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Factors related to the distribution and prevalence of the fungal pathogen *Batrachochytrium dendrobatidis* in *Rana cascadae* and other amphibians in the Klamath Mountains

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ABSTRACT

The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), which causes the disease chytridiomycosis, has been associated with declines and extinctions of montane amphibians worldwide. To gain insight into factors affecting its distribution and prevalence we focus on the amphibian community of the Klamath Mountains in northwest California. The Cascades frog (*Rana cascadae*), one of the most common amphibians in these mountains, experienced increased mortality as a result of *Bd* exposure in laboratory trials and has experienced recent, dramatic declines in other parts of California. We surveyed 112 sites in the Klamaths, all of which supported *R. cascadae* between 1999 and 2002, for amphibians and *Bd* to (1) determine the distribution of *Bd*, (2) evaluate changes in the distribution of *R. cascadae*, and (3) assess associations between potential biotic and abiotic drivers and *Bd* infection. *Bd* was widely distributed in the Klamath Mountains – we detected the pathogen at 64% of sites. *R. cascadae* was found at 79% of sites, and was often infected with *Bd*. These results suggest that *Bd* has not caused dramatic declines in *R. cascadae* in the Klamaths in recent years. Subadult *R. cascadae* had a higher *Bd* prevalence than other *R. cascadae* life stages (subadults: 36%, adults: 25%, metamorphs: 4%, larvae: 1%), and while the probability of infection decreased over the season for adults, it did not for subadults, suggesting that subadults may be more vulnerable to chytridiomycosis than other *R. cascadae* life stages. *Bd* prevalence in *R. cascadae* was highest early in the season at high-elevation sites, which may indicate that populations inhabiting high elevation sites may have a greater risk of being affected by chytridiomycosis. Three other common amphibian species also tested positive for *Bd*: Pacific chorus frog (*Pseudacris regilla*), western toad (*Anaxyrus boreas*), and rough-skinned newt (*Taricha granulosa*).

1. Introduction

Chytridiomycosis, the disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), has been associated with extinctions and declines in amphibians worldwide (Skerratt et al., 2007; Fisher et al., 2009; Kilpatrick et al., 2010). For example, outbreaks of chytridiomycosis have caused the extinction of hundreds of populations of mountain yellow-legged frog (*Rana sierrae*/*muscosa*) in California's Sierra Nevada Mountains (Briggs et al., 2005; Rachowicz et al., 2006; Vredenburg et al., 2010). A closely-related species, the Cascades frog (*Rana cascadae*), has experienced severe declines in nearby mountain ranges in northern California (Fellers et al., 2008). Laboratory experiments have shown that exposure to *Bd* increases mortality in juvenile *R. cascadae* (Garcia et al., 2006), and *Bd* was discovered in multiple *R. cascadae* populations in California in 2006 (Pope et al., unpublished data), suggesting that chytridiomycosis may be a causal agent in the observed declines. The purpose of this study was to examine the distribution of *Bd* and *R. cascadae* in California's Klamath Mountains and identify biotic and abiotic factors associated with *Bd* infection.

Bd infects the keratinized tissue of amphibians, including the mouthparts of larvae and the skin of post-metamorphic animals (Berger et al., 1998, 2005). The effects of *Bd* on larvae tend to be minor, although sublethal and lethal effects have been described (Parris and Cornelius, 2004; Blaustein et al., 2005; Garner et al., 2009). For post-metamorphic amphibians, there is a great deal of variation among species in the effects of *Bd*, with some species experiencing 100% mortality within weeks of exposure and others experiencing

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few negative effects of infection (Skerratt et al., 2007; Fisher et al., 2009; Kilpatrick et al., 2010). Environmental variables can also play an important role in the outcome of *Bd* infection. For example, laboratory experiments have demonstrated that elevated temperatures can ameliorate the effects of *Bd* on infected individuals and help them clear infections (Retallick and Miera, 2007; Andre et al., 2008), and animals that behaviorally warm themselves can clear infections (Richards-Zawacki, 2010). In addition, field studies have found lower prevalence of *Bd* at warmer sites (Woodhams and Alford, 2005; Drew et al., 2006; Kriger et al., 2007; Walker et al., 2010). Elevation has also been linked to outbreaks of chytridiomycosis. Many of the most dramatic amphibian die-offs have been observed in montane areas (Fisher et al., 2009), and elevation is correlated with *Bd* prevalence in field studies (Woodhams and Alford, 2005; Muths et al., 2008; Pearl et al., 2009b; Adams et al., 2010, but see Knapp et al., in press). As elevational changes are associated with variation in temperature regimes, it is likely that these patterns are at least in part explained by differences in temperature. Seasonal patterns in *Bd* prevalence are also likely to be influenced by temperature changes, although seasonal changes in the abundance of susceptible amphibian life stages may also be important (Pearl et al., 2009b; Adams et al., 2010; Russell et al., 2010; Kinney et al., 2011).

In addition to environmental drivers, the impact of *Bd* on host populations may be linked to the density of amphibian hosts and the diversity of the host community. Host-pathogen dynamics are often strongly dependent on host density, with increases in density expected to lead to higher transmission rates and faster spread of pathogens through populations (Anderson and May, 1981). Host diversity can influence the prevalence of a pathogen by changing the density of host species, altering host behavior, and allowing poorly reservoir-competent host species to dilute the force of infection or strongly reservoir-competent hosts to increase it (Keesing et al., 2006, 2010). However, while amphibian populations often exhibit dramatic variation in density and *Bd* susceptibility (Fisher et al., 2009; Kilpatrick et al., 2010), few studies have explored how amphibian density and community composition influence patterns of infection in the field. In the mountains of northern California *R. cascadae* commonly co-occurs with at least four other amphibian species. One of these, the western toad (*Anaxyrus* [*Bufo*] *boreas*), has been adversely affected by *Bd* in laboratory trials (Blaustein et al., 2005; Carey et al., 2006; Garcia et al., 2006; Murphy et al., 2009), while another, the Pacific chorus frog (*Pseudacris regilla*), did not experience enhanced mortality when exposed to *Bd* in the laboratory (Garcia et al., 2006).

