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NOTE

Amphibian chytrid prevalence on boreal toads in SE Alaska and NW British Columbia: tests of habitat, life stages, and temporal trends

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ABSTRACT: Tracking and understanding variation in pathogens such as *Batrachochytrium dendrobatidis* (Bd), the agent of amphibian chytridiomycosis which has caused population declines globally, is a priority for many land managers. However, relatively little sampling of amphibian communities has occurred at high latitudes. We used skin swabs collected during 2005–2017 from boreal toads *Anaxyrus boreas* (n = 248), in southeast Alaska (USA; primarily in and near Klondike Gold Rush National Historical Park [KLGO]) and northwest British Columbia (Canada) to determine how Bd prevalence varied across life stages, habitat characteristics, local species richness, and time. Across all years, Bd prevalence peaked in June and was >3 times greater for adult toads (37.5%) vs. juveniles and metamorphs (11.2%). Bd prevalence for toads in the KLGO area, where other amphibian species are rare or absent, was highest from river habitats (55.0%), followed by human-modified upland wetlands (32.3%) and natural upland wetlands (12.7%) — the same rank-order these habitats are used for toad breeding. None of the 12 Columbia spotted frogs *Rana luteiventris* or 2 wood frogs *R. sylvatica* from the study area tested Bd-positive, although all were from an area of low host density where Bd has not been detected. Prevalence of Bd on toads in the KLGO area decreased during 2005–2015. This trend from a largely single-species system may be encouraging or concerning, depending on how Bd is affecting vital rates, and emphasizes the need to understand effects of pathogens before translating disease prevalence into management actions.

KEY WORDS: Disease · Chytridiomycosis · Habitat · Stream · Isolation · Community · Species richness · National Park

1. INTRODUCTION

The aquatic fungus *Batrachochytrium dendrobatidis* (Bd) causes amphibian chytridiomycosis, which can reduce survival of hosts (Berger et al. 1998, Briggs et al. 2010, Russell et al. 2019). Given its global role in amphibian population declines, management agencies often emphasize monitoring Bd, especially when there is concern that host species are rare or vulnerable (Grant et al. 2018). However, relatively few data are available on Bd prevalence from areas that host few amphibian species, such as many

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high-elevation and, especially, high-latitude areas (Seimon et al. 2007, Reeves 2008, Slough 2009). Disease prevalence and dynamics can be affected by local climate and habitat, depending in part on physiological tolerances of pathogens and their hosts, as well as how changes to community structure and abundance of hosts alter transmission (Stewart 1995, Adams et al. 2010, Voyles et al. 2017). Management options for many diseases are also easier to identify and implement if there is only a single host vs. multiple hosts (May & Anderson 1983, Grant et al. 2018).

To measure and track prevalence of Bd on amphibians in southeast Alaska (USA) and northwest British Columbia (Canada), 248 boreal toads *Anaxyrus boreas*, 12 Columbia spotted frogs *Rana luteiventris*, and 2 wood frogs *R.* [*Lithobates*] *sylvatica* were sampled during 2005–2017. During 2005–2006, sampling was focused in 5 general areas: (1) the Skagway and Taiya River valleys (Alaska) and Lindeman (British Columbia), including areas managed by the Klondike Gold Rush National Historical Park and Chilkoot Trail National Historic Site (hereafter, collectively called KLGO); and the (2) Haines, (3) Juneau, and (4) Prince of Wales Island areas in Alaska (Adams et al. 2007) (Fig. 1). Staff at KLGO continued sampling toads for Bd during most years through 2017, because of concerns about population declines, and because toads have local cultural significance (Thornton 2004). We analyzed the collected data to provide greater understanding of ecological variation, potential management links, and temporal trends in Bd prevalence in this under-sampled region.

