Herpetological Review, 2011, 42(2), 209–211.

Prevalence and Distribution of *Batrachochytrium dendrobatidis* at Montane Sites in Central Washington State, USA

The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) causes mortality in some amphibians (Berger et al. 1998; Nichols et al. 2001) and has been implicated as one cause of amphibian declines (Pounds et al. 2006; Stuart et al. 2004). Recent studies have found *Bd* in Oregon, Alaska, and Idaho, USA, as well as in British Columbia, Canada (Adams et al. 2007; Garner et al. 2006; Pearl et al. 2007; Reeves 2008; http://www.spatialepidemiology.net/Bd-Maps/), but few data are available on its distribution in Washington, USA. *Bd* has been detected in samples collected in western, central and eastern Oregon but was absent from two sites in Washington (Pearl et al. 2007). *Bd* has been detected in the Oregon Spotted Frog (*Rana pretiosa*) in two other sites in southwestern Washington (Hayes et al. 2009; Pearl et al. 2009). In addition, the fungus has been isolated from amphibians associated with large die-offs in the Cascade Range of Washington (Snoqualmie Pass area; R. S. Wagner and J. E. Johnson, unpubl. data). We investigated the prevalence and distribution of *Bd* on Table Mountain in the Blewett Pass area of central Washington (Fig. 1) to assess the presence of *Bd* in the region and to determine the prevalence for local anurans.

We collected anurans from three sites on Table Mountain, Washington (elevation 1430–1550 m), between June and September 2008. The area is characterized by dry coniferous forest with heavy snowfalls (typically 2–3 m). Species collected were the Columbia Spotted Frog (*Rana luteiventris)*, Cascades Frog (*Rana cascadae)* and Northern Pacific Treefrog (*Pseudacris regilla)*. Animals were hand collected during visual surveys and held individually in clean plastic bags until processing. The sample size at each site was determined by plotting the cumulative number of sampled individuals within a species (x-axis) versus cumulative infection rate (y-axis) for that site. Sampling ended once this plot had stabilized at a plateau, because further sampling would have had little effect on the overall estimate of prevalence.

After capture, skin cells were collected from each frog by swabbing its ventral surface vigorously for fifteen seconds. Swabs were stored in sterile 1.5 ml mirocentrifuge tubes filled with 70% ethanol, and kept at -20°C until they were processed. We also recorded snout–vent length, sex, presence of secondary sexual characteristics, and the presence of typical signs of *Bd* infection (e.g., redness, skin lesions, lethargy, etc.; as in Pessier et al. 1999) for each individual. In addition, the digit second from the outside on the right hind limb of each frog was removed to prevent resampling. Toes were preserved in 70% ethanol and frozen at -80°C. Field gear was sterilized between sites and a clean pair of powder-free latex gloves was used per frog to prevent the spread of infection.

To identify the presence of *Bd* in the samples, skin cells were dislodged from swabs by vortexing for 15 sec. The swabs were removed and the remaining mixture was centrifuged at 13,400 rpm for 7 min. The supernatant was removed and the pellet was resuspended in 20 mL distilled water. Approximately 10 ml of this mixture was then mounted on a glass slide and examined using differential interference contrast microscopy for *Bd* zoosporangia. A sample was considered

CHRISTOPHER A. GAULKE*
JASON T. IRWIN
R.STEVEN WAGNER
Department of Biological Sciences, Central Washington University, MS-7537
400 East University Way, Ellensburg, Washington 98926-7537, USA

**Corresponding author; current address:*
Department of Medical Microbiology and Immunology
University of California Davis, 3146 Tupper Hall
451 Health Sciences Drive, Davis, California 95616, USA
**Corresponding author; e-mail: cagaulke@ucdavis.edu*

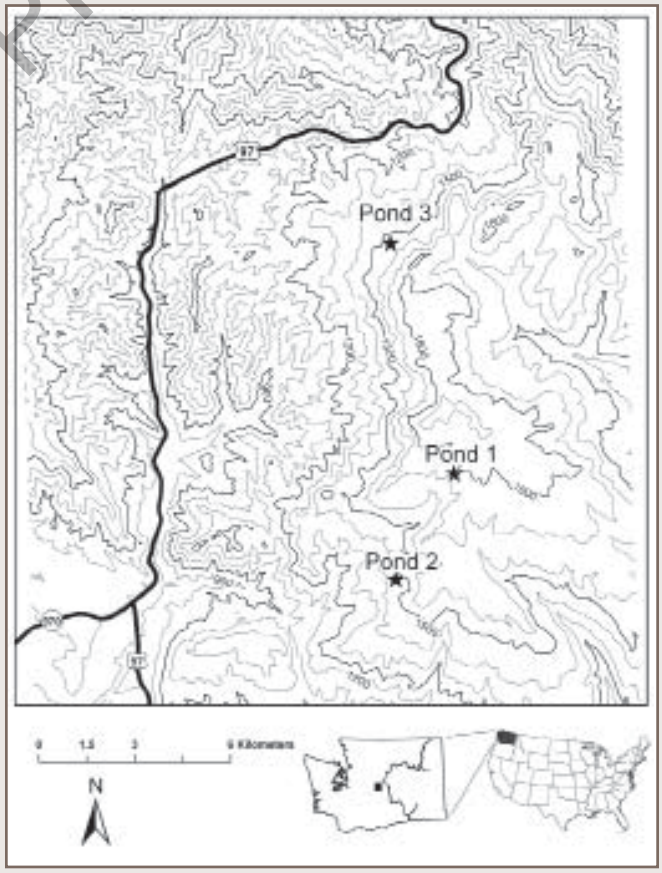

FIG. 1. Sites surveyed for *Batrachochytrium dendrobatidis* on Table Mountain in central Washington, USA, west of the Columbia River (see inset) and east of Highway 97.

