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Amphibian Surveys at Klondike Gold Rush NHP

2015 Summary

Natural Resource Report NPS/KLGO/NRR—2018/1655

ON THE COVER

Photograph of an adult boreal toad being measured at breeding site TR01 on June 4th, 2015 by an NPS Biological Science Technician.

Photograph courtesy of the National Park Service/ S. Millard.

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Shelby L. Surdyk and Jami Belt

National Park Service
Department of Natural Resources
Klondike Gold Rush NHP
P.O. Box 517
Skagway, Alaska 99840

May 2018

U.S. Department of the Interior
National Park Service
Natural Resource Stewardship and Science
Fort Collins, Colorado

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Please cite this publication as:

Surdyk, S. L., and J. Belt. 2018. Amphibian surveys at Klondike Gold Rush NHP: 2015 summary. Natural Resource Report NPS/KLGO/NRR—2018/1655. National Park Service, Fort Collins, Colorado.

NPS 461/145404, May 2018

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Abstract

Amphibian monitoring has been conducted annually at Klondike Gold Rush National Historical Park since 2004 with the primary goal to monitor long-term changes in amphibian distribution, abundance, reproduction, and survival at core breeding sites. The Park has two confirmed amphibian species, the boreal toad (*Bufo boreas)* and the Columbia spotted frog *(Rana luteiventris)*; however, toads are the primary focus of monitoring efforts due to their relative abundance in the Taiya River watershed. In 2015, the monitoring season spanned a four month period from May 1 to August 28. A total of 55 routine Visual Encounter Surveys were conducted at eight intensive core breeding sites in Dyea, three intensive non-core sites in Dyea and West Creek, and four extensive sites along the Chilkoot and Laughton Glacier Trails. Breeding activity was observed at three of the core sites. A total of 27 adult boreal toads (4 of which were in amplexus), 7 egg masses, ~ 60,000 tadpoles, 4 juveniles, and ~19,000 of metamorphs were observed. Two western toads were also fitted with radio-transmitters and tracked to identify upland habitat use, hibernation sites, and migration corridors.

Acknowledgments

The fieldwork conducted this year would not have been possible without the support of many dedicated volunteers: Joshua De Guzman, Nicole Kovacs, Julene Brown, Curtis Rowley, Cody Burnham, Courtney Mason, Erica Harris, and others. A special thanks to the SCA interns Kevin Hauser and Shannon Millard, who bush-whacked through 6ft tall Devil's Club with me to search for toads in the West Creek valley! And to Jami Belt, for encouraging me to delve into statistical models and ArcGIS, and for being an all-around great biologist and leader!

I would also like to acknowledge all of the former wildlife technicians who initiated, expanded, and improved the amphibian monitoring program at Klondike Gold Rush NHP and whose research and data are reflected in this report.

Introduction

National Park managers use many indicators to understand and help maintain the integrity of park natural resources. Amphibians are considered good indicators of ecosystem health because of their sensitivity to environmental change. E.O. Wilson, a Harvard biologist said, “We ourselves could not have devised a better early-warning device for general environmental deterioration than the frog,” (2002).Worldwide, 32% of amphibian species are now threatened with extinction while 43% exhibit some form of population decrease (Stuart et al. 2004). Amphibians are far more threatened and declining more rapidly than either mammals or birds, with many amphibians on the brink of extinction (Stuart et al. 2004). A recent study published by USGS scientists and collaborators found that amphibians in the United States are disappearing at a rate of 3.7% each year with the greatest declines observed on National Park Service Lands (Adams et al. 2013). Primary hypotheses to explain global amphibian declines include habitat degradation, climate change, contaminants, and disease as well as other unknown stressors (Adams et al. 2013). Chytrid fungus (*B. dendrobatitis*) is an invasive pathogen responsible for amphibian declines around the globe and was first confirmed in Klondike Gold Rush National Historic Park (KLGO) in 2005.

Efforts to monitor amphibian populations in KLGO are part a worldwide effort over the last twenty years to study and protect amphibian biodiversity (Anderson 2004). In 2000, the National Park Service in Alaska prioritized amphibians as a taxonomic group to be inventoried and monitored. A program was established at KLGO in 2004 and began by surveying wetlands to assess the presence and absence of species. Since then, it has evolved from a purely inventory-based research strategy to a mid-level monitoring effort based on the USGS Amphibian Research and Monitoring Initiative (ARMI). The current objectives are to monitor long-term changes in amphibian abundance, reproduction, and survival at core breeding sites in Dyea, determine the presence and distribution of chytrid fungus, and assess upland habitat use.

