Boreal Toad (*Anaxyrus boreas*) Monitoring Program in Klondike Gold Rush National Historical Park

Survey design recommendations and trends in wetland occupancy and amphibian chytrid

Thierry Chambert[1,2], Jami Belt[3], Blake R. Hossack[4*]

[1]Calao Wildlife Consulting, Montpellier, 34090 France

[2]University of Montana, College of Forestry & Conservation, Missoula, Montana, 59812 USA

[3]Glacier National Park, National Park Service, West Glacier, Montana, 59936 USA

[4]U.S. Geological Survey, Northern Rocky Mountain Science Center, Missoula, Montana, 59812 USA

Peter Frank, NPS

*Please contact: Blake Hossack (blake_hossack@usgs.gov)

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1 Executive summary

Boreal Toads (*Anaxryus boreas*, previously *Bufo boreas*) have been monitored in and around Klondike Gold Rush National Historical Park (KLGO) since 2004. Because of their significant cultural and ecological importance, and due to threats of habitat change and the presence of the pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*), the surveillance of Boreal Toad populations has become a priority. We analyzed data collected by the park during 2005–2018 to assess the global trend of toads' occupancy in KLGO. To provide insight into the likelihood that sites produce metamorphs, we also estimated survival of larvae from early in the season (June-July) to late season (July-August) at 8 core sites that were surveyed intensively during most years. In addition, we used simulations and statistical power analyses to make recommendations on how to improve the sampling design of this monitoring program.

The analyses of the historical data (2005 – 2018) provided the following results:

- Based on the 8 core sites, there was a 72% probability that a site occupied by toad larvae early in the summer had toad metamorphs (i.e., larvae transformed and left the water). This estimate was stable over time, indicating there has not been a large increase in complete die-offs of larvae over time, as commonly occurs if a site dries or is flushed by high river flows.

- The detection probability of larvae was high (81%), while that of metamorphs was much lower (44%) at the 8 core sites.

- For larvae survival estimation, robust data can be obtained with only 2 larvae surveys performed in June-July and 5 metamorph surveys performed in July-August.

- Based on 62 sites across a broader area than the core sites, toad occupancy has been mostly stable within the KLGO area since 2005. The data suggest a slight increase of 3.7% per year, but that trend was not statistically significant.

- Analysis of 248 amphibian chytrid swabs collected from the KLGO area and other areas in southeast Alaska indicated chytrid prevalence was highest on adult toads (37.5%) in river-associated habitats (55.0%; where toads were most abundant), and lowest on juveniles and metamorphs (11.2%) in natural upland wetlands (12.7% where toads were uncommon).

- The prevalence of the amphibian chytrid fungus on toads in the KLGO area decreased during 2005–2015. Based on a linear trend model, the odds that a sampled toad was chytrid-positive decreased by 0.34 annually (95% CI = 0.15–0.78 [reduction]).

For future monitoring, as a minimal sampling design, we recommend the following:

- **Select between 8 and 12 core breeding sites** to be monitored every year. These core sites will serve as sentinels for the detection of trends in occupancy and they will also be used to provide information on whether sites represent viable breeding habitats (i.e., high survival of larvae to metamorphosis). The park currently monitors 8 core sites: TR01, DY03, DY13, DY14, DY19, DY33, WC02, WC04.

  Each core site must be visited (at least) twice between approximately June 01 and July 31 (larvae sampling window) and five times between July 01 and August 31 (metamorph sampling window), for a total of 7 repeated surveys per site, per year. These survey windows can be adjusted as needed to account for changes in timing of breeding or development, such as during early, warm springs or years with persistent snowpack at high elevations. These sentinel core sites are not randomly selected but chosen among the sites with highest known occupancy rate, and where breeding occurs regularly.

- **Randomly select 90 additional non-core sites**, which will be monitored using a 3-year rotating panel design. A total of 30 non-core sites will thus be surveyed twice every year. To maximize detection probabilities, surveys should be timed so they coincide with the expected presence of larvae, roughly between June 01 and August 31 (depending upon elevation, aspect, and other factors). Each non-core site will be revisited every 3 years. Based on changes in which types of data are collected and how the data are collected, our recommendations will result in less total effort than has occurred in most years during 2005 – 2018.

  Because detection of toad larvae is so high, doing more than 2 surveys at these sites will not provide much improvement in terms of statistical power (for trend detection purposes). Also, it is important to use a 3-year rotating panel design, as statistical power can decrease substantially under different rotation schedules. This recommendation is based on customized power analyses built for this specific KLGO monitoring program.

At the end of this report, we provide an example data sheet modified from the Cooperative Amphibian Monitoring Protocol for the Greater Yellowstone Network (Bennetts et al. 2013; Appendix 1). This data sheet is structured to help ensure data are collected in a format appropriate for occupancy analyses.

2 Introduction

The Boreal Toad (*Anaxryus boreas*, previously *Bufo boreas*) is a species of conservation concern that has experienced widespread population declines throughout much of its range (Carey 1993, Corn et al. 1997, Hossack et al. 2015). Some declines have been associated with the spread of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis)* (Muths 2003, Russell et al. 2019), but many declines remain unexplained (Hossack et al. 2015). Little is known about population status or trends of Boreal Toads in Canada and Alaska. Boreal Toads in Alaska, which are at the northernmost edge of the species' range, are of particular interest because the rate of climate change is greatest at high latitudes (Cohen et al. 2014, IPCC 2014).

Boreal Toads in southeast Alaska are suspected to have experienced large population declines since the 1970s (Carstensen et al. 2003a). Southern populations of Boreal Toads (e.g., in Colorado and Wyoming) are primarily found at high elevations, while Boreal Toads in Alaska are typically found closer to sea level; thus, in Alaska, important toad habitat is at a higher risk of being damaged by human development or affected by habitat fragmentation. Because the Boreal Toad is an important regional indicator species and because it has local cultural significance[1](Thornton and McBride 2004), the National Park Service's Southeast Alaska Network Inventory and Monitoring Program (SEAN) and Klondike Gold Rush National Historical Park (KLGO; Figure 1) selected it as a Secondary Vital Sign that could serve as an indicator of ecosystem health. KLGO initiated wetlands surveys in 2004, following peer-reviewed and published protocols, with the intention of assessing population status and trends. Little is known about the species' habitat requirements or demographics in this spatially disjunct sub-population. Preliminary analysis of wetland monitoring data suggested that breeding site occupancy declined during 2004–2017; however, it is unclear if these preliminary estimates are reliable indicators of true population trends.

Selection of the Boreal Toad as a Secondary Vital Sign provides strong justification for developing a more rigorous long-term monitoring protocol and refining the ability to detect trends. As a Secondary Vital Sign, Klondike National Historical Park is expected to take the lead in developing this protocol but needs additional resources, support and expertise. Despite this goal and interest from the park, all of SEAN's funding and resources are currently dedicated to developing protocols for their backlog of Core Vital Signs.

Concurrent with the initiation of wetland monitoring, the park also began monitoring prevalence of amphibian chytrid fungus (hereafter, Bd) because it represents a potential threat to toad populations (Russell et al. 2019a). Park staff and collaborators collected Bd samples over 8 years between 2005–2017, with 38% of the 103 samples testing positive for the fungus. Like the wetland monitoring data, these samples have not been analyzed to determine if the probability of a toad testing Bd-positive is related to animal sex or size or if animals in specific habitats (e.g., river floodplain vs. upland wetland) seem especially prone to infection. If analysis reveals that toads in certain populations or habitats are less likely to be infected by Bd, then managers may use that information to prioritize habitat protection or restoration in ways that can minimize harm from the disease, such as by targeting wetlands that have the lowest prevalence of chytrid fungus infection for conservation efforts.

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[1]According to oral histories from the local Tagish and Tlingit peoples as well as interviews with Skookum Jim's family, a frog aided discovery of the Klondike gold. After Skookum Jim helped a frog escape a deep hole near Dyea, Alaska, the frog later helped heal Jim when he was severely injured. The frog, who either represented the powerful Wealth Woman herself or was her helper, also told Jim where he would find his fortune, directing him over the mountains into the Yukon. Given that frogs per se, based on current American English vernacular, are not present in the Dyea area and we are unaware of any historical records of their presence, the frog in the oral histories could have been a boreal toad. Regardless, frogs and toads are important to the origin legends of the Inland Tlingit and Tagish peoples; because of their dependence on both water and land, frogs aptly represent "inbetweenness." (Footnote based on quotes and summaries in McClellan 1963 and Thornton & McBride 2004).

Because it is important to determine if the current data collection methods provide an effective and efficient use of park resources to identify critical Boreal Toad habitat or to detect important population changes, in 2018 the National Park Service's Focused Condition Assessment provided funding to analyze the existing data and assess evidence for trends (decline or growth) in local populations between 2004 and 2018. In addition to analyzing existing data, we conducted a series of related simulations to provide a range of monitoring scenarios that managers can use to detect specified trends (e.g., a 40% decline over 10 years). Based on the combination of analyzing already-collected data and simulating a wide range of sampling scenarios, we provide recommended data collection procedures that will help managers ensure they are using resources wisely to meet conservation goals. Further, understanding the status and population trends for Boreal Toads will enable managers to incorporate toad habitat conservation into local planning, including restoration planning and development of new trails and infrastructure. Quantitative information about the status and role of breeding areas on non-park land within the National Historic Landmark will also inform collaborative conservation efforts among a diversity of landowners.

The main objective of this work was to assess the status of Boreal Toad and its threats in KLGO since 2004 and to propose an updated monitoring strategy. We addressed objectives in 4 main steps:

1. Assess an existing monitoring program for Boreal Toads at KLGO and analyze data that have been collected by this program since 2004. Conduct a thorough occupancy analysis of existing data at core breeding sites to determine if there is evidence of a trend in wetland occupancy and if local habitat factors drive trends in wetland occupancy.
2. Incorporate information on the relationship between the hydrology of the Taiya River and the status of core breeding sites used by Boreal Toads, with the goal of determining how toads specifically will be affected by changes to the river hydrology and how to best mitigate those impacts if the Dyea area is developed or restored.
3. Conduct a series of related data simulations to identify and recommend optimal monitoring methods to detect specified trends.
4. Assess how sex, size, and site-specific factors (e.g., river floodplain vs. upland wetland) are related to the prevalence of Bd on toads.

To make it easier for readers, we structured the report into 2 major sections. The first section is focused on analyzing the toad occupancy data collected during 2005-2018, power analyses, and recommendations for monitoring design. The second section is focused on analysis of the amphibian chytrid data collected during 2005-2017. The section on amphibian chytrid has been submitted for publication as a stand-alone research note.

3 Occupancy Methods

3.1 Monitoring design

The first amphibian surveys in KLGO (Figure 1) started in 2004. During that first year, the goal was simply to determine which amphibian species were present in the park (Wetherbee 2009); only Boreal Toads were found. Starting in 2005, the park thus started a monitoring program targeted specifically for the toads. The monitoring has slightly evolved over years, including expansion into neighboring non-NPS lands in cooperation with stakeholders (see Wetherbee [2009] for more details) to ensure that all potential breeding areas for this small and tenuous population are monitored. Overall, the approach used by the park has been based on the following protocol.

Every year, a selected set of wetlands (hereafter, "sites") identified as potentially suitable habitats for toads were visited to assess the presence of the species. The monitoring of sites was divided in 2 components, referred to as (i) the "high intensity stratum" and (ii) the "low intensity stratum" (Wetherbee 2009). The high intensity stratum consists of core sites that were confirmed, or highly-suspected, as important breeding sites for toads. By 2018, there were a total of 8 core sites included in the monitoring design (Figure 2). These core sites have been monitored (almost) every year, using several survey occasions each year (mean = 10, SD = 8.9, max = 51). These sites provide the type of repeated data required for occupancy analyses (Chelgren 2005; see section 3.2 below). The low intensity stratum consists of other (non-core) sites where there was no prior evidence of breeding. These wetlands were scheduled to be surveyed every 4 years, based on a 4-year rotating panel design (Wetherbee 2009), with low annual monitoring intensity. Most of these sites were visited only once during a season. By 2018, 252 non-core sites had been surveyed since the beginning of the monitoring program. Among these, 208 sites were actually visited only once, in 2008. The other 44 non-core sites were visited between 2 and 10 times since 2007.

Sites were always visited between April and September. The presence of the species was determined based on visual encounters of any of its life stages: egg masses, tadpoles, metamorphs, juveniles or adults. In addition, some toads were caught and swabbed to detect the presence of chytrid fungus.

For the analyses presented in this report, we differentiated 3 sampling periods, corresponding to the time windows of peak detection for egg masses, larvae, and metamorphs, respectively. These periods were established from the observed temporal detections of different life stages, across the core of the reproductive season (May – August). The egg sampling window was defined as the period between May 01 and June 30, the larvae sampling window between June 01 and July 31, and the metamorph sampling window between July 01 and August 31. Although detection of different life stages such as larvae occurred across multiple time windows, the names of respective windows simply reflect the propensity to detect specific life stages.

