Herpetological Conservation and Biology 6(2):99−106.
Submitted: 28 December 2010; Accepted: 18 March 2011.

ASSESSMENT OF THE VULNERABILITY OF THE OREGON SPOTTED FROG (*RANA PRETIOSA*) TO THE AMPHIBIAN CHYTRID FUNGUS (*BATRACHOCHYTRIUM DENDROBATIDIS*)

GRETCHEN E. PADGETT-FLOHR[1] AND MARC P. HAYES[2]

[1]Department of Zoology, Southern Illinois University-Carbondale, Carbondale, Illinois 62901-2594, USA, e-mail:gpadgettflohr@aol.com
[2]Science Division, Habitat Program, Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, Washington 98501-1091, USA

***Abstract.*—The Oregon Spotted Frog (*Rana pretiosa*) is at risk across its geographic range. Discovery of the chytridiomycete fungus, *Batrachochytrium dendrobatidis* (*Bd*) in declining populations of *R. pretiosa* suggested that this etiological agent might be a contributor to these declines. We experimentally examined sensitivity of *R. pretiosa* to *Bd* by exposing juvenile *R. pretiosa* (4–6 g) to two strains of *Bd.* Over a 90-day post-exposure period, all individuals in the exposed groups became infected, but no frog in either group died or showed behavioral or morphological manifestations of disease. Moreover, by the end of the exposure period, nearly all frogs cleared their infections. Skin sloughing, hypothesized to play a role in clearing infections, appeared minimal. Minimal skin sloughing argues for investigating other mechanisms, such as antimicrobial peptide activity, as the basis for the clearing of *Bd* infections in *R. pretiosa*. However, frogs in both exposed groups gained significantly less mass than frogs in the control group. This experiment, and the recent discovery of *Bd* in non-declining populations of *R. pretiosa*, suggests that the species is *Bd* resistant. We remain cautious in this conclusion, as we obtained tested animals from a population in decline; so tested frogs may be descendants of adults surviving a catastrophic epizootic.**

Key Words.—amphibian chytridiomycosis; amphibian declines; amphibians; anurans; *Batrachochytrium dendrobatidis*; Oregon Spotted Frog; *Rana pretiosa*; Washington

INTRODUCTION

Prominent among etiological agents believed responsible for global amphibian declines is the amphibian chytridiomycete fungus, *Batrachochytrium dendrobatidis* or *Bd* (Berger et al. 1999; Daszak et al. 1999). Notably, the disease manifestation of *Bd*, amphibian chytridiomycosis, has been increasingly associated with declines and local or regional disappearances among diverse species of amphibians (Berger et al. 1998; Alford and Richards 1999; Rachowicz et al. 2006; Skerratt et al. 2007; Lips et al. 2008). Yet, research has also demonstrated that the outcome of *Bd* infection is species-specific in that neither mortality nor manifestation of disease may result (Davidson et al. 2003; Blaustein et al. 2005; Woodhams and Alford 2005; Padgett-Flohr 2008). The conditional nature of infection outcome was a major reason that led us to investigate the sensitivity of the Oregon Spotted Frog (*Rana pretiosa*) to *Bd.* Our study species, *R. pretiosa* (Fig. 1), is a highly aquatic ranid frog endemic to the Pacific Northwest that uses seasonally warm stillwater habitats characterized by large areas of low emergent marsh vegetation (Hayes, M.P. 1997. Status of the Oregon Spotted Frog [*Rana pretiosa sensu stricto*] in the Deschutes Basin and selected other systems in Oregon and northeastern California with a rangewide synopsis of the species' status. Unpublished report prepared for the Nature Conservancy, Portland, Oregon. 57 p. Available from U.S. Fish and Wildlife Service, 26000 SE 98th Avenue, Portland, Oregon 97266, USA; Cushman, K.A., and C.A. Pearl. 2007. A conservation assessment for the Oregon Spotted Frog [*Rana pretiosa*] Unpublished report. USDA Forest Service and USDI

FIGURE 1. Adult female Oregon Spotted Frog (*Rana pretiosa*) from Conboy Lake National Wildlife Refuge, Klickitat County, Washington, USA . (Photographed by Marc Hayes)