The goals of our study were to examine the distribution of *Bd* and *R. cascadae* in the Klamath Mountains, the home of most of the remaining *R. cascadae* populations in California, and evaluate several potential biotic and abiotic drivers of *Bd* prevalence. We surveyed amphibians and sampled for *Bd* in over one hundred sites in the Klamath Mountains, all of which were found to be inhabited by at least one *R. cascadae* in surveys conducted between 1999 and 2002. We assessed site occupancy by *R. cascadae* and used hierarchical models to address the following questions concerning *Bd*: (1) Is there a geographical pattern in the distribution of *Bd* in the region? (2) Do different amphibian species and life stages have different patterns of *Bd* infection? (3) Are conspecific density and community composition associated with *Bd* infection in *R. cascadae*? (4) Are there associations between environmental variables and *Bd* infection?

2. Material and methods

2.1. Study system and sampling design

In northern California *R. cascadae* inhabits two mountain ranges – the southern Cascades and the Klamath Mountains. Severe declines have occurred in the southern Cascades, and the few remaining populations consist of relatively small numbers of frogs (Fellers et al., 2008). In contrast, there were still hundreds of *R. cascadae* populations found in the Klamath Mountains in surveys conducted 1999–2002 (Welsh et al., 2006; Welsh and Pope, unpublished data). The Klamath Mountains comprise a series of ranges in northwestern California (Fig. 1). Elevations range from 730 m to 2750 m, the dominant habitat types are mixed conifer and subalpine forests, and the climate is characterized by moderately cold winters with heavy snowfall, and warm summers with limited rainfall. The amphibian fauna of the Klamath Mountains includes five lentic-breeders (Cascades frog: *R. cascadae,* Pacific chorus frog: *P. regilla* [the boundary between *P. regilla* and its congener, *Pseudacris sierra*, is in the vicinity of our study region but its location has not been precisely determined (Recuero et al., 2006b,a), so there is some taxonomic uncertainty associated with this species designation], western toad: *A. boreas*, long-toed salamander: *Ambystoma macrodactylum*, rough-skinned newt: *Taricha granulosa*) and two lotic-breeders (coastal tailed frog: *Ascaphus truei*, Pacific giant salamander: *Dicamptodon tenebrosus*). While we sampled all amphibians encountered, surveys focused primarily on lentic waters, so few lotic-breeding amphibians were sampled.

We sampled sites in the Klamath Mountains that were found to support *R. cascadae* in previous, comprehensive surveys of 915 lentic waters in the Klamath Mountains (Welsh et al., 2006; Welsh and Pope, unpublished data) using a geographically-stratified random design . We first divided the 436 sites in the Klamath Mountains found to support *R. cascadae* in the 1999–2002 surveys (Welsh et al., 2006; Welsh and Pope, unpublished data) into six geographic units (Marble Mountains Wilderness, Russian Wilderness, Trinity Alps Wilderness, Shasta-Trinity National Forest [outside wilderness areas], and Castle Crags Wilderness and State Park; Fig. 1). We then randomly selected sites such that the sample contained the same proportion of sites from each geographic region as the population from which the sample was drawn.

2.2. Data collection

We surveyed 112 sites–105 sites were surveyed between June and September 2008 and seven sites in the Marble Mountains were surveyed in June 2009. We conducted the 2009 surveys because a portion of the Marble Mountains Wilderness was inaccessible in 2008 due to several large forest fires. Thirty of the selected sites were surveyed twice in order to enhance our ability to detect seasonal patterns and to estimate the detection probability for *R. cascadae* (MacKenzie et al., 2006). As in a previous study (Welsh et al., 2006), our estimate of detection probability was high for *R. cascadae* ($p = 0.94$) so we present our raw occurrence findings. We found amphibians and collected *Bd* samples at 106 sites, including sites in all of the targeted geographic areas.

During each survey, we sampled the main water body and all smaller water bodies associated with the site. Visual encounter surveys (VES; Crump and Scott, 1994) were used to estimate the density of each amphibian species at a site. VESs were conducted during the warmest part of the day (between 10:00 and 18:00), when *R. cascadae* primarily occupy shoreline and near-shore habitats and larvae of all species would be visible in the warm, shallow water (Welsh et al., 2006). Crews counted amphibians in each life stage by searching the shoreline and littoral habitats and by looking under banks and logs and in the littoral zone substrate. While numbers of animals observed during a survey are an underestimate of the site's population, we used these values as coarse site-level estimators of density. Multiple resurveys of 16 sites over 4 years showed consistent single-survey density patterns relative to true population values for *R. cascadae* (Pope, 2008; Pope,