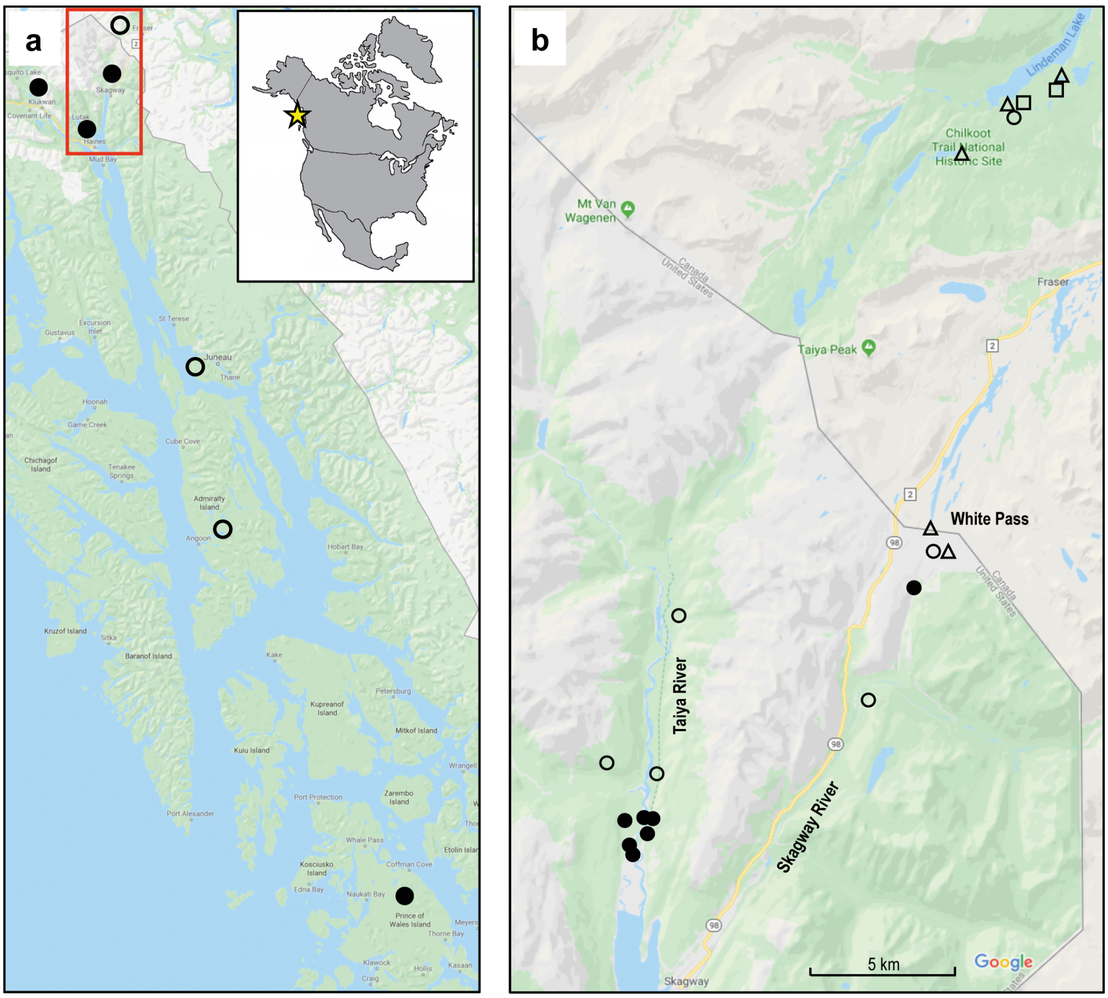

Fig. 1. (a) General areas where amphibians were sampled for chytrid fungus *Batrachochytrium dendrobatidis* (Bd) in southeast Alaska (USA) and northwest British Columbia (Canada) during 2005–2017; and (b) site-level Bd results from 248 boreal toads *Anaxyrus boreas* (circles), 12 Columbia spotted frogs *Rana luteiventris* (triangles), and 2 wood frogs *R. sylvatica* (squares) sampled in Klondike Gold Rush National Historical Park and neighboring areas (red box in panel a). For both panels, solid symbols indicate that Bd was detected

2. MATERIALS AND METHODS

To test for Bd, the pelvic area and undersides of legs and feet of toads and frogs were sampled with a sterile swab, using standardized, clean procedures (Adams et al. 2007). Animals were located during visual encounter surveys and captured by hand or net. Selection of animals to sample was haphazard and effort varied among years, although effort during 2005–2006 primarily targeted known toad breeding sites (Fig. 1a). Swabs were air-dried for 30 min and stored in microtubes (2005–2006) or in ethanol-filled microtubes (2007, 2010, 2012, 2014–2017). Samples collected during 2005–2006 were analyzed for Bd DNA via real-time Taqman qPCR assay (Boyle et al. 2004) at the USGS National Wildlife Heath Center (Wisconsin, USA). Samples collected during 2007–2017 were analyzed by Pisces Molecular (Colorado, USA) using methods described by Annis et al. (2004) (years 2007–2012) or real-time Taqman qPCR (Boyle et al. 2004) (2015–2017). For all swabs, detection of any Bd DNA above the assay threshold was considered a positive.