TABLE 1. Adult and juvenile amphibians that tested positive (+) or negative (–) for *Batrachochytrium dendrobatidis* in central Washington, USA. Species were *Rana cascadae* (RACA), *Rana luteiventris* (RALU), and *Pseudacris regilla* (PSRE).

Site	Latitude / Longitude	Elevation (m)	Total Sample Size	Species	No. Adults		No. Juveniles	
					+	–	+	–
Pond 1	47.14223°N, 120.34485°W	1760	39	RACA	12	10	2	7
				PSRE	3	5	0	0
Pond 2	47.12467°N, 120.36016°W	1430	22	RACA	0	5	11	4
				PSRE	0	0	1	1
Pond 3	47.18142°N, 120.35890°W	1540	31	RACA	10	8	0	2
				RALU	7	6	1	**1**

negative if no *Bd* structures were found within 10 min. of searching.

We found *Bd* at all sites and in all species sampled. Overall, 49% of the anurans tested positive for *Bd* (Table 1). *Pseudacris regilla* had the lowest infection rate of 40%, whereas *R. cascadae* and *R. luteiventris* had infection rates of 52% and 53%, respectively. Fifty percent of juveniles and 52% of adults tested positive for *Bd*. Across sites, the adult infection rate ranged between 50% and 63%, and the juvenile infection rate ranged between 22% and 50%. None of the differences in infection rate between species, sex, pond site, and life stages were significantly different when compared using contingency tables. No frogs showed the typical signs of infection (i.e., redness, bleeding, lethargy, etc.) and no dead frogs were found during collection.

We report some of the first published accounts of *Bd* in central Washington State, USA and our data are consistent with other studies within the state (Hayes et al. 2009; Pearl et al. 2009), but we observed a higher prevalence than samples from the Northeastern USA (Longcore et al. 2007). Histological methods that sample from clipped toes are known to be less sensitive than PCR methods for detection of *Bd* (Hyatt et al. 2007). We maximized sensitivity by swabbing ventral skin, where zoosporangia are more likely to be found (Berger et al. 2005). Whereas our methods are very unlikely to yield false positives, it is possible that false negatives occurred when infection loads were very light. Considering our conservative methods, our infections rates are very high and consistent with the data of Pearl et al. (2009) for *R. pretiosa*. The high infection rates are very similar between the sites and among the species used in our study. More study is needed to determine if any significant differences exist in *Bd* prevalence at larger spatial and temporal scales (Kriger and Hero 2007). Given that the infected individuals appeared asymptomatic and no dead frogs were found (again similar to the observations of Pearl et al. 2009), the current impact of *Bd* on Washington amphibians is unclear; more study is needed to accurately determine the extent of the threat posed by this pathogen.

Acknowledgments.—This work was financially support by the McNair Scholars program. We thank J. Lamperth for creating the map and Z. Lessig, G. Galindo, J. Galindo, A. Barreca, and B. Hill for help in the field. This work was approved by the CWU Institutional Animal Care and Use Committee (#A09081).

LITERATURE CITED

ADAMS, M. J., S. GALVAN, D. REINITZ, R. A. COLE, S. PYARE, M. HAHR, AND P. GOVINDARAJULU. 2007. Incidence of the fungus *Batrachochytrium dendrobatidis* in amphibian populations along the Northwest Coast of North America. Herpetol. Rev. 38:430–431.

BERGER, L., R. SPEARE, 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. Proc. Natl. Acad. Sci. 95:9031–9036.

———, ———, AND L. F. SKERRATT. 2005. Distribution of *Batrachochytrium dendrobatidis* and pathology in the skin of green tree frogs *Litoria caerulea* with severe chytridiomycosis. Dis. Aquat. Org. 68:76–70.

GARNER, T. W. J., M. W. PERKINS, P. GOVINDARAJULU, D. SEGLIE, S. WALKER, A. A. CUNNINGHAM, AND M. C. FISHER. 2006. The emerging pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. Biol. Lett. 2:455–459.

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, USA. Northwest. Nat. 90: 148–151.

HYATT, A. D., D. G. BOYLE, V. OLSEN, D. B. BOYLE, L. BERGER, D. OBENDORF, A. DALTON, K. KRIGER, M. HERO, H. HINES, R. PHILLOTT, R. CAMPBELL, G. MARANTELLI, F. GLEASON, AND A. COLLING. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis. Aquat. Org. 73:175–192.