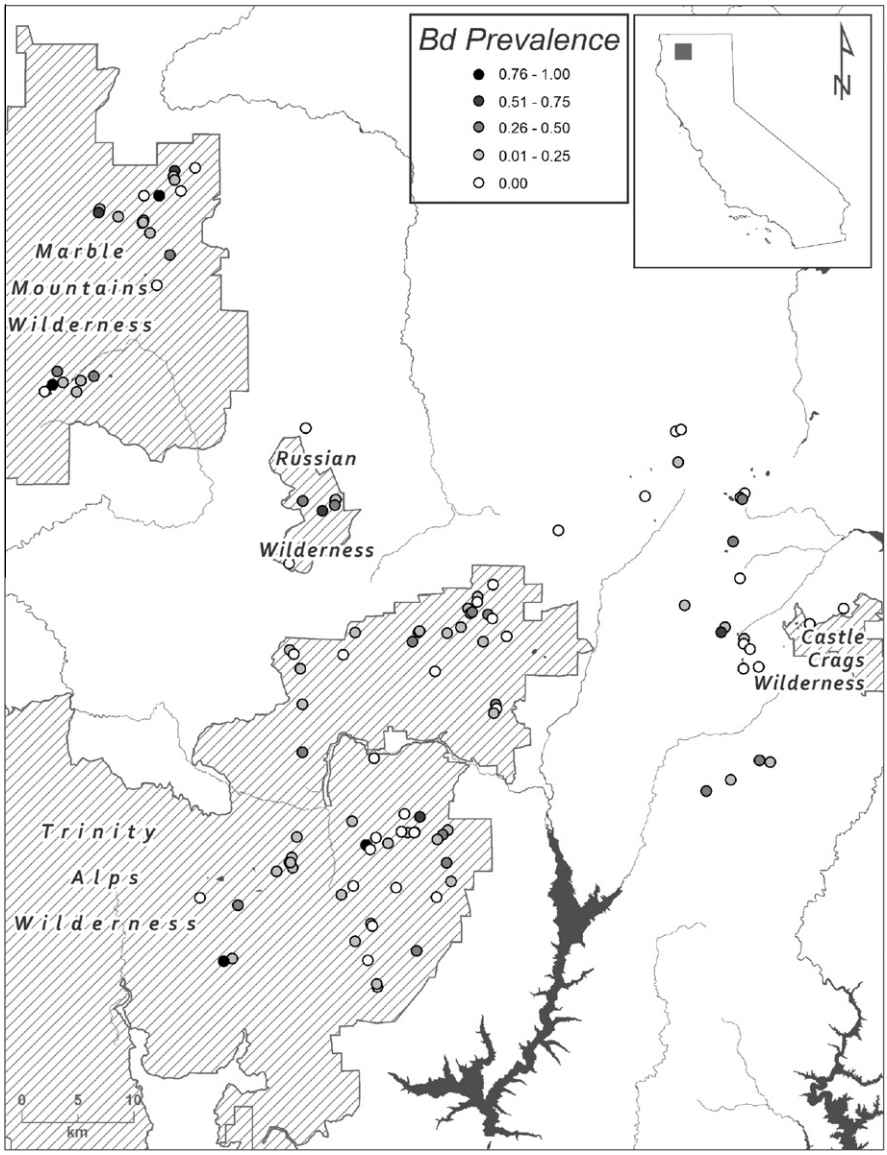

Fig. 1. Prevalence of *Bd* across the study area. Each symbol represents a site, with the degree of shading corresponding to the prevalence of *Bd* (see legend on figure) (map created by Shannon Chapin).

unpublished data). In each water body, we captured up to 20 individuals of each life stage of each species for *Bd* sampling. These included post-metamorphic individuals and late-stage larvae encountered during VESs. In addition, because some species of amphibians are more active at night, minnow traps were set overnight at 55 of the surveyed sites. Six minnow traps, baited with glow sticks (Grayson and Roe, 2007), were set within 2 m of the shoreline at regular intervals. Traps were placed at the surface of the water with an air space for captured animals to breathe. Since minnow traps were not set at all sites, animals captured in minnow traps were not included in density estimates, which used only data from VESs.

Captured animals were sexed (where possible), weighed, measured [post-metamorphic animals: snout-urostyle length (SUL); larvae: total length], and swabbed for *Bd* following the technique described by Boyle et al. (2004). We ran a sterile rayon swab (Medical Wire & Equipment) along the potentially infected tissues of animals. For post-metamorphic frogs and toads, we swabbed each of the following areas five times: the ventral surface of the abdomen, the left and right inner thighs, and webbing of each hind foot. For larval frogs and toads, we gently inserted the swab into the mouth and rotated the swab 15 times. Lateral and ventral body surfaces of larval and post-metamorphic salamanders (*A. macrodactylum* and *T. granulosa*) were swabbed five times. Swabs were air dried away from direct sunlight and placed in a sterile vial. These vials were placed at −20 °C upon return from the field and remained frozen until DNA extraction and PCR analysis could take place. To prevent moving *Bd* between sites, all sampling gear was disinfected using a 0.1% solution of quaternary ammonia between surveys at different sites (Johnson et al., 2003).

DNA was extracted from swab samples using PrepMan Ultra (Applied Biosystems) following established protocols (Retallick et al., 2006). We used a real-time PCR assay to test for the presence of *Bd* in each sample following standard protocols (Boyle et al., 2004; Hyatt et al., 2007), with the exception that samples were analyzed singly rather than in triplicate (Kriger et al., 2006) and reaction volumes were scaled down to 12.5 μL from 25 μL. Either 1 or 2.5 μL of undiluted DNA was added to each 12.5 μL reaction. Preliminary analyses indicated that the volume of DNA added did not affect the probability of PCR amplification, so DNA volume was not included as a predictor in subsequent analyses. DNA

volume did, however, appear to influence the quantitative measure of the amount of *Bd*, so we do not present data on infection intensity. Real-time PCR assays were conducted using an Applied Biosystems StepOnePlus real-time PCR system. Animals were considered infected if any amplification was visible in the PCR. Negative controls (water blanks) were run on each plate; amplification was never detected in negative controls.