Bureau of Land Management, Oregon. 46 p.). The species, which has undergone significant reductions in its historic range (McAllister et al. 1993; Hayes *op. cit*), is listed as Endangered in British Columbia (British Columbia Ministry of Water, Land, and Air Protection. 2002. Wildlife in British Columbia at risk: Oregon Spotted Frog. British Columbia Ministry of Water, Land, and Air Protection, Victoria, British Columbia, Canada. 6 pp. [unnumbered] http://www.env.gov.bc.ca/wld/documents/spotted_frog.pdf) and Washington State (McAllister and Leonard 1997) and is a federal candidate for listing as endangered or threatened (U.S. Fish and Wildlife Service 2010). Because *Bd* has been detected in *R. pretiosa* populations that have undergone at least short-term declines (Hayes et al. 2009), and *Bd* infections appear more prevalent in *R. pretiosa* than among other amphibian species in the Pacific Northwest (Pearl et al. 2007, 2008), *Bd* might be contributing to declines. However, we lack information on sensitivity of *R. pretiosa* to *Bd*. As a first step in assessing *R. pretiosa* sensitivity to *Bd*, we conducted a controlled laboratory exposure of *R. pretiosa* to *Bd* that was modeled after similar investigations of the California Red-Legged Frog (*R. draytonii*) and the California Tiger Salamander (*Ambystoma californiense*; Padgett-Flohr 2008).

MATERIALS AND METHODS

Background.—We collected 20 *Rana pretiosa* eggs from each of 20 egg masses from Conboy Lake National Wildlife Refuge, Klickitat County, Washington (UTM Zone 10, 625223-635180E, 5086652-5095491N, WGS84; elevation 552 to 576 m) on 26 March 2009. We transported eggs to the Woodland Park Zoo (Seattle, Washington) on 27 March 2009, where they were reared as a cohort to metamorphosis. During early rearing, we fed larvae a mixed diet of wilted romaine lettuce and kale with *Spirulina*. After metamorphosis, we fed frogs crickets dusted with calcium and vitamin supplement.

In June 2009, we randomly selected 28 of these recently metamorphosed *R. pretiosa* and transported them to the laboratory at Southern Illinois University-Carbondale. We initially housed each animal in a covered 9.5-L aquarium that was raised slightly at one end and filled with aged (24 h) tap water to a depth of approximately 2.5 cm at the lower end. As an added precaution, we treated the aged tap water with Amquel® (Kordon LLC, Hayward, California, USA) with the standard dosage of 4.9 ml per 56.8 L (Lovich 2007) to ensure that it was completely dechlorinated prior to use. We placed sphagnum moss at the slightly elevated (1.5 cm) end of the tank to avoid drowning crickets fed to the frogs; however, even this area was water-saturated to ensure that the frogs were always in contact with water. As the frogs grew, we concomitantly lowered the raised ends of the tanks and increased the water levels gradually until each tank was level and holding water approximately 75 mm deep. Once tanks were level, we removed the sphagnum and placed a 100 mm × 60 mm piece of rubberized matting that floated on the water to provide a resting place for frogs and crickets. Throughout the experiment, we used new Nitrile gloves to handle each animal and sterilized all equipment prior to and between handling of animals using a 20% bleach solution. We replaced tank water every other day and sterilized all floating mats in the bleach solution at the same time. We initially fed all post-metamorphic animals appropriately-sized crickets every other day, but as the frogs grew larger and could consume larger crickets, feedings were gradually reduced to twice weekly. We housed the animals in an environmentally controlled chamber held at 19.0˚ C, the mid-range of the optimal temperatures for *Bd* growth (Longcore et al. 1999; Piotrowski et al. 2004) and maintained them initially on a photoperiod of 8D:16L, which was gradually changed to 12D:12L mimicking summer and fall climatic conditions in the field.

Bd exposure.—Prior to experimental exposure to *Bd*, we tested each animal for *Bd* infection via PCR analysis to verify that all frogs were initially negative for the pathogen. We obtained samples by rubbing a Medical Wire™ (VWR, West Chester, Pennsylvania, USA) fine-tipped cotton swab ≥ 25 times over all ventral surfaces, with particular attention paid to the undersides of the feet. Assay swabs were stored in leak-proof vials in 70% ethanol and shipped to Pisces Molecular (Boulder, Colorado, USA). PCR analysis followed the procedure outlined in Annis et al. (2004) with the following modifications: we increased 35-cycle to 45-cycle, we increased annealing temperature from 15.6˚ C to 18.3˚ C, and $[Mg^{2+}]$ concentration was also increased from 1.5 mM to 3.5 mM (John Wood , unpubl. data). Each PCR run included controls of positive DNA, negative DNA, and contamination detection. The PCR assay is highly specific for the *Bd* ribosomal RNA Intervening Transcribed Sequence and the test is very sensitive as it will detect the presence of < 10 *Bd* zoospores in a 2 µl sample (Annis et al. 2004). We found that all *R. pretiosa* were *Bd* free before experimental exposure. We measured (snout-vent length [SVL]) and weighed frogs 36 days prior to *Bd* exposure.

