Journal of Herpetology, Vol. 41, No. 1, pp. 145–149, 2007

Occurrence of the Amphibian Pathogen *Batrachochytrium dendrobatidis* in the Pacific Northwest

CHRISTOPHER A. PEARL,[1,2] EVELYN L. BULL,[3] DAVID E. GREEN,[4] JAY BOWERMAN,[5] MICHAEL J. ADAMS,[1] ALEX HYATT,[6] AND WENDY H. WENTE[1]

[1]*USGS Forest and Rangeland Ecosystem Science Center, 3200 SW Jefferson Way, Corvallis, Oregon 97331, USA*
[3]*USDA Forest Service, PNW Research Station, 1401 Gekeler Lane, La Grande, Oregon 97850, USA*
[4]*USGS National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711-6223, USA*
[5]*Sunriver Nature Center, Box 3533, Sunriver, Oregon, 97707, USA*
[6]*CSIRO, Livestock Industries, Australian Animal Health Laboratory, 5 Portarlington Road, Geelong Victoria 3220, Private Bag 24, Australia*

ABSTRACT.—**Chytridiomycosis (infection by the fungus *Batrachochytrium dendrobatidis*) has been associated with amphibian declines in at least four continents. We report results of disease screens from 210 pond-breeding amphibians from 37 field sites in Oregon and Washington. We detected *B. dendrobatidis* on 28% of sampled amphibians, and we found ≥ 1 detection of *B. dendrobatidis* from 43% of sites. Four of seven species tested positive for *B. dendrobatidis*, including the Northern Red-Legged Frog (*Rana aurora*), Columbia Spotted Frog (*Rana luteiventris*), and Oregon Spotted Frog (*Rana pretiosa*). We also detected *B. dendrobatidis* in nonnative American Bullfrogs (*Rana catesbeiana*) from six sites in western and central Oregon. Our study and other recently published findings suggest that *B. dendrobatidis* has few geographic and host taxa limitations among North American anurans. Further research on virulence, transmissibility, persistence, and interactions with other stressors is needed to assess the potential impact of *B. dendrobatidis* on Pacific Northwestern amphibians.**

Chytridiomycosis is a recently described disease caused by the fungal pathogen *Batrachochytrium dendrobatidis*, and has been linked with amphibian mortality or population declines in at least four continents (Berger et al., 1998; Bosch et al., 2001; Green et al., 2002; Lips et al., 2006). The origins of the pathogen are not well known (see Rachowicz et al., 2005), but some evidence suggests it is novel to many amphibians and has spread rapidly after recent introductions (Daszak et al., 1999; Morehouse et al., 2003; Weldon et al., 2004). This disease has the potential to impact amphibian communities directly through local extirpations (Berger et al., 1998; Bosch et al., 2001; Pounds et al., 2006) and indirectly through altering interactions among extant species (Parris and Beaudoin, 2004; Parris and Cornelius, 2004).

The origin, geographic distribution, and effects of *B. dendrobatidis* in North America remain poorly understood. Increased attention and sampling for the disease since the mid-1990s have revealed its presence in eastern Canada (Ouellet et al., 2005), the desert southwest (Bradley et al., 2002), California (Fellers et al., 2001; Green et al., 2002), and the Rocky Mountains (Green et al., 2002; Muths et al., 2003; Green and Muths, 2005). Chytridiomycosis may be linked to local declines of Boreal Toad (*Bufo boreas*) in Colorado (Muths et al., 2003; Scherer et al., 2005), and Yosemite Toad (*Bufo canorus*) and Mountain Yellow-Legged Frog (*Rana muscosa*) in California (Fellers et al., 2001; Green and Kagarise Sherman, 2001; Green et al., 2002; Briggs et al., 2005), as well as the near extinction of the Wyoming Toad (*Bufo baxteri*) in Wyoming (Taylor et al., 1999).