2.3. Statistical modeling and hypothesis evaluation

We developed hierarchical models to assess the prevalence of *Bd* in two data sets: (1) all life stages of the four common species (*A. boreas*, *P. regilla*, *R. cascadae*, and *T. granulosa*), and (2) adults and subadult *R. cascadae*. We fit generalized linear mixed models to the data and used multi-model inference based on information-theoretic approaches to assess the effect of various predictor variables on the probability of *Bd* infection for each of the two datasets. Fitting mixed models allowed us to account for non-independence of samples collected from the same site or water body and simultaneously evaluate individual-level, water-body-level, and site-level predictors. By calculating model-averaged parameter estimates and standard errors, we account for both sampling variance and model-selection uncertainty.

We used a combination of biological, environmental, and geographical predictor variables in our models (Table 1). While there was some collinearity between summer maximum temperature and elevation ($\rho = 0.62$), we chose to keep both of those predictors in the model, as previous studies have shown associations between both of these factors and *Bd* prevalence (Drew et al., 2006; Muths et al., 2008; Adams et al., 2010). There was little evidence of correlation between other pairs of continuous predictors ($\rho < 0.35$ in all cases). All continuous predictors were *z*-transformed to facilitate comparisons between effect sizes and interpretation of interaction terms. Site was a random factor, and because there was often more than one water body at a site, water body was also a random factor.

Bd presence/absence was modeled as a Bernoulli random variable with binomial errors and a logit link function. We assembled a set of balanced (sensu Anderson, 2008) candidate models for each analysis based on *a priori* hypotheses concerning *Bd* prevalence. In order to maintain a reasonable number of candidate models predictor variables were grouped into categories – individual-level predictors (e.g. life stage) and interactions involving these terms, water body-level biological predictors (e.g. density), water body-level environmental predictors (e.g. temperature), and water body-level geographical predictors (latitude and longitude) – with sets of candidate models including all possible combinations of these groups of predictors (Appendix A). In addition to the simple effects of the predictors in Table 1, we included a perimeter $*$ air temperature interaction term because water body size and air temperature are likely to be the most important determinants of water temperature and a latitude $*$ longitude interaction term to allow for the detection of a greater range of spatial patterns.

For models of all common species, species–life stage was included as a combined variable as not all life stages were sampled for all species, and ordinal date was not included, as all life stages of all species were not present throughout the season. For models of *R. cascadae* adults and subadults, we included ordinal date as a predictor and date $*$ life stage and date $*$ elevation interaction terms in order to compare seasonal patterns of infection across life stages and elevations. We also included the presence/absence of the other three common amphibian species as water-body level predictors in order to assess whether these species influence *Bd* presence/absence in *R. cascadae* adults and subadults.

Akaike's Information Criterion with a second-order bias correction (AICc) and AICc-based model probabilities, or "Akaike weights", were calculated for every model in each candidate set (Anderson, 2008). Model-averaged parameters and standard errors were estimated according to formulae in Lukacs et al. (2010). Each model-averaged parameter estimate is a weighted average of parameter estimates from each of the candidate models, with weights provided by the model probabilities and a value of zero assigned for models in which the parameter being estimated does not appear (Anderson, 2008; Lukacs et al., 2010). Similarly, the unconditional variance of each parameter is estimated using a weighted average of the sampling variance given a model combined with the variation due to model selection uncertainty for each model in the candidate set (Anderson, 2008; Lukacs et al., 2010). Approximate 95% confidence intervals (CI) for each parameter were calculated as the model-averaged mean ± two times the model-averaged standard errors (Anderson, 2008; Lukacs et al., 2010). Interaction terms that were not strongly supported (i.e., approximate 95% confidence intervals overlap zero) in preliminary analyses were removed from the models in order to facilitate interpretation of the main effects of the predictors involved. When interaction terms were included, simple effects were estimated using inferential simulations (Gelman and Hill, 2007, Chapter 7). Point estimates and 95% confidence intervals for the appropriate linear combination of parameters were based on the parameter estimates and associated standard errors from the best-fitting model. For example, in the presence of a life stage $*$ ordinal date interaction, the relationship between ordinal date and subadult probability of infection would be represented by the sum of the ordinal date parameter and the ordinal date $*$ life stage (subadult) parameter.

Table 1
Predictor variables used in analyses of *Bd* infection probability and intensity.

Covariate	Level	Type	Range	Description
Species	Individual	Biological	–	*Rana cascadae*, *Anaxyrus boreas*, *Pseudacris regilla*, *Taricha granulosa*
Life stage	Individual	Biological	–	Larva, metamorph, subadult, adult[a]
Date	Individual	Temporal	164–248 days	Ordinal date, sampling was conducted late June through early September
Density	Water body	Biological	0–1.86 m^{-1}	Density of adult and subadult amphibians combined[b]
Perimeter	Water body	Environmental	7–2385 m	Distance around water body[c]
Elevation	Water body	Environmental	1414–2423 m	Meters above sea level
Longitude	Water body	Geographical	480080–551771	UTM E[d]
Latitude	Water body	Geographical	4532242–4603590	UTM N[d]
Temperature	Site	Environmental	12.9–22.4 °C	Average daily maximum air temperature during June, July, and August 2008[e]

[a] We used the following thresholds in snout-urostyle length to distinguish subadults from adults – *A. boreas*: 70 mm, *P. regilla*: 30 mm, *R. cascadae*: 45 mm, *T. torosa*: 60 mm (Pope et al., unpublished data; Stebbins, 2003). Metamorphs are defined as individuals that metamorphosed in the same season in which they were sampled, and were identified by the presence of residual larval traits (e.g. tail stub) and size.
[b] Density was obtained by dividing the number of adults and subadults seen in visual encounter surveys by the perimeter of the surveyed water body.
[c] Water body perimeters were measured from USGS 7.5 min quads using GIS or estimated in the field when the water body was too small to be mapped on 7.5 min quads.
[d] UTMs were recorded in NAD27, zone 10.
[e] Temperature data was obtained from PRISM (www.prismclimate.org).

