a productive way to detect *Bd* in *R. pretiosa* populations.

Table 4. Parameter Estimates for Terms in the Best Ranked Model for Factors Associated with Condition of Juvenile *R. pretiosa*

	Beta	SE	95% Confidence limits
Intercept	0.991	0.012	0.968, 1.013
Bd	−0.021	0.012	−0.044, 0.002
Juvenile year	−0.042	0.012	−0.065, −0.019
Bd × juvenile year	−0.024	0.012	−0.046, −0.001
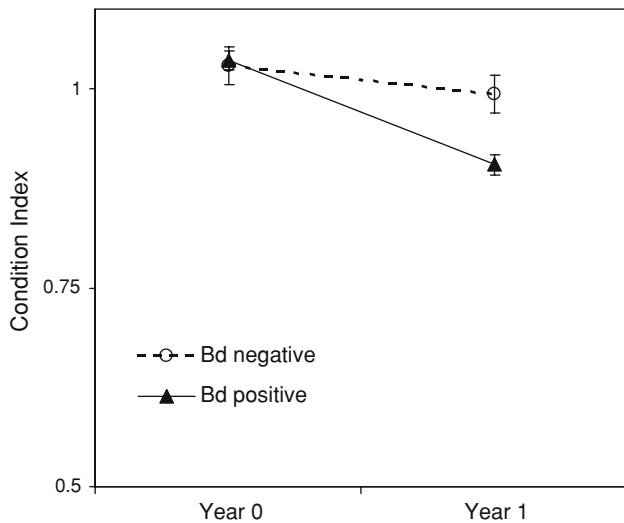
Figure 4. Body condition (mean ± SE) and *Bd* status of juvenile *R. pretiosa* sampled in the summer of their metamorphosis (Year 0) and the year after first winter as a frog (Year 1). Sample sizes are in Table 1. We did not monitor marked frogs

Several field surveys that have used PCR to detect *Bd* on a focal anuran have reported relatively high occupancy (e.g., 77% of *Litoria lesueuri* sites [Kriger et al., 2007]; 64% of *B. boreas* site clusters [Muths et al., 2008]), but we are unaware of reports of 100% occupancy. Moreover, most reports of field prevalence of *Bd* range from 10% to 40% of individuals within populations where *Bd* is present (Retallick et al., 2004; Ouellet et al., 2005; Adams et al., 2007; Kriger and Hero, 2007a; Kriger et al., 2007; Longcore et al., 2007; Muths et al., 2008). Reports of *Bd* prevalence that approach or exceed 50% of individuals are infrequent, but season-specific (Kriger and Hero, 2007b) and site-specific (Schlaepfer et al., 2007) cases have been reported. Our high rate of *Bd* prevalence is consistent with a smaller sample of *R. pretiosa* (12/21 [57%]; Pearl et al., 2007), and with two studies of the closely related *R. luteiventris* (20/50 [40%], Muths et al., 2008; 32/68 [47%], Pearl et al., 2007). We also found high *Bd* prevalence in introduced *R. catesbeiana* (*n* = 62 postmetamorphs), another anuran that is highly aquatic and is found in lentic

habitats similar to *R. pretiosa*. Our bullfrog results are consistent with other data suggesting high *Bd* prevalence from bullfrogs' native and introduced ranges (Ouellet et al., 2005; Garner et al., 2006; Longcore et al., 2007; Pearl et al., 2007).