Pathogen culture followed the standardized protocol described in Longcore et al. (1999). We used two isolates of *Bd* strain 284: a cultured strain (JEL) provided by J.E. Longcore and a wild strain (FB) provided by F. Brem (University of Memphis). We grew *Bd* isolates (JEL and FB) on 1% tryptone agar incubated at 23˚ C. We flooded each of 15 agar plates with 2–3 mL of sterile distilled water and decanted after 30 min to collect zoospores. Mean zoospore concentration calculated by counting zoospores contained in 1.0 mL of

TABLE 1. Thirty-six day pre-exposure summary data and comparison of mass among juvenile *R. pretiosa* populations targeted for control and treatment groups. Groups were wild strain (FB), cultured strain (JEL; to be exposed to FB and JEL *Bd* strains, respectively), and control group (C). Measurement values are in grams. Contrasts analysis used Fisher's PLSD.

Group	n	$\bar{X}$	*SD*	minimum	maximum
FB	9	1.5	0.4	1.0	2.2
JEL	9	2.1	0.3	1.7	2.6
C	10	1.9	0.6	1.1	2.9

Category	Sum of Squares	df	*F*	*P*
Group	1.688	2	4.097	0.029
Error	5.149	25		

Contrast	$\bar{X}$ Difference	Critical Difference	*P*
FB,JEL	-0.611	0.441	0.009
FB,C	-0.339	0.429	0.117
JEL,C	0.272	0.429	0.204

TABLE 2. Thirty-six day pre-exposure summary data and comparison of SVL among juvenile *R. pretiosa* populations targeted for control and treatment groups. Groups and their sample sizes are as in Table 1. Measurement values are in millimeters. Contrasts analysis used Fisher's PLSD.

Group	$\bar{X}$	*SD*	minimum	maximum
FB	26.9	1.9	25	31
JEL	29.3	0.9	28	30
C	28.1	2.9	25	33

Category	Sum of Squares	df	*F*	*P*
Group	26.890	2	3.118	0.062
Error	107.789	25		

Contrast	$\bar{X}$ Difference	Critical Difference	*P*
FB,JEL	-2.444	2.016	0.020
FB,C	-1.211	1.965	0.216
JEL,C	1.233	1.965	0.208

solution from each plate using a hemocytometer, was 1.52 x 10^6/mL and 1.65 x 10^6/mL, respectively. Prior to exposing *R. pretiosa* to *Bd*, we weighed each animal, measured its SVL and randomly assigned it to one of three treatment groups: (1) control (0 zoospores/mL); (2) JEL (1 x 10^6 zoospores/mL); or (3) FB (1 x 10^6 zoospores/mL). We assigned 10 frogs to the control group and nine to each *Bd* treatment group. To expose the frogs to *Bd* zoospores, we individually housed each animal for 24 h in a coded 118-mL plastic container with a top perforated with 10-mm holes. Each container held the minimum volume of water that enabled contact with all ventral body parts, but allowed the animal to keep its upper torso and head above the water surface. We released a quantity of inoculums sufficient to achieve target zoospore concentration directly into the water in the container. At the end of the 24-h exposure period, we transferred all animals back into their individual aquaria that were coded on their bottoms, and then randomly arranged them using a double-blind protocol. We changed the tank arrangement weekly using a random number generator for the duration of the experiment to minimize any potential tank placement effects. The chamber was environmentally controlled and the temperature was maintained at 19.0˚C throughout the study.

We monitored *R. pretiosa* visually for approximately 2 h every other day to assess for behavioral changes commonly associated with *Bd* infection, including excessive skin sloughing, lethargy, inappetence, loss of righting reflex, and avoidance of the ponded area in the tanks (Berger et al. 1999, Voyles et al. 2007). We tested for *Bd* infection via PCR assay 11, 40, and 90 days post-exposure. We also re-measured and re-weighed all frogs 40 and 90 days post-exposure.