In the model of all common species, the species–life stage factor was the only predictor included in the top-ranked model of *Bd* presence/absence (Appendix A). Because of this, we used a likelihood ratio test on the top-ranked model to evaluate the significance of this factor and Tukey post-hoc tests to compare species and life stages. Tukey comparisons were deemed significant when the 95% confidence intervals for the difference between groups did not overlap zero.

All analyses were conducted in R Development Core Team (2008). Mixed models were fit using the 'glmer' function in the lme4 package (Bates and Maechler, 2010). Post-hoc comparisons were performed using the 'glht' function in the 'multcomp' package (Hothorn et al., 2008).

3. Results

R. cascadae was found at 79% of the sites surveyed (88 out of 112), all of which were previously found to support the species in the 1999–2002 surveys. *Bd* was found at 64% of sites where samples were collected (68 out of 106) and in 16% of individuals (309 out of 1926). *R. cascadae*, *P. regilla*, *T. torosa*, and *A. boreas* were the most common amphibian species encountered, and all four species tested positive for the pathogen. We collected *Bd* samples from three other species – *A. macrodactylum* (17 larvae, four adults), *D. tenebrosus* (five larvae, one subadult, two adults), and *A. truei* (two adults) – and did not detect *Bd* in any of these samples.

The analysis of the four common species indicated that there were clear differences between species and life stages in the probability of *Bd* infection (species–life stage effect – likelihood ratio test: $\chi^2_{13} = 220$, $P < 0.0001$; Table 2 and Fig. 2). Subadult *R. cascadae* had higher probability of infection than all other *R. cascadae* life stages (post-hoc Tukey tests, Table 2 and Fig. 2). The infection probability for *R. cascadae* subadults was also greater than that for larvae and metamorphs of *P. regilla*, and adults of *T. granulosa* (post-hoc Tukey tests, Table 2 and Fig. 2). For both frog species (*R. cascadae* and *P. regilla*), young of the year (i.e., larvae and metamorphs) had lower probabilities of infection than later life stages (i.e., adults and subadults). In addition, *R. cascadae* larvae were less likely to be infected than adults and subadults of *T. granulosa*, as well as metamorphs and subadults of *A. boreas*. Similar results were found for larvae of *P. regilla*, although the difference between the larvae and *A. boreas* metamorphs was not significant (Table 2 and Fig. 2). We did not detect *Bd* in *A. boreas* larvae ($n = 28$) or adults ($n = 23$) (Fig. 2).

As in the analysis of all four common species, the analysis of *R. cascadae* adults and subadults alone indicated that subadults had a higher probability of infection than adults [Table 3, Data set 2: Life stage (subadult) parameter]. However, this analysis also indicated that seasonal patterns of infection differed between subadult and adult *R. cascadae* [Table 3, Data set 2: Ordinal date * life stage (subadult) parameter; Fig. 3]. For adults the probability of infection diminished over the course of the season (estimate: −0.74, 95% CI: [−1.33, −0.19]; Fig. 3), while no such decline was evident for subadults (estimate = 0.34, 95% CI = [−0.27, 0.94]; Fig. 3). For *R. cascadae* adults and subadults seasonal patterns of infection changed with elevation, with the highest probability of infection occurring early in the season at high elevation (Table 3, Data set 2: elevation * date interaction; Fig. 4). At the highest elevation (2389 m) the probability of infection decreased over the course of the season (estimate = −1.64, 95% CI = [−3.31, −0.05]), but there was no seasonal pattern of infection at the lowest elevation (1414 m: estimate = 0.55, 95% CI = [−1.66, 3.00]). In addition, *R. cascadae* adults and subadults tended to have a lower prevalence of *Bd* infection at sites with *A. boreas* and a lower probability of infection at higher latitudes (Table 3, Data set 2; these patterns were not significant for $\alpha = 0.05$, but were for $\alpha = 0.10$). There were not significant associations between conspecific density, *P. regilla* and *T. granulosa* presence, temperature, water body perimeter, and geographic variables and *Bd* in *R. cascadae* adults and subadults (Table 3, Data set 2).

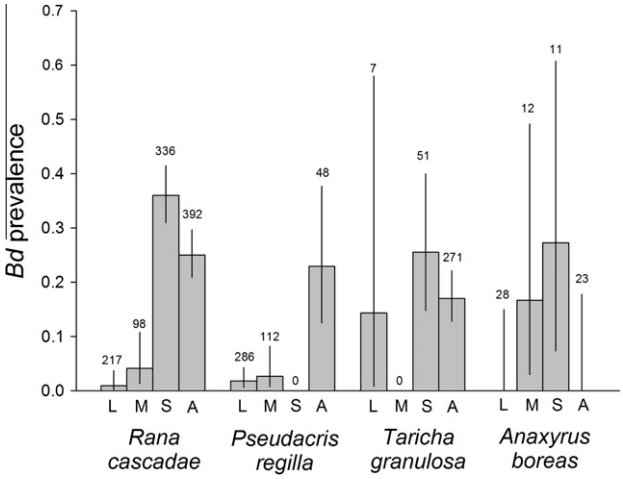

Fig. 2. Prevalence of *Bd* in four common amphibian species in the Klamath Ranges. Life stages are coded as follows: L = larvae, M = metamorphs, S = subadults, A = adults. Error bars denote 95% confidence intervals from the raw prevalence data and numbers above each bar denote the number of individuals sampled for each group.

