***Batrachochytrium dendrobatidis* surveillance in British Columbia 2008-2009, Canada**

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Abstract

Opportunistic surveys for *Batrachochytrium dendrobatidis* (Bd) in 2008-2009 indicates that it is widely distributed in all regions of British Columbia and in all the anurans tested. Rough-skin newts were the only salamander that tested positive of Bd. Average prevalence was 11.6% (range: 0% to 71%) and was highly variable among species and among regions. Future surveys will focus on rapid detection and characterization of amphibian mass mortality events to assess the role of Bd in these events.

Introduction

Since the emergence of chytridiomycosis as a key threat to amphibian populations, conservation and wildlife management agencies have felt an urgent need to implement prevention and mitigation measures (Department of Environment and Heritage 2006, Hyatt et al. 2007). As a first step, once presence is established, an understanding of the distribution pattern of *Batrachochytrium dendrobatidis* (Bd) within the region is essential. If Bd is patchily distributed and only present in a few isolated areas, then the management action should focus on quarantine measures to attempt to limit further spread. If *Bd* is widely distributed throughout the management region, then management actions will need to focus on increasing surveillance and documenting the effects on amphibian populations through identifying mass mortalities, understanding regional strain differences in *Bd* and species specific impacts on native amphibians. Although the two strategies are not mutually exclusive, the focus of management efforts is different in the two scenarios.

Our primary goal in this project was to rapidly and cost-effectively assess the distribution of *Bd* in British Columbia, Canada using an opportunistic and collaborative sampling design.

Methods

There are 11 native and 2 introduced anurans in almost 1 million square kilometres of British Columbia. Systematic sampling would have been ideal (Hyatt et al. 2007) but given the urgency of information needed and constraints in funding, we implemented an opportunistic sampling program. We developed a standardized protocol for collecting swab samples from post-metamorphic amphibians. We also assembled sampling kits, with a standard set of swabs, vials, gloves, callipers, a copy of the collection protocol and the British Columbia hygiene protocol for amphibian researchers (http://www.env.gov.bc.ca/wld/frogwatch/ecology/diseases.htm). We developed a list of contacts using personal networks and by contacting people applying for scientific permits under the BC Wildlife Act for research on or surveys of amphibians. Once a person agreed to participate in our survey, we sent them a sampling kit with detailed instructions. We asked for a maximum of 30 samples per site, but emphasized that even one or two samples for a site were important because one positive result was still important information. A total of 22 people participated in this survey (listed in acknowledgements). In addition, the primary author opportunistically collected swab and tadpole tissue samples while working on other research projects in various parts of the province. Over 1000 samples were collected between February 2008 and November 2009, but some were rejected due to inappropriate methods or excess duplication. A total of 956 samples were analyzed.

Samples were collected from all anuran species, except for the two tailed frog species (*Aschapus truei* and *montanus*) and the Boreal Chorus Frog (*Pseudacris maculata*). An independent study has been assessing the prevalence of *Bd* in Northern Leopard Frogs since 2003 (Voordouw et al., 2010), and therefore those samples were not included our analysis. Our primary focus was on anurans because our previous work has failed to obtain positive swabs from aquatic or terrestrial salamanders, except for rough-skin newts. Another exception to the anuran focus was the inclusion of swabs from the Pacific Giant Salamander, a red listed species in BC.

All samples were extracted and amplified following the real-time PCR procedure of Boyle et al. (2004).

Results

In total, 147 of the 956 samples tested positive for Bd and the positive samples were widely distributed over the entire province (Map 1). We obtained positive Bd tests for all anuran species tested (Table 1). Where there were sufficient samples, both post-metamorphic and tadpole stages of anurans tested positive. Roughskin newts tested positive for Bd but larval Pacific Giant salamanders did not. Rigorous comparison of prevalence rates is not justified due to opportunistic sampling design and widely varying samples sizes.

Discussion

This single season survey indicated that Bd is widely prevalent over British Columbia with no discernable regional pattern. All native anuran species tested positive for Bd, with the Wood Frog (71%) and the Columbia Spotted Frog (41%) exhibiting some of the highest prevalence rates overall (Table 1). Bd caused mortality has only been observed in the Northern Leopard Frog (*Rana pipiens*), a red-listed frog in British Columbia (Voordouw et al., 2010). Although we confirmed Bd presence in another red-listed species, the Oregon Spotted Frog, we have not recorded mortality from chytridiomycosis in this species.

Of the introduced frog species, this study confirmed Bd presence in Green Frogs. Previous research has shown that introduced Bullfrog populations almost always test positive for Bd in British Columbia (Garner et al. 2006, unpublished data PG 2006). Introduced frogs such as Bullfrogs have been suggested as potential vectors for the spread of Bd. In British Columbia, Bullfrogs and Green Frogs are found only in a limited area in the south west of the province, and cannot therefore be the vectors for the widespread prevalence of Bd in British Columbia.

The information provided by this Bd prevalence survey clarifies the direction of management actions for British Columbia. Based on this survey, it is reasonable to assume that *Bd* is potentially present in all regions and anuran species in British Columbia. Ongoing efforts will focus on determining the genetic relatedness of regional Bd strains and assessing the relative pathogenicity of these strains. Future surveys will focus on rapid detection and characterization of amphibian mass mortality events to assess the role of Bd in these events.