To estimate how Bd prevalence for toads varied based on life stage, habitat characteristics, and over time in the KLGO area (Fig. 1b), we used generalized linear mixed-effects models (binomial distribution, logit link) and likelihood-ratio tests to test the effects of month (May–August) and year (2005–2017) of sampling, life stage of host (adult vs. juvenile), habitat features, and an area term (Taiya River Valley vs. other sites) that served as a proxy for single- vs. multi-host systems (Table 1). We did not include toad sex or size as predictors because that information was not recorded for >45% of observations. Most of the 2007–2017 samples were from toads because they are the only amphibian species documented at low elevations (<1000 m; Fig. 1b) in the KLGO area, where surveys were concentrated (Surdyk & Waldo 2018). Columbia spotted frogs are present at high elevations, and wood frogs occur throughout the sampled area except for most of the Skagway and

Table 1. Summary of the number of positive *Batrachochytrium dendrobatidis* (Bd+) samples and number of animals sampled by species, life stage (toads only), and habitat type in southeast Alaska (USA) and northwest British Columbia (Canada), 2005–2017. Because of missing information on life stages for some samples, the numbers in this table do not sum to those in the text; na: not available

Site	WGS84		Habitat type	No. Bd+ / no. sampled			
	Lat. (°N)	Long. (°W)		Boreal toads		Columbia spotted frogs	Wood frogs
				Adult	Juvenile		
Bare Loon Lake	59.7958	135.0370	Upland, natural	0/0	0/0	0/2	0/1
CT01	59.5933	135.3265	Upland, natural	0/0	0/1	0/0	0/0
CT11	59.5255	135.3435	Upland, natural	0/2	0/0	0/0	0/0
CTCAN1	59.7664	135.1197	Upland, natural	0/0	0/0	0/5	0/0
CTCAN2	59.7776	135.0871	Upland, natural	0/2	0/0	0/1	0/1
DY02	59.5106	135.3442	River, natural	3/6	8/10	0/0	0/0
DY03	59.5101	135.3486	Upland, human	5/8	3/8	0/0	0/0
DY13	59.4999	135.3616	River, natural	2/2	0/0	0/0	0/0
DY14	59.4986	135.3617	River, natural	6/8	1/3	0/0	0/0
DY19	59.5109	135.3621	Upland, natural	0/0	2/11	0/0	0/0
HAIN01	59.2274	135.4581	Upland, human	7/15	0/10	0/0	0/0
HAIN02	59.2459	135.5253	Upland, human	4/17	0/0	0/0	0/0
HAIN03	59.4154	135.9503	Upland, natural	4/17	0/0	0/0	0/0
JNU01	58.2998	134.6727	Upland, natural	0/17	0/0	0/0	0/0
LAUGHTON	59.5504	135.1106	Upland, natural	0/1	0/0	0/0	0/0
POW3	55.5750	132.6423	Upland, natural	3/5	7/10	0/0	0/0
PRIN01	55.9269	132.7679	Upland, natural	5/19	0/1	0/0	0/0
PRIN02	55.6883	132.6350	River, natural	6/22	0/0	0/0	0/0
SKAG01	57.5753	134.3961	Upland, natural	0/0	0/10	0/0	0/0
TR01	59.5058	135.3507	River, natural	7/16	0/3	0/0	0/0
WC02	59.5286	135.3691	Upland, natural	0/2	0/2	0/0	0/0
WC03	59.5371	135.4317	Upland, natural	0/0	0/2	0/0	0/0
WC04	59.6113	135.1463	Upland, human	2/7	0/0	0/0	0/0
WP01	59.6237	135.1381	Upland, natural	0/0	0/0	0/2	0/0
WP02	59.6130	135.1444	Upland, natural	0/1	0/0	0/1	0/0
WP03	na	na	Upland, natural	0/0	0/0	0/1	0/0
WPC01	59.5619	135.1898	Upland, natural	0/0	0/0	0/0	0/0

Taiya River valleys (Carstensen et al. 2003; http://vertnet.org). Because of small sample sizes and potential for species-specific effects that we could not estimate, we excluded the 12 Columbia spotted frogs and 2 wood frogs from models but included those data in summaries (Table 1).