Table 2
Differences in probability of *Bd* infection between species and life stages. Tukey groups for species and life stages are from the best-fitting model of *Bd* presence/absence in all common species – species–life stage groups that do not share a letter are significantly different from one another.

Species	Life stage	Tukey groups					
R. cascadae	Larva	a					
R. cascadae	Metamorph	a			d		f
R. cascadae	Subadult		b				
R. cascadae	Adult			c			
P. regilla	Larva	a				e	
P. regilla	Metamorph	a			d		
P. regilla	Adult		b	c			f
T. granulosa	Larva	a	b	c	d		
T. granulosa	Subadult		b	c	d		
T. granulosa	Adult			c	d		
A. boreas	Larva	a	b	c	d		
A. boreas	Metamorph		b	c	d	e	
A. boreas	Subadult		b	c	d		
A. boreas	Adult	a	b	c	d		

4. Discussion

Our study indicates that *Bd* is distributed throughout the Klamath Mountains and is present in all common lentic amphibian species. Although *R. cascadae* has exhibited increased mortality in response to *Bd* exposure in the laboratory (Garcia et al., 2006), the apparent persistence of this species between 1999–2002 and 2008–2009 at the majority of sites surveyed suggests that widespread die-offs associated with *Bd* did not occur during this period. Thus, while it is possible that *Bd* may have led to the extinction of large numbers of *R. cascadae* populations prior to the late 1990s, many extant populations appear to be coexisting with the pathogen. Similarly, *R. cascadae* does not appear to have experienced recent, broad population losses in the Oregon Cascades (Pearl et al., 2009a), despite the presence of *Bd* (Pearl et al., 2007, 2009b; Adams et al., 2010). Furthermore, *R. sierrae*/*muscosa*, a closely-related montane amphibian, has experienced dramatic declines as a result

Table 3
Model-averaged parameter estimates and approximate 95% confidence intervals from the sets of candidate models described in the text and Appendix A. The common species are *Anaxyrus boreas*, *Pseudacris regilla*, *Rana cascadae*, and *Taricha granulosa*. The models for *Rana cascadae* adults and subadults are parameterized such that adults serve as the reference level (see footnotes for details). Asterisks indicate parameter estimates whose approximate 95% confidence intervals do not overlap zero.

Data set	Parameter	*Bd* presence/absence		
		Estimate	95% Confidence intervals	
			Lower	Upper
1. Common species	Species–life stage (13 parameters)[a]	–	–	–
	Density[b]	0.001	−0.291	0.293
	Elevation	0.02	−0.208	0.248
	Temperature	0.001	−0.161	0.163
	Perimeter	0.013	−0.125	0.151
	Longitude	−0.09	−0.548	0.368
	Latitude	−0.081	−0.523	0.361
2. *Rana cascadae* adults and subadults	Density[c]	−0.103	−0.543	0.337
	Anaxyrus boreas[d]	−0.965	−1.953	0.023
	Pseudacris regilla[d]	0.039	−0.445	0.523
	Taricha granulosa[d]	−0.274	−1.042	0.494
	Life stage (subadult)[e]	0.692*	0.318	1.066
	Ordinal date (adults)[f]	−0.704*	−1.3	−0.108
	Elevation	0.596	−0.052	1.244
	Temperature	−0.01	−0.358	0.338
	Perimeter	0.132	−0.288	0.552
	Longitude	0.052	−0.49	0.594
	Latitude	−0.804	−1.666	0.058
	Ordinal date * life stage (subadult) [g]	1.054*	0.298	1.81
	Ordinal date * elevation	−1.37*	−2.422	−0.318

[a] Significance for this group of parameters was assessed using a likelihood ratio test (see Section 2).
[b] Density of adults and subadults from all common species.
[c] Density of *R. cascadae* adults and subadults.
[d] Presence of named species.
[e] Deviation from adults.
[f] Effect of ordinal date on adults.
[g] Deviation from ordinal date effect for adults.

of *Bd* in the southern Sierra Nevada (Rachowicz et al., 2006; Vredenburg et al., 2010), but is still relatively widespread in the central Sierra Nevada, despite a high prevalence of *Bd* (Knapp et al., in press).

Subadult *R. cascadae* were more likely to be infected with *Bd* than other *R. cascadae* life stages and their probability of infection did not change over the course of the summer season. In contrast to subadults, the probability of *Bd* infection decreased in adult *R. cascadae* as the season progressed. Seasonal changes in the strength of immunity may help explain these patterns. Multiple gains and losses of *Bd* infection have been observed in individual adult *R. cascadae* in the southern Cascades (Pope et al., unpublished manuscript), and in persistent populations of *R. sierrae* (Briggs et al., 2010), suggesting that adults of these species may be able to clear infections. Since amphibian immune capacity is temperature-sensitive (Raffel et al., 2006), as the season warms adults may have a greater ability to resist or clear infection, leading to reduced prevalence later in the summer. In contrast, juvenile frogs may have reduced immune function or experience more pronounced trade-offs between immune function and other physiological requirements (Fisher et al., 2009), and therefore might not be able to resist or clear infections as effectively as adults. Furthermore, animals that have a greater ability to clear infections may be more likely to survive to adulthood.