KRIGER, K. M., AND J. M. HERO. 2007. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. J. Zool. 271:352–359.

LONGCORE, J. R., J. E. LONGCORE, A. P. PESSIER, AND W. A. HALTEMAN. 2007. Chytridiomycosis widespread in anurans of northeastern United States. J. Wild. Manage. 71:435.

NICHOLS, D. K., E. W. LAMIRANDE, A. P. PESSIER, AND J. E. LONGCORE. 2001. Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. J. Wildl. Dis. 37:1–11.

PEARL, C. A., J. BOWERMAN, M. J. ADAMS, AND N. D. CHELGREN. 2009. Widespread occurrence of the chytrid fungus *Batrachochytrium dendrobatidis* on Oregon spotted frogs (*Rana pretiosa*). EcoHealth 6:209–218.

———, 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. J. Herpetol. 41:145–149.

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*). J. Vet. Diagn. Investig. 11:194–199.

Pounds, A. J., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf , S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439:161–167.

Reeves, M. K. 2008. *Batrachochytrium dendrobatidis* in wood frogs (*Rana sylvatica*) from three national wildlife refuges in Alaska, USA. Herpetol. Rev. 39:68–70.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Herpetological Review, 2011, 42(2), 211–214.

First Detection of Ranavirus in *Lithobates pipiens* in Quebec

Ranaviral disease and chytridiomycosis are emerging infectious diseases implicated in mortality events among wild and captive amphibians (Chinchar 2002; Daszak et al. 1999; Longcore et al. 1999). Ranaviral disease is caused by infection with members of the genus *Ranavirus* and afflicts both larval and adult amphibians (Gray et al. 2009). Chytridiomycosis is a cutaneous disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) that occurs only in postmetamorphic amphibians, although larvae can be infected (Berger et al. 1998). Both were recently listed as notifiable diseases by the World Organization for Animal Health (Fisher et al. 2009; Gray et al. 2009).

In Canada, amphibian die-offs associated with ranaviruses have occurred in the provinces of Saskatchewan, Manitoba, Ontario, and New Brunswick (Bollinger et al. 1999; Charbonneau 2006; Greer et al. 2005; Forzán et al. 2009; Jancovich et al. 2005; Schock et al. 2008). *Bd* is present in several Canadian provinces and may have caused population declines in the past (Carey et al. 1999; Deguise and Richardson 2009; Ouellet et al. 2005; Schock et al. 2009). This report describes the incidental detection of ranaviral infection in frogs during their collection for experimental use, the first record of infection of this type in amphibians from Quebec, Canada.

Methods.—In late July 2007, Northern Leopard Frogs (*Lithobates pipiens*) intended for experimental use were caught with nets, in grass and near a pond situated in a privately-owned wildlife preserve in Boucherville, Quebec (45.6477°N, 73.4350°W). Upon capture, small groups were temporarily held in moistened cotton bags and transferred to iceboxes containing pond water. A total of 400 metamorphs and 5 adults were screened for deformities, and except for four unilaterally anophthalmic (missing one eye) and two ectromelic (missing leg below the femur) metamorphs (Table 1), all animals appeared clinically healthy. As only 175 young-of-the-year frogs were required for the planned experiment, these were measured (mean snout–vent length ± SD: 29.95 ± 1.31 mm), transferred to a single icebox holding pond water, and transported to the laboratory. The additional animals were released on site.

In a laboratory held at 22 ± 0.9°C, the froglets were housed in groups of 10 inside tilted 33-L aquariums that contained 2 L of dechlorinated water, to acclimate prior to experimental use. Feces were removed and live crickets were provided daily. Within three days of capture, 7 out of 175 frogs (4%) died; five among them exhibited erythema (i.e., reddening) of the thighs and ventrum. To stabilize the animals, the survivors were individually isolated in 1.84-L plastic shelters containing 50 ml of dechlorinated water, along with platforms to allow them to exit the water. They were also fed crickets injected with tetracycline (50 mg/kg frog weight dissolved in double distilled water) to eliminate potential pathogenic bacterial infections. However, frogs only received half of the recommended dose (i.e. once rather than twice daily) due to

LINDA J. PAETOW
Department of Biology, Concordia University
Montreal, Quebec H4B 1R6, Canada
e-mail: linda.paetow@gmail.com
BRUCE D. PAULI
Environment Canada, National Wildlife Research Centre, Carleton University
Ottawa, Ontario K1A 0H3, Canada
e-mail: bruce.pauli@ec.gc.ca
J. DANIEL McLAUGHLIN
Department of Biology, Concordia University
Montreal, Quebec H4B 1R6, Canada
e-mail: mcljd@alcor.concordia.ca
JULIE BIDULKA
Ministry of Agriculture, Animal Health Centre
Abbotsford, British Columbia V3G 2M3, Canada
e-mail: julie.bidulka@gov.bc.ca
DAVID J. MARCOGLIESE
Fluvial Ecosystem Research Section,
Aquatic Ecosystem Protection Research Division,
Water Science and Technology Directorate, Science and Technology Branch
Environment Canada
Montreal, Quebec H2Y 2E7, Canada
e-mail: david.marcogliese@ec.gc.ca