Analyses.—We used JMP® software Version 8 for all analyses (SAS Institute Inc., Cary, North Carolina, USA). Summary statistics are means ($\bar{X}$), standard deviations (*SD*), and ranges. We used normal probability plots and Lilliefors tests to evaluate distributions and compared variances with F tests. For all treatment-group populations, SVL and mass were normally distributed and variances were homogeneous. We used a one-factor ANOVA on SVL and mass to determine whether differences existed among pre-treatment group populations, and then used Fisher's Protected Least Significant Difference (Fisher's PLSD) to evaluate between-group contrasts. These ANOVAs revealed a difference in mass (Table 1), but not SVL (Table 2). Furthermore, probability in the latter test being close to the rejection criterion led us to examine contrasts for both analyses. In both cases, each treatment-group population targeted for *Bd* exposure did not differ from the control group, whereas the treatment-group populations differed from one another in both mass (Table 1) and SVL (Table 2). Though we were primarily interested in the responses of the *Bd*-treated groups relative to the control group, these differences led us to focus our subsequent analyses on changes in mass and SVL over the two intervals for which we had data: the 36 day pre- to 40 day post-exposure interval and the 41–90 day post-exposure interval (hereafter, pre-40 and post-40). This approach enabled comparing the *Bd*-treated groups under the assumption that intrinsic growth rates of juvenile *R. pretiosa* across the 25–33 mm SVL size range between these groups at the start of this experiment did not differ. We have independent data on similarly raised frogs indicating that this is a valid assumption for juvenile *R. pretiosa* originating from the same wild population over this size range (Marc Hayes, unpubl. data).

For our focal comparison, we used a Repeated-Measures ANOVA to examine whether differences in change in SVL (ΔSVL) or mass (Δmass) existed among treatment groups over the pre-40 and post-40 time intervals. Because we identified a significant interaction

TABLE 3. Comparison of changes in mass (ΔMass) among juvenile *R. pretiosa* in control and *Bd*-exposed treatment groups. The pre-40 interval was 36 days pre-exposure to 40 days post-exposure and the post-40 interval was 41-90 days post-exposure. Groups and their sample sizes are as in Table 1. Measurement values are in grams/day. Contrasts analysis used Fisher's PLSD.

Interval	Group	$\bar{X}$	*SD*	minimum	maximum
pre-40	FB	0.087	0.009	0.089	0.099
	JEL	0.074	0.009	0.054	0.085
	C	0.097	0.010	0.083	0.111
post-40	FB	0.145	0.016	0.119	0.169
	JEL	0.179	0.022	0.138	0.210
	C	0.166	0.025	0.131	0.197

Group	Sum of Squares	df	*F*	*P*
Group	0.002	2	4.348	0.024
Subject (Frog)	0.007	25		
ΔMass	0.083	1	288.459	<0.001
ΔMass × Group	0.006	2	9.823	<0.001
ΔMass × Subject	0.007	25		

Interval	Contrast	$\bar{X}$ Difference	Critical Difference	*P*
pre-40	FB,JEL	0.014	0.009	0.007
	FB,C	-0.010	0.009	0.031
	JEL,C	-0.024	0.009	<0.001
post-40	FB,JEL	-0.034	0.021	0.003
	FB,C	-0.021	0.021	0.042
	JEL,C	0.013	0.021	0.207

term in one of these analyses, we opted to evaluate contrasts using Fisher's PLSD separately for each interval. We also tested for differences in the prevalence of infection at each of 40 and 90 days post-treatment with a Fisher's Exact Test. Last, we examined whether differences existed in SVL or mass for those individuals still infected at 90 days in contrast to uninfected individuals in the same groups with a one-sample t-test. The rejection criterion (α) was set at 0.05 for all tests.

RESULTS

Pre-Exposure.—We scored pre-exposure frogs as either floating in the water, hiding under the moss, or later, resting on the floating mats. When a researcher entered the environmental chamber, most frogs on the mats would leap into the water. Frogs were also consistent in their responses during feeding times. Each animal, whether resting on a pad or in water, would respond by jumping into the water or making an in-water movement, respectively, indicating disturbance. After post-disturbance settling, each frog began hunting and consuming crickets. We observed no differences in the frequency or character of these behaviors among pre-exposure treatment groups.

Post-Exposure.—All exposed *R. pretiosa* became infected with *Bd* regardless of strain type and tested positive for the pathogen within 11 days post-exposure. However, no animals died nor did any display clinical signs of disease. Moreover, at the end of the study (90 days post-exposure), 15 of the 18 infected frogs had cleared their infection and tested negative for the pathogen. However, clearance rates varied by *Bd* strain. At the 40-day mark, eight of nine frogs in the JEL strain group versus only one of nine frogs in the FB strain group had cleared their infection (Fisher's Exact Test: $P = 0.003$). By the 90-day mark, the difference between the two infected groups had essentially vanished: the lone infected frog in the JEL treatment still tested positive for *Bd*, but only two frogs in the FB group remained positive (Fisher's Exact Test: $P > 0.999$). Control animals remained negative for *Bd* except for one frog that tested positive 40 days post-exposure; however, this animal tested negative at the completion of the study, 90 days post-exposure.