Bd prevalence was low in larvae (relative to adults and subadults) for both *R. cascadae* and *P. regilla*. *Bd* infects keratinized epidermal cells in amphibians (Pessier et al., 1999; Piotrowski et al., 2004). Only the mouthparts of larval anurans are keratinized, whereas the entire skin of post-metamorphic frogs is keratinized. Thus, larvae may be less likely to be colonized by the pathogen, less likely to sustain high levels of infection, or more likely to clear infections than post-metamorphic individuals. In laboratory trials, *Bd* did not increase mortality in larval *R. cascadae* (Blaustein et al., 2005; Han et al., 2008, 2011; Searle et al., 2010) and *P. regilla* (Blaustein et al., 2005), and larvae of other anuran species are also less likely to be adversely affected by *Bd* than later life stages (Kilpatrick et al., 2010). In addition to being less vulnerable to chytridiomycosis, larvae have not been exposed to the pathogen for as long as later life stages, which should reduce the probability of developing detectable infections. Both *R. cascadae* and *P. regilla* have larval periods of approximately 2–3 months in the Klamath Mountains. Other anuran species that metamorphose the same year they hatch also have low prevalence of *Bd* infection in larvae (Pearl et al., 2007, 2009b; Adams et al., 2010; Russell et al., 2010). In contrast, *R. sierrae*, a montane frog with a larval period of 1–4 years shows consistently high *Bd* prevalence in larvae, and prevalence tends to increase during the course of development (Briggs et al., 2010), suggesting that longer duration of exposure may lead to higher prevalence even in the absence of the dramatic physiological changes associated with metamorphosis.

While low prevalence in larvae seems to be the rule for anurans that complete metamorphosis within one season, high prevalence and load is common for newly post-metamorphic anurans (Briggs et al., 2010; Russell et al., 2010; Walker et al., 2010, but see Murphy et al., 2009; Kinney et al., 2011). We found that metamorphs of *R. cascadae* and *P. regilla* had a lower prevalence of *Bd* infection than later life stages. This could be the result of rapid mortality of infected individuals during metamorphosis (Garner et al., 2009), removing them from the sampling pool, or low rates of transmission and pathogen growth, such that infections take a while to build up on animals early in life. In laboratory studies *R. cascadae* experienced elevated mortality within 1 week of exposure to *Bd* while metamorphs of *P. regilla* did not experience enhanced mortality (Garcia et al., 2006). This finding suggests that rapid mortality of infected metamorphs could be an explanation for low *Bd* prevalence in *R. cascadae* metamorphs but is unlikely to explain low prevalence in metamorphs of *P. regilla*. We did not, however, observe any evidence of widespread mortality in metamorphic *R. cascadae* at our field sites.

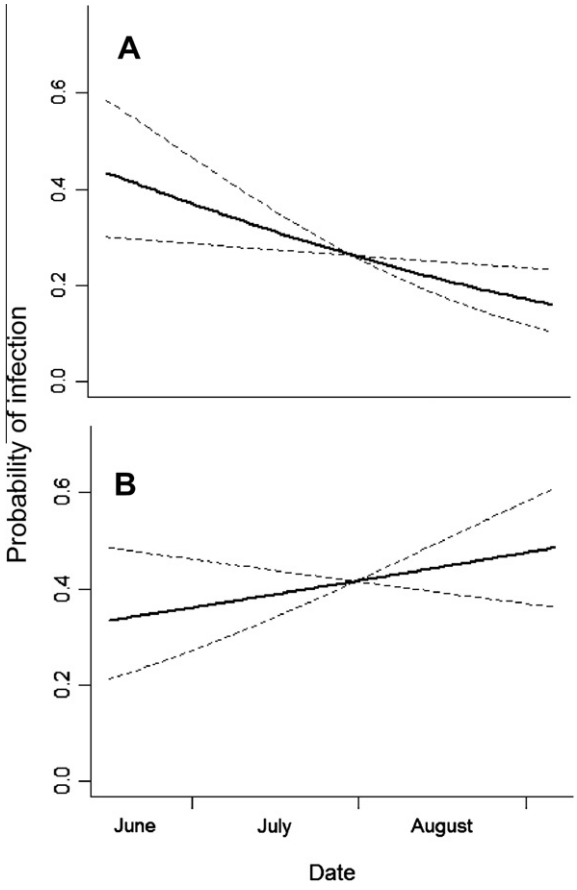

Fig. 3. The effect of date on the probability of *Bd* infection for *Rana cascadae* (A) adults and (B) subadults. Lines represent the predicted relationship (and 95% confidence intervals) between ordinal date and probability of infection from the model with the lowest AICc (Appendix A: Table A2).

Of the water body-level predictors, we found evidence for an interactive effect of elevation and date, with pronounced seasonal differences apparent at higher elevation but not at lower elevation. In contrast, previous studies of *Bd* in western North America suggested that seasonal differences were more pronounced at lower elevations (Pearl et al., 2009b; Adams et al., 2010). This discrepancy is likely due to the fact that our study featured higher elevations than previous studies (1414–2423 m in the current study; 4–2222 in Adams et al., 2010 and 35–1650 in Pearl et al., 2009b). We hypothesize that cool temperatures at high elevation sites early in the season were associated with lower immune activity and enhanced susceptibility to *Bd* infection, leading to higher *Bd* prevalence. We found stronger evidence for effects of elevation and season on *Bd* prevalence than for an effect of average summer temperature suggesting that temperatures during critical periods of the season may be more important for *Bd* infection than average temperatures.