[2]Corresponding Author. E-mail: christopher_pearl@usgs.gov

The Pacific Northwest (PNW) is a region of known and suspected amphibian declines, which are most commonly noted among anurans (Blaustein and Wake, 1990; McAllister et al., 1993; Wente et al., 2005). Causes of amphibian declines in the PNW are likely to be complex (Adams, 1999) and declines around the western USA have been attributed to habitat modification and the introduction of a variety of introduced predators (Fisher and Shaffer, 1996; Adams, 1999; Knapp and Matthews, 2000). The role of *B. dendrobatidis* in PNW declines is currently unknown, but concern is increasing as a result of studies in other parts of the western United States (e.g., Fellers et al., 2001; Green and Kagarise Sherman, 2001; Muths et al., 2003). Information on the distribution of *B. dendrobatidis*–infected amphibian populations in the region is sparse: the only published reference we were able to locate was a single Bullfrog (*Rana catesbeiana*) collected in Oregon's Willamette Valley (Pearl and Green, 2005). Here, we report results of our broad sampling to further define the distribution of *B. dendrobatidis* in PNW anurans. We also put these findings in context of recent published surveys in North America that add to our understanding of the geographic and taxonomic distribution of the disease.

MATERIALS AND METHODS

Sample Collection and Diagnostic Methods.—We opportunistically collected samples for *B. dendrobatidis* screening during ongoing amphibian research and monitoring projects during 2001–2006. We used visual surveys, dip netting, and aquatic trapping to capture individuals. All sampled amphibians were live with the exception of four *Rana pretiosa* and four *R. catesbeiana* in central Oregon. We observed no outward signs of infection in swabbed amphibians

prior to sampling. Sample sites ranged from 59–2249 m elevation. Samples reported in this paper were examined with one of three standardized techniques for determining *B. dendrobatidis* presence: (1) histological examinations by D. E. Green and colleagues at the USGS National Wildlife Health Center (NWHC) in Madison, Wisconsin (N = 134 larvae, juveniles, and adults from all regions of Oregon and Washington; 13 *B. boreas*, 28 *Pseudacris regilla*, 11 *Rana aurora*, 44 *R. catesbeiana*, 21 *Rana cascadae*, five *Rana luteiventris*, 12 *Rana pretiosa*); (2) PCR analysis by J. Wood and colleagues at Pisces Molecular Labs in Denver, Colorado (N = 63 adult *R. luteiventris* from northeastern Oregon); and (3) TaqMan real-time PCR performed by Dr. Alex Hyatt and colleagues at The Australian Animal Health Laboratory, CSIRO (N = 13 juveniles and adults from central Oregon; four *R. catesbeiana*, nine *R. pretiosa*). Histological examinations followed techniques described in Muths et al. (2003) and Green and Muths (2005). Histological exams of infected tadpoles typically detected chytridiomycosis in deformed tooth rows, whereas postmetamorphic stages were diagnosed via presence of thalli among epithelial cells. With four exceptions (2 deceased adult *R. aurora*; single deceased adults of *R. catesbeiana* and *R. pretiosa*), animals examined histologically were collected and shipped alive to NWHC in sealed plastic containers in bleach-cleaned coolers. We held animals in clean plastic containers or sterilized aquaria for ≤ 3 days prior to shipping. At NWHC, live amphibians were euthanized in a 2% solution of tricaine methanesulfonate ("MS-222") and necropsied under a dissecting microscope within 10 days of receipt.

For the samples analyzed by both PCR techniques, we swabbed postmetamorphic animals 10–20 times across the ventral skin on the thighs and abdomen (the pelvic patch or "drink patch") and interdigital webbing of the hind limbs. We swabbed live animals at the site of collection, and the eight dead amphibians were swabbed immediately upon returning to the lab. Swabs where packaged in sealed sterile vials per lab-specified procedures for shipping, and were generally processed within two days (CSIRO) and 14 days (Pisces Molecular) of receipt. Techniques for Pisces Molecular and CSIRO lab PCR analyses are described in Annis et al. (2004) and Boyle et al. (2004), respectively.

Statistical Comparisons.—We used Chi-square tests of independence to compare *B. dendrobatidis* infection frequencies among (1) region (eastern Oregon [Upper Columbia River basin], central Oregon [Upper Deschutes basin], western Oregon [Willamette and Umpqua basins], and Olympic National Park (Washington); (2) amphibian species; (3) life stages (larval, metamorphic/juvenile [within the year of transformation], and adult); and (4) season of sample collection (winter/spring = 1 November to 30 June; summer = 1 July to 31 October). We used the Yates' Continuity Correction for variables compared with 2 × 2 contingency tables (season) per Zar (1999). We considered tests significant if $P < 0.05$. We selected these predictors based on other work that has hypothesized their influence on the occurrence of *B. dendrobatidis* (e.g., Berger et al., 1998; Retallick et al., 2004; Blaustein et al., 2005; McDonald et al., 2005).