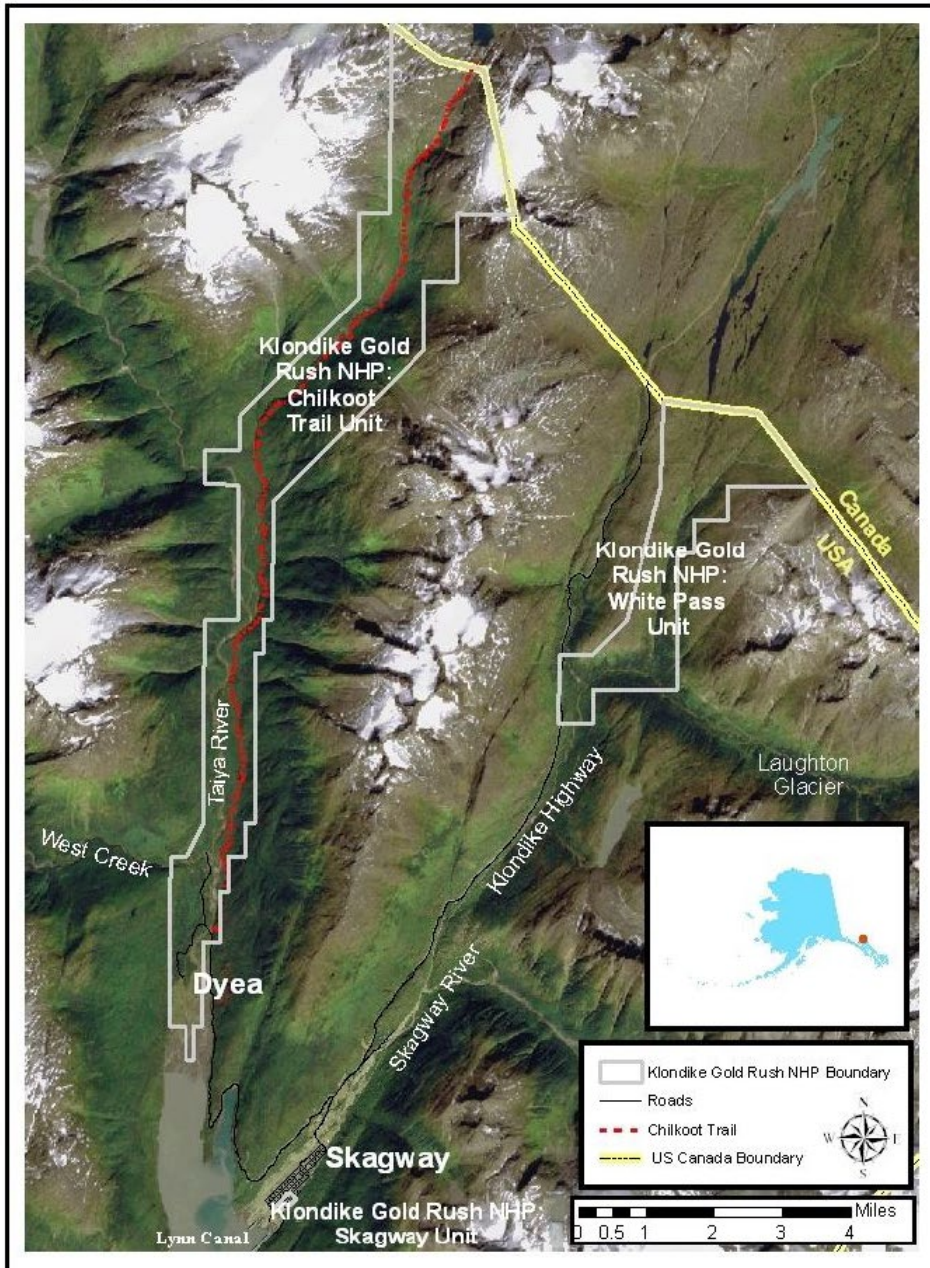## Klondike Gold Rush National Historical Park
Skagway, Alaska

Figure 1. Klondike Gold Rush National Historical Park (KLGO; Skagway, Alaska) and neighboring areas where Boreal Toads have been monitored by KLGO staff since 2004. (Map is from Welfelt 2012).

3.2 Modeling overview

Each analysis presented below addresses a specific question, but they are all based on the same multi-season occupancy framework (MacKenzie et al. 2003, 2006). Occupancy is defined as the proportion of sample units (sites) occupied by a species of interest. For a single season (e.g., June–August), this parameter can be estimated without bias if imperfect detection is taken into account, using repeated surveys data at each site (MacKenzie et al. 2002). For occupancy studies, a sampling design typically consists of within-season repeated surveys (K), performed at a specific number of sites (n). During each survey, one must simply record whether the focal species was detected or not, as was done here for Boreal Toads at various wetlands within the KLGO. These detection/non-detection data can then be analyzed with a likelihood approach, using specific programs such as PRESENCE or the R package *unmarked*, to estimate two basic parameters: site occupancy (ψ) and detection probability (p). An important assumption of the single season occupancy model is that the occupancy status of any site does not change between any of the surveys (i.e., the site is "closed" to changes in occupancy).

When multiple seasons are involved, the basic model is extended to relax the closure assumption, thus allowing for changes in occupancy status from one season to the next. Changes in occupancy are modelled with two additional parameters: a local extinction (ε) and a colonization (γ) probability. Colonization is the transition from a site being unoccupied to being occupied. A site will thus remain unoccupied with probability $1-\gamma$. Extinction is the transition from being occupied to becoming unoccupied. A site will thus remain occupied with probability $1-\varepsilon$, a parameter which we also refer to as the *site survival probability* ($\varphi = 1-\varepsilon$). This latter parameter was the primary focus of our first analysis on larvae survival.

We emphasize that the occupancy framework we recommend relies solely on the detection of at least 1 individual of any particular life stage. For example, for surveys focused on the larval sampling period (described below), the detection of 1 larva is treated the same as the detection of 1000 larvae. Much effort has been expended in the past on attempts to document detailed events such as when breeding first occurred, how many egg strings were deposited, or counting the number of metamorphs or larvae observed. If there are specific objectives for these quantitative data, they can be useful. But in general, count or event data for amphibians are typically very biased and difficult to use unless methods are highly standardized across all sites (e.g., a standardized trapping regime with known number of traps per wetland perimeter) and methods and effort are consistent over time. In the long run, the KLGO Boreal Toad monitoring program will likely be more maintainable and viable if crews focus on simple, efficient metrics like determining presence rather than trying to collect quantitative data of questionable reliability.

We provide an example of a more detailed analysis based on survival from larval to metamorph stages (i.e., *Larval survival* below). We make this suggestion because we think it is the most feasible method to collect detailed information from sites that are likely important to the toad meta-population in the area. Note that the time windows we define for surveys can be modified as needed, especially if breeding is especially early or late in a given year. We used fixed time windows in this demonstration analysis in part to keep the example simple.
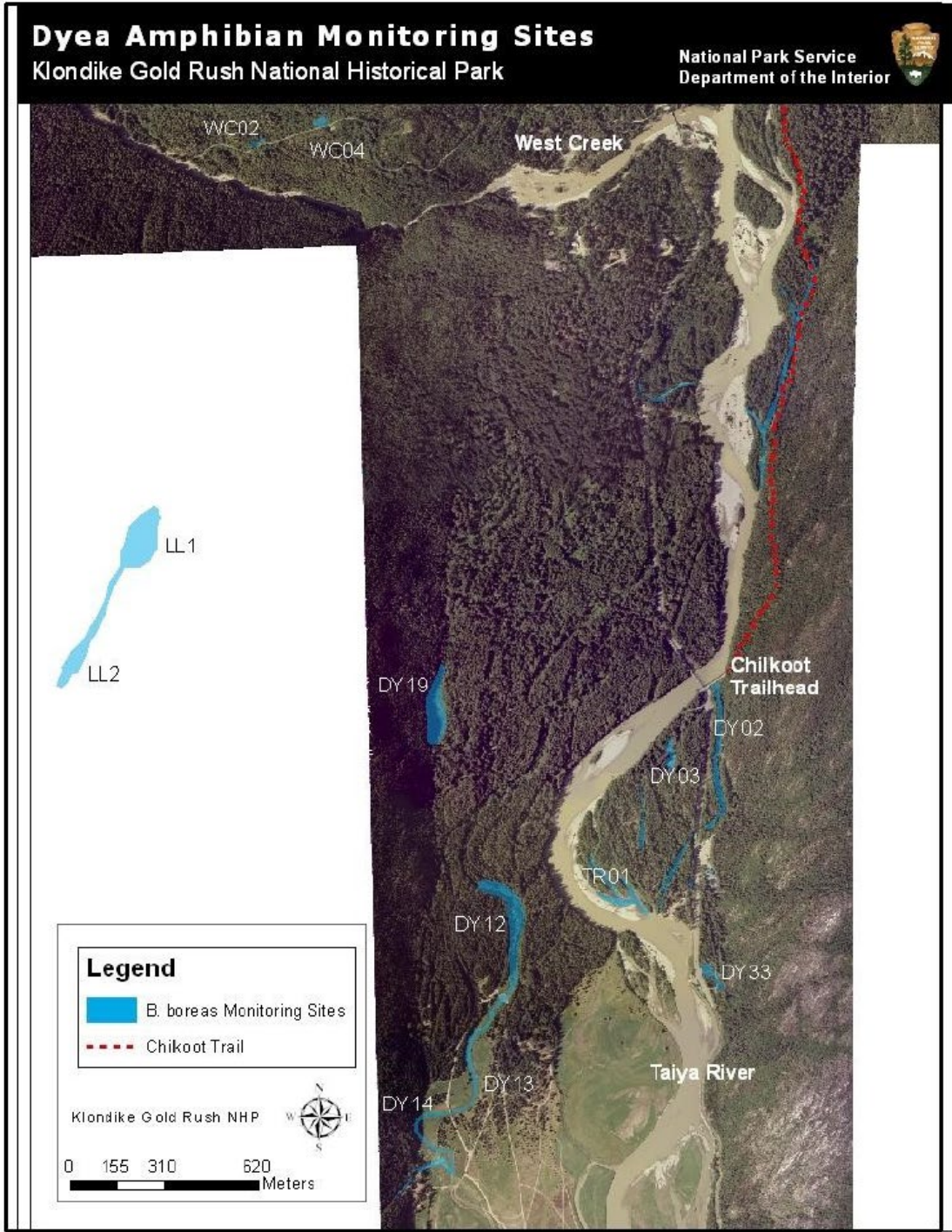
Figure 2. Location of the 8 intensively-monitored core sites, located around Dyea, Alaska. (Sites LL1 and LL2 are not core sites. Map is from Welfelt 2012.)

3.3 Larval survival

We used life-stage detection data from the 8 core sites only (TR01, DY03, DY13, DY14, DY19, DY33, WC02, WC04; Figure 2; see Surdyk and Waldo 2018 for more information on these sites) to estimate *site-level survival* from the larva to the metamorph stage. In the absence of detection data at the individual level (e.g., marking), we cannot estimate individual survival rates. Here, survival is thus estimated for a whole site and refers to the probability that a site that held larvae, around June-July, also held metamorphs later in the season. In other words, it is the probability that *at least some* larvae have survived and metamorphosed. Failed recruitment of 100% of the larvae is not uncommon in the area because sites occasionally encounter major drying or flushing events from the river. Note that this analysis could be modified to include both late-stage larvae and metamorphs detected between July 01 and August 31. However, because of concern that some sites frequently host breeding but larvae often get stranded from drying or flushed from high river flows, we focused on evidence of survival throughout the entire larval stage. To estimate this survival rate, we used a multi-season occupancy model with 2 seasons, each one corresponding to a specific life-stage period: (i) the larvae sampling window, going from June 01 to July 31 and (ii) the metamorph sampling window, going from July 01 to August 31. Within each period, we only retained detections from the corresponding life stage. All adult detections were thus removed, as well as any other off-window detection (e.g., any larvae detection occurring after July 31).

Here, occupancy during the first period is thus interpreted as the presence of larvae at a site, while occupancy during the second period during the same summer strictly corresponds to the presence of metamorphs. Survival from the larvae to the metamorph stage *during the same summer* can then be modeled as a non-extinction event, with probability $\varphi = 1 - \varepsilon$. Indeed, an extinction event between periods 1 and 2 means the death of all larvae before they could complete metamorphosis. For the sake of simplicity, models were built and run using parameter ε (as implemented in software PRESENCE), and the survival probability was derived as $\varphi = 1 - \varepsilon$. In this case, site colonization would be interpreted as a site transitioning from no larvae to some metamorphs *during the same summer*. To estimate survival, we have to assume sites are not colonized by metamorphs from neighboring sites *during the same summer* (i.e., they are "closed" to colonization during a summer, but *can be colonized between years*), we fixed this parameter at $\gamma = 0$. Under this framework, the initial occupancy parameter ψ_0 corresponds to the probability of larvae being present during the first sampling period. Finally, we note that, as in any occupancy model, the estimation of all parameters takes imperfect detection into account, which is modeled using a detection probability parameter p.

Besides estimating larvae survival, our goal was also to assess how habitat and river flow affect this parameter. For habitat, we included a categorical covariate that described (i) whether a site was located upland (Up) or connected to the river (Riv), and (ii) whether the site was mostly natural (Nat) or human-created (Hum). We combined these 2 variables into a single habitat factor taking 3 different categories: upland and natural (UpNat), riverine and natural (RivNat), upland and human-created (UpHum). Among the 8 core sites, there was no human-created river site, so there was no "RivHum" category. For river discharge, we used values of July's minimal and maximal flow from the Taiya River station near Skagway (USGS Station 15056210), originally measured

in cubic feet per second (cfs), which we standardized. Discharge in the Taiya River is similar during June and July. We used July flow data because it better captures the transition from the larval sampling window to the metamorph sampling window and larvae that were stranded or flushed during July are less likely to be replaced by a late breeding event compared to June, when breeding still occurred commonly.

These analyses were done in program PRESENCE (Hines 2006). The starting model considered all 3 parameters (ψ_0, ε and p; γ being fixed at 0) as constant. Then, using a model selection approach based on the Akaike Information Criterion (AIC), we added various effects to identify relevant sources of parameter variation and test the effect of covariates on survival. Initial occupancy ψ_0 was always assumed constant. For detection p, we tested the effect of the life stage sampling window and the effect of year. Finally, for survival ($\varphi = 1 - \varepsilon$), we assessed annual variation, as well as the river flow and habitat covariates. These latter effects were tested separately first, then together in an additive effect model (river flow + habitat). Sample sizes were too small to assess the interactive effect model (river flow × habitat).

3.4 Occupancy trend

Using data from both core and non-core sites, we investigated annual variations of occupancy between 2005 and 2018. The primary objective was to assess whether there was any global trend (decline or growth) at the scale of the entire park. In addition, this analysis, based on a typical multi-season occupancy approach (MacKenzie et al. 2003), allowed us to assess variation in the species' detection probability (seasonal effects and annual variation).

Analyses were all performed in program PRESENCE (Hines 2006). We used the multi-season parameterization based on the following 3 basic parameters: seasonal occupancy (ψ_t), extinction (ε) and detection (p) probabilities. This parameterization has the advantage of directly modelling annual occupancy ψ_t, making the investigation of its variability, and especially trend models, more straightforward. In addition, initial test runs revealed that this parameterization was slightly better supported by the data than the classic parameterization based on 4 basic parameters (ψ_0, ε, γ and p).