Bd was detected in all common amphibian species in lentic waters in the Klamath Mountains, indicating that all of these species can contribute to the total pathogen pool in any water body. However, we found no evidence for a link between our indices of amphibian density and *Bd* prevalence. Given that amphibians are not evenly distributed within a water body, it is possible that patterns of aggregation within a water body have a larger effect on *Bd* prevalence than differences in density between water bodies. The trend for lower *Bd* prevalence in *R. cascadae* at sites with *A. boreas* may be related to the fact that *Bd* can increase mortality in *A. boreas* (Blaustein et al., 2005; Carey et al., 2006; Garcia et al., 2006; Murphy et al., 2009). Thus, the presence of *A. boreas* may indicate that a particular site is not conducive to the growth of *Bd*.

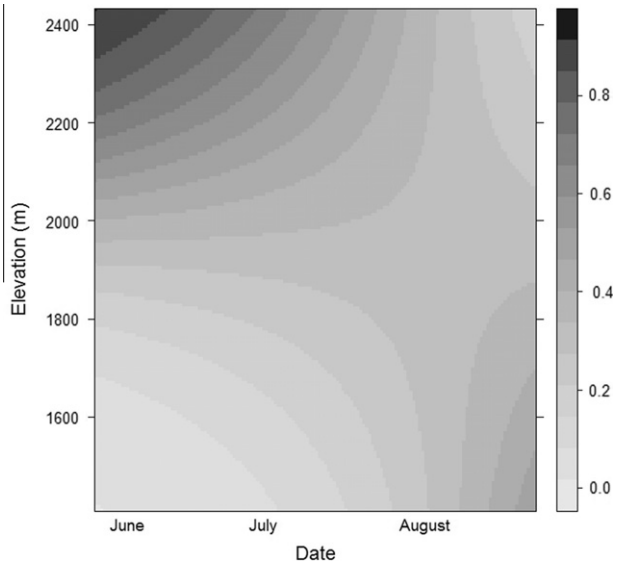

Fig. 4. The effect of ordinal date and elevation on *Bd* infection probability for *Rana cascadae* adults and subadults. The surface depicts the predicted probability of infection as a gray-scale gradient (see side bar). Predictions were generated using the model with the lowest AICc (Appendix A: Table A2).

R. cascadae and other amphibians in the mountains of northern California are threatened by a number of factors including the introduction of non-native trout (Welsh et al., 2006; Pope, 2008; Pope et al., 2008; Joseph et al., 2011) and changing hydrological conditions (Fellers et al., 2008, Pope et al. unpublished data). Our results indicate that *Bd* should also be considered a potential threat, as the pathogen is widespread in the Klamath Mountains and species that are known to be adversely affected by *Bd*, such as *R. cascadae*, may be vulnerable. While widespread die-offs do not appear to have occurred in the Klamath Mountains in the last 6–10 years, we have observed repeated recruitment failures for *R. cascadae* at three out of seven long-term monitoring sites in the Trinity Alps Wilderness that cannot be attributed to introduced trout or changing hydrology (Pope et al., unpublished data), and dramatic declines have occurred in this species in other parts of California (Fellers et al., 2008). Our results suggest that assessing the vulnerability of *R. cascadae* to chytridiomycosis will require a more detailed understanding of the effects of *Bd* on particular life stages under field conditions. Future research should focus on the effects of *Bd* on *R. cascadae* individuals and populations, with particular attention paid to young, post-metamorphic frogs.

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Appendix A

A.1. Candidate models

See Tables A1 and A2.

Table A1
Summary of candidate models for *Bd* presence/absence in four common amphibian species: *Rana cascadae*, *Pseudacris regilla*, *Taricha granulosa*, and *Anaxyrus boreas*.

Rank	Species–life stage[a]	Density[b]	Elevation	Temperature	Perimeter	Latitude	Longitude	AICc	ΔAICc	AICc weight
1	x							1351.8	0.0	0.77
2	x	x				x	x	1354.8	3.0	0.17
3	x	x	x	x	x			1357.7	5.9	0.04
4	x	x	x	x	x	x	x	1359.1	7.2	0.02
5						x	x	1549.2	197.4	0
6			x	x	x	x	x	1550.7	198.9	0
7			x	x	x			1547.1	195.2	0
8		x						1547.5	195.7	0
9								1545.6	193.7	0

[a] Larvae, metamorphs (where available), subadults (where available), and adults of each common species.
[b] Adults and subadults of all common species.

Table A2
Summary of candidate models for *Bd* presence/absence in *Rana cascadae* adults and subadults.

Rank	Life stage	Density[a]	*Anaxyrus boreas*[b]	*Pseudacris regilla*[b]	*Taricha granulosa*[b]	Ordinal date	Elev.	Temp.	Perimeter	Latitude	Longitude	Ordinal date * life stage interaction	Ordinal date * elevation interaction	AICc	ΔAICc	AICc weight
1	x	x	x	x	x	x	x					x	x	845.7	0.0	0.45
2	x	x	x	x	x	x	x	x	x	x	x	x	x	846.6	0.9	0.29
3	x					x	x			x	x	x	x	848.4	2.8	0.11
4	x	x	x	x	x	x	x	x	x			x	x	849.4	3.8	0.07
5	x	x	x	x	x	x	x					x	x	849.9	4.3	0.05
6	x					x	x	x	x	x	x	x	x	852.1	6.5	0.02
7		x	x	x	x			x	x	x	x			871.8	26.2	0
8								x	x	x	x			871.4	25.8	0
9	x					x	x	x	x			x	x	860.2	14.5	0
10		x	x	x	x					x	x			870.2	24.5	0
11		x	x	x	x			x	x					871.9	26.3	0
12										x	x			868.2	22.6	0
13								x	x					877.6	31.9	0
14	x					x	x					x	x	857.0	11.4	0
15		x	x	x	x									871.3	25.7	0
16														875.5	29.8	0

[a] *Rana cascadae* adults and subadults.
[b] Presence of species.

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