All frogs evaluated in this experiment increased in mass and length through time regardless of treatment. However, repeated measures ANOVAs revealed differences in Δmass (Table 3) but not in ΔSVL among treatments groups (Table 4). For both the pre- and post-40 intervals, all groups differed from one another in Δmass except for the JEL control group contrast in the post-40 interval. Differences were time dependent: for the pre-40 interval, control > FB > JEL, whereas for the post-40 JEL ≅ control > FB (Table 3). At the end of the experiment (90 days post-exposure), the three juvenile *R. pretiosa* that tested positive for *Bd* were either heavier (N = 2) or not significantly different in mass (N = 1) from remaining uninfected members of their respective exposure groups.

We also observed no differences in feeding or typical postural behavior among experimental groups. Scoring of these behaviors revealed no differences in frequency or character over the entire post-exposure period among treatment groups. Visual inspections of tanks revealed almost no skin sloughing among experimental groups.

DISCUSSION

Our results indicate that juvenile *R. pretiosa* are easily infected with *Bd*. However, that infection neither led to mortality nor an overt disease state, at least under the laboratory conditions provided. Mortality in vulnerable species typically occurs fairly rapidly (< 45 days) in infected post-metamorphic animals (Nichols et al. 2001; Carey et al. 2006), so we are confident that any lag effects should have manifested within our long (90 day) post-exposure observation period. Further, our inability to detect either clinical signs of infection or behavioral changes commonly associated with *Bd* infections supports

TABLE 4. Comparison of changes in SVL (ΔSVL) among juvenile *R. pretiosa* in control and *Bd*-exposed treatment groups. The pre-40 interval was 36 days pre-exposure to 40 days post-exposure and the post-40 interval was 41-90 days post-exposure. Groups and their sample sizes are as in Table 1. Measurement values are in millimeters/day. Contrasts analysis used Fisher's PLSD.

Interval	Group	$\bar{X}$	*SD*	minimum	maximum
pre-40	FB	0.249	0.034	0.089	0.099
	JEL	0.241	0.035	0.054	0.085
	C	0.259	0.028	0.083	0.111
post-40	FB	0.122	0.070	0.119	0.169
	JEL	0.102	0.041	0.138	0.210
	C	0.114	0.039	0.131	0.197

Category	Sum of Squares	df	*F*	*P*
Group	0.003	2	0.999	0.383
Subject (Frog)	0.031	25		
ΔSVL	0.262	1	109.121	<0.001
ΔSVL × Group	0.001	2	0.181	0.835
ΔSVL × Subject	0.060	25		

Interval	Contrast	$\bar{X}$ Difference	Critical Difference	*P*
pre-40	FB,JEL	0.007	0.031	0.634
	FB,C	-0.011	0.030	0.476
	JEL,C	-0.018	0.030	0.234
post-40	FB,JEL	0.020	0.050	0.415
	FB,C	0.008	0.048	0.730
	JEL,C	-0.012	0.048	0.621

the hypothesis that *R. pretiosa* is refractory to *Bd*. Species mortally affected by *Bd* generally exhibit a suite of behavioral symptoms, which include inappetence, lethargy, loss of the righting reflex, and abnormal postures (Daszak et al. 1999; Banks and McCracken 2002; Bradley et al. 2002). Infected *R. pretiosa,* when contrasted to uninfected (control) animals, showed no disinclination to eat and were active (floating, moving, swimming) in their enclosures in a manner that characterized normal behavior.

Collectively, our results support the general finding that the outcome of *Bd* infection is species-specific (Davidson et al. 2003; Blaustein et al. 2005; Woodhams and Alford 2005; Padgett-Flohr 2008), and emphasizes the need for species-specific testing, as done here.

Though exposure did not result in moribundity or mortality, we emphasize that *Bd* infection was not devoid of effects. Infected animals gained significantly less weight, evident 40 days post-exposure. This pattern agrees with the field observations of Pearl et al. (2008), who recorded that *Bd*-infected juvenile *R. pretiosa* averaged less in mass than uninfected animals. Moreover, Davidson et al. (2007) obtained similar results in an experiment involving a controlled exposure in the Foothill Yellow-legged Frog (*Rana boylii*) to *Bd* and the carbamate pesticide carbaryl. In that experiment, Davidson et al. (2007) also observed no mortality, but *Bd* exposed animals averaged less in mass, regardless of whether the frogs were exposed to *Bd* alone or to *Bd* and carbaryl combined. The combined results of Davidson et al. (2007) and our study suggest that *Bd* infection carries an energetic cost. Whether that energetic cost translates into any sublethal effects, such as compromising reproduction, deserves investigation.