The significance of annual and seasonal effects were evaluated using a model selection approach based on the AIC. We sequentially selected the best parameterization for detection probability first, extinction probability next, and finally site occupancy probability. For detection probability, we assessed the effects of year and sampling period (egg vs. larvae vs. metamorph sampling windows; see definition in section 3.1). For extinction probability, we simply assessed the effect of year, modeled as a fixed effect. Finally, for site occupancy probability, we evaluated the influence of year as a fixed effect, as well as a trend effect.

3.5 Power analyses

To help optimize the monitoring design, we used simulations to conduct power analyses. Here, the main goal of the monitoring is to maintain some baseline surveillance of Boreal Toad populations, so we framed the objective as "allowing the detection of some level of decline D (e.g. a decline of 30%) within a given time frame T (e.g., over 10 years)". There is necessarily a trade-off between the effort invested in the monitoring and the precision of estimations obtained from the resulting data. However, there are no universal thresholds for defining the objectives of a surveillance monitoring program, and the effort available is often variable and adaptable. Therefore, instead of using a unique objective regarding the magnitude of the decline (D/T) that must be detectable or fixing a specific level of sampling effort, we ran a range of scenarios to assess what objectives could be achieved under varying levels of effort.

Following the current KLGO strategy, we considered 2 components of the monitoring design: core breeding sites and non-core sites. Core sites are monitored every year, and more intensely than non-core sites. Because they have higher occupancy rates, core sites provide more power for the detection of local trends and can thus be used as *sentinel* sites. It is however important to realize that, because they are not randomly selected, core sites are not representative of the overall species occupancy status in the KLGO area. The bigger picture on the overall occupancy dynamics in KLGO is provided by the second component of the design, which consists of a larger number of non-core sites that are randomly selected among all the wetlands available for monitoring. Here, the main difference with the current design is that non-core sites are preselected and monitored in a systematic way, at regular intervals (but not necessarily every year), rather than opportunistically. The additional data obtained from these sites will allow estimating representative values of overall occupancy within the KLGO area and they will provide further information to assess trends at a larger scale. In the analyses presented below, we investigated both design components separately, using the same criteria of trend detection (i.e. the "objective", as described above) to assess the performance of different sampling designs. We note that core sites could also be used to gather detection data aimed at estimating larvae survival, as described above, which requires very little modification from the designs investigated here (see section 4.2 below).

3.5.1 Sampling frame and scenarios assessed

For all scenarios, the sampling design was defined by 2 variables: (1) the number of sites surveyed (n) and (2) the number of replicated surveys (K) performed each season. The total effort allocated to monitoring, each year, corresponds to the product of these 2 components (nK) and it forms the basis for the comparison of cost among different sampling designs. For all the designs assessed, we used a systematic sampling scheme, meaning that all sites included are surveyed on a regular annual or inter-annual basis (e.g., every 2 years, every 3 years, etc.) and at least twice (K = 2) within each year of survey (within-season replication). We first assessed a large set of scenarios for the non-core site component, which provide more generalizability (due to random selection) than the non-random core site component. Then, based on these first results, we ran a smaller set of selected scenarios to investigate design optimization for the core site component.

3.5.1.1 Non-core sites

Non-core sites do not necessarily need to be surveyed every year. The goal here is to be spatially representative, hence to try to maximize the number of sites included without degrading power to detect temporal trends (which requires surveying the same sites across years). Two different sampling strategies were thus assessed. The first strategy consists of monitoring the same sites every year, which is the design classically used in occupancy studies. We refer to this strategy as the *standard fixed design*. The second strategy is a *rotating panel design* (Figure 3), in which different sites are surveyed in consecutive years, but all sites included in the sampling scheme are resampled at regular intervals R (e.g., every 3 years for a 3-year rotation; R = 3). This design allows surveying more sites in total, for a fixed amount of annual effort expended. For instance, with a 3-year rotation of n = 20 sites surveyed per year, there will a total of N = 60 sites surveyed (N = n × R). With the standard design, which equates a 1-year rotation (R = 1), the same level of annual effort (n = 20) would lead to only N = 20 sites (N = 20 × 1) being surveyed in total. On the other hand, the rotating panel design does not allow estimation of transition rates (extinction, colonization) from one year to the next. Instead, these transition rates would be estimated at the same scale as the rotation-period R (e.g., 3-year), which might not be an issue if the goal is to assess multi-year trends.

Regarding the number of replicated surveys performed within each season, because detection was high ($p \approx 0.8$) during the larva and metamorph sampling windows (see section 4.3), we only assessed scenarios consisting of K = 2 and K = 3 replicated surveys. This choice followed the recommendations of previous studies (Guillera-Arroita et al. 2010) that have shown that when detection is that high, the optimal number of surveys is K = 2. Indeed, with 2 surveys, the overall

Sites	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Site 1	✔			✔		
Site 2	✔			✔		
Site 3	✔			✔		
Site 4		✔			✔	
Site 5		✔			✔	
Site 6		✔			✔	
Site 7		✔			✔	
Site 8			✔			✔
Site 9			✔			✔
Site 10			✔			✔

Figure 3. Example of a rotating design over 3 years, with 10 sites. A check mark (✔) is present when a site is surveyed a given year.

detection probability ($p* = 1 - (1-p)^K$) is already very high ($p* = 0.96$) and it makes more sense to invest the effort available in surveying more sites. There would be virtually no benefits, in terms of power gains, to add more surveys to sites previously surveyed that same summer. This was confirmed by some early simulations we ran, assessing cases with K going from 2 to 5.

Power analyses were performed in 2 steps. First, we investigated monitoring performance for a large range of ecological situations and sampling strategies (168 scenarios total) under the *standard fixed design*. Then, we selected the 28 most relevant scenarios to investigate *rotating panel designs* for rotations values of R = {1, 2, 3, 4 and 5} years, hence a total of 140 scenarios. In the first step, the 168 scenarios assessed consisted of combinations of the following variables: (i) declines of D = {20%, 30%, 40% and 50%}, occurring over (ii) a time frame of T = {5, 10 and 20} years; and, sampling designs of n = {10, 20, 30, 40, 50, 75 and 100} sites surveyed each year, with K = {2 and 3} replicated surveys per season. As discussed above, we anticipated very similar performances for K = 2 ($p* = 0.96$) and K = 3 ($p* = 0.99$), but we still decided to assess sampling designs with K = 3 to ensure we would not miss any substantial gain of power from increasing K. In the second step, we limited the time horizon to T = 10 years and the number of sampling repetitions to K = 2 surveys per season. We also slightly modified the range of annual number of sites surveyed, removing the most extreme values (10 and 100) and adding some intermediate low values (15 and 25). We thus assessed n = {15, 20, 25, 30, 40, 50 and 75}. We kept the same 4 values of declines D = {20%, 30%, 40% and 50%} and assessed 5 different rotation strategies, R = {1, 2, 3, 4 and 5} years, for a total of 140 scenarios. We remind that, for a given sampling strategy {n, K}, the annual level effort (nK) remains the same independently from the rotation chosen because the same number of sites (n) is being surveyed each year. For instance, with n = 30 and K = 2, the annual effort will be of nK = 60 both with a 3-year and a 5-year rotation. In the first case, only N = 90 sites (30 × 3) will be surveyed in total vs. N = 150 sites (30 × 5) in the latter case. In all scenarios, we used an initial occupancy value of $\psi_0 = 0.3$, which corresponds to the average value estimated from the general occupancy analysis, based on all sites ($\widehat{\psi}_1 = 0.30$ [0.21 – 0.39]; see section 4.3).

3.5.1.2   Core sites

For the sentinel core sites component, we investigated the same scenarios of decline as above, using values D = {20%, 30%, 40% and 50%} over a time frame of T = 10 years. Here, instead of fixing $\psi = 0.3$, we assessed several scenarios of initial occupancy using a range of values [ψ_0 = {0.6, 0.7, 0.8, 0.9 and 1.0}) representative of the higher rate of occupancy observed at breeding core sites (current estimate: $\widehat{\psi}_1 = 0.80$ [0.69 – 0.92]). In terms of sampling scenarios, we assessed n = {8, 10 and 12}, with K = 2, giving a grand total of 60 scenarios. Because sentinel core sites must be surveyed every year, we only assessed the standard fixed design (i.e., no rotating panel).

3.5.2   Simulations

The data were simulated under a typical multi-season occupancy model, which involves 4 basic parameters (section 3.2): initial occupancy (ψ_0), site colonization (γ), site survival probability ($\varphi = 1 - \varepsilon$), and detection probability (p). Initial occupancy values were set at $\psi_0 = 0.3$ for non-core

sites and between 0.6 and 1.0 for core sites, based on preliminary analyses of the full data set (see section 4.3 below). Colonization was assumed constant and its value was set at $\gamma = 0.1$. Site survival rates (φ) varied annually and each year's value (φ_t) was calculated to match the level of decline defined for a given scenario, while also accounting for the effect of site colonization. The calculation of the φ_t's was done as follows. First, the level of decline was translated in an annual growth rate (λ):

$$\lambda = e^{log(1+D)/T-1}$$

where D is the value of the decline (e.g., D = -0.2 for a 20% decline) and T is the time horizon (in years) over which that decline occurs. We note that in the case of a decline (i.e. a negative trend), λ is necessarily < 1. Then, we calculated the annual occupancy values (ψ_t) for years 2 to T, as:

$$\psi_{t+1} = \psi_t * \lambda$$

Finally, we calculated the corresponding annual values of survival rate (φ_t) from year t to t+1, as using the following formula:

$$\phi_t = \frac{\psi_{t+1} - (1-\psi_t)\gamma}{\psi_t}$$

The final parameter that was defined is p, the per-survey detection probability. The multi-season occupancy analysis (section 4.3) revealed that detection was highest for surveys done after June 01, during the larva and metamorph stages. Indeed, detection probability increases from $p \approx 0.53$ before June 01 to $p \approx 0.80$ later in the season. In the simulations, we thus used an average detection of $p = 0.80$, assuming surveys will not start before June. We also included inter-annual variability in p based on our findings from that same multi-season occupancy analysis. The coefficient of variation estimated, and thus used, was CV = 0.19. Therefore, for each year simulated, we drew a random value of p_t from a distribution having a mean of 0.80 and a SD of 0.15 (CV of 0.19). This random drawing was done from a Normal distribution on the logistic scale, and values were back-transformed to the probability scale to ensure that p_t remained constrained between 0 and 1.

Once all parameters were defined for a given scenario, we simulated the site occupancy trajectories of every site, followed by the simulation of the detection data for each replicated survey. Then, we analyzed this simulated dataset with the package *unmarked*, using a multi-season occupancy model (function '*colext*') where parameters ψ_0 and γ were assumed constant while φ and p varied annually. Next, the presence of a trend was assessed with a linear regression using the approach recommended by Weir et al. (2009). This process was repeated 1,000 times for each scenario to calculate statistical power.

3.5.3 Statistical power

In the context of this analysis, statistical power is defined as the probability that a decline of known magnitude (e.g. -20% over 10 years) is detected at a given statistical significance threshold α (e.g. $\alpha = 0.05$). In simpler terms, it is our capacity at detecting a specific magnitude of decline with a given sampling effort. Power was calculated, from 1,000 repeated simulations, as the proportion

of times (x/1000) that the statistical test used to assess a decline (one-sided test) was significant at a given significance threshold (α). Statistical power is positively related to (i) sample size, (ii) the magnitude of decline (effect size), (iii) the number of years of monitoring and (iv) the significance threshold α used for the statistical test. The value of α actually represents the risk we accept for making a type 1 error, which is itself defined as the probability of detecting a false trend.

Ideally, we want to minimize this risk (hence we want to use a small value for α) but there is a trade-off to be found between minimizing α and maximizing power. Indeed, statistical power is the complement of the type 2 error risk (called β), which corresponds to the probability of *not detecting* a real trend (power is the probability of *actually detecting* a real trend; thus, $1 - β$). With a fixed amount of data, we cannot minimize both types of error risks α and β at the same time, so we must find the best trade-off. For statistical power, it is common practice to define a target of $1 - β = 80\%$, which corresponds to a 20% risk of making a type 2 error (e.g. not detecting the decline). But our ability to reach this target with affordable effort strongly depends on the threshold α that we choose. In basic science, it is common to use small values of α (typically α = 0.05), at the cost of smaller power (<80%), because detecting false effects (type 1 error) is worse than failing to detect a real effect (type 2 error). The main goal in science is to build reliable knowledge. For wildlife conservation purposes, however, failing to detect a decline can have disastrous consequences (e.g. inaction), so it is often more sensible to use higher rates of α to ensure we can reach the desired power. In the literature, it has been suggested that equating both types of error risks, by setting α = β, was a sensible approach in conservation (Di Stefano 2003). Here, we calculated power at 4 different α levels (0.05, 0.10, 0.15 and 0.20) to determine the smallest α level that could be used, under reasonable sampling effort, to detect sensible trends with a power of ≥80%.

4 Occupancy Results

4.1 *Data summary*

Between 2004 and 2018, there were a total of 1719 surveys, which led to 962 Boreal Toad detection events (all life stage included). The earliest survey date within any season was on April 21 (year of 2005) and the latest date occurred on October 03 (year of 2008). However, surveys in April, September and October were very rare — 97% of surveys occurred between May 01 and August 31. These are thus the threshold dates we used to define the different life stage sampling windows (see section 3.1).

When we filter out data from April and October, there were a total of 1677 surveys with 957 Boreal Toad detections (all life stages included) and 720 non-detections. The number and distribution of each life stage's detection by month are provided in Table 1 (number of detections) and Table 2 (proportion of detections). Note that the majority of detections were of larvae (44%; Table 2), during the larvae sampling window especially (55% in June, 52% in July) but also during the metamorph sampling window (39%, in August). On the other hand, metamorphs represent the

minority of detections (only 7 % of all detections). Even during the metamorph sampling period, they only represented 31% of the detections made, a lower contribution than larvae detections. Egg masses, juveniles and adults had intermediate contribution levels (15%-18%). As expected, egg masses were especially prevalent early in the season (mostly May, some in June). Juveniles and adults were detected fairly evenly between May and August.

Table 1. Number of detections of each life stage (rows) by month (columns) for all sites (top table) and core sites only (bottom table).