Probability of *Bd* was inversely related to body length of *R. pretiosa*, a pattern seen in several other field-sampled anurans (Beard and O'Neill, 2005; Kriger et al., 2006, 2007). Possible explanations for this association include: (1) greater susceptibility to contracting *Bd* in younger stages (e.g., as a function of ontogenetic differences in habitat use or immune capacity), (2) *Bd* infection results in slower growth, and (3) frogs can clear *Bd* infections as they age (Beard and O'Neill, 2005; Briggs et al., 2005; Kriger et al., 2006, 2007; Rachowicz et al., 2006). Early stages after metamorphosis can suffer high mortality associated with *Bd* infection in several North American anurans (Garcia et al., 2006; Rachowicz et al., 2006). In addition, larvae and juveniles of several species experimentally infected with *Bd* show reduced growth compared to uninfected controls (Parris and Cornelius, 2004; Davidson et al., 2007; Retallick and Miera, 2007). This is relevant since size is a strong predictor of survival in anurans such as *R. pretiosa* (Chelgren et al., 2008).

Our analysis also indicated that *Bd* presence on *R. pretiosa* was influenced by an interaction between sampling date and elevation (Figure 3). This interaction suggests that *Bd* increases as summer progresses for low-elevation *R. pretiosa*, but that this trend diminishes at higher elevations. A positive association between *Bd* and elevation has been reported elsewhere (McDonald et al. 2005; Woodhams and Alford, 2005; but see Muths et al., 2008). The trend of increasing prevalence with date in summer (which we found at lower elevations) contrasts with work in tropical and subtropical areas that found higher prevalence in cooler seasons, such as spring (Berger et al., 2004; Retallick et al., 2004; Kriger and Hero, 2007b). Some other species of frogs can clear *Bd* infections later in summer and with higher temperatures (Woodhams et al., 2003; Kriger and Hero, 2006; Retallick and Miera, 2007). However, we did not

resample individuals so cannot address whether this occurs in *R. pretiosa*, and we had few *R. pretiosa* sites (*n* = 5) that were <1000 m. This small number of sites at lower elevations is a reflection of asymmetrical losses in *R. pretiosa* in lowland parts of their range; the bulk of *R. pretiosa* extant range is >1200 m (Hayes, 1994, 1997). A broader temporal range of sampling at low elevation sites will be necessary to understand dynamics of *Bd* in those systems.

Few studies have examined body condition in relation to amphibian diseases such as *Bd*, but this variable may offer a useful metric if *Bd* is acting as a stressor. Body condition in some Australian species varied with season and elevation, but did not significantly differ between *Bd*-positive and *Bd*-negative frogs (Woodhams and Alford, 2005). Our analysis is exploratory, but provides some evidence of a cost associated with *Bd* for juvenile *R. pretiosa* over winter; however, there was little differentiation among models and we only assessed *Bd* presence in the summer. It is also not clear that modest differences in condition early in the summer would affect survival of juvenile *R. pretiosa*. The potential for such sublethal effects deserves further study, which would benefit from refining variables that improve model prediction of juvenile body condition. For example, we did not quantify factors, such as food availability and severity of preceding winter, which can affect frog condition (e.g., Reading, 2007).

Our data do not directly address whether *Bd* had a role in the range contraction of *R. pretiosa*. The high prevalence and site occupancy by *Bd* where *R. pretiosa* persist, along with the lack of observed morbidity or mortality, indicate that *R. pretiosa* can persist with *Bd* at least temporarily. However, a better understanding of local *R. pretiosa* population trends is needed to more fully address potential relationships to declines. The highly aquatic life history of *R. pretiosa* probably exposes all *R. pretiosa* life stages to zoospores where *Bd* is present, so further information is needed to understand mechanisms by which *R. pretiosa* coexist with *Bd* and what conditions might result in *Bd* effects on *R. pretiosa*. Recapture studies of marked frogs could reveal whether individuals are capable of clearing *Bd* during the summer (e.g., Retallick et al., 2004). Also, peptide and other antimicrobial compounds afford some amphibians protection from chytridiomycosis (Woodhams et al., 2006). The closely related Columbia spotted frog (*R. luteiventris*; Goraya et al., 2000) and the more distantly related foothill yellow-legged frog (*R. boylii*; Davidson et al., 2007) possess some skin peptides that have antimicrobial properties, but defenses in *R. pretiosa* have not been studied.

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