The mechanism whereby *R. pretiosa* cleared their infection is unclear. Davidson et al. (2003) found that *Ambystoma tigrinum* from southern Arizona could clear *Bd* infection and speculated that the rapid skin sloughing they noted might contribute to that ability. Though a hyperplastic response involving accumulation of unsloughed skin layers is a frequent pattern in *Bd*-susceptible species (Pessier et al. 1999; Bradley et al. 2002; Berger et al. 2005), examples of anurans clearing infections via skin sloughing remain undocumented. Admittedly, such documentation may be difficult because anurans often consume their shed skin and do so fairly rapidly (Weldon et al. 1993). Our juvenile *R. pretiosa* also cleared their infections, but the loss probably cannot be ascribed to excessive skin sloughing as we observed little sloughing. Whether this pattern reflects differences in phylogeny or environmental conditions between laboratory exposures is unclear. Though the mechanism whereby *R. pretiosa* clear their infection is not understood, our results imply that a non-skin sloughing alternative should be investigated. Selected frog skin peptides have high activity against *Bd* (Rollins-Smith et al. 2002; Rollins-Smith et al. 2005; Woodhams et al. 2006a, 2006b), so some understanding of the *R. pretiosa* skin peptides may be useful. Moreover, because the production of skin peptides represents an energetic cost (Rigby and Jokela 2000; Ahmed et al. 2002; Carey 2005), exploration of how that cost may affect the investment needed to maintain body mass may be revealing.

This study adds to those discussed by Marquez et al. (2010) in which an anuran species is able to clear a *Bd* infection under conditions ideal for *Bd* growth *in vitro*. However, we caution against overextending the results of our study. The frogs used in this study were collected from a population that had already manifested a substantial decline (Hayes et al. 2009), so we cannot exclude the possibility that tested individuals represent the descendants of individuals selected for resistance following a catastrophic epizootic. Nonetheless, the fact that *Bd* has been detected in most known population of *R. pretiosa* (Pearl et al. 2007, 2008), a number of which do not appear to be declining, would seem to support the notion that the species is *Bd* resistant. Confirming that notion will require exposing animals from a non-declining population.

Acknowledgments.—Funding for this project was provided under USFWS Competitive State Wildlife

Grant (CFDA #15.634) and the Department of Zoology, Southern Illinois University-Carbondale. Collection of *R. pretiosa* eggs at Conboy Lake National Wildlife Refuge was done under a U.S. Fish and Wildlife Service Research/Monitoring Special Use Permit provided through Howard Browers (Mid-Columbia National Wildlife Refuge Complex). Handling of state-endangered *R. pretiosa* was conducted under a Programmatic Scientific Permit for employees of the Washington State Department of Fish and Wildlife. The Southern Illinois University-Carbondale Institutional Animal Care and Use Committee approved our experimental protocol (Protocol #09-010). Kimberly Cooper and Mike Teller assisted with rearing frogs at the Woodland Park Zoo. We thank Joyce E. Longcore and Forrest Brem for *Bd* isolates; Eric Schauber for guidance, and Aimee McIntyre and Frithiof Waterstrat for comments on this manuscript.

LITERATURE CITED

Alford, R.A., and S.J. Richards. 1999. Global amphibian declines: A problem in applied ecology. Annual Review of Ecology and Systematics 30:133–165.

Ahmed, A.M., S.L. Baggott, R. Maingon, and H. Hurd. 2002. The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. Oikos 97:371–377.

Annis, S.L., F.P. Dastoor, H. Ziel, P. Daszak, and J.E. Longcore. 2004. A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. Journal of Wildlife Diseases 40:420–428.

Banks, C., and H. McCracken. 2002. Captive management and pathology of Sharp Snouted Dayfrogs, *Taudactylus acutirostris*, at Melbourne and Taronga zoos. Pp. 94–102 *In* Frogs in the Community: Proceedings of the Brisbane Symposium of the Queensland Frog Society, East Brisbane. Natrass, A.E.O. (Ed.). Queensland Frog Society, East Brisbane, Australia.

Berger, L., R. Spear, P. Daszak, D.E. Green, A.A. Cunningham, C.L. Goggin, R. Slocombe, M.A. Ragan, A.D. Hyatt, K.R. McDonald, H.B. Hines, K.R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences 95:9031–9036.

Berger, L., R. Speare, and A. Hyatt. 1999. Chytrid fungi and amphibian declines: Overview, implications and future directions. Pp. 21–31 *In* Declines and Disappearances of Australian Frogs. Campbell, A. (Ed.). Environment Australia, Canberra, Australia.

