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PREVALENCE OF THE CHYTRID FUNGUS
(*BATRACHOCHYTRIUM DENDROBATIDIS*) IN WESTERN TOADS IN
SOUTHWESTERN BRITISH COLUMBIA, CANADA

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ABSTRACT—The Western Toad (*Anaxyrus boreas*) is an IUCN red-listed species affected by habitat destruction and emerging infectious diseases. We tested for the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) in a breeding population of toads in southwestern British Columbia. Although the toads showed no physical signs of infection, 28% of 32 toads tested positive for *Bd*. To our knowledge, this study provides the first evidence of the chytrid fungus in a population of Western Toads in British Columbia. We also compared body condition to infection prevalence and found no significant relationship. Monitoring population trends and further testing for *Bd* prevalence are required throughout the province.

Key words: Western Toad, *Anaxyrus boreas*, chytrid fungus, *Batrachochytrium dendrobatidis*, *Bd* prevalence, protected area, body condition, British Columbia, Canada

Nearly half of all amphibian species around the world have experienced population declines, making amphibians one of the most threatened groups of vertebrates, more so than birds and mammals (Alford and Richards 1999; Stuart and others 2004). Species are even declining in relatively undisturbed habitats and protected areas worldwide (Lips and others 2006). Although significant amphibian losses have resulted from familiar and obvious threats such as habitat loss and the introduction of invasive species, disease has recently emerged as a major contributor to global declines (Daszak and others 1999; Stuart and others 2004). Specifically, emergence of the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) and its associated pathology, chytridiomycosis (a cutaneous infection caused by a zoosporic chytrid), has been attributed as the proximate cause of numerous mass mortality events (Berger and others 1998; Muths and others 2003; Lips and others 2006).

The Western Toad (*Anaxyrus boreas*) is known to be susceptible to *Bd* infection (Blaustein and others 2005). Although widely distributed from Baja California north to Alaska, the Western Toad has suffered severe population declines in the southeastern part of the species' range in New Mexico, Colorado, and Wyoming (Carey 1993; Muths and others 2003). *Bd* has been iden-

tified from Western Toads in Colorado, Wyoming, Oregon, and Washington (Muths and others 2003; S. Wagner, pers. comm.), and mass mortality events have been attributed to chytridiomycosis (Muths and others 2003; Scherer and others 2005). In Canada, there has only been 1 report of *Bd* infection in a single Western Toad from northeastern British Columbia (Raverty and Reynolds 2001).

We tested a population of breeding Western Toads from a protected area (provincial park) in southwestern British Columbia, Canada, for the presence of *Bd*. We also tested the hypothesis that infection prevalence is related to body condition of adult toads, as it is well documented that disease can negatively affect body condition of the host, directly and indirectly (Moller and others 1998; Sacks and Blejwas 2000).

METHODS

Study Site

Male toads were collected during the breeding season in Alice Lake Provincial Park, near Squamish, British Columbia (UTM: Zone 10 N, 492229 E, 5513837 N, WGS84; elevation 275 m) within the Coastal Western Hemlock biogeoclimatic zone (Meidinger and Pojar 1991). The 396-ha park is composed primarily of Western Hemlock (*Tsuga heterophylla*), Douglas-fir (*Pseu-*

dotsuga menziesii), and Western Redcedar (*Thuja plicata*). Toads were captured at Edith Lake, a location accessible only by hiking trails and a private road. The lake is approximately 25 ha, with a mean seasonal depth of 6 m and maximum depth of 13 m. In 1950, the lake was stocked with Rainbow Trout (*Onchorhynchus mykiss*), and with Brook Trout (*Salvelinus fontinalis*) in 1965 (BC Ministry of Environment Fisheries Inventory Data). Toads were found breeding in the northern corner of Edith Lake, in a sandy shallow area approximately 2 m² in extent and 0.3 m deep. Riparian and aquatic vegetation included Salal (*Gaultheria shallon*), sedges (*Carex* spp.), and young willows (*Salix* spp.).

Testing Procedures

A total of 32 adult male toads were captured by hand from Edith Lake and kept in individual containers. Powder-free latex gloves were worn during collection and changed for each toad. All individuals were inspected thoroughly for visual signs of chytridiomycosis, including lethargy, redness on the legs, and sloughing skin (Berger and others 1998; Ouellet and others 2005). Toe-clips were taken from each toad using a simple numeric system (Hero 1989) and stored in 70% ethanol in individual 2 ml screw-cap tubes. Scissors were dipped in 70% ethanol and flamed between toe-clipping of each toad to minimize the chance of disease transfer between toads and destroy any residual DNA. Powder-free latex gloves were changed after each clip and all field equipment was thoroughly disinfected in a 5% bleach solution.

Toe-clips were transported in a cooler to the British Columbia Animal Health Centre in Abbotsford, BC, and tested for the presence of *Bd* using real-time Taqman PCR assays (Boyle and others 2004). DNA was extracted from samples using the QIAamp DNA mini kit from Qiagen (Valencia, USA). The primers ITS1-3 Chytr and 5.8S Chytr and the probe Chytr MGB2 were used for PCR analysis.