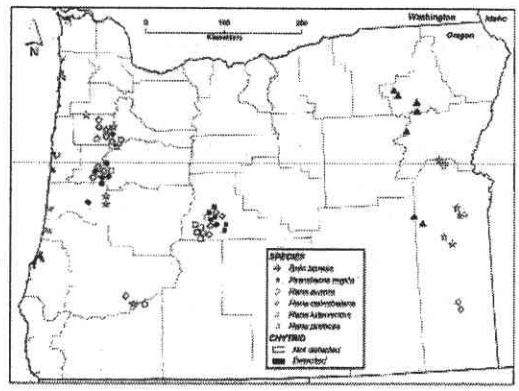

FIG. 1. Detections of *Batrachochytrium dendrobatidis* in Oregon. Filled symbols indicate at least 1 detection of *B. dendrobatidis* among sampled amphibians of the same species, stage, sample date, and diagnostic method at a site. Map includes samples from 37 sites in Oregon; samples from two sites in Olympic National Park, Washington are described in text.

RESULTS

We examined 210 field-sampled amphibians from Oregon (178 individuals from 35 sites) and Washington (32 individuals from two sites; Fig. 1). Samples came from one nonnative (*R. catesbeiana*) and six native anurans (Table 1). All of our *R. cascadae* (21 larvae from two sites) and 11 of our 13 *B. boreas* larvae (1 site) were from Olympic National Park, Washington. Our examinations detected *B. dendrobatidis* in 28.6% (60/210) of samples. We found ≥ 1 detection of *B. dendrobatidis* in 43% (16/37) of sampled sites. We were able to sample two amphibian species at seven sites: four sites had at least one *B. dendrobatidis* detection in a single species and three sites had no *B. dendrobatidis* detections in either species. We did not detect *B. dendrobatidis* in either of the sites in Olympic National Park.

Batrachochytrium dendrobatidis was detected in 14.9% (20 of 134) of specimens examined with histology, 28.6% (18 of 63) examined by Pisces PCR, and 92.3% (12 of 13) examined by TaqMan PCR. We have no comparison of detection techniques using the same specimen.

Detection of *B. dendrobatidis* was not uniform among species ($\chi^2_6 = 46.80$, $P < 0.001$; Table 1). We detected *B. dendrobatidis* in native *R. aurora* (9.0%), *R. luteiventris* (47.0%), and *R. pretiosa* (57.1%), as well as nonnative *R. catesbeiana* (31.2%). We did not detect *B. dendrobatidis* in 13 *B. boreas*, 28 *P. regilla* or 21 *R. cascadae*. We detected *B. dendrobatidis* in all three life stages and its occurrence differed with respect to stage ($\chi^2_2 = 14.24$, $P < 0.001$), with the highest percentage in adults. Frequency of *B. dendrobatidis* was also unequal among regions ($\chi^2_3 = 22.46$, $P < 0.001$). We found *B. dendrobatidis* in all three regions of Oregon but not in the two sites in Olympic National Park, Washington. Detection of *B. dendrobatidis* was higher in winter and spring than summer sampling ($\chi^2_1 = 21.61$, $P < 0.001$).

TABLE 1. Detections of *Batrachochytrium dendrobatidis* in PNW anurans in relation to selected predictors (N = 210 amphibians). *Rana catesbeiana* is introduced in the PNW.

Variable	Category	% (number) of samples testing positive for *Batrachochytrium dendrobatidis*	χ^2 value	df	P
Species	*B. boreas*	0% (0/13)	46.80	6	< 0.001
	P. regilla	0% (0/28)			
	R. aurora	9% (1/11)			
	R. cascadae	0% (0/21)			
	R. catesbeiana	31.2% (15/48)			
	R. luteiventris	47.0% (32/68)			
	R. pretiosa	57.1% (12/21)			
Stage	Larvae	14.3% (12/84)	14.24	2	< 0.001
	Met/Juv	34.5% (10/29)			
	Adult	39.2% (38/97)			
Season	Winter/Spring	38.6% (56/145)	21.61	1	< 0.001
	Summer	6.15% (4/65)			
Region	Olympic NP, WA	0% (0/32)	22.46	3	< 0.001
	Western OR	22.6% (12/53)			
	Central OR	50% (16/32)			
	Eastern OR	34.4% (32/93)			