The first model we fit included the terms month, year (standardized), life stage, habitat type, and area and was based on all samples. The second model had the same predictor variables but was used to determine if there was a linear or quadratic inter-annual trend in Bd prevalence in the KLGO area, the only area sampled for the duration of the study. We excluded data from 2016–2017 from the trend models because only 1 toad was sampled each year. For all models, we included site as a random effect to account for correlation in Bd status among individuals from the same location. Temperature summaries were generated from the Moore Creek Bridge weather station near Skagway, Alaska (https://wcc.sc.egov.usda.gov/nwcc/site?sitenum=1176&state=ak).

For the habitat type variable, we grouped sites into 3 broad habitat categories based on origin and dominant hydrological features: upland/natural (13 sites, 105 swabs), upland/human (4 sites, 66 swabs), and riverine/natural (5 sites, 77 swabs) (Christensen et al. 2004). Riverine sites are influenced primarily by changes in river hydrology, whereas upland sites are mostly isolated from variation in river flows. Natural sites were formed by and are still largely controlled by natural forces. Human sites were created by or mostly transformed by human actions, including former gravel quarries and a mitigation pond. No sites were coded as riverine/human, although some river sites have been affected by human alteration.

3. RESULTS AND DISCUSSION

Of the 248 boreal toads sampled for Bd from 2005–2017, 79 (31.9%) tested positive. Detection of Bd on toads varied seasonally (χ^2 = 10.92, 3 df, p = 0.012), with highest estimated prevalence in June (56.5%) and lowest during August (7.1%; Fig. 2a). Seasonal variation in detection of Bd is common, partly because growth of most strains of Bd is reduced above approximately 27°C (Voyles et al. 2017). However, summer air temperatures in southeast Alaska (July mean maximum air temperature in Skagway = 19.1°C; https://wrcc.dri.edu) are well within the optimum growth temperatures for most strains of Bd. Similar patterns of reduced summertime prevalence of Bd on boreal toads and other amphibians are evident in other areas of western North America, such as the US Pacific Northwest (Pearl et al. 2007, Adams et al. 2010), which suggests that temperature is not the lone driver of the pattern. The strong seasonal pattern highlights the importance of understanding temporal variation to maximize sampling efficiency and accurately describe the distribution and prevalence of Bd.

Adults toads were >3 times as likely to test Bd-positive (37.5%) as juvenile and metamorph toads (11.15%) (Fig. 2b; χ^2 = 6.21, 1 df, p = 0.013). This large difference is surprising, especially because juvenile toads are often more aquatic than adults (Bartelt et al. 2004), which could increase exposure or infection intensity to an aquatic pathogen such as Bd (Murphy et al. 2009, Hossack et al. 2013). Estimates of variation in Bd prevalence across life stages of boreal toads vary considerably among studies. For example, Bd prevalence was higher for adult boreal toads than for juveniles in Oregon and northern California (USA) (Adams et al. 2010), but in Montana (USA), female boreal toads had lower Bd prevalence than males or juveniles (Hossack et al. 2013). The lack of detailed demographic data and small number of samples from some life stages precluded us from generating sex- and life-stage estimates, but these differences in prevalence make it critical to understand how Bd affects vital rates of different sexes and life stages.

Toads from river/natural habitats (55.0%) were more likely to be Bd-positive than toads from upland/human-transformed (32.3%) or upland/natural habitats (12.7%; Fig. 2c; χ^2 = 8.31, 2 df, p = 0.016). Riverine vs. upland sites are of particular management interest because river-associated sites provide some of the most important toad habitat in the region (Christensen et al. 2004, Surdyk & Waldo 2018), because there is potential for hydropower development that could affect riverine wetlands, and because there are likely fewer management options for rivers. Notably, Bd prevalence corresponded with the frequency that these habitat types are used for toad breeding. Toads in the Taiya River Valley area are most abundant in riverine habitats, followed by upland/human habitats. No documented breeding has occurred in upland/natural habitat in recent years (see Surdyk & Waldo 2018 and prior annual reports referenced therein), where Bd prevalence was lowest.