Berger, L., R. Speare, and L. Skerratt. 2005. Distribution of *Batrachochytrium dendrobatidis* and pathology in the skin of Green Tree Frogs (*Litoria caerulea*) with severe chytridiomycosis. Diseases of Aquatic Organisms 68:65–70.

Blaustein, A.R., J.M. Romansic, E.A. Scheesle, B.A. Han, A.P. Pessier, and J.E. Longcore. 2005. Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. Conservation Biology 19:1460–1468.

Bradley, G.A., P.C. Rosen, M.J. Sredl, T.R. Jones, and J.E. Longcore. 2002. Chytridiomycosis in native Arizona frogs. Journal of Wildlife Diseases 38:206–212.

Carey, C. 2005. How physiological methods and concepts can be useful in conservation biology. Integrative and Comparative Biology 45:4–11.

Carey, C, J.E. Bruzgul, L.J. Livo, M.L. Walling, K.A. Kuehl, B.F. Dixon, A.P. Pessier, R.A. Alford, and K.B. Rodgers. 2006. Experimental exposures of Boreal Toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). EcoHealth 3:5–21. DOI: 10.1007/s10393-005-0006-4.

Daszak, P., L. Berger, A.A. Cunningham, A.D. Hyatt, D.E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. Emerging Infectious Diseases 5:735–748.

Davidson, C., M.F. Benard, H.B. Shaffer, J.M. Parker, C. O'Leary, J.M. Conlon, and L.A. Rollins-Smith. 2007. Effects of chytrid and carbaryl exposure on survival, growth and skin peptide defenses in Foothill Yellow-legged Frogs. Environmental Science and Technology 41:1771–1776.

Davidson, E.W., M. Parris, J.P. Collins, J.E. Longcore, A.P. Pessier, and J. Brunner. 2003. Pathogenicity and transmission of chytridiomycosis in Tiger Salamanders (*Ambystoma tigrinum*). Copeia 2003:601–607.

Hayes, M.P., C.J. Rombough, G.E. Padgett-Flohr, L.A. Hallock, J.E. Johnson, R.S. Wagner, and J.D. Engler. 2009. Amphibian chytridiomycosis in the Oregon Spotted Frog (*Rana pretiosa*) in Washington State. Northwestern Naturalist 20:148–151.

Lips, K.R., J. Diffendorfer, J.R. Mendelson III, and M.W. Sears. 2008. Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. PLOS Biology 6:e72.

Longcore, J.E., A.P. Pessier, and D.K. Nichols. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227.

Lovich, K. 2007. Husbandry manual for *Rana muscosa*: 2007 final version. Pp. 110–120 *In* Amphibian Conservation Resource Manual. Grow, S., and V.A. Poole (Eds.). Association of Zoos and Aquariums, Silver Spring, Maryland, USA.

Marquez, M., F. Nava-González, D. Sánchez, M. Calcagno, and M. Lampo. 2010. Immunological clearance of *Batrachochytrium dendrobatidis* infection at a pathogen-optimal temperature in the hylid frog *Hypsiboas crepitans*. EcoHealth DOI: 10.1007/s10393-010-0350-x.

McAllister, K.R., W.P. Leonard, and R.M. Storm. 1993. Spotted Frog (*Rana pretiosa*) surveys in the Puget Trough of Washington, 1989–1991. Northwestern Naturalist 74:10–15.

McAllister, K.R., and W.P. Leonard. 1997. Washington State Status Report for the Oregon Spotted Frog. Washington Department of Fish and Wildlife, Olympia, Washington, USA. 38 p.

Nichols, D.K., E.W. Lamirande, A.P. Pessier, and J.E. Longcore. 2001. Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. Journal of Wildlife Diseases 37:1–11.

Padgett-Flohr, G.E. 2008. Pathogenicity of *Batrachochytrium dendrobatidis* in two threatened California amphibians: *Rana draytonii* and *Ambystoma californiense*: Fungal infection. Herpetological Conservation and Biology 3:182–191.

Pearl, C.A., E.L. Bull, D.E. Green, J. Bowerman, M.J. Adams, A. Hyatt, and W.H. Wente. 2007. Occurrence of the amphibian pathogen *Batrachochytrium dendrobatidis* in the Pacific Northwest. Journal of Herpetology 41:145–149.

Pearl, C.A., J. Bowerman, M.J. Adams, and N.D. Chelgren. 2008. Widespread occurrence of the chytrid fungus *Batrachochytrium dendrobatidis* on Oregon Spotted Frogs (*Rana pretiosa*). EcoHealth 6:209–218.

Pessier A.P., D.K. Nichols, J.E. Longcore, and M.S. Fuller. 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's Tree Frogs (*Litoria caerulea*). Journal of Veterinary Diagnostic Investigation 11:194–199.

