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Article *in* Northwestern Naturalist · September 2009

DOI: 10.1898/NWN08-28.1

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NORTHWESTERN NATURALIST 90:148–151 AUTUMN 2009

AMPHIBIAN CHYTRIDIOMYCOSIS IN THE OREGON SPOTTED FROG (*RANA PRETIOSA*) IN WASHINGTON STATE, USA

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Key words: Oregon Spotted Frog, *Rana pretiosa*, chytrid fungus, *Batrachochytrium dendrobatidis*, chytridiomycosis, Washington State, Conboy Lake National Wildlife Refuge, Trout Lake Natural Area Preserve, American Bullfrog, *Lithobates catesbeianus*

In recent years *Batrachochytrium dendrobatidis* (*Bd*), a chytridiomycete fungus pathogenic to amphibians (Longcore and others 1999), has been implicated as the proximate cause of amphibian declines around the world (Berger and others 1998; Daszak and others 2003; Muths and others 2003; Pounds and others 2006). Despite the insidious nature of *Bd* (Green and others 2002), few published data exist addressing its occurrence in the Pacific Northwest (PNW). When Pearl and others (2007) opportunistically examined 7 PNW amphibian species, they found *Bd* most often (57% of 21 individuals from 14 sampled populations) in the highly aquatic Oregon Spotted Frog (*Rana pretiosa*), an Endangered Species in Washington State (WDFW 2009). However, all *R. pretiosa* they sampled were from Oregon, and *Bd* was not detected in the *Rana cascadae* (Cascades Frog) and *Anaxyrus boreas* (Western Toad) specimens they sampled from Washington. Here, we report the detection of *Bd* in *R. pretiosa* from Washington.

Since 1997 and 1998, respectively, we have been monitoring *R. pretiosa* populations at the Trout Lake Natural Area Preserve (TLNAP: UTM Zone 10, 610857-612950E, 5095880-5097574N, WGS84; elev. 594 to 599 m) and Conboy Lake National Wildlife Refuge (CLNWR: UTM Zone 10, 625223-635180E, 5086652-5095491N, WGS84; elev. 552 to 576 m), in Klickitat County, Washington. These sites represent 2 of only 3 areas where *R. pretiosa* is known to occur in Washington (McAllister and Leonard 1997). At both sites, monitoring included egg mass surveys during the annual breeding season following snowmelt (late February to mid-March at CLNWR; mid-March to early April at TLNAP). Surveys involved area-specific counts of individual egg masses and egg mass groups, each of which was marked with flags and geo-referenced using a Geographic Positioning System (GPS) to avoid double-counting. Sampling at CLNWR addressed 4 hydrologically distinct units, which were surveyed repeatedly until no new egg masses were found; at TLNAP, 3 units consistently used for breeding were sampled. We inferred a 1:1 correspondence between egg mass numbers and number of breeding females based on a combination of our direct observations of oviposition ($n = 13$), the recapture of non-gravid females known to have laid eggs ($n = 84$), and the relatively short interval over which the laying of new egg masses occurs (about 3 wk). Collectively, these data indicate that females lay only 1 clutch annually, and that egg mass numbers reflect the effective population numbers of adult females. Decline in egg mass numbers in 3 of the 4 surveyed units at CLNWR from 2004 to 2005 (Fig. 1) and 2 of the 3 units at TLNAP over the same period (Fig. 2), coupled with the increasing recognition of *Bd* as a cause of amphibian declines, motivated us to collect dead frogs found during 2006 and test them for *Bd*.

Five dead adult *R. pretiosa* were tested for *Bd*; 1 from CLNWR and 4 from TLNAP. The CLNWR specimen collected on 13 March 2006 displayed feeble vital signs, minimal response to touch, and righting response was lacking. The frog died within 20 min of discovery, was preserved in 10% formalin, and then stored in 70% ethanol before histological examination using a standard wet-mount preparation of its epidermal tissue (Berger and others 1999), which was sloughing extensively as multiple epidermal layers. A dead adult female *R.*

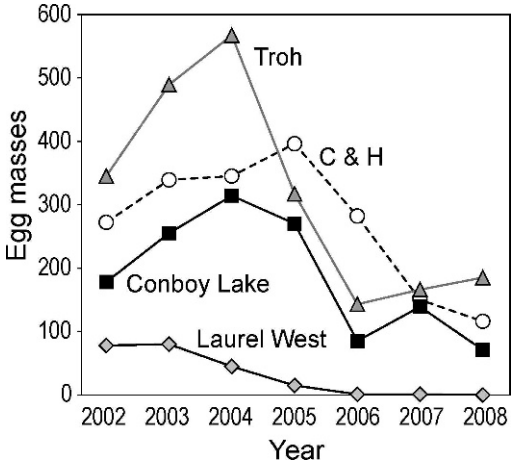

FIGURE 1. *Rana pretiosa* egg mass counts at the 4 surveyed units at Conboy Lake National Wildlife Refuge, 2002–2008.