All sites (core + non-core sites)

Month	May	June	July	August	September	Total
Sampling Period	Egg	Egg + Larvae	Larvae + Metamorph	Metamorph	Metamorph	
Egg mass	107	30	3	0	0	**140**
Larva	53	195	128	36	5	**417**
Metamorph	0	3	35	29	3	**70**
Juvenile	35	74	35	9	0	**153**
Adult	70	52	35	19	1	**177**
None detected	208	162	191	155	4	**720**
Total	**473**	**516**	**427**	**248**	**13**	**1677**

Core sites only

Month	May	June	July	August	September	Total
Sampling Period	Egg	Egg + Larvae	Larvae + Metamorph	Metamorph	Metamorph	
Egg mass	107	30	3	0	0	**140**
Larva	53	195	122	36	5	**411**
Metamorph	0	3	34	29	3	**69**
Juvenile	31	66	31	9	0	**137**
Adult	69	46	24	17	1	**157**
None detected	165	87	36	21	1	**310**
Total	**425**	**427**	**250**	**112**	**10**	**1224**

Table 2. Proportions of total detections represented by each life stage (rows), for each month (columns), and for all sites (top table) and core sites only (bottom table).

All sites (core + non-core sites)

Month	May	June	July	August	September	Total
Sampling Period	Egg	Egg + Larvae	Larvae + Metamorph	Metamorph	Metamorph	
Egg mass	40%	8%	1%	0%	0%	15%
Larva	20%	55%	54%	39%	56%	44%
Metamorph	0%	1%	15%	31%	33%	7%
Juvenile	13%	21%	15%	10%	0%	16%
Adult	26%	15%	15%	20%	11%	18%

Core sites only

Month	May	June	July	August	September	Total
Sampling Period	Egg	Egg + Larvae	Larvae + Metamorph	Metamorph	Metamorph	
Egg mass	41%	9%	1%	0%	0%	15%
Larva	20%	57%	57%	40%	56%	43%
Metamorph	0%	1%	16%	32%	33%	7%
Juvenile	12%	19%	14%	10%	0%	14%
Adult	27%	14%	11%	19%	11%	16%

4.2 Larval survival

Life stage data from the 8 core sites (2004 – 2018) provided a total of 707 surveys, during which 340 detections (1's) were made. The remaining 367 surveys consisted of non-detections (0's). Among these surveys, 447 occurred during the sampling window for larvae, which led to a total of 276 detections and 171 non-detections. There were 260 surveys performed during time, for a total of 64 detections and 196 non-detections.

The data clearly supported the effect of life stage sampling period (larva vs. metamorph) on detection probability (ΔAIC = -52.22; Table 3). Detection was almost twice as high during the larvae sampling period ($\hat{p}$ = 0.81, 95% CI = [0.77, 0.85]) than during the metamorph period ($\hat{p}$ = 0.44, 95% CI = [0.35, 0.53]; Table 4). When a fixed year effect was included on parameter p, the likelihood optimization algorithm (i.e. model run) was not able to reach a solution, suggesting an issue of over-parameterization. In other words, including a fixed year effect adds too many

parameters for the sample size available; the model is not identifiable. We thus decided to exclude this effect for the rest of the analyses.

Table 3. Model selection results from the life stage analysis (section 4.2). The covariate assessed include: (i) the effect of life stage sampling window on detection parameter p; and (ii) the effects of year, river flow (minimum [min] and maximum [max] flow in July) and habitat (hab) on survival (φ), which is modeled as the complementary of extinction probability ($\varphi = 1 - \varepsilon$).

Model	AIC	ΔAIC	AIC weight	Number of parameters
$\psi(.)$, $\varphi(.)$, p(life stage)	700.27	0.00	0.49	4
$\psi(.)$, φ(max flow), p(life stage)	702.00	1.73	0.21	5
$\psi(.)$, φ(min flow), p(life stage)	702.27	2.00	0.18	5
$\psi(.)$, φ(hab), p(life stage)	704.24	3.97	0.07	6
$\psi(.)$, φ(hab + max flow), p(life stage)	705.94	5.67	0.03	7
$\psi(.)$, φ(hab + min flow), p(life stage)	706.24	5.97	0.02	7
$\psi(.)$, φ(year), p(life stage)	710.00	9.73	0.00	18
$\psi(.)$, $\varphi(.)$, p(.)	752.49	52.22	0.00	3

Regarding survival, the year effect was not supported by the data (ΔAIC = 9.73; Table 3), which suggests survival of larvae to the metamorph stage has been relatively stable during the monitoring program. In addition, we did not find support for an effect of habitat type (ΔAIC = 3.97) or river flow (minimum July flow: ΔAIC = 2.00; maximum July flow: ΔAIC = 1.73;). Similarly, the model including an additional effect of habitat type and river flow was not supported by the data (ΔAIC = 5.97; ΔAIC = 5.67). Based on crew observations, it is clear that variation in discharge of the Taiya River affects survival of eggs and larvae in site TR01. Across the 8 core sites, however, minimum and maximum flows during July were not strongly associated with the probability that larvae present during June and July resulted in metamorphs during August.

The best model was thus the model with ψ_0 and ε (hence survival $\varphi = 1 - \varepsilon$) constant, and with an effect of life stage sampling window on detection probability p (see above). With this model, the probability of presence of larvae is estimated at 0.64 [0.54, 0.74], which means that about 64% (~ 5 of 8 sites) of the core sites had larvae each year (Table 4). The survival probability from the larvae to the metamorph stage was estimated at 0.72 [0.56, 087], meaning that about 72% of sites that held larvae early in the season held metamorphs later that same summer. In other words, about 28% of sites where breeding occurs (with successful egg development) experienced a complete mortality event (i.e. 100% of the larvae die) before metamorphosis could be completed. As we mentioned above, the lack of support for including a year covariate for survival suggests that across this time series there have not been large changes in the fraction of sites where larvae present early in the summer do not survive to metamorphosis later in the summer.

These results demonstrate that it is possible to estimate life stage survival at the level of the site if repeated survey data are available within the sampling window of each life stage of interest (here: larvae and metamorphs). For the future, we suggest KLGO staff continue surveying core breeding sites every year and to do so in a standardized fashion, using the same number of repetitions and similar survey dates, within a given period, for all core sites. Given the high detection probability of larvae ($\hat{p}$ = 0.81), 2 surveys would be enough to reach a high overall detection rate (p* = 0.96). For the metamorph sampling window, we recommend doing 4 or 5 surveys (p* = 0.90 and 0.94, respectively) considering that the detection probability of metamorphs is lower ($\hat{p}$ = 0.44). With 2 surveys in June-July for larvae, and 5 additional surveys in July-August for metamorphs, one would obtain robust data to estimate site-level larvae survival probability with only 56 surveys in total (8 sites × 7 surveys).

To reduce travel time to sites (core and non-core sites) and increase overall project efficiency, we recommend using a double, independent observer survey technique, where 2 field technicians conduct independent surveys during the same visit. Under this design, each survey is treated as a full surveys, so 2 surveys would be completed with a single visit to a site. To keep observations independent, it is important that observers do not communicate (or even give obvious clues) survey results until both surveys are done. If needed, surveyors could be coded based on experience (e.g., experienced technician vs. volunteer) to account for potential differences in detection probabilities. See Gould et al. (2012) for more details of this survey method where travel to sites is expensive.

Table 4. Parameter estimates from the top model of the life stage analysis, which only includes the effect of the life stage sampling window on detection. The survival parameter (φ) is defined as the probability that a site occupied with larvae (tadpoles) transitions to the status "occupied with metamorphs".

Parameter	Estimate	Standard Error	Lower limit 95% C.I.	Upper limit 95% C.I.
Presence of Larvae (ψ_0)	0.64	0.050	0.54	0.74
Survival (φ)	0.72	0.080	0.56	0.87
Detection (p) during the larvae stage	0.81	0.022	0.77	0.85
Detection (p) during the metamorph stage	0.44	0.046	0.35	0.53

4.3 Occupancy trend

To estimate trends in wetland occupancy across the entire sampled area, we had a total of 62 sites and 427 survey dates distributed over 14 years (2005-2018). A total of 1,201 individual surveys (site × occasion) were actually performed, among which 636 had a positive detection (1) of the species, while the remaining 565 observation were non-detections (0). Overall, naïve occupancy (i.e., occupancy probability not corrected for imperfect detection) varied between 0.22 and 0.57

over the 2005-2018 time period, with an average value of 0.35. When considering core sites only, the naïve rate of occupancy varied between 0.5 and 1.0, with an average at 0.71.

The basic model considering all parameters as constant provided an estimated average occupancy rate of 0.30 [0.21, 0.39], an annual extinction rate of 0.24 [0.15 – 0.32], and an average detection probability of 0.69 [0.66, 0.72] (Table 5). The derived estimate for colonization rate was 0.10 [0.05, 0.15], a value that offsets the rate of local extinction $[(1-\psi) \times \gamma = \psi \times \varepsilon = 0.07]$. Each year, on average, about 7% of all sites experienced an extinction, but 7% of sites were colonized. The annual growth rate was thus estimated as 1.0, suggesting the absence of any trend in occupancy rate over the years. These first results provide a very broad overview for Boreal Toad occupancy in the KLGO area, but inference from this simple model is limited as it ignores any source of variation in all parameters. We now discuss the sources of temporal variation assessed during the analysis.

Table 5. Parameter estimates obtained from the basic model, which considers all parameters as constant.

Parameter	Estimate	Standard Error	Lower limit 95% C.I.	Upper limit 95% C.I.
Seasonal occupancy (ψ_t)	0.30	0.047	0.21	0.39
Extinction (ε)	0.24	0.043	0.15	0.32
Detection (p)	0.69	0.016	0.66	0.72
Colonization[‡] (γ)	0.10	0.025	0.05	0.15
Growth rate[‡] (λ)	1.00	0.000	1.00	1.00

[‡] derived parameters

Including annual variation on detection probability improved model support substantially, relative to the constant model (ΔAIC = -41.67; Table 6), but the effect of the sampling period was even stronger (ΔAIC = -84.12). Although both these effects might be relevant, the sample size was too small to allow running a model including both factors in combination. We thus favored the effect of the sampling period, which was most supported by the data and was more biologically relevant. Indeed, we expected the detection of egg masses to be different from that of tadpoles and metamorphs. Detection appeared to be much lower during the egg sampling period (May; $\hat{p} = 0.51$ [0.46, 0.56]; Table 7), than during the larvae-only sampling period (June; $\hat{p} = 0.79$ [0.74, 0.83]) and the metamorph sampling period (July-August; $\hat{p} = 0.83$ [0.78, 0.87]). We note that, unlike in the larvae survival analysis (section 4.2), detection was fairly high during the so-called metamorph sampling window (p $\approx$ 0.8) and very similar to that of the larvae sampling window. This is explained by 2 differences with the larvae survival analysis. First, what we called the metamorph sampling window here includes July, which actually overlaps the larvae sampling window. We did this in part because detection of larvae in high-elevation sites (e.g., White Pass area) would likely be higher during July than in June. Second, in this analysis we included detections based on any life stages (including juveniles and adults) during a survey, in contrast to the results reported in section 4.2. As discussed in the data summary section (4.1), there were still a lot of larvae

detections (a life stage that is highly detectable), as well as adult detections, occurring in July and August (see also Table 1 and Table 2). This explains the high detection probability estimated here for July-August, which includes detections from several life stages. However, this does not mean that metamorphs or juveniles and adults are as detectable as larvae.

The model that included year variation on extinction probability was not supported by the data (ΔAIC = 15.53). We thus kept this parameter constant to assess the effect of year on occupancy. When modeled as a fixed effect, annual variation of site occupancy probability was clearly not supported (ΔAIC = 11578). When modeled as a trend, it was almost supported (ΔAIC = 0.83). Although not statistically significant (95% C.I. = [-0.04, 0.14]), the trend estimated by this latter model (β_{trend} = +0.05, SE = 0.047; see Table 8 and Figure 4) suggests a slight increase of occupancy over time, from 0.22 [0.10, 0.34] in 2005 to 0.36 [0.17, 0.54] in 2018. This represents a positive trend of about 3.7% per year, which equals an increase of 0.01 occupancy probability each year. Given the amount of among-year variability in occupancy (SD = 12%, CV = 35%), it is not surprising that such a small growth does not appear quite statistically significant.

Table 6. Model selection results from the occupancy trend analysis.

Model	AIC	ΔAIC	AIC weight	Number of parameters
ψ(.), ε(.), p(Sampling Periods)	1263.73	0.00	0.60	5
ψ(trend), ε(.), p(Sampling Periods)	1264.56	0.83	0.40	6
ψ(.), ε(.), p(year)	1305.40	41.67	<0.01	16
ψ(.), ε(.), p(.)	1347.85	84.12	<0.01	3
ψ(trend), ε(.), p(.)	1348.38	84.65	<0.01	4
ψ(.), ε(year), p(.)	1363.38	99.65	<0.01	15
ψ(year), ε(.), p(.)	12926.48	11662.75	<0.01	16

Table 7. Estimated detection probabilities for each sampling period.

Sampling Period	Primary life stage present	Probability of detection	Lower limit 95% C.I.	Upper limit 95% C.I.
Before June 01	Eggs	0.51	0.46	0.56
June 01 to July 01	Larvae	0.79	0.74	0.83
After July 01	Metamorphs	0.83	0.78	0.87

Table 8. Annual estimates of occupancy from the trend model.