The highest prevalence of Bd in riverine habitats, which are considered the most critical and perhaps most threatened environments in the KLGO area, is concerning. The parallels between frequent habitat

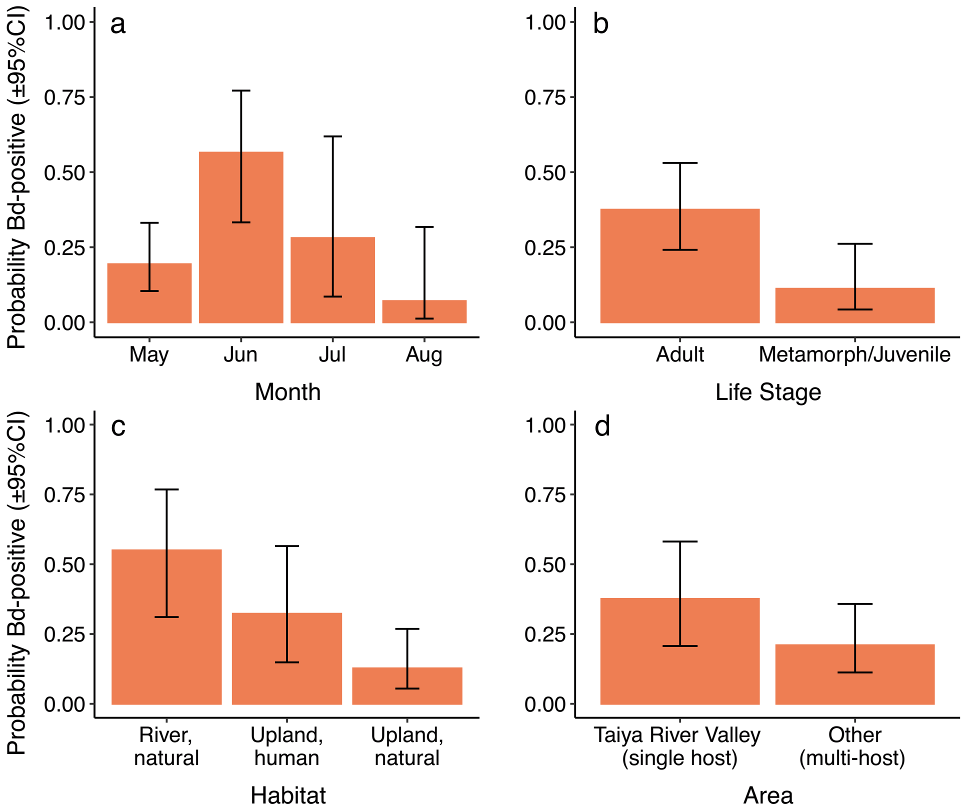

Fig. 2. Estimated mean probability (±95% CI) that boreal toads *Anaxyrus boreas* tested positive for amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd) according to (a) month sampled, (b) life stage, (c) habitat type, and (d) whether the toad was from the mostly single-host Taiya River Valley area (Alaska, USA) or a multi-host community elsewhere in the study area. All estimates are marginal means from the habitat model

use and high Bd prevalence suggest that prevalence might be driven partly by abundance and reliable presence of hosts, especially in the lower Taiya River, where toads are the only amphibian species. There was less variation in Bd prevalence among habitat types in the Haines, Juneau, and Prince of Wales Island samples, where naive prevalence ranged from 24–27% across habitat types; however, most of those samples were from one-time sampling events and those areas have other amphibian species that host Bd (Adams et al. 2007, Reeves 2008).

Estimated mean prevalence of Bd on toads in the Taiya River Valley (37.6%; N = 107) was nearly twice that of toads from areas where other amphibian species co-occur (21.0%; N = 141; Fig. 2d). This difference suggests that local species richness might affect Bd prevalence of toads, but the large variance around the estimates precludes that conclusion (χ^2 = 1.71, 1 df, p = 0.191). Because our data come from only 1 single-host area (although from 10 distinct sites) and sampling intensity from single- vs. multi-host areas was uneven across time, our data cannot distinguish between the species richness hypothesis and other sources of spatial variation. Notably, much of the highest-elevation areas of the Sierra Nevada Mountains in California (USA) only have a single amphibian species, and it is one of the best-documented systems in which chytridiomycosis has caused population declines (Briggs et al. 2010).

