Batrachochytrium dendrobatidis infection dynamics in the Columbia spotted frog Rana luteiventris in north Idaho, USA

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ABSTRACT: The pathogenic chytrid fungus Batrachochytrium dendrobatidis (Bd) is contributing to amphibian declines worldwide. Temperature plays an important role in both pathogen growth and host immune function, but little is known about seasonal dynamics of Bd infection in north temperate regions. Our objective was to increase understanding of Bd disease ecology by investigating patterns of Bd infection of Columbia spotted frogs Rana luteiventris across seasons, age classes, and sexes in north Idaho, USA. We collected skin swabs from 223 R. luteiventris in spring, summer, and fall 2009 at 7 ponds in the Palouse region and quantified Bd zoospores for each sample using quantitative PCR. Across seasons, Bd prevalence of adults was higher in summer than in spring or fall, suggesting that individuals may be clearing low-level infections over the summer. Among age classes, all but one late stage tadpole (Gosner stage 43–45) tested negative for Bd. Conversely, 100% of metamorphs tested positive for Bd and had the highest Bd loads of all age classes, suggesting they may be the most vulnerable age class. Adult R. luteiventris had high infection prevalence (>60%) in all seasons, indicating that Bd infection is maintained within populations and that adults likely serve as disease reservoirs across seasons. Among adults, we also found weak evidence for females having higher infection prevalence than males. Further laboratory and field studies are needed to determine whether there are individual and population impacts from Bd on R. luteiventris and other amphibians in north Idaho.

KEY WORDS: Amphibian declines · Batrachochytrium dendrobatidis · Chytrid · Idaho · Rana luteiventris

INTRODUCTION

Chytridiomycosis is a skin disease of amphibians caused by the chytrid fungus Batrachochytrium dendrobatidis (hereafter Bd). This chytrid pathogen has infected hundreds of amphibian species worldwide and has caused mass mortalities, population declines, and extinctions (e.g. Berger et al. 1998, Lips et al. 2006, Schloegel et al. 2006). The origin of Bd is unknown, but evidence suggests that Bd is an emerging pathogen (Morehouse et al. 2003, James et al. 2009) and can spread quickly after introductions to new regions (Mazzoni et al. 2003, Pearl et al. 2007, Kriger & Hero 2008, Picco & Collins 2008).

Temperature is an important driver of Bd dynamics because both Bd growth and frog immune response are temperature dependent. In laboratory settings, Bd has an optimal range of temperature conditions for growth (17 to 25°C; Piotrowski et al. 2004), and field studies have shown that infection dynamics in nature can be correlated with temperature and climatic conditions (Berger et al. 2004, Kriger & Hero 2008). Bd does not easily survive sustained temperatures above 30°C (Piotrowski et al. 2004), and laboratory experiments have shown that some frog species are capable of clearing Bd infection at temperatures above 29°C (Woodhams et al. 2003). Amphibian immune responses are also strongly affected by temperature (Maniero &
Many of the most dramatic natural populations. Differences in infection dynamics across demographic groups in temperature play an important role as well as pathogen growth. Regions with highly seasonal climates can be especially informative for understanding the role of temperature in mediating Bd host–pathogen dynamics. In seasonal temperate environments, there is often a narrow window in which climatic conditions are optimal for Bd growth (although microhabitat use may extend this window to some extent). Additionally, in seasonal environments amphibian immune function is likely to be suppressed during and immediately following hibernation due to lower temperatures (Maneiro & Carey 1997). Thus, it is unclear if there is a predictable net effect of seasonality on Bd infection dynamics. Many of the most dramatic Bd-related die-offs have occurred in tropical environments (e.g. Lips et al. 2006), but there are also documented die-offs in highly seasonal temperate environments, e.g. Rana muscosa in California, USA (Briggs et al. 2005), and Bufo boreas in Colorado, USA (Muths et al. 2003). To achieve a more complete understanding of the relationship between seasonality, host response and pathogen growth it is important to quantify the dynamics of Bd infection in additional natural settings.