Two amphibian species have been confirmed in the Park, the boreal toad, *Bufo boreas*, and the Columbia spotted frog, *Rana luteiventris*. Prior to 2007, with the discovery of Columbia spotted frogs in the White Pass Unit of KLGO, the boreal toad was the only confirmed amphibian species within Park boundaries. KLGO also falls within the expected range of three additional amphibian species, the northwestern salamander, *Ambystoma gracile*, rough-skinned newt, *Taricha granulosa*, and wood frog, *Rana sylvatica* (Alaska National Heritage Program 2001); however, their presence has not yet been confirmed.

Boreal toads, due to their relative abundance in the Taiya River watershed, are the primary focus of amphibian monitoring efforts at KLGO. This species, historically widespread throughout the western United States and Canada, has shown abrupt declines in abundance and distribution through large portions of its range over the last several decades (Hallock and McAllister 2005). In 2003, boreal toads were classified as a species of concern for Southeast Alaska by the Alaska Department of Fish and Game, however they have revised their ranking system since that time and no longer use the “species of concern” classification (Carstensen et al. 2003). In 2003, boreal toads were also recognized as a rare and uncommon species in Alaska by the Natural Heritage Program (AKNHP) and The Nature Conservancy Incomplete information on boreal toad distribution, population size, and habitat range in Southeast Alaska prompted the development of a long-term monitoring protocol for KLGO and adjacent lands (Carstensen et al. 2003). As of 2013, the boreal toad conservation status was classified as “IX. Blue, low status and low biological vulnerability and action need” by the AKNHP Alaska Species Ranking System.

Since 2004, KLGO has monitored boreal toad occupancy and development at known breeding sites; however, effective conservation requires an understanding of habitat use across all seasons and life stages. Toads use shallow water for breeding, but depend on terrestrial habitats to feed and hibernate. Outside of their brief breeding period in late spring and early summer, adults are very difficult to detect as they spend most of the time in upland habitats. Information on the spatial behavior and movement patterns of individuals is necessary for a complete understanding of the species’ ecology and can be obtained using radio-telemetry.

Initiated in 2012, the radio-tracking study at KLGO aims to provide information about the use of upland habitat by boreal toads and locate key habitat elements such as hibernacula, movement corridors and additional breeding locations that are required to facilitate the conservation of the species. Data on upland habitat use can be used to inform the planning process for any infrastructure development or enhancement proposals for Dyea.

Methods

Standard Operating Procedures

The field methods and data handling procedures referred to in this report have been developed as Standard Operating Procedures (SOPs) and are subject to revision as methodology is refined over time. All of the SOPs are available from the Integrated Resource Management Application (IRMA – www.irma.npg.gov). As of this publication date, the current SOPs are:

Standard Operating Procedure 1: Routine Amphibian Survey Field Methods
Standard Operating Procedure 2: Field Preparation
Standard Operating Procedure 3: Hygiene Protocol for Control of Disease Transmission between Amphibian Study Sites
Standard Operating Procedure 4: Collecting Samples for Chytrid fungus testing
Standard Operating Procedure 5: Data Entry, Verification and Editing
Standard Operating Procedure 6: Data Management
Standard Operating Procedure 7: Amphibian Handling Guidelines
Standard Operating Procedure 8: Amphibian Radio Tagging Methods

Visual Encounter Surveys

The amphibian monitoring program at KLGO is organized into intensive and extensive components to focus efforts appropriately given the relative abundance of amphibians. The lower Taiya River and West Creek watersheds, with their multiple western toad breeding sites, have been considered the intensive component (Appendix A1). In this area, core sites (those where breeding has been documented in the past) are visited multiple times throughout each season to monitor toad presence and tadpole development, while non-core sites (those with potential activity) are surveyed less frequently. Over the years, more non-core sites have been reclassified to core sites as breeding activity has been detected.

The extensive component was given a stratified design in which the greater areas of White Pass, the remaining sections of the Upper Skagway River watershed, select areas of the lower Taiya River watershed and the entire upper Taiya River watershed, were divided into Panels 1-4, one to two of which are monitored per given season (Appendix A2). Unlike the intensive component sites, each site within a panel of the extensive component is visited only once per season (SOP 1. Routine Amphibian Survey Field Methods, 1.1.2 Seasonal Monitoring Schedule).