Piotrowski, J.S., S.L Annis, and J.E. Longcore. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15.

Rachowicz, L.J., R.A. Knapp, J.A.T. Morgan, M.J. Stice, V.T. Vredenburg, J.M. Parker, and C.J. Briggs. 2006. Emerging infectious disease as a proximate cause of amphibian mass mortality. Ecology 87:1671–1683.

Rigby M.C., and J. Jokela. 2000. Predator avoidance and immune defence: costs and trade-offs in snails. Proceedings of the Royal Society of London B 267:171–176.

Rollins-Smith, L.A., C. Carey, J.E. Longcore, J.K. Doersam, A. Boutte, J.E. Bruzgal, and J.M. Conlon. 2002. Activity of antimicrobial skin peptides from ranid frogs against *Batrachochytrium dendrobatidis*, the chytrid fungus associated with global amphibian declines. Developmental and Comparative Immunology 26:471–479.

Rollins-Smith, L.A., and J.M. Conlon. 2005. Antimicrobial peptide defenses against chytridiomycosis, an emerging infectious disease of amphibian populations. Developmental and Comparative Immunology 29:589–598.

Skerratt, L.F., L. Berger, R. Speare, S. Cashins, K.R. MacDonald, A.D. Phillott, H.B. Hynes, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134.

U.S. Fish and Wildlife Service. 2010. Endangered and threatened wildlife and plants; Review of native species that are candidates for listing as endangered or threatened; Annual notice of findings on resubmitted petitions; Annual description of progress on listing actions; Proposed rule. Federal Register 75:69222–69294.

Voyles, J., L. Berger, S. Young, R. Speare, R. Webb, J. Warner, D. Rudd, R. Campbell, L.F. Skerratt. 2007. Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. Diseases of Aquatic Organisms 77:113–118.

Weldon, P.A., B.J. Demeter, and R. Rosscoe. 1993. A survey of shed skin-eating (dermatophagy) in amphibians and reptiles. Journal of Herpetology 27:219–228.

Woodhams, D.C., and R.A. Alford. 2005. Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. Conservation Biology 19:1449–1459.

Woodhams, D.C., L.A. Rollins-Smith, C. Carey, L. Reinert, M.J. Tyler, and R.A. Alford. 2006a. Population trends associated with skin peptide defenses against chytridiomycosis in Australian frogs. Oecologia 146:531–540.

Woodhams, D.C., J. Voyles, K.R. Lips, C. Carey, and L.A. Rollins-Smith. 2006b. Predicted disease susceptibility in a Panamanian amphibian assemblage based on skin peptide defenses. Journal of Wildlife Diseases 42:207–218.

GRETCHEN E. PADGETT-FLOHR earned her doctorate at Southern Illinois University-Carbondale with support from National Science Foundation and James R. Walker Fellowships. She obtained her B.S. and M.S. in Organismal and Conservation Biology at San Jose State University conducting research on endangered small mammal ecology and conservation. Her doctoral research has significantly augmented the understanding of amphibian chytridiomycosis in central California. She documented the earliest record of *Bd* in California and developed a geospatial model for its distribution through time across central California. Her most recent work was focused on landscape epidemiology of the pathogen, where she examined landscape processes and land use practices that may influence the distribution of *Bd*. Now employed in the private sector, Gretchen is nonetheless continuing her research working with the Washington Department of Fish and Wildlife addressing *Bd* sensitivity in the critically endangered Oregon Spotted Frog. Her research interests focus on conservation biology and disease ecology of amphibians and small mammals. (Photographed by Mark Jennings)

MARC P. HAYES is a Senior Research Scientist with the Washington Department of Fish and Wildlife, where he directs the Forests and Fish adaptive management science research program that focuses on amphibian research in headwater streams. He obtained a B.A. at the University of California at Santa Barbara, an M.A. at California State University Chico, and his doctorate at the University of Miami (Florida), where he worked on parental care on Costa Rican glass frogs (Centrolenidae) with support from a National Science Foundation Doctoral Dissertation Improvement Grant. He has been involved in amphibian and reptile research for 38 years, with a strong research emphasis in the conservation and ecology of western North American ranid frogs. Most recently, much of this part of his research has focused on the at-risk Oregon Spotted Frog. The foci of the latter research include investigating experimental approaches to control Reed Canarygrass (*Phalaris arundinacea*) to enhance Oregon Spotted Frog oviposition habitat, modeling the distribution of Oregon Spotted Frog to define areas of the historic distribution that remain unrecognized, and understanding the sensitivity of Oregon Spotted Frogs to *Bd*. (Photographed by Mark Leppin)