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Figure 1

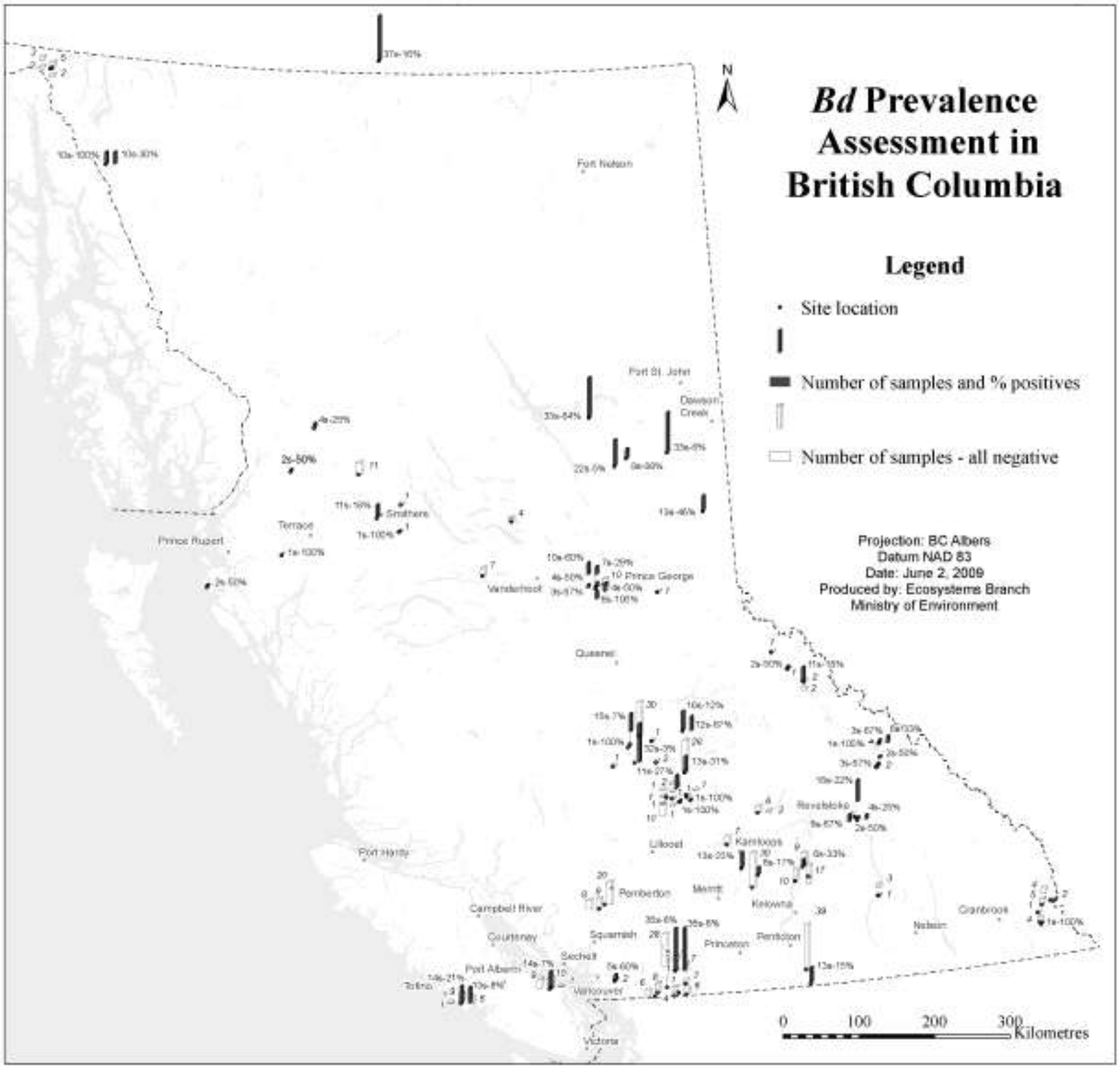
N
Bd Prevalence
Assessment in
British Columbia
Legend
Site location
Number of samples and % positives
Number of samples - all negative
Projection: BC Albers
Datum NAD 83
Date: June 2, 2009
Produced by: Ecosystems Branch
Ministry of Environment
0
100
200
300
Kilometres

Table 1

Species	Life-stage/sample	Sample size	Prevalence %	95% Confidence Interval	
				Lower	Upper
Native Anurans					
Western Toad	Metamorph	28	0	0%	12%
	Post-metamorphic	238	20%	16%	26%
	Tadpoles	217	2%	1%	5%
Spea Intermontanus Great Basin Spadefoot	Post-metamorphic	19	0%	0%	16%
	Tadpoles	35	14%	6%	30%
Pacific Chorus Frog Pseudacris regilla	Post-metamorphic	24	4%	1%	20%
	Tadpoles	53	4%	1%	13%
Redlegged Frog Rana aurora	Post-metamorphic	46	4%	1%	14%
	Tadpoles	8	0%	0%	32%
Oregon Spotted Frog Rana pretiosa	Post-metamorphic	5	20%	4%	62%
Columbia Spotted Frog Rana lutieventris	Post-metamorphic	130	41%	33%	50%
	Tadpoles	17	6%	1%	27%
Wood Frog Rana sylvatica	Post-metamorphic	28	71%	53%	85%
Introduced Anurans					
Bullfrog Rana catesbeiana	Post-metamorphic	1	0%	0%	79%
Green Frog Rana clamitans	Post-metamorphic	21	14%	5%	35%
	Tadpoles	38	8%	3%	21%
Salamanders					
Roughskin Newt	Post-metamorphic	20	1%	3%	30%
Pacific Giant Salamander Dicamptodon	Larval	27	0%	0%	12%