During Visual Encounter Surveys (VES), habitat and amphibian data were collected on hard copy data forms (Appendix D). The VES was performed by visually scanning the shoreline and all wade-able areas, counting or estimating the number of amphibians present, and recording growth and development attributes. Age class was defined according to Carstensen et al. (2003) with some minor changes as follows: metamorph- according to presence and timing at known tadpole locations; juvenile- <45mm SVL; and adult- >45mm. Counts for amplexing pairs, adults, and juveniles are "memory-less," meaning it was assumed that the same individual was never detected twice and that there were no recaptures, as recommended by Nate Chelgren from USGS ARMI in 2005. Numbers for tadpoles and metamorphs are sums from the single survey counts with the greatest estimates.

To reduce the transmission of chytrid fungus or other diseases between study sites, a strict hygiene protocol was followed. All footwear and field gear were disinfected using a diluted bleach solution. The exact procedure is outlined in the SOP 3 of the Amphibian Monitoring Protocol.

The 2015 amphibian monitoring season focused on nine core sites (Table 1), at which a total of 47 routine Visual Encounter Surveys (VES) were conducted from May through August. During active breeding times, sites were visited more often in an effort to detect egg laying and hatching. Additionally, two intensive non-core sites (Table 2) and four extensive sites (Table 3) were surveyed this season.

Table 1. Intensive core sites visited during the 2015 monitoring season. Coordinates are in UTM Zone8 NAD83.

Site Number	Easting (meters)	Northing (meters)
Taiya River 01 (TR01)	0480075	6596476
Dyea 03 (DY03)	0480267	6596890
Dyea 13 (DY13)	0479529	6595772
Dyea 14 (DY14)	0479518	6595625
Dyea 19 (DY19)	–	–
Dyea 33 (DY33)	0480408	6596190
West Creek 02 (WC02)	0479147	6598956
West Creek 04 (WC04)	0491745	6608133
West Creek 03 (WC03)	0475585	6599939

Table 2. Intensive non-core sites visited during the 2015 monitoring season. Coordinates are in UTM Zone8 NAD83.

Site Number	Easting (meters)	Northing (meters)
Lost Lake (LL1)	0478471	6597508
Dyea 02 (DY02)	0480408	6597121

Table 3. Extensive sites monitored in 2015. Chilkoot Trail (Lower Taiya River) sites are located in extensive Panel 3: Basin A. The Laughton Glacier trail and sites along it are located in extensive Panel 4: Basin C. Coordinates are in UTM Zone8 NAD83.

Site Number	Easting (meters)	Northing (meters)
Chilkoot Trail 01 (CT01)	0481566	6606160
Chilkoot Trail 03 (CT03)	0481521	6604689
Chilkoot Trail 11 (CT11)	0480567	6598617
Laughton Glacier (LG) 2015 Pond survey site	0493745	6601348

Site Occupancy Model

To assess the status of boreal toads in KLGO, one or more attributes (state variables) must be selected and measured each year (Scherer, 2011), and ideally compared to a reference condition (Bernatz, 2011). State variables could potentially include population size, breeding productivity (number of eggmasses or clutch size), breeding success (number of metamorphs, or proportion of tadpoles that develop into metamorphs), adult survivorship, or other demographic parameters. However, due to imperfect detection, and the lack of success in conducting a capture-recapture study in KLGO, the usefulness of monitoring such demographic parameters is limited and the results are difficult to interpret (see Results: Visual Encounter Surveys).

Therefore, this year site occupancy (a non-demographic parameter) was selected as a state variable for assessing the status of boreal toads in KLGO. More commonly called Percent Area Occupied (PAO), this was the primary state variable assessed by Carstensen et al (2003) in their study of amphibian habitat use in Northern Southeast, Alaska. The PAO that they reported for boreal toads in Juneau was very low (0.07, pg. 63). It led them to "a bleak prognosis" and a conclusion that "something has eliminated them [the boreal toads] from the majority of their range [in Juneau]" (pg.64).

It is important to note that Carstensen et al (2003) calculated a *naïve* PAO (definitely not saying that these great herpetologists are naïve in the colloquial sense, only that their PAO was naïve in the technical sense, because it was uncorrected for imperfect detectability). The detectability of boreal toads is known to be < 1 (meaning when present, the species is not necessarily detected 100% of the time). So, nondetection at a site does not mean that the species is absent (Mackenzie et al, 2002). MacKenzie at al (2002) coined the term "naïve PAO," referring to a calculation of PAO determined by "number of sites where a species is detected" divided by "total number of sites searched." Because naïve PAOs assume a detectability of 1, they are inherently an *underestimate* of the *actual* PAO.

To address this, MacKenzie and colleagues invented a statistical model and software called PRESENCE for determining the actual PAO (psi). PRESENCE estimates the psi for a set