Year	Occupancy Estimate	Standard Error	Lower limit 95% C.I.	Upper limit 95% C.I.
2005	0.22	0.06	0.10	0.34
2006	0.23	0.06	0.12	0.34
2007	0.24	0.05	0.14	0.34
2008	0.25	0.05	0.16	0.34
2009	0.26	0.05	0.17	0.35
2010	0.27	0.04	0.18	0.36
2011	0.28	0.04	0.19	0.37
2012	0.29	0.05	0.20	0.38
2013	0.30	0.05	0.20	0.40
2014	0.31	0.06	0.20	0.42
2015	0.32	0.06	0.20	0.45
2016	0.33	0.07	0.19	0.48
2017	0.34	0.08	0.18	0.51
2018	0.36	0.09	0.17	0.54
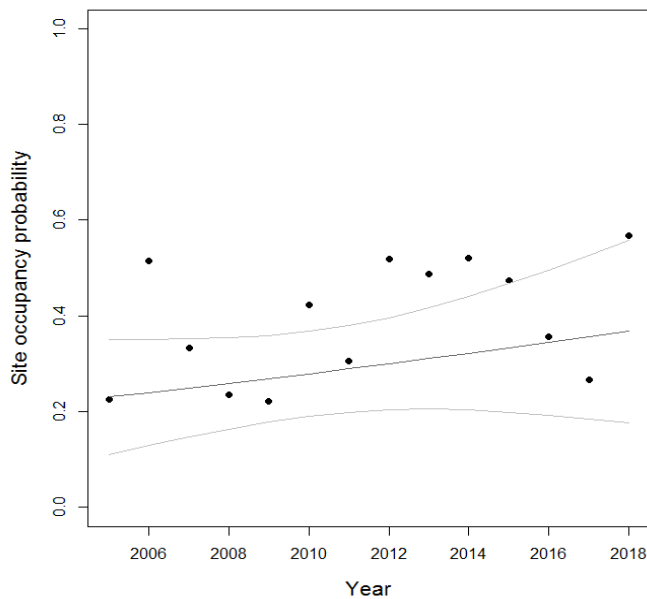
Figure 4. Observed annual occupancy (black dots, naïve values) and estimated trend (black line) with the 95% confidence interval limits (grey lines). This positive trend (βtrend = +0.05) was not statistically significant (95% C.I. = [-0.04, 0.14]). Note that, unlike the trend line, the naïve values are not adjusted for detection uncertainty.

Overall, the best supported model of the analysis only includes an effect of the sampling season (egg *vs.* larvae *vs.* metamorph periods) on detection probability. All other parameters seemed relatively constant over the years (see average estimates in Table 9). We only found suggestive evidence for a small increasing trend (3.7% per year) in site occupancy.

Table 9. Parameter estimates from the top model.

Parameter	Estimate	Standard Error	Lower limit 95% C.I.	Upper limit 95% C.I.
Seasonal occupancy (ψ_t)	0.27	0.044	0.19	0.36
Extinction (ϵ)	0.24	0.043	0.16	0.33
Colonization[‡] (γ)	0.09	0.022	0.05	0.14
Growth rate[‡] (λ)	1.00	0.000	1.00	1.00

[‡] derived parameters

4.4 Power analyses

4.4.1 Non-core sites

4.4.1.1 General results from the standard fixed design

Choice of a relevant α level

Based on simulations done in the R package *unmarked*, if we were to use a threshold $\alpha = 0.05$ with a standard fixed design, we could only reach the target power of 80% for high level of declines and using a large amount of survey effort (Table A1). For instance, detecting a decline of 50% over 10 years would require surveying ~100 sites per year (Table 10). At the strict level of $\alpha = 0.05$, detecting even large declines would thus require survey effort that seems unrealistic. Using α levels of 0.10 or 0.15, we could detect slightly smaller declines (40% over 10 years) but the same effort of n = 100 would still be required. At lower efforts (e.g., n = 75), we never reach the 80% power target even for the largest decline considered. Only when using $\alpha = 0.20$, do we start detecting similar levels of declines with substantially smaller efforts. For the detection of a 40% decline over 10 years, the statistical power of a study design of n = 75 sites and K = 2 repeated surveys (nK = 150) improves from 0.59 to 0.80 when we increase the tolerance for type 1 error from 5% to 20% (Table 10). Taken together, these results strongly support recommendations from previous authors (Di Stefano 2003) in setting $\alpha = \beta$ (see also section 3.5.3). In the rest of the report, we will thus discuss results based on a level α of 0.20.

Table 10. Selected results of the power analysis illustrating the effect of the type 1 error α level. These results are based the *standard fixed design* simulation study. See table A1 below (in Appendix) for the full results table.

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Total Effort	Power at α = 5%	Power at α = 10%	Power at α = 15%	Power at α = 20%
-40%	10	10	2	20	26%	32%	37%	41%
-40%	10	20	2	40	36%	45%	50%	55%
-40%	10	30	2	60	43%	51%	57%	62%
-40%	10	40	2	80	49%	57%	63%	68%
-40%	10	50	2	100	54%	64%	68%	72%
-40%	10	75	2	150	59%	70%	76%	80%
-40%	10	100	2	200	70%	80%	84%	87%
-50%	10	10	2	20	30%	38%	43%	46%
-50%	10	20	2	40	43%	53%	58%	62%
-50%	10	30	2	60	51%	61%	68%	73%
-50%	10	40	2	80	61%	68%	74%	77%
-50%	10	50	2	100	63%	73%	78%	81%
-50%	10	75	2	150	75%	84%	88%	90%
-50%	10	100	2	200	82%	88%	92%	94%

Choice of a relevant time horizon

The use of longer time horizons in the quantitative definition of the decline to be detected (the objective) allows detecting smaller declines with less effort (Table 11), as we would expect. For instance, a decline of 50% occurring over only 5 years ($\lambda = 0.841$, thus a 16% annual decline) would require monitoring of n = 75 sites annually. The same decline of 50% occurring over 10 or 20 years, which actually corresponds to a slower annual rate of decline (7.4% or 3.6%, respectively), would require monitoring only 50 or 30 sites per year, respectively. More time thus gives us more power, but using large time horizons to calibrate our effort increases the risk of delaying our ability to detect declines and the opportunity for taking action. It is thus important that the choice of the optimal design be based on a relevant time horizon, taking the trade-off between detection delay and required effort into account.

Here, we suggest that a time horizon of T = 10 years is a good option. A metapopulation declining at a rate of 50% every 10 years ($\lambda = 0.926$; annual decline of 7.4%) would have its occupancy rate divided by 10 after 31 years (hereafter, we refer to this as the "quasi-extinction time"). Such a decline could be detected over 10 years with fairly reasonable effort (n = 50). For a time horizon of 5 years, a 50% decline would lead to a quasi-extinction time of only 15 years, and it would require higher effort to be detected. A time horizon of 5 years thus seems too short to allow reaching decent detection power with realistic effort. On the other hand, using T = 20 years would provide higher power, but it is too long of a delay to be considered operational for conservation purposes. In the rest of this report, we will thus be using T = 10 year as the reference time horizon for comparing sampling strategies.

Table 11. Selected results of the power analysis illustrating the effect of the time horizon.

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Total Effort	Power at α = 20%
-50%	5	10	2	20	39%
-50%	5	20	2	40	51%
-50%	5	30	2	60	60%
-50%	5	40	2	80	67%
-50%	5	50	2	100	70%
-50%	5	75	2	150	81%
-50%	5	100	2	200	85%
-50%	10	10	2	20	46%
-50%	10	20	2	40	62%
-50%	10	30	2	60	73%
-50%	10	40	2	80	77%
-50%	10	50	2	100	81%
-50%	10	75	2	150	90%
-50%	10	100	2	200	94%
-50%	20	10	2	20	59%
-50%	20	20	2	40	70%
-50%	20	30	2	60	83%
-50%	20	40	2	80	86%
-50%	20	50	2	100	90%
-50%	20	75	2	150	96%
-50%	20	100	2	200	98%

Choice of a relevant number of repeated surveys

As mentioned in section 3.5.1.1, given the high detection rates of Boreal Toads ($p \approx 0.80$), we anticipated it would be optimal to use only 2 repeated surveys per site every year (K = 2) to maximize power while minimizing effort. This was demonstrated by previous authors (Guillera-Arroita et al. 2010), and we confirmed it with a small set of simulations (Table A2, Appendix). Within the 168 scenarios we ran in the first simulation study (Table A1), in no instance did we find any substantial improvement of power when using 3 surveys per site (instead of K = 2). The ≥50% increase of effort this would represent cannot be justified for the objective of trend detection. As a reminder, if field technicians typically work in pairs, both surveys for the year can be accomplished during a single visit to the site, using a double independent observer approach we described earlier (Gould et al. 2012). It would be much more useful to spend any additional effort on surveying more sites 2 times each rather than surveying fewer sites more times. The following scenarios we ran were thus all based on K = 2 surveys per year.

We note however that this recommendation is only valid for the purpose of detecting declines, and it is a minimalist approach aimed at reducing total effort while fulfilling this simple objective. Doing more than 2 surveys (for the same number of sites) would not impede this objective.

Once we determined that 2 surveys were sufficient, the main focus of the non-core site power analyses was then to (i) determine if a rotating panel design would be better than a standard fixed design, (ii) optimize the number of sites to survey every year and (iii) determine what level of decline D (over 10 years) we could hope to detect with a realistic effort.

4.4.1.2   Optimal sampling strategies: fixed vs. rotating panel designs

With a standard fixed design applied to non-core sites ($\psi = 0.3$), we could expect to detect declines of -40% over 10 years by following n = 75 sites each year (Table 10). We used this scenario (D = -40%, n = 75) as a basis for comparison with other sampling strategies (hereafter "basis scenario"). Increasing effort to n = 100 did not allow reaching the desired power of 80% for smaller declines. For a 30% decline, the highest power reached was 67% (for K = 2), and for a 20% decline it was only 58% (table A1). A decline of 50% could be detected with less effort, using only between 40 and 50 sites.

Regarding the rotating panel design, it seems that using a 3-year rotation can substantially improve power (Table 12). Higher rotations (R = 4, 5) did not further improve power here, for an objective defined over a 10-year time horizon (table A2); the 3-year rotation was clearly the optimum here. As a comparison with the fixed design, the 3-year rotating panel design allows detecting smaller declines (here: -30%, vs. -40% with a fixed design) with a similar sampling effort (n = 75) as used in the basis scenario. Moreover, the same objective of detecting a 40% decline could now be detected with much less effort, monitoring only 30-40 sites per year instead of 75 (Table 12). The use of 30 sites provides a power of 78%, which is close enough to our 80% target to be considered acceptable. At 40 sites, we reach 81%, so we can surmise that the 80% mark would be attained around n = 35-37 sites. Despite these improvements, it is still not possible to detect small declines of 20% with any level of effort under n = 100 sites per year (at n = 100, power is only 70%).

Based on these results, we make the following recommendations regarding the non-core site sampling component. First, one should not expect to detect small declines such as 20% over 10 years, unless more effort could be devoted to the monitoring of non-core sites. Even a 30% decline would require a high level of effort, monitoring 75 sites each year under a 3-year rotating panel design. We thus recommend making decisions about sampling design calibration based on the objective of detecting declines around 40% – 50% over 10 years. To provide some frame of reference, we translated these different values of decline magnitude in terms of (i) annual rate of decline, (ii) annual growth rate and (iii) time to quasi-extinction (Table 13). Declines of 20% or 30% over 10 years are actually fairly slow rates of declines, so it is not too surprising that they are so hard to detect. It is thus probably sufficient to target rates of declines in the 40% –50% range. We also recommend using a 3-year rotating panel design, as this strategy allows improving power by 23% (and up to 36%) in comparison to the standard fixed design. Especially, for a recommended

objective of D = -40%, using a 3-year rotating panel design allows decreasing the effort required from n = 75 to n ≈ 30 sites.

Table 12. Selected results of the power analysis to illustrate the effect of the rotating panel design on statistical power. Rotation over 1 and 3 years are shown for comparison. See table A2 (Appendix) for the full results of the rotating panel design analysis.

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Rotation	Total Effort	Power at $\alpha = 20\%$
-40%	10	15	2	1	30	52%
-40%	10	15	2	3	30	62%
-40%	10	20	2	1	40	53%
-40%	10	20	2	3	40	67%
-40%	10	25	2	1	50	60%
-40%	10	25	2	3	50	73%
-40%	10	30	2	1	60	62%
-40%	10	30	2	3	60	78%
-40%	10	40	2	1	80	67%
-40%	10	40	2	3	80	81%
-40%	10	50	2	1	100	72%
-40%	10	50	2	3	100	85%
-40%	10	75	2	1	150	80%
-40%	10	75	2	3	150	91%

Table 13. Summary statistics for the expected population trajectory under each scenario of decline.

Summary statistics	Rate of decline (D) over 10 years			
	-50%	-40%	-30%	-20%
Annual rate of decline	7.4%	5.5%	3.9%	2.4%
Annual growth rate (λ)	0.926	0.945	0.961	0.976
Time to quasi-extinction (years)	31	42	60	94

To summarize, we recommend monitoring 30 non-core sites every year with a 3-year rotating panel design and doing 2 surveys per site per year. This represents an annual effort of 60 surveys (nK = 30 × 2) for non-core sites. Implementing this strategy requires the (random) selection of 90 sites, initially, that will be included in the rotating panel design. Each site will then be monitored every 3 years only. This strategy will provide the data required to estimate trends equivalent to a 40% decline occurring over a 10-year period, and representative of the dynamics occurring at the scale of the whole KLGO-area wetland landscape.

To help reach a total of 90 sites, park staff could consider splitting some very large sites into multiple sub-sites that are selected, surveyed, and tracked independently. Although the sub-sites would not be totally independent, this would be an acceptable approach to increasing the number of sites monitored as long as closure can be assumed (i.e., as long as there are no movements of larvae or metamorphs between these sub-sites within a given year). For example, the large beaver pond complex along the Chilkoot Trail could likely be divided into >10 sub-sites, with buffer zones in between sub-sites that are not surveyed, to help ensure larvae do not move from one surveyed sub-site to another during a summer.

4.4.2 Sentinel Core Sites

This third set of simulations revealed that the use of high-occupancy breeding core sites as sentinels is an efficient way of ensuring the detection of declines, although the observed trend might not be representative of the entire KLGO area given that these sites were not randomly selected. Nevertheless, the information it provides is worth the investment, especially since the core sites represent most known Boreal Toad sites in the KLGO area.

With 8 core sites having an initial occupancy rate of 0.8, which is close to the current situation in the KLGO area, we would be able to detect a decline of 50% after 10 years (power = 83%, Table 14; see also Table A3, Appendix). This would only require doing 2 surveys per year, hence a total annual effort of 16 surveys across the 8 sites. In comparison, with the non-core site strategy it would require three times more effort (50 surveys) to reach similar power. If a few more core sites could be added, such dividing the Chilkoot Trail beaver pond complex into several sub-sites, the sentinel strategy becomes even more efficient and we could now even detect declines of 40% with only 10 or 12 sites (power = 78% and 80%, respectively). If initial occupancy happens to be even higher (e.g. $\psi = 0.9$) the resulting power will be even better (Table A3).

Table 14. Selected results of the power analysis on sentinel core sites. See Table A3 (Appendix) for full results.

