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### Herpetological Review

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# Amphibian Chytrid Fungus in Western Toads (Anaxyrus boreas) in British Columbia and Yukon, Canada

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The amphibian chytrid fungus, Batrachochytrium dendrobatidis (Bd), appears to have a patchy pattern of occurrence, particularly at the northernmost extent of its distribution (Aanensen et al. 2009). In northwestern North America, Bd has been detected in Western Toads, Anaxyrus boreas, in southeast Alaska, USA (Adams et al. 2007), the Peace River district in northeast British Columbia, Canada (Raverty and Reynolds 2001), and in southwest British Columbia (Deguise and Richardson 2009). Bd also was found in Wood Frogs, Lithobates sylvaticus, in Kenai National Wildlife Refuge, Alaska (Reeves and Green 2006, Reeves 2008), and in Northern Red-legged Frogs, Rana aurora, from Vancouver Island, British Columbia (Adams et al. 2007). Conversely, Bd was not detected in Columbia Spotted Frogs (Rana luteiventris), Wood frogs, or Western Toads collected at several locations including the Chilkoot Trail National Historic Site, northwest British Columbia (Adams et al. 2007). Likewise, Bd was not found in Wood Frogs in Denali National Park (Chestnut et al. 2008), Innoko National Wildlife Refuge, or Tetlin National Wildlife Refuge, Alaska (Reeves 2008). However, small sample sizes and low prevalence may have affected detection at some sites (see Skerratt et al. 2008).

Amphibians within a large portion of the range of Western Toads at its northern limit in northern British Columbia (Matsuda et al. 2006) and the southeast Yukon Territory, Canada (Slough and Mennell 2006) have not been sampled for *Bd*. To address this gap, I sampled amphibians for *Bd* from 4 distinct populations in this region in 2007 and 2008, including Tutshi Lake, Atlin Lake and the Nakina River in northwest British Columbia, and the Coal River in the southeast Yukon Territory (Table 1).

Amphibians were located using visual encounter surveys and were captured by hand or dip net, which had been cleaned and dried between captures and disinfected with bleach between sites. Each animal was held separately prior to swabbing and was handled with a new pair of vinyl medical examination gloves and swabbed with a sterile 15 cm cotton-tipped swab (AMG Medical Inc., Montréal, QC) 5-times each below the throat, between the toes of the front feet, along the belly, thighs, around the drink patch and cloaca and between the toes of the hind feet. Each swab was air-dried for 5 min and placed tip down in a 1.5 ml microcentrifuge tube. Excess handle-length of the swab was broken off and the tube was sealed and stored frozen. Swabs were analyzed for Bd with a real-time PCR assay at either the British Columbia Ministry of Agriculture, Fisheries and Food, Animal Health Centre (2007 samples) or the University of Victoria, British Columbia (2008 samples) using the technique described by Boyle et al. (2004).

In 2007, seven of 20 toads from Atlin Lake and the Nakina River tested positive for *Bd*, and the Tutshi Lake samples were *Bd*-negative (Fig. 1). A Wood Frog from Atlin Lake and two Long-toed



Fig. 1. Locations in British Columbia and Yukon, Canada where amphibians were sampled for the presence (+) of *Batrachochytrium dendrobatidis* (*Bd*) in 2007 and 2008. "—" indicates locations where *Bd* was not detected.

Salamanders (*Ambystoma macrodactylum*) from the Nakina River also tested negative. One of the *Bd*-positive Western Toads from Atlin Lake had sloughing skin on a thigh; otherwise no other individuals had visual signs of the disease such as lethargy or redness on the legs. In 2008, all 10 toad metamorphs from Atlin Lake, an adult toad, and six Wood Frogs from the Coal River, Yukon Territory, tested *Bd*-positive. Western Toad tadpoles from the Coal

River were *Bd*-negative.

The pattern of patchy *Bd* occurrence at high northern latitudes is supported by these additional data from northern British Columbia and Yukon Territory. *Bd* was present in three Western Toad populations sampled in northwestern British Columbia and Yukon Territory but was not detected at Tutshi Lake (Fig. 1), the northwestern-most site surveyed, which coincidentally is only 10 km east of the Chilkoot Trail National Historic Site where amphibians previously sampled also were *Bd*-negative (Adams et al. 2007).

There is concurrent Western Toad breeding site monitoring at one of the study sites, the Atlin Warm Springs, at Atlin Lake, where there were eggs, larvae or metamorphs present demonstrating breeding activity in survey years 1988, 1996, 1998, 1999, 2001, 2005 and 2008, but no breeding activity or adults were observed in 2006, 2007 and 2009 (B. Slough, unpubl. data). This is a small population, with no more than 25 adults and 8 egg clutches observed at one time, however it has demonstrated long-term persistence. Other observations of toads at this site were made in 1924 (Slevin 1928; MVZ 9478–9483, Museum of Vertebrate Biology, University of California, Berkeley), 1952 (Cook 1977), 1962 (RBCM 1273, Royal British Columbia Museum, Victoria), 1979 (CMNAR 21852, Canadian Museum of Nature, Ottawa), 1980 (RBCM 1389) and 1993 (Mennell 1997). These data indicate that the breeding population may have suffered recent losses, and although Bd may be contributing to this, several alternative or interacting factors warrant consideration (e.g., climate change; Slough 2009), especially due to the location of this area at the margin of the species' range. The general status rank of the Western Toad is *Sensitive* in British Columbia and the Yukon (Canadian Endangered Species Conservation Council 2006), and it is protected under the federal Species at Risk Act after being designated Special Concern (COSEWIC 2002). The effect of Bd on northern amphibian populations, especially

TABLE 1. Incidence of *Batrachochytrium dendrobatidis (Bd)* in amphibians sampled in northwest British Columbia and Yukon, Canada. The species examined were *Anaxyrus boreas* (ANBO), *Lithobates sylvaticus* (LISY), and *Ambystoma macrodactylum* (AMMA).

Location	Date	Latitude °N, Longitude °W	Positive <i>Bd</i> Samples	Negative <i>Bd</i> Samples
Tutshi River uplands	20 June 2007	59.772, -134.931	0	1 juvenile ANBO
Tutshi Lake	3 June 2007	59.817, -134.799	0	8 adult ANBO
Atlin Lake, Torres Channel	23 June 2007	59.398, -133.951	1 adult ANBO	0
Atlin Lake, near Griffith Island	23 & 28 June 2007	59.286, -133.824 59.284, -133.823	2 adult ANBO	1 adult ANBO
Atlin Lake, 12-Mile Point, Teresa Island	28 June 2007	59.436, -133.695	2 juvenile 1 adult ANBO	1 adult ANBO
Atlin Lake, Warm Springs Homestead	19 April 2007	59.406, -133.579	0	1 adult ANBO
Atlin Lake, Warm Springs Homestead	26 June 2007	59.406, -133.579	0	1 adult LISY
Atlin Lake, Warm Springs	10 May 2008	59.404, -133.573	10 metamorph ANBO	0
Nakina River, near confluence with Sloko River	1 July 2007	59.011, -133.141 59.008, -133.151	1 adult ANBO	1 adult ANBO 2 adult AMMA
Coal River Springs and Coal River	23–24 July 2008	60.156, -127.435	1 adult ANBO 6 adult LISY	30 ANBO larvae

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