Following toe-clipping, all toads were weighed to the nearest gram and snout-to-vent length was measured to the nearest mm. The mass to length ratio was used as a measure of body condition (Woodhams and Alford 2005). A *t*-test was used to compare differences in mass between infected and uninfected toads.

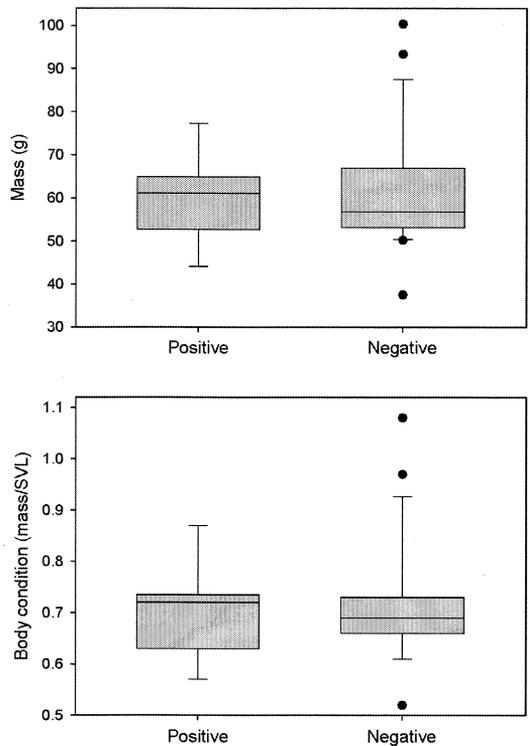


FIGURE 1. Box plots showing mass and body condition of Western Toads that tested positive ($n = 9$) or negative ($n = 23$) for *Batrachochytrium dendrobatidis* (*Bd*). The center line in each box represents the median, the box outlines the 25th and 75th percentiles, the whiskers show the 10th and 90th percentiles, and the dots are outliers.

To determine the relationship between infection status and body condition, a logistic regression was conducted. PROC TTEST and PROC LOGISTIC in SAS v 9.0 was used for statistical analyses (SAS Institute, Cary, USA).

RESULTS

Of the 32 toads tested, 9 individuals (28.1%) tested positive for the presence of *Bd*. None of the toads showed any visual signs of infection. Individuals that tested positive had a mean mass of 59.7 g (range: 37.5 g to 100.3 g), and individuals that tested negative had a mean mass of 60.8 g (range: 44.1 g to 77.3 g) (Fig. 1). The difference in mass among individuals with different disease status was not significant ($t = 0.203$, $df = 30$, $P = 0.84$). There was no relationship between disease status and body condition.

dition ($r^2 = 0.005$, Wald $X^2 = 0.164$, $df = 1$, $P = 0.6851$).

DISCUSSION

Our results provide the first evidence of *Bd* in a population of Western Toads in Canada. The amphibian chytrid fungus has been documented as the proximate cause of declines in Western Toad populations in the United States, but in some populations other agents are also implicated in declines (Muths and others 2003). Given that toads have been present at our study site for numerous years with no reports of mass mortality events (C Platz, pers. comm.), *Bd* may occur naturally without lethal outbreaks. However, mortality events can easily be unnoticed, especially in remote areas.

It is not entirely surprising that *Bd* is present in Western Toads in Canada. Ouellet and others (2005) found that chytrid infection was widespread in various amphibians across Canada and the United States. They documented infection in 13.1% of individuals in pooled data for 12 common species but did not find any evidence of infection in Western Toads. Garner and others (2006) found very high prevalence levels of *Bd* in Bullfrogs (*Lithobates catesbeianus*) on Vancouver Island, BC. Further testing of Western Toads from Canadian populations is needed to properly document the extent of *Bd* infection in this species.

It has been well documented in various vertebrate species that host vulnerability to infection is correlated with body condition, a proxy for individual health (Moller and others 1998; Sacks and Blejwas 2000). Moller and others (1998) found a negative correlation between body condition and disease status in several species of birds. Conversely, body condition is known to affect susceptibility to various stressors, including disease (Brown and Brown 1998). However, this may not be the case for amphibians and *Bd*. In our study, mass was not significantly different between infected and uninfected toads, and there was no relationship between body condition and infection prevalence. These results are similar to those by Woodhams and Alford (2005), who also found no correlation between infection status and body condition. The lack of a relationship may suggest only trivial consequences for host fitness, but this has not been well explored in amphibians and warrants further research.

Western Toads are widespread in western Canada, but the status of most populations is poorly known. Declines have been noted in some areas where toads were historically abundant on Vancouver Island and in the Lower Fraser Valley (Davis and Gregory 2003; unpublished data cited in COSEWIC 2002; I Deguise and S Wood, unpublished data). Given the susceptibility of Western Toads to *Bd* infection (Blaustein and others 2005) and the fact that the prevalence of chytridiomycosis can increase very rapidly (Lips and others 2006), the population of Western Toads at Edith Lake and surrounding areas should be monitored closely. On a global scale, cross-disciplinary programs and the coordination of research and conservation priorities are promising developments for the preservation of amphibian biodiversity (Lips and Mendelson 2008).

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