Decline	Time Horizon	Initial Occupancy	Number of Sites Surveyed	Number of Survey Occasions	Total Effort	Power at $\alpha = 20\%$
-20%	10	0.8	8	2	16	56%
-20%	10	0.8	10	2	20	56%
-20%	10	0.8	12	2	24	62%
-30%	10	0.8	8	2	16	68%
-30%	10	0.8	10	2	20	68%
-30%	10	0.8	12	2	24	73%
-40%	10	0.8	8	2	16	75%
-40%	10	0.8	10	2	20	78%
-40%	10	0.8	12	2	24	80%
-50%	10	0.8	8	2	16	83%
-50%	10	0.8	10	2	20	83%
-50%	10	0.8	12	2	24	88%

Based on these results, in addition to the non-core site strategy described previously, we thus recommend monitoring between 8 and 12 core sites per year in addition to the 30 non-core sites that are surveyed annually. These sites would serve as sentinel to further improve the detection of potential declines occurring within KLGO, in addition to providing data to estimate larva survival probabilities, as discussed above (section 4.2). This latter goal will actually require doing more surveys every season (we recommend 7), which will increase the effort invested only slightly, especially if a double observer survey method is adopted.

We remind readers that core sites will not be selected randomly, but instead should be known breeding sites that have shown the highest rates of occupancy and are easily accessible. If selection of core sites is biased towards those that are already used for breeding by toads, there would be little capacity for detecting *increases* in toad occupancy. This trade-off seems like a reasonable approach, however, especially if the main concern is detecting *decline* of potentially sensitive species.

We recommend the park include the 8 current core sites (DY03, DY13, DY14, DY19, DY33, TR01, WC02 and WC04) in future monitoring programs. In addition to these sites, it would be worth trying to identify 2 to 4 more known toad breeding sites that are easily accessible to add. Based on the current data available, we suggest that site DY02 would be a good candidate. In addition, it would worth investigating the possibility for either one of sites CT11, WC03, CT07 or DY12 to also be added as a core site in the monitoring design.

5 Amphibian Chytrid Fungus: Introduction and Methods

Note: As of October 2019, this section on amphibian chytrid fungus was accepted as a stand-alone publication. Citation: Hossack, B. R., M. J. Adams, R. K. Honeycutt, J. J. Belt, and S. Pyare. In press. Variation in amphibian chytrid prevalence on boreal toads: tests of habitat, life stages, and temporal trends in southeast Alaska and northeast British Columbia. *Diseases of Aquatic Organisms*.

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. 2019b). 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, there is still relatively little data on Bd prevalence from areas that host few amphibian species, such as many 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 and Anderson 1983, Grant et al. 2018).

To measure and track prevalence of Bd on amphibians in southeast Alaska and northwest British Columbia, 248 boreal toads (*Anaxyrus boreas*), 12 Columbia spotted frogs (*Rana luteiventris*), and 2 wood frogs (*R. sylvatica*) were sampled during 2005–2017. During 2005–2006, sampling was focused in 5 general areas: (1) the Skagway and Taiya river valleys (Alaska, USA) and Lindeman (British Columbia, Canada), 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) (Figure 5). We included data collected during 2005–2006 and previously published in Adams et al. (2007) because it allowed us to better examine differences in Bd prevalence among habitat types in the region. The majority of Bd swabs collected by KLGO staff during 2005–2017 came from < 10 sites. 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.

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 (Figure 5a). Swabs were air-dried for 30 min and stored in microtubes (2005–2006) or were stored 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 result for Bd infection.

To estimate how Bd prevalence for toads varied based on life stage, habitat characteristics, and over time in the KLGO area (Figure 5b), 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, an area term (Taiya River Valley vs. other) that served as a proxy for single- vs. multi-host systems (Table 15). 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 (e.g., < 1000 m elev.; Figure 5b) in the KLGO area, where surveys were concentrated (Surdyk and Waldo 2018; http://vertnet.org). Columbia spotted frogs are present at high elevations, and wood frogs occur throughout the sampled area except for most of the Skagway and 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 15).

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 three 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.

6   Amphibian Chytrid Fungus: Results and Discussion

Of the 248 boreal toads sampled for Bd from 2005 through 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%; Figure 6a). 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 summer-time 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 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%) (Figure 6b; 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%; Figure 6c; 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 and 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. There has not been any documented breeding in upland/natural habitat in recent years (see Surdyk and 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 (Christensen et al. 2004, Surdyk and Waldo 2018), is concerning. The parallels between frequent habitat use and high Bd prevalence suggest 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% to 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 5d).

This difference suggests 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 one 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 (Figure 5). 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 and Waldo 2018 and prior annual reports referenced therein), which suggests host density is low. Our results, along with samples from boreal toads approximately 30–40 km from our White Pass study (Slough 2009), suggest 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 (Figure 7; χ^2 = 6.483, 1 df, P = 0.011), but there was insufficient evidence to include a quadratic term in the model (z = 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 days 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 and 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. 2019b). Collectively, these results emphasize the need to understand Bd is affecting populations before managers can translate pathogen prevalence into risk and make informed actions.

Table 15. 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.

Site	WGS84 Lat.	WGS84 Long.	Habitat Type	No. Bd+/No. Sampled			
				Adult Boreal Toads	Juvenile Boreal Toads	Columbia Spotted Frogs	Wood Frogs
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	N/A	N/A	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
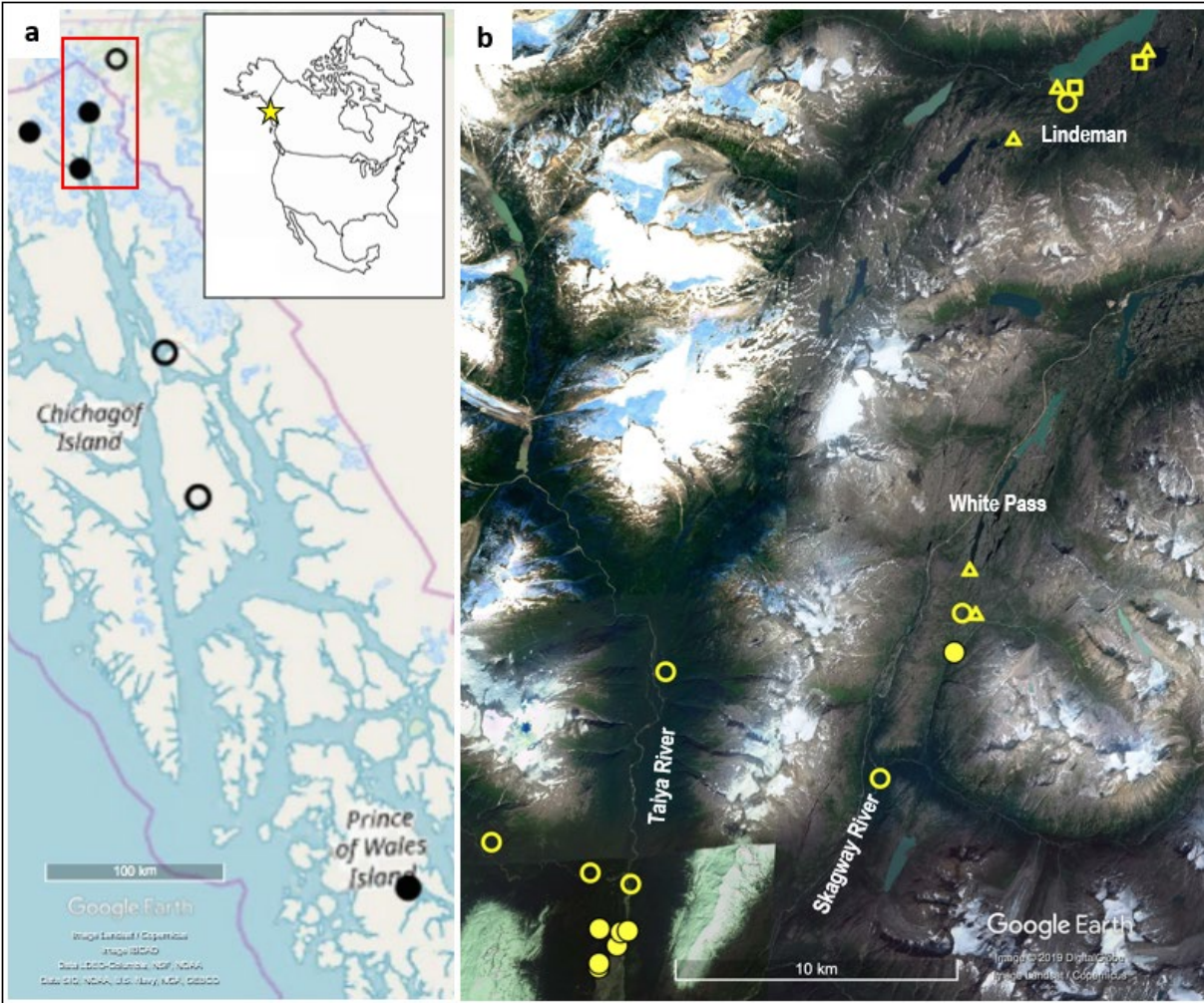
Figure 5. (a) General areas where amphibians were sampled for chytrid fungus (*Batrachochytrium dendrobatidis*; Bd) in southeast Alaska (USA) and northwest British Columbia (Canada) during 2005–2017; and (b) site-level Bd results from 248 boreal toads (*Anaxyrus boreas*; circles), 12 Columbia spotted 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 1a). For both panels, solid symbols indicate Bd was detected.
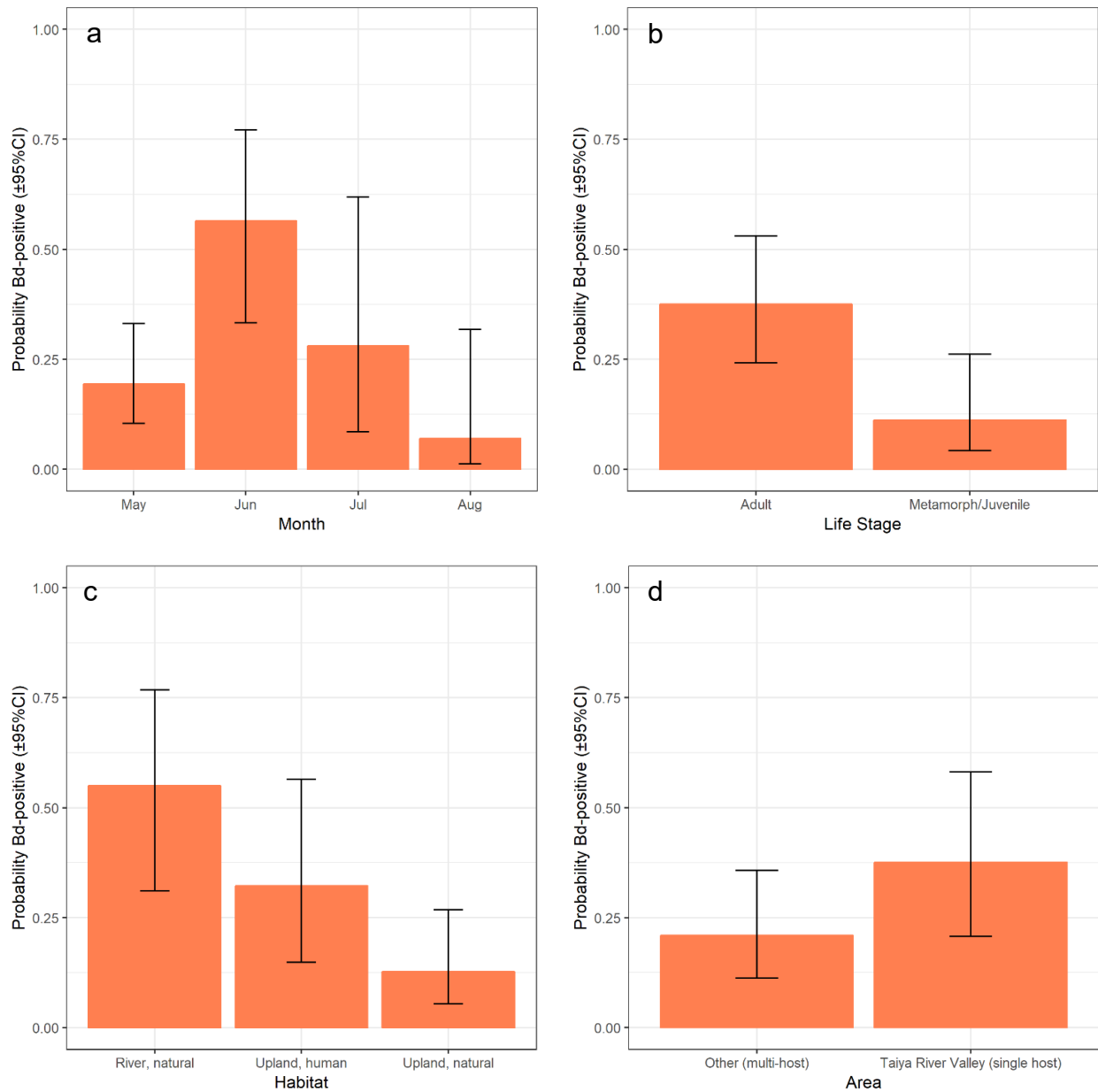
Figure 6. Estimated mean probability (± 95% CI) that boreal toads (*Anaxyrus boreas*) tested positive for amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; Bd) according to (a) month sampled, (b) life stage, (c) habitat type, and (d) whether the toad was from the mostly single-host Taiya River Valley area (Alaska, USA) or a multi-host community elsewhere in the study area. All estimates are marginal means from the habitat model.
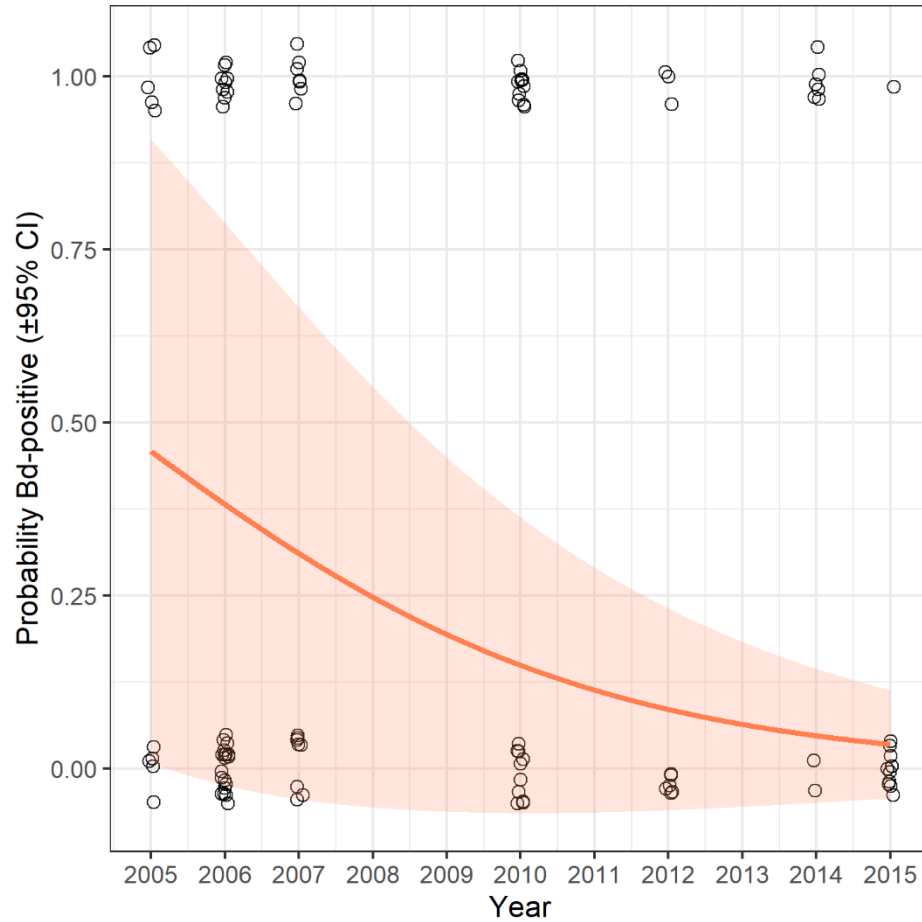
Figure 7. Model-estimated trend (2005–2015; ± 95% CI) in prevalence of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; Bd) on boreal toads (*Anaxyrus boreas*) in the Klondike Gold Rush National Historical Park area. Each open circle represents a positive (1) or negative (0) detection of Bd. The trend estimate is the marginal mean after accounting for variation in sampling date, life stage, and habitat.