DISCUSSION

Our examinations of field-collected samples provide evidence that *B. dendrobatidis* is currently widespread geographically and taxonomically in PNW anurans. We report what appear to be the first confirmations of *B. dendrobatidis* in *R. aurora*, *R. luteiventris*, and *R. pretiosa*. Concern about *B. dendrobatidis* first became acute in Central America, but our findings add to others that show *B. dendrobatidis* to be widespread in U.S. anurans. Published reports of chytridiomycosis now exist for at least three species of Bufonidae, three Hylidae, and 16 Ranidae (Green et al., 2002; Carey et al., 2003; Ouellet et al., 2005). Our detection of *B. dendrobatidis* in 28.6% of samples is similar to the 30.5% (47/154) reported from field-collected anurans in and around Colorado's Rocky Mountain National Park (Green and Muths, 2005). Ouellet et al. (2005) reported *B. dendrobatidis* in 13.1% (383/2931) of museum specimens collected primarily since 1960 from eastern Canada and the United States. Both the aforementioned studies used histological examinations, and differences in detectability of *B. dendrobatidis* by method are currently incompletely understood. The broad occurrence of *B. dendrobatidis* across regions and host species suggests there may be few limits to its potential distribution among North American anurans.

We found correlative evidence that suggests that species, life stage and season of sample collection may affect *B. dendrobatidis* occurrence in the Pacific Northwest. We caution that our sampling was limited and nonrandom: these results should be considered only a preliminary assessment of factors that may affect occurrence and detection of *B. dendrobatidis*. The higher occurrence of *B. dendrobatidis* in our winter and spring samples is consistent with other work suggesting detection and effects of *B. dendrobatidis* can be more pronounced during cool seasons (Bradley et al., 2002; Retallick et al., 2004; Woodhams and Alford, 2005; but see Green et al., 2002). *Batrachochytrium dendrobatidis* appears to survive and grow better at low and moderate (14–21°C) temperatures than in warmer water (Woodhams et al., 2003; Piotrowski et al., 2004).

Occurrence of *B. dendrobatidis* also varied among the anurans we sampled. Interspecific variation in susceptibility to *B. dendrobatidis* among anurans has been implied in other portions of North America as well as Australia and Central America (Retallick et al., 2004; Blaustein et al., 2005; Ouellet et al., 2005; Pounds et al., 2006). We detected *B. dendrobatidis* more frequently in highly aquatic species (43.1% of 137 *R. catesbeiana*, *R. luteiventris*, and *R. pretiosa*) than in the species with more terrestrial adult stages and shorter larval periods (1.4% of 73 *B. boreas*, *P. regilla*, *R. aurora*, and *R. cascadae*). This is consistent with other data that suggest anurans with highly aquatic habitat use and life history may experience elevated exposure and infection by *B. dendrobatidis* (Woodhams and Alford, 2005; Bosch and Martinez-Solano, 2006; Lips et al., 2006). Of concern is our detection of *B. dendrobatidis* in adults of several native amphibians that have declined in most (*R. pretiosa*; McAllister et al., 1993; Pearl and Hayes, 2005) or parts (*R. luteiventris*; Reaser, 1997; Wente et al., 2005) of their historic range. For most pond-breeding anurans, elevated mortality in postmetamorphic stages (compared to eggs or larvae) is more likely to contribute toward population declines (Biek et al., 2000).

We found *B. dendrobatidis* in nonnative Bullfrogs in two of the three regions (central and western Oregon) where we sampled the species. Bullfrogs have invaded lowlands throughout the western United States, including much of the ranges of the *R. aurora* and *R. pretiosa* (McAllister et al., 1993; Pearl et al., 2004). We detected *B. dendrobatidis* in Bullfrogs that cooccur with *R. aurora* in western Oregon and *R. pretiosa* in central Oregon. Bullfrogs may host *B. dendrobatidis* with limited negative effects, raising the concern that they could serve as disease reservoirs for native anurans (Daszak et al., 2004; Hanselmann et al., 2004). Transmission of *B. dendrobatidis* between postmetamorphic Bullfrogs is suspected (Hanselmann et al., 2004) and has been documented between life