None of the 4 boreal toads, 12 Columbia spotted frogs, or 2 wood frogs from the Lindeman area tested positive for Bd (Fig. 1). To our knowledge, Bd has yet to be detected from Columbia spotted frogs at the northern end of their range, where our sampling occurred, but they are often infected farther south, where the pathogen has caused mortality events (Pearl et al. 2007, Hossack et al. 2013, Patla et al. 2016). Bd is present on wood frogs at high latitudes in Alaska and northwestern Canada (Reeves 2008, Slough 2009, Schock et al. 2010), although it seems less common than in other areas of the frog's range (e.g. Longcore et al. 2007, Martinez Rodriguez et al. 2009). Extensive surveys in the Lindeman area have not detected evidence of amphibian breeding (see Surdyk & Waldo 2018 and prior annual reports referenced therein), which suggests a low host density.

Our results, along with samples from boreal toads approximately 30–40 km from our White Pass study (Slough 2009), suggest that Bd is still patchily distributed in this isolated, steep landscape compared to areas that have greater abundance and richness of amphibians and greater human influence.

Based on 2005–2015 samples, Bd prevalence on toads in the KLGO area decreased over time (Fig. 3; $\chi^2 = 6.483$, 1 df, p = 0.011), but there was insufficient evidence to include a quadratic term in the model (χ^2 = 1.865, 1 df, p = 0.172). Based on the linear trend model, the odds that a sampled toad was Bd-positive decreased by 0.34 annually (95% CI = 0.15–0.78). Including average temperature for the 30 d preceding the mean sampling date each year did not affect the trend in Bd prevalence (odds ratio 0.47 [95% CI = 0.17–1.30]). Reduced prevalence of a lethal pathogen seems encouraging, but it is difficult to interpret without knowing effects on vital rates and host abundance. This trend could occur if Bd is highly virulent and transmission is reduced after a reduction in host density, or if hosts are evolving resistance to infection (May & Anderson 1983, Briggs et al. 2010). For example, at another boreal toad site in Wyoming (USA), the reduction in survival attributable to Bd has increased during the last decade, opposite the pattern expected if hosts are adapting to a pathogen (Russell et al. 2019). Collectively, these results emphasize the need to understand the how Bd is affecting populations before managers can translate pathogen prevalence into risk and make informed actions.

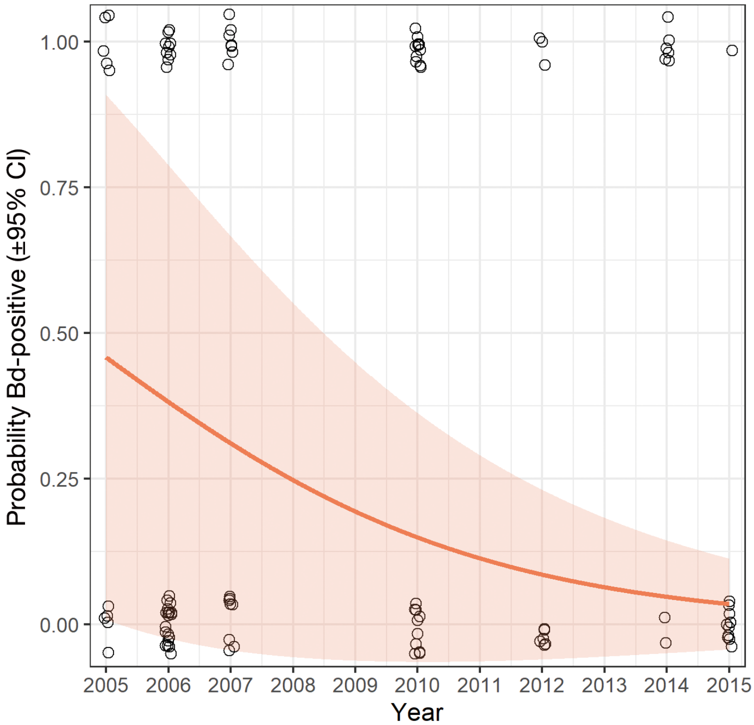

Fig. 3. Model-estimated trend (2005 2015; ±95% CI) in prevalence of amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd) on boreal toads *Anaxyrus boreas* in the Klondike Gold Rush National Historical Park area. Each open circle represents a positive (1) or negative (0) detection of Bd. The trend estimate is the marginal mean after accounting for variation in sampling date, life stage, and habitat

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