7 References

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Appendix 1. Example data sheet

(modified from the Cooperative Amphibian Monitoring Protocol for the Greater Yellowstone Network)

Site Code:_____   Recorder: _____

Date: _____   Arrival time: _____ Survey? YES   NO

Why Not Surveyed?   Dry   Too shallow   Slope/Seep   Thermal   Hazard   Other

New Site?   YES   Easting: _____   Northing: _____   NAD 27 or 83

First Survey   Searcher(s)_____ Start Time: _____

Perimeter Searched %:   1 – 25%;   26 – 50%;   51 – 75%;   76 – 99%;   100%

End Time: _____   Total Minutes of Search: _____

****

Weather   Air Temp (˚C): _____   Water Temp (˚C): _____

Skies: Clear (0 -10% cloud cover); Partly Cloudy (11 – 80% clouds);   Overcast (>80% clouds)

Winds: None   Light   Moderate   Strong   Wind Speed (m/s): _____

Precipitation during survey: None   Rain   Snow/hail

****

Habitat   Type: lake/pond/pool;   marsh/wet meadow;   stream/backwater

Beaver Pond?   YES   NO

Maximum Water Depth (m):   ≤0.5;   0.5 – 1.0;   1.0 – 2.0;   >2.0

Wetland Length (m): _____   Wetland Width (m):_____

GPS Wetland Area (m²): _____ (preferred measurement)

Fish Detected?   YES   NO

%Shallows (< 0.5 m deep):   0;   1 – 10;   11 – 25;   26-50;   51 – 75;   76 – 100

% Aquatic Vegetation Cover: 0;   1 – 10;   11 – 25;   26-50;   51 – 75;   76 – 100

Dominant Aquatic Veg: sedge/rush/grass;   bulrush/cattail;   pond lily;   shrubs;   other

Dominant Aquatic Substrate: mud/silt/veg;   sand;   gravel;   cobble;   boulder/bedrock

****

Second Survey   Searcher(s)_____ Start Time: _____

Perimeter Searched %:   1 – 25%;   26 – 50%;   51 – 75%;   76 – 99%;   100%

End Time: _____   Total Minutes of Search: _____

Site Code:_____   Date: _____

Survey No.	Species	Life Stage	Notes

Species:
ANBO = Boreal Toad; RALU = Columbia Spotted Frog; LISI = Wood Frog; define others as needed

Life Stages:
EGG = egg (masses); LAR = Larva (tadpoles); MET = Metamorph; JUV = Juvenile; ADU = Adult

Notes:

Photo(s):   **Note: List UTMs for new photos, or if different from those provided.**

Frame #: _____   Bearing: _____

Easting: _____   Northing: _____ ± _____ m

Frame #: _____   Bearing: _____

Easting: _____   Northing: _____ ± _____ m

Frame #: _____   Bearing: _____

Easting: _____   Northing: _____ ± _____ m

Appendix 2. Supplementary tables

Table A1. Full results from the power analysis based on a standard fixed design.

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Total Effort	Power at α = 5%	Power at α = 10%	Power at α = 15%	Power at α = 20%
-20%	5	10	2	20	15%	21%	25%	31%
-20%	5	10	3	30	15%	23%	28%	36%
-20%	5	20	2	40	17%	24%	30%	34%
-20%	5	20	3	60	14%	21%	26%	33%
-20%	5	30	2	60	16%	25%	32%	36%
-20%	5	30	3	90	17%	27%	32%	37%
-20%	5	40	2	80	16%	27%	33%	38%
-20%	5	40	3	120	15%	24%	31%	36%
-20%	5	50	2	100	21%	30%	36%	42%
-20%	5	50	3	150	19%	28%	34%	39%
-20%	5	75	2	150	23%	36%	44%	49%
-20%	5	75	3	225	21%	33%	40%	45%
-20%	5	100	2	200	23%	33%	42%	46%
-20%	5	100	3	300	24%	36%	42%	48%
-30%	5	10	2	20	15%	21%	24%	32%
-30%	5	10	3	30	14%	22%	27%	34%
-30%	5	20	2	40	20%	29%	36%	41%
-30%	5	20	3	60	18%	29%	34%	39%
-30%	5	30	2	60	22%	33%	39%	44%
-30%	5	30	3	90	19%	31%	39%	44%
-30%	5	40	2	80	23%	35%	43%	49%
-30%	5	40	3	120	26%	38%	47%	52%
-30%	5	50	2	100	22%	33%	41%	48%
-30%	5	50	3	150	26%	39%	47%	53%
-30%	5	75	2	150	32%	46%	52%	58%
-30%	5	75	3	225	31%	46%	54%	59%
-30%	5	100	2	200	32%	45%	53%	60%
-30%	5	100	3	300	35%	51%	61%	68%
-40%	5	10	2	20	15%	23%	28%	34%
-40%	5	10	3	30	16%	26%	30%	37%
-40%	5	20	2	40	23%	35%	42%	47%
-40%	5	20	3	60	20%	33%	40%	46%
-40%	5	30	2	60	27%	37%	46%	52%
-40%	5	30	3	90	26%	38%	46%	55%
-40%	5	40	2	80	30%	42%	52%	58%
-40%	5	40	3	120	33%	46%	54%	61%
-40%	5	50	2	100	32%	44%	53%	59%
-40%	5	50	3	150	36%	50%	59%	64%

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Total Effort	Power at α = 5%	Power at α = 10%	Power at α = 15%	Power at α = 20%
-40%	5	75	2	150	38%	55%	64%	70%
-40%	5	75	3	225	42%	57%	66%	72%
-40%	5	100	2	200	45%	62%	71%	75%
-40%	5	100	3	300	48%	65%	72%	78%
-50%	5	10	2	20	18%	27%	33%	39%
-50%	5	10	3	30	17%	30%	36%	44%
-50%	5	20	2	40	24%	37%	44%	51%
-50%	5	20	3	60	26%	40%	48%	54%
-50%	5	30	2	60	30%	43%	54%	60%
-50%	5	30	3	90	29%	45%	55%	61%
-50%	5	40	2	80	36%	51%	60%	67%
-50%	5	40	3	120	37%	54%	63%	69%
-50%	5	50	2	100	41%	55%	65%	70%
-50%	5	50	3	150	43%	57%	68%	75%
-50%	5	75	2	150	47%	65%	75%	81%
-50%	5	75	3	225	52%	68%	76%	82%
-50%	5	100	2	200	56%	72%	81%	85%
-50%	5	100	3	300	62%	78%	86%	89%
-20%	10	10	2	20	22%	28%	32%	35%
-20%	10	10	3	30	21%	28%	32%	36%
-20%	10	20	2	40	25%	31%	36%	39%
-20%	10	20	3	60	25%	30%	34%	38%
-20%	10	30	2	60	28%	34%	39%	42%
-20%	10	30	3	90	27%	35%	39%	43%
-20%	10	40	2	80	29%	35%	39%	43%
-20%	10	40	3	120	27%	36%	41%	45%
-20%	10	50	2	100	32%	38%	43%	46%
-20%	10	50	3	150	31%	39%	44%	48%
-20%	10	75	2	150	33%	41%	45%	49%
-20%	10	75	3	225	35%	45%	50%	54%
-20%	10	100	2	200	40%	49%	54%	58%
-20%	10	100	3	300	40%	48%	54%	58%
-30%	10	10	2	20	26%	32%	35%	39%
-30%	10	10	3	30	23%	31%	36%	38%
-30%	10	20	2	40	27%	36%	40%	43%
-30%	10	20	3	60	29%	36%	40%	45%
-30%	10	30	2	60	35%	43%	48%	52%
-30%	10	30	3	90	35%	44%	50%	53%
-30%	10	40	2	80	40%	48%	53%	58%
-30%	10	40	3	120	38%	46%	51%	56%
-30%	10	50	2	100	41%	49%	55%	59%
-30%	10	50	3	150	42%	50%	56%	61%
-30%	10	75	2	150	48%	57%	63%	67%

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Total Effort	Power at α = 5%	Power at α = 10%	Power at α = 15%	Power at α = 20%
-30%	10	75	3	225	48%	57%	64%	69%
-30%	10	100	2	200	50%	59%	64%	67%
-30%	10	100	3	300	57%	65%	69%	74%
-40%	10	10	2	20	26%	32%	37%	41%
-40%	10	10	3	30	29%	36%	42%	45%
-40%	10	20	2	40	36%	45%	50%	55%
-40%	10	20	3	60	35%	45%	51%	55%
-40%	10	30	2	60	43%	51%	57%	62%
-40%	10	30	3	90	41%	49%	54%	59%
-40%	10	40	2	80	49%	57%	63%	68%
-40%	10	40	3	120	46%	56%	61%	65%
-40%	10	50	2	100	54%	64%	68%	72%
-40%	10	50	3	150	53%	63%	68%	72%
-40%	10	75	2	150	59%	70%	76%	80%
-40%	10	75	3	225	64%	72%	77%	80%
-40%	10	100	2	200	70%	80%	84%	87%
-40%	10	100	3	300	74%	80%	84%	86%
-50%	10	10	2	20	30%	38%	43%	46%
-50%	10	10	3	30	31%	38%	43%	47%
-50%	10	20	2	40	43%	53%	58%	62%
-50%	10	20	3	60	42%	51%	57%	62%
-50%	10	30	2	60	51%	61%	68%	73%
-50%	10	30	3	90	51%	61%	67%	71%
-50%	10	40	2	80	61%	68%	74%	77%
-50%	10	40	3	120	62%	71%	76%	80%
-50%	10	50	2	100	63%	73%	78%	81%
-50%	10	50	3	150	65%	76%	80%	83%
-50%	10	75	2	150	75%	84%	88%	90%
-50%	10	75	3	225	78%	86%	89%	91%
-50%	10	100	2	200	82%	88%	92%	94%
-50%	10	100	3	300	86%	91%	93%	95%
-20%	20	10	2	20	29%	34%	38%	41%
-20%	20	10	3	30	24%	31%	35%	37%
-20%	20	20	2	40	29%	35%	39%	42%
-20%	20	20	3	60	29%	35%	39%	42%
-20%	20	30	2	60	32%	38%	42%	44%
-20%	20	30	3	90	32%	39%	42%	45%
-20%	20	40	2	80	33%	42%	47%	50%
-20%	20	40	3	120	36%	42%	46%	49%
-20%	20	50	2	100	42%	49%	53%	56%
-20%	20	50	3	150	37%	44%	49%	51%
-20%	20	75	2	150	40%	47%	52%	56%
-20%	20	75	3	225	44%	51%	55%	59%

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Total Effort	Power at $\alpha = 5\%$	Power at $\alpha = 10\%$	Power at $\alpha = 15\%$	Power at $\alpha = 20\%$
-20%	20	100	2	200	47%	54%	59%	63%
-20%	20	100	3	300	49%	56%	60%	63%
-30%	20	10	2	20	30%	35%	39%	42%
-30%	20	10	3	30	31%	37%	42%	46%
-30%	20	20	2	40	38%	43%	48%	51%
-30%	20	20	3	60	39%	46%	49%	54%
-30%	20	30	2	60	44%	51%	55%	59%
-30%	20	30	3	90	44%	52%	56%	58%
-30%	20	40	2	80	46%	55%	61%	64%
-30%	20	40	3	120	49%	57%	63%	66%
-30%	20	50	2	100	50%	57%	62%	66%
-30%	20	50	3	150	54%	62%	66%	69%
-30%	20	75	2	150	59%	67%	71%	73%
-30%	20	75	3	225	64%	70%	74%	76%
-30%	20	100	2	200	66%	74%	78%	81%
-30%	20	100	3	300	69%	75%	79%	81%
-40%	20	10	2	20	35%	43%	47%	50%
-40%	20	10	3	30	35%	44%	50%	53%
-40%	20	20	2	40	47%	54%	59%	63%
-40%	20	20	3	60	49%	54%	58%	62%
-40%	20	30	2	60	57%	63%	69%	72%
-40%	20	30	3	90	58%	65%	68%	71%
-40%	20	40	2	80	63%	68%	73%	76%
-40%	20	40	3	120	62%	69%	74%	76%
-40%	20	50	2	100	65%	72%	77%	81%
-40%	20	50	3	150	68%	75%	79%	81%
-40%	20	75	2	150	79%	85%	88%	90%
-40%	20	75	3	225	81%	86%	89%	90%
-40%	20	100	2	200	84%	89%	91%	93%
-40%	20	100	3	300	87%	93%	94%	95%
-50%	20	10	2	20	44%	51%	55%	59%
-50%	20	10	3	30	40%	47%	53%	56%
-50%	20	20	2	40	56%	62%	67%	70%
-50%	20	20	3	60	57%	64%	68%	72%
-50%	20	30	2	60	70%	76%	80%	83%
-50%	20	30	3	90	69%	76%	79%	83%
-50%	20	40	2	80	74%	80%	84%	86%
-50%	20	40	3	120	77%	82%	86%	88%
-50%	20	50	2	100	81%	85%	88%	90%
-50%	20	50	3	150	81%	87%	90%	92%
-50%	20	75	2	150	91%	94%	95%	96%
-50%	20	75	3	225	91%	94%	96%	96%
-50%	20	100	2	200	95%	97%	98%	98%
-50%	20	100	3	300	96%	98%	99%	99%

Table A2. Full results from the power analysis based on a rotating panel design.