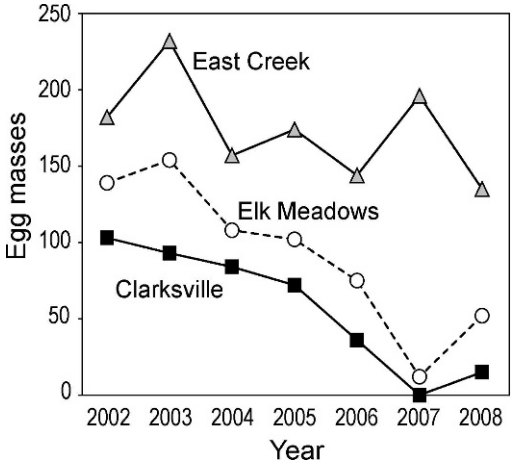

FIGURE 2. *Rana pretiosa* egg mass counts at the 3 surveyed units at Trout Lake Natural Areas Preserve, 2002–2008.

pretiosa that was not collected was observed at Elk Meadows at TLNAP on 22 March 2006. Six dead adult *R. pretiosa* were recorded at TLNAP on 3 April 2006; based on location, the dead female found on 22 March is unlikely to have represented 1 of the latter 6 frogs. When found, 4 of the frogs (2 females and 2 males), including at least 1 from each of the 3 survey units, were individually bagged, placed on ice, and taken to the laboratory for histological and genetic analyses. The 2 remaining frogs (1 female and 1 male), which were in the later stages of decomposition, were not collected. We used the polymerase chain reaction (PCR) assay to amplify an internal transcribed spacer gene fragment specific for *Bd* (Annis and others 2004).

The *R. pretiosa* from CLNWR was infected with *Bd*. Examination of the initial wet-mount epidermal preparation revealed multiple epidermal layers and numerous *Bd* thalli, many with discharge papillae. Though necropsy of the animal was not performed, the level of *Bd* infection in this specimen is characteristic of that attributed to *Bd*-induced mortality in other anurans (Berger and others 1998; Bradley and others 2002; Berger and others 2005; Carey and others 2006). Moreover, behavior of this specimen coupled with its multiple epidermal layers is consistent with the behavior of *Bd*-compromised animals and the hyperplastic tissue response characteristic of *Bd* infection (Pessier and others 1999; Bradley and others 2002; Berger and others 2005).

Because the 4 *R. pretiosa* collected from TLNAP were beginning to decompose, the PCR diagnosis was inconclusive, as the resulting amplifications were weak or non-existent. However, microscopy of their epidermal tissue revealed *Bd* zoosporangia.

Additionally, all 4 units at CLNWR for which we obtained *R. pretiosa* egg mass counts showed declines between 2005 and 2006 (Fig. 1). Three had sharp declines in that interval, while the fourth declined from 15 egg masses to 1. Before 2006, the 3 most populous units generally had egg mass counts >200, but after 2006, all 4 units had <200 egg masses and 1 unit, Laurel West, declined to zero (Fig. 1). The latter unit was resurveyed beyond the regular 3 surveys to confirm that no egg masses were present. Similarly, all 3 TLNAP breeding areas showed declines between 2005 and 2006 (Fig. 2). In 2007, egg mass counts at 2 of the 3 breeding areas, Elk Meadows and East Creek, continued to decline. The Clarksville unit at which no egg masses were found was resurveyed to determine whether oviposition might have been delayed. The resurvey also recorded no egg masses.

Although the magnitude and direction of the changes in annual egg mass numbers at CLNWR prior to 2006 varied among the units, the 2004 to 2005 decline in egg mass numbers in

the Troh unit (567 to 317 egg masses, respectively) may have been a precursor to declines in the number of egg masses we observed across units in 2006 (Fig. 1). Moreover, at CLNWR we observed no overt indications of pathogenic activity during sampling prior to 2006 (that is, we found no dead or moribund frogs not clearly attributable to predation). The only dead *R. pretiosa* collected at CLNWR after 2006 was the remains of an adult female with a Mink (*Mustela vison*) predation signature. At TLNAP, except for the probably 7 whole dead *R. pretiosa* observed during the 2006 surveys, no more than 6 additional dead *R. pretiosa* were recorded over the interval 2002 through 2008. Four independent observations were of adults (2 females and 2 males) in 2002, of which 2 may have represented the same individual. One observation was a female that 3 males appeared to have drowned during attempted amplexus; cause of death for the others was unknown. The remaining 2 adults (1 unknown sex and 1 adult male from 2005 and 2008, respectively) had trauma associated with predation.

Given the chronology of events at CLNWR and TLNAP, we suspect *Bd* was a contributor to the declines we observed. At both sites, a rapid reduction in egg mass numbers (presumably reflecting reduced numbers of breeding females) were associated with few observed dead frogs, a pattern reported for *Bd* elsewhere (Green and others 2002). This pattern may be due either to significant mortality occurring in refugia or overwintering sites that are unknown or inaccessible to human observers (Green and others 2002), or to the rapid removal of dead or moribund amphibians by predators and scavengers. CLNWR, in particular, covers around 2600 ha, is hydrologically complex, and harbors many frog predators (Hayes and others 2005, 2006). Moreover, *Bd*-related mortality in North American ranids immediately after overwintering is often reported (for example, Bradley and others 2002; Johnson and others 2006). Thus, the pattern we observed, coupled with the unknown susceptibility of *R. pretiosa* to *Bd*, is a cause for concern. This concern is heightened by the fact that CLNWR is the only place where *R. pretiosa* and *Lithobates catesbeianus* (American Bullfrog) have successfully co-existed for over 60 y. Because *L. catesbeianus* is known to carry *Bd* asymptomatically (Daszak and others 2004; Garner and others 2006), the potential for *Bd* transmission within and among species at CLNWR could be high.

Acknowledgments.—Amphibians were handled under permits from the Washington Department of Fish and Wildlife (WDFW). We thank the WDFW, the United States Fish and Wildlife Service (USFWS), and many volunteers, including those led by David Shepherdson of the Oregon Zoo, for logistic support. A WDFW grant provided support for work in 2006. Permission to conduct research was granted by the Washington Department of Natural Resources and the USFWS. Susan Van Leuven clarified information on dead Oregon spotted frogs at Trout Lake. William Muhlach provided support through the Department of Zoology, Southern Illinois University (Carbondale). Robert Hoffman and 2 anonymous reviewers made valuable suggestions on the manuscript. This is contribution No. 19 of the WDFW Habitat Program Amphibian Research Group.

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