In addition to understanding the effects of seasonality, further data are needed to understand differences in infection dynamics across demographic groups in natural populations. Differences in Bd infection dynamics among age classes are likely given developmental differences in skin composition and immune function. For example, amphibian skin is not keratinized in tadpoles (Gosner 1960), and immune function is not mature until well after metamorphosis (Flajnik et al. 1987). Documenting differences in Bd dynamics across amphibian age classes is thus important for understanding life-stage specific disease vulnerability. Similarly, differences in Bd infection dynamics between sexes are possible in some amphibian species because of sex-specific behaviors that can increase disease transmission, such as differential use of substrates or aggregation during the breeding season (e.g. Rowley & Alford 2007). Differences in Bd impacts between sexes could have important implications for reproduction and inbreeding, especially for species with small population sizes and/or biased sex ratios. Thus, studying patterns of Bd infection across sexes and age classes can provide information critical to predicting disease-related impacts on amphibian populations.

The Columbia spotted frog Rana luteiventris is an informative model species for studying Bd patterns across seasons, sexes, and age classes in north temperate habitats. R. luteiventris has an extensive distribution in western North America (including southern Alaska, British Columbia, western Alberta, Oregon, Washington, Idaho, Montana, Wyoming, Utah, and Nevada), occupies a variety of habitats ranging from sagebrush benches to subalpine forests, and is found at elevations from sea level to 2700 m (Reaser & Pilliod 2005). The conservation status of R. luteiventris varies regionally; populations in some regions are relatively large while those in other regions have been considered for listing under the Endangered Species Act (Reaser & Pilliod 2005). In the Palouse region of north Idaho, breeding populations are often small and isolated, which may make R. luteiventris vulnerable to the genetic consequences of inbreeding and extirpation by pathogens (Davis & Verrell 2005). Breeding sites in this area were first tested for Bd in 2004; Bd was detected at all 10 sites sampled (C. S. Goldberg & L. P. Waits unpubl. data) although no amphibian population declines have been documented in this region. Opportunities for Bd transmission between populations may be frequent, as R. luteiventris has relatively high levels of movement among ponds in this area (Goldberg & Waits 2010). Additionally, R. luteiventris inhabits a highly seasonal environment and the large differences in temperature among seasons in this region may mediate Bd infection dynamics. Given its variable conservation status and the documented presence of Bd, field studies are important for understanding the role Bd plays in the population dynamics of this species and other pond-breeding amphibians in seasonal environments. Thus, we examined the prevalence and intensity of Bd infection in R. luteiventris populations in north Idaho ponds. Specifically, we evaluated Bd prevalence and intensity across (1) seasons, (2) age classes, and (3) sexes.

**MATERIALS AND METHODS**

**Sampling.** We sampled 223 Rana luteiventris (adults, metamorphs, and late stage tadpoles) from 7 ponds in Latah County, Idaho over 3 seasons in 2009 (spring, summer, and fall; Table 1, see Fig. 1). We chose study sites to cover a range of elevations (774 to 1341 m; determined using a 30 m digital elevation model). Lower elevation sites were surrounded by wheat fields, and

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<th>Season</th>
<th>Adult</th>
<th>Late stage tadpole</th>
<th>Metamorph</th>
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<tr>
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Table 1. Rana luteiventris. Summary of sample sizes swabbed for Batrachochytrium dendrobatidis by developmental stage and sampling season. Samples were collected in 7 ponds in Latah County, Idaho, USA, in 2009.
higher elevation sites were surrounded by coniferous forest. The ponds were all less than 1 acre (ca. 0.4 ha) in area and varied in volume seasonally (2 ponds were ephemeral). We collected *R. luteiventris* using minnow traps and dip-nets. We handled frogs with unused, non-powdered latex gloves to prevent disease transmission and swab contamination. We sampled *R. luteiventris* at each pond once during each of the 3 seasons with a target of sampling 10 to 15 frogs per pond per season. To ensure that frogs were not sampled more than once in a season, sampling was generally restricted to a single day per pond. We held all frogs in individual plastic bags until all sampling was complete for the day, and if we reached our target sample size, no further sampling took place. If fewer than 10 frogs were caught in a single day, we marked captured frogs by clipping one toe-tip to ensure it would not be resampled if we recaptured it the same season. Pond water temperature was measured during each visit using a thermometer placed 1 ft (ca. 30 cm) below the water surface.

We sampled frogs in 2 broad age classes: adults and young of the year. Adults had undergone at least 1 yr of overwintering, although they may not be sexually mature until 2 to 6 yr of age (Reaser & Pilliod 2005). We sexed adults when diagnostic characteristics were apparent. Males typically are much smaller in size compared to females and have large nuptial pads at the base of their thumbs (Patla & Keinath 2005). Young of the year were further categorized as ‘late stage tadpoles’ and ‘metamorphs’. The late stage tadpoles were Gosner stage 43–46; these late stage tadpoles had a developed mouth and both hind and forelimbs, but still possessed a tail or tail stub (Gosner 1960). *Bd* attacks only the keratinized tissues of the frog; at stage 43–46 the skin is only keratinized in localized areas on the sides, feet, and tail (Berger et al. 1998, Marantelli et al. 2004). Although detectability of *Bd* may vary by developmental stage, late stage tadpoles exposed to *Bd* consistently show infection on their limbs detectable by swabbing and quantitative PCR (qPCR) (Garner et al. 2009). Other studies have shown that high *Bd*-related mortality typically occurs soon after metamorphosis (Rachowicz et al. 2006, Kriger & Hero 2008, Garner et al. 2009). Therefore, we were particularly interested in metamorphs and adults as the primary reservoirs for *Bd* and metamorphs as a potentially vulnerable age class. Late stage tadpoles were also included because we were interested in *Bd* dynamics during the transition from late stage tadpole to early metamorph.

We sampled in each of 3 seasons: spring, summer, and fall. Our spring sampling (March–June) coincided with the *Rana luteiventris* breeding season. Because breeding time varies by elevation, we sampled each pond at the peak of its breeding season. During our spring surveys, only adults were sampled because young of the year were not yet present. Our summer sampling (July–August) occurred during tadpole metamorphosis. During our summer surveys adults and late stage tadpoles were sampled. Our fall sampling (October) was just prior to overwintering. Thus, during our fall surveys, adults and metamorphs were sampled (all tadpoles had completed metamorphosis). Therefore, adults were collected in all 3 seasons, late stage tadpoles were only present in the summer and metamorphs were only present in the fall.

For each frog sampled, we recorded season, sex and age class and took a swab sample to measure *Bd* infection load. We swabbed each frog by firmly running a sterile swab (MW 100-100, Medical Wire & Equipment) 10 times over the frog’s dorsal surface, each of the frog’s sides (from groin to armpit), and the ventral surface and 5 times on the undersides of each thigh and on the undersides of each foot, for a total of 70 strokes per individual (Kriger et al. 2006a). The swabs were placed in individual sterilized tubes and frozen at −20°C within 6 h of sampling to prevent DNA degradation.

**Laboratory methods.** We used established qPCR techniques to assess whether *Bd* was present on frogs in each pond sampled (prevalence) and the number of zoospore equivalents on infected frogs (intensity). We extracted DNA from swabs using a Prepman Extraction kit (Boyle et al. 2004; Applied Biosystems) and used an Applied Biosystems 7500 Fast Real-Time quantitative PCR System (Applied Biosystems) to quantify *Bd* intensity following a protocol modified from Boyle et al. (2004). The following changes were made to the conditions in Boyle et al. (2004): total reaction volume was 10 µl, probe concentration was 125 nM, and 1 out of the 3 wells for each sample contained the internal control recommended by Hyatt et al. (2007) to ensure there was no inhibition of the reaction. The Australian Animal Health Laboratory (Geelong, Victoria, Australia) provided the quantification standards. Any sample yielding ambiguous results (testing positive for 1 or 2 replicates) was rerun and considered positive only if another positive detection occurred (following Kriger et al. 2006b). We report intensity as the mean number of zoospores detected in all replicates for an individual swab. A negative control with all standard reagents was included in each extraction to confirm that there was no contamination.

**Data analysis.** We analyzed patterns of *Bd* infection (i.e. prevalence and intensity) between sexes and among age classes and seasons using generalized linear models (GLMs) with pond as a random effect. We conducted GLMs for *Bd* prevalence and *Bd* intensity separately. The objective of the GLM analyses was to determine the contribution of each fixed effect (i.e. sex, age class, season) after accounting for the random
effect (i.e. differences between ponds). Because age class for young of the year was confounded with season (i.e. tadpoles were only present in summer and metamorphs were only present in fall), we analyzed only adult samples in tests of differences among seasons. We determined significance for each fixed effect separately using a likelihood ratio test between the model with both the fixed and random effect and a model with just the random effect. We conducted our GLM analyses separately for each fixed effect but we also evaluated whether age class and season jointly explained patterns of Bd infection better than either variable alone. To determine whether both age class and season contributed to explaining patterns of Bd prevalence and intensity (while accounting for uneven sampling from ponds), we constructed a set of candidate hypotheses and ranked the evidence for each using Akaike’s Information Criterion (AIC) (Burnham & Anderson 2003). Finally, we used simple linear regression to test if water temperature or elevation could explain variation in Bd prevalence and average intensity at ponds. We determined 95% confidence intervals (CI) for prevalence data using the relationship between the F distribution and the binomial distribution (Zar 1984). For analyses of intensity, we log-transformed the data to better meet assumptions of normality. Statistical analyses were performed using JMP software (SAS, Cary, NC, USA) and package lme4 in R (Bates 2005, R Development Core Team 2008).

**RESULTS**

We found that *Rana luteiventris* from all 7 surveyed pond sites were infected with *Bd*, with prevalence varying from 35 to 100% (Fig. 1). We found strong evidence that both age class and season affected patterns of *Bd* infection, weak evidence that females had higher *Bd* prevalence than males, and no evidence of a relationship between patterns of *Bd* and environmental variables. These findings are detailed below.

**Sex**

We found weak evidence that *Bd* prevalence varied between sexes after accounting for pond differences ($\chi^2_1 = 3.09, p = 0.08$). Females had an average prevalence of 87.0% (95% CI = 66.4 to 97.2%) and males had an average prevalence of 65.3% (95% CI = 56.4 to 73.6%). There was no evidence that intensity differed between the sexes ($\chi^2_1 = 0.015, p = 0.90$; mean for females = 94 zoospores, 95% CI = 37 to 240 zoospores; mean for males = 69 zoospores, 95% CI = 44 to 107 zoospores).

**Age class**

We found that both *Bd* prevalence and intensity varied among age classes. *Bd* prevalence showed strong differences among life stages after accounting for pond differences ($\chi^2_2 = 44.39, p < 0.001$) with metamorphs having the highest infection prevalence (Fig. 2). Averaging across all seasons, 67.6% of the adults (121/179), 100% of metamorphs (23/23), and 4.8% of late stage tadpoles (1/21) tested positive for *Bd*. *Bd* intensity for infected individuals also differed among age classes after accounting for pond ($\chi^2_2 = 0.015, p < 0.001$; Fig. 3). Metamorphs had a higher infection intensity (mean = 490 zoospores, 95% CI = 212 to 1133 zoospores) than adults (80 zoospores, 95% CI = 55 to 116 zoospores) and late stage tadpoles ($n = 1$ positive, 10 zoospores).

![Fig. 1. Geographic distribution of ponds sampled for *Batrachochytrium dendrobatidis* (*Bd*) in Latah County, north Idaho, 2009. For each pond, the number of adult *Rana luteiventris* sampled (N) is shown adjacent to a pie chart displaying the proportion of *Bd*-infected adults](image-url)
Among adults, there was some evidence that Bd prevalence but not intensity varied seasonally. Bd prevalence showed weak evidence of a relationship with season after accounting for pond differences ($\chi^2 = 5.71$, p = 0.06; Fig. 2). Prevalence was higher in the summer (83.0%, 95% CI = 70.2 to 91.9%) than in the breeding season (60.2%, 95% CI = 50.3 to 69.5%); estimates for the fall had a large variance due to smaller sample sizes (66.7%, 95% CI = 41.0 to 86.7%). We did not detect a difference in intensity of adult Bd infection among seasons after accounting for pond ($\chi^2 = 2.11$, p = 0.35; Fig. 3), although mean Bd intensity increased through the seasons; we found a mean intensity of 52 zoospores in the breeding season (95% CI = 31 to 86 zoospores), 117 zoospores in the summer (95% CI = 66 to 208 zoospores), and 203 zoospores in the fall (95% CI = 64 to 650 zoospores).

Although single-effect GLM analyses did not provide strong evidence that Bd dynamics varied seasonally, our AIC model selection analyses suggested that seasonality helps explain Bd infection dynamics. The most supported models of both Bd prevalence and intensity included both season and age class (after accounting for the random effect of pond, evidence weights >0.70), indicating that both factors are likely important for explaining Bd patterns in this system (Table 2).

### Environmental variables

We did not detect a relationship between seasonal water temperature and Bd prevalence ($F_{1,17} = 1.42$, p = 0.25) or infection intensity ($F_{1,17} = 1.64$, p = 0.22). Average water temperature was 8.7°C in the spring (95% CI = 8.2 to 9.2°C), 19.8°C in summer (95% CI = 18.2 to 21.4°C), and 8.9°C (95% CI = −3.6 to 14.2°C) in the fall season. Similarly, no relationship was detected between elevation and Bd prevalence ($F_{1,6} = 0.018$, p = 0.90) or intensity ($F_{1,6} = 3.49$, p = 0.12).

### DISCUSSION

We investigated the prevalence and intensity of Bd infection of *Rana luteiventris* populations in northern Idaho to better understand disease dynamics across seasons, sexes, and age classes in a seasonal north temperate environment. Although we did not observe obvious clinical symptoms of chytridiomycosis in this species, we recorded the relatively ubiquitous presence of Bd in the focal *R. luteiventris* populations. All ponds sampled were infected with Bd in every season. We also observed differences in Bd prevalence and intensity among seasons, and in Bd prevalence and intensity across frog age classes, providing information on disease dynamics in the north temperate zone.

Patterns of infection in adult *Rana luteiventris* suggest that Bd is widespread and persists year to year in northern Idaho. Although our study occurred during a single year, prior surveys indicate that Bd has been present in this system since at least 2004. In our 2009 surveys Bd was present in at least 60% of adults sampled in each season. The high prevalence across seasons suggests that individual frogs may maintain their Bd loads over time and/or are highly susceptible to reinfection. The high Bd prevalence (if relatively low intensity) in the early spring also suggests that Bd persists through overwintering and that adults function as disease reservoirs across years.
Patterns of prevalence and intensity in adults also provide insight into seasonal disease dynamics. *Bd* prevalence in adults was highest in the summer and lower in the spring and fall. When the adults emerge in the spring, *Bd* likely proliferates quickly because temperatures are increasing (favoring *Bd* growth) while frog immunity can still be suppressed from overwintering (Maniero & Carey 1997). Some adult frogs may carry *Bd* through hibernation and adult prevalence may increase during the breeding season when many adults come into direct skin contact. The underlying mechanism causing decreased *Bd* prevalence in the fall remains unclear. It is possible that warmer summer temperatures allow for maximum immune activity to fight *Bd* infection (Anver & Pond 1984) and/or that frogs may exhibit thermoregulatory basking behavior, which could reduce *Bd* infection (Richards-Zawacki 2010). While prevalence decreased in the fall, frogs that were infected in the fall had higher infection intensities than in other seasons. It is possible that only those frogs with lower *Bd* intensity were able to clear their infections in the warm summer months.

Comparisons across age classes revealed life-stage specific patterns of infection. Adults exhibited intermediate infection prevalence and intensity relative to late stage tadpoles (low infection) and metamorphs (high infection). The differences between late stage tadpoles and metamorphs were particularly striking. Only a single late stage tadpole tested positive for *Bd* with a low intensity of 10 zoospores. In contrast, metamorphs were infected with relatively high intensity (average of 488 zoospores, more than double the average adult infection intensity). This rapid transition from low prevalence and intensity to high prevalence and intensity following metamorphosis is dramatic and is consistent with observations in other studies (e.g. Marantelli et al. 2004, Rachowicz et al. 2006, Garner et al. 2009). There are 2 possible explanations for the rapid increase of infection prevalence and intensity in young of the year from summer to fall. Late stage tadpoles may harbor *Bd* infection not only in their keratinized mouthparts but also on their newly keratinized hind feet (Marantelli et al. 2004). Therefore, an existing *Bd* infection could increase within a single host as metamorphosis completes and skin becomes highly keratinized. Alternatively, late stage tadpoles may not harbor *Bd* infections but may become infected after metamorphosis is complete. Distinguishing between these alternatives would require isolating late stage tadpoles in a laboratory setting, swabbing their mouthparts and tracking infection progression through metamorphosis. Either way, our data demonstrate a dramatic increase in *Bd* infection immediately following metamorphosis, with metamorphs exhibiting a much higher *Bd* infection prevalence and intensity than adults. It is possible that the metamorph immune system, which is not yet mature, is less able to control *Bd* infection than the adult immune system. Amphibians such as *Rana luteiventris* that undergo metamorphosis quickly (to avoid desiccation in ephemeral ponds) may be at a further disadvantage with low white blood cell counts and increased susceptibility to pathogens (Gervasi & Foufopoulos 2008). Metamorphs, thus, will enter hibernation with relatively high infection intensities and clearly represent a vulnerable age class.

The ecological effects of *Bd* infection on *Rana luteiventris* populations remain unknown. The molecular *Bd* assay does not differentiate between the presence of *Bd* on the skin and a clinical infection that impacts the animal’s health (Smith 2007). Ranid frogs can exhibit a variety of responses when infected with *Bd*. Some species, such as the mountain yellow-legged frog *R. muscosa* in the Sierra Nevada Mountains of California, are highly susceptible to chytridiomycosis and have experienced massive *Bd*-related die-offs.

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**Table 2. Rana luteiventris.** Evidence weights for mixed-effects models predicting prevalence and intensity of *Batrachochytrium dendrobatidis* infection at ponds in north Idaho. ‘Age’ is age class (late stage tadpole, metamorph, adult); ‘Season’ is when sampling occurred (spring, summer, fall; ‘Pond’ is a random effect. AIC: Akaike’s Information Criterion

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<th>Intensity</th>
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(Briggs et al. 2005). Other species, such as the invasive American bullfrog *Lithobates catesbeiana* are more resistant and may even serve as vectors for new Bd introductions (Daszak et al. 2004). Field studies of the Oregon spotted frog *R. pretiosa*, sister species to *R. luteiventris*, found that first-year metamorphs infected with *Bd* had poorer body condition than uninfected metamorphs, indicating that infection causes stress during overwintering (Pearl et al. 2009). Further ecological and laboratory studies are needed to determine whether there are lethal or sub-lethal effects of *Bd* on *R. luteiventris*. The high *Bd* prevalence, relatively moderate *Bd* intensity, lack of clinical symptoms of chytridiomycosis and no reported *Bd*-related die-offs suggest that *R. luteiventris* is persisting with *Bd*. However, *Bd* infection could make *R. luteiventris* more sensitive to other diseases or environmental stressors. Further study across years will be required to determine the stability of this host–pathogen interaction.

If, as it appears, *Rana luteiventris* is persisting with *Bd*, there are several non-mutually exclusive alternative explanations for tolerance to *Bd* infection. First, local north Idaho *Bd* isolates may have attenuated virulence, as differences between *Bd* isolate virulence have been reported (Berger et al. 2005, Fisher et al. 2009). Second, *R. luteiventris* may be a relatively resistant host species; as discussed above, the susceptibility of ranid frogs to chytridiomycosis varies. Laboratory studies are required to determine the relative virulence of local north Idaho *Bd* to specific hosts and the clinical effects of *Bd* infection on *R. luteiventris*. Finally, environmental conditions in north Idaho may explain the high prevalence of *Bd* and its persistence with *R. luteiventris*. As in other highly seasonal north temperate environments (e.g. Rocky Mountains; Muths et al. 2008), *Bd* growth is likely suppressed in the winter when ponds are frozen over. However, local thermal conditions are overall quite advantageous for *Bd*, with ambient air and water temperatures rarely high or low enough to be lethal. In fact, the active season for local amphibians corresponds almost exactly to the optimal temperature for *Bd* growth. Particularly in the summer, the ponds we sampled had an average water temperature of 19.9°C, ideal thermal conditions for *Bd* to thrive (Piotrowski et al. 2004).

If additional research demonstrates that *Rana luteiventris* is a relatively *Bd*-resistant species, then *R. luteiventris* could be a reservoir for pathogen transmission to other more ecologically sensitive species. For example, the boreal toad *Anaxyrus boreas* is sympatric with *R. luteiventris* in much of Idaho. *A. boreas* is known to be experiencing *Bd*-related die-offs in other parts of its range (Muths et al. 2003), so monitoring *Bd* effects in Idaho populations is a priority. Currently, *Bd* has not been detected in some species in this area, such as the Coeur d’Alene salamander *Plethodon idahoensis* (C. S. Goldberg, L. P. Waits & E. B. Rosenblum unpubl. data) and Rocky Mountain tailed frog *Ascaphus montanus* (Hossack et al. 2010) but more rigorous field surveys are needed to understand the community level impacts of *Bd* in this region.

**Acknowledgements.** This project was conducted under a State of Idaho Department of Fish and Game Wildlife Collecting Permit (#040217) and an approved Animal Care and Use Protocol (#2008-55). We thank the Rosenblum, Harmon and Waits laboratories of the University of Idaho for field assistance and comments on the manuscript. Funding was provided by a Berkland Undergraduate Research Award and a DeVleig Grant.

**LITERATURE CITED**


Submitted: April 22, 2010; Accepted: September 15, 2010

Proofs received from author(s): November 4, 2010

Editorial responsibility: Cynthia Carey, Boulder, Colorado, USA