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Rotation	Total Effort	Power at α = 5%	Power at α = 10%	Power at α = 15%	Power at α = 20%
-50%	10	15	2	1	30	35%	44%	51%	55%
-50%	10	15	2	2	30	36%	43%	49%	52%
-50%	10	15	2	3	30	49%	57%	64%	68%
-50%	10	15	2	4	30	47%	57%	64%	68%
-50%	10	15	2	5	30	49%	60%	65%	70%
-50%	10	20	2	1	40	43%	53%	59%	63%
-50%	10	20	2	2	40	36%	44%	50%	55%
-50%	10	20	2	3	40	58%	68%	73%	77%
-50%	10	20	2	4	40	58%	66%	72%	77%
-50%	10	20	2	5	40	56%	66%	72%	77%
-50%	10	25	2	1	50	48%	57%	62%	66%
-50%	10	25	2	2	50	35%	43%	51%	55%
-50%	10	25	2	3	50	64%	74%	79%	83%
-50%	10	25	2	4	50	62%	73%	79%	82%
-50%	10	25	2	5	50	59%	71%	76%	81%
-50%	10	30	2	1	60	51%	60%	65%	69%
-50%	10	30	2	2	60	29%	39%	45%	52%
-50%	10	30	2	3	60	67%	77%	82%	86%
-50%	10	30	2	4	60	63%	73%	79%	84%
-50%	10	30	2	5	60	64%	76%	81%	84%
-50%	10	40	2	1	80	59%	70%	75%	79%
-50%	10	40	2	2	80	42%	54%	62%	67%
-50%	10	40	2	3	80	77%	84%	87%	90%
-50%	10	40	2	4	80	71%	79%	84%	87%
-50%	10	40	2	5	80	74%	83%	87%	90%
-50%	10	50	2	1	100	62%	73%	78%	81%
-50%	10	50	2	2	100	56%	64%	70%	75%
-50%	10	50	2	3	100	81%	87%	91%	93%
-50%	10	50	2	4	100	77%	84%	88%	90%
-50%	10	50	2	5	100	79%	86%	89%	92%
-50%	10	75	2	1	150	75%	84%	88%	90%
-50%	10	75	2	2	150	67%	76%	83%	88%
-50%	10	75	2	3	150	89%	93%	95%	96%
-50%	10	75	2	4	150	87%	91%	94%	96%
-50%	10	75	2	5	150	87%	91%	94%	96%
-40%	10	15	2	1	30	34%	43%	49%	52%
-40%	10	15	2	2	30	23%	28%	32%	35%
-40%	10	15	2	3	30	42%	51%	57%	62%
-40%	10	15	2	4	30	38%	48%	55%	60%
-40%	10	15	2	5	30	39%	49%	55%	60%

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Rotation	Total Effort	Power at α = 5%	Power at α = 10%	Power at α = 15%	Power at α = 20%
-40%	10	20	2	1	40	34%	43%	49%	53%
-40%	10	20	2	2	40	22%	27%	33%	38%
-40%	10	20	2	3	40	48%	56%	63%	67%
-40%	10	20	2	4	40	45%	54%	59%	64%
-40%	10	20	2	5	40	45%	55%	61%	65%
-40%	10	25	2	1	50	41%	51%	57%	60%
-40%	10	25	2	2	50	18%	24%	30%	37%
-40%	10	25	2	3	50	54%	64%	70%	73%
-40%	10	25	2	4	50	49%	59%	65%	70%
-40%	10	25	2	5	50	50%	59%	65%	71%
-40%	10	30	2	1	60	43%	51%	57%	62%
-40%	10	30	2	2	60	13%	21%	27%	33%
-40%	10	30	2	3	60	61%	70%	75%	78%
-40%	10	30	2	4	60	51%	61%	68%	72%
-40%	10	30	2	5	60	52%	62%	69%	73%
-40%	10	40	2	1	80	50%	59%	65%	67%
-40%	10	40	2	2	80	22%	30%	37%	42%
-40%	10	40	2	3	80	66%	74%	79%	81%
-40%	10	40	2	4	80	59%	68%	73%	77%
-40%	10	40	2	5	80	61%	72%	78%	81%
-40%	10	50	2	1	100	54%	64%	68%	72%
-40%	10	50	2	2	100	28%	35%	42%	47%
-40%	10	50	2	3	100	72%	79%	82%	85%
-40%	10	50	2	4	100	63%	71%	77%	82%
-40%	10	50	2	5	100	65%	74%	79%	81%
-40%	10	75	2	1	150	59%	70%	76%	80%
-40%	10	75	2	2	150	39%	47%	55%	60%
-40%	10	75	2	3	150	80%	85%	90%	91%
-40%	10	75	2	4	150	73%	81%	85%	88%
-40%	10	75	2	5	150	74%	80%	85%	88%
-30%	10	15	2	1	30	26%	34%	38%	41%
-30%	10	15	2	2	30	12%	15%	19%	22%
-30%	10	15	2	3	30	33%	40%	45%	50%
-30%	10	15	2	4	30	27%	36%	42%	47%
-30%	10	15	2	5	30	30%	39%	45%	50%
-30%	10	20	2	1	40	30%	36%	41%	44%
-30%	10	20	2	2	40	12%	14%	18%	23%
-30%	10	20	2	3	40	39%	46%	51%	55%
-30%	10	20	2	4	40	32%	41%	46%	50%
-30%	10	20	2	5	40	36%	45%	51%	56%
-30%	10	25	2	1	50	32%	41%	46%	51%
-30%	10	25	2	2	50	7%	10%	13%	18%
-30%	10	25	2	3	50	41%	50%	57%	62%

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Rotation	Total Effort	Power at α = 5%	Power at α = 10%	Power at α = 15%	Power at α = 20%
-30%	10	25	2	4	50	38%	47%	53%	58%
-30%	10	25	2	5	50	37%	48%	56%	61%
-30%	10	30	2	1	60	36%	45%	50%	54%
-30%	10	30	2	2	60	4%	8%	13%	17%
-30%	10	30	2	3	60	48%	55%	61%	66%
-30%	10	30	2	4	60	36%	46%	52%	58%
-30%	10	30	2	5	60	39%	50%	58%	63%
-30%	10	40	2	1	80	36%	46%	51%	55%
-30%	10	40	2	2	80	8%	11%	16%	20%
-30%	10	40	2	3	80	55%	63%	68%	71%
-30%	10	40	2	4	80	41%	50%	57%	64%
-30%	10	40	2	5	80	49%	58%	63%	68%
-30%	10	50	2	1	100	43%	51%	57%	60%
-30%	10	50	2	2	100	9%	14%	19%	23%
-30%	10	50	2	3	100	57%	64%	69%	72%
-30%	10	50	2	4	100	46%	54%	60%	65%
-30%	10	50	2	5	100	49%	59%	66%	70%
-30%	10	75	2	1	150	47%	56%	62%	65%
-30%	10	75	2	2	150	11%	17%	22%	27%
-30%	10	75	2	3	150	67%	73%	78%	81%
-30%	10	75	2	4	150	50%	59%	65%	70%
-30%	10	75	2	5	150	59%	68%	74%	78%
-20%	10	15	2	1	30	24%	30%	34%	37%
-20%	10	15	2	2	30	8%	11%	13%	15%
-20%	10	15	2	3	30	28%	36%	41%	45%
-20%	10	15	2	4	30	21%	28%	33%	36%
-20%	10	15	2	5	30	20%	29%	35%	40%
-20%	10	20	2	1	40	26%	31%	36%	39%
-20%	10	20	2	2	40	6%	8%	11%	12%
-20%	10	20	2	3	40	33%	39%	44%	48%
-20%	10	20	2	4	40	22%	29%	34%	39%
-20%	10	20	2	5	40	24%	33%	39%	45%
-20%	10	25	2	1	50	24%	32%	37%	40%
-20%	10	25	2	2	50	3%	5%	7%	9%
-20%	10	25	2	3	50	34%	41%	46%	50%
-20%	10	25	2	4	50	24%	31%	36%	42%
-20%	10	25	2	5	50	30%	38%	44%	48%
-20%	10	30	2	1	60	28%	35%	40%	43%
-20%	10	30	2	2	60	2%	4%	6%	8%
-20%	10	30	2	3	60	36%	42%	49%	52%
-20%	10	30	2	4	60	22%	30%	37%	41%
-20%	10	30	2	5	60	33%	43%	49%	53%
-20%	10	40	2	1	80	27%	35%	39%	43%

Decline	Time Horizon	Number of Sites Surveyed	Number of Survey Occasions	Rotation	Total Effort	Power at α = 5%	Power at α = 10%	Power at α = 15%	Power at α = 20%
-20%	10	40	2	2	80	3%	5%	7%	10%
-20%	10	40	2	3	80	41%	49%	54%	58%
-20%	10	40	2	4	80	25%	35%	41%	46%
-20%	10	40	2	5	80	34%	44%	52%	57%
-20%	10	50	2	1	100	31%	39%	44%	48%
-20%	10	50	2	2	100	2%	3%	5%	7%
-20%	10	50	2	3	100	47%	55%	60%	62%
-20%	10	50	2	4	100	26%	33%	40%	45%
-20%	10	50	2	5	100	35%	45%	50%	54%
-20%	10	75	2	1	150	32%	40%	45%	49%
-20%	10	75	2	2	150	2%	4%	6%	9%
-20%	10	75	2	3	150	49%	57%	61%	64%
-20%	10	75	2	4	150	32%	42%	47%	53%
-20%	10	75	2	5	150	40%	49%	55%	61%

Table A3. Full results from the power analysis based on the monitoring of sentinel core sites. Power is only shown for α levels of 5% and 20%.

Decline	Time Horizon	Initial Occupancy	Number of Sites Surveyed	Number of Survey Occasions	Total Effort	Power at α = 5%	Power at α = 20%
-20%	10	0.6	8	2	16	35%	48%
-20%	10	0.6	10	2	20	36%	50%
-20%	10	0.6	12	2	24	38%	51%
-20%	10	0.7	8	2	16	39%	50%
-20%	10	0.7	10	2	20	43%	57%
-20%	10	0.7	12	2	24	45%	57%
-20%	10	0.8	8	2	16	44%	56%
-20%	10	0.8	10	2	20	44%	56%
-20%	10	0.8	12	2	24	48%	62%
-20%	10	0.9	8	2	16	49%	60%
-20%	10	0.9	10	2	20	55%	66%
-20%	10	0.9	12	2	24	56%	68%
-20%	10	1	8	2	16	52%	64%
-20%	10	1	10	2	20	56%	68%
-20%	10	1	12	2	24	61%	73%
-30%	10	0.6	8	2	16	45%	56%
-30%	10	0.6	10	2	20	46%	57%
-30%	10	0.6	12	2	24	46%	60%
-30%	10	0.7	8	2	16	46%	58%
-30%	10	0.7	10	2	20	50%	62%
-30%	10	0.7	12	2	24	55%	68%
-30%	10	0.8	8	2	16	56%	68%
-30%	10	0.8	10	2	20	55%	68%
-30%	10	0.8	12	2	24	62%	73%
-30%	10	0.9	8	2	16	60%	72%
-30%	10	0.9	10	2	20	66%	78%
-30%	10	0.9	12	2	24	67%	78%
-30%	10	1	8	2	16	66%	77%
-30%	10	1	10	2	20	71%	82%
-30%	10	1	12	2	24	75%	84%
-40%	10	0.6	8	2	16	48%	61%
-40%	10	0.6	10	2	20	52%	65%
-40%	10	0.6	12	2	24	57%	70%
-40%	10	0.7	8	2	16	54%	68%
-40%	10	0.7	10	2	20	60%	72%
-40%	10	0.7	12	2	24	66%	77%
-40%	10	0.8	8	2	16	64%	75%
-40%	10	0.8	10	2	20	69%	78%
-40%	10	0.8	12	2	24	72%	80%
-40%	10	0.9	8	2	16	68%	80%

Decline	Time Horizon	Initial Occupancy	Number of Sites Surveyed	Number of Survey Occasions	Total Effort	Power at α = 5%	Power at α = 20%
-40%	10	0.9	10	2	20	72%	83%
-40%	10	0.9	12	2	24	79%	88%
-40%	10	1	8	2	16	77%	85%
-40%	10	1	10	2	20	79%	88%
-40%	10	1	12	2	24	83%	92%
-50%	10	0.6	8	2	16	55%	69%
-50%	10	0.6	10	2	20	61%	74%
-50%	10	0.6	12	2	24	63%	76%
-50%	10	0.7	8	2	16	64%	75%
-50%	10	0.7	10	2	20	66%	79%
-50%	10	0.7	12	2	24	75%	84%
-50%	10	0.8	8	2	16	72%	83%
-50%	10	0.8	10	2	20	74%	83%
-50%	10	0.8	12	2	24	79%	88%
-50%	10	0.9	8	2	16	75%	87%
-50%	10	0.9	10	2	20	79%	90%
-50%	10	0.9	12	2	24	85%	93%
-50%	10	1	8	2	16	83%	93%
-50%	10	1	10	2	20	87%	93%
-50%	10	1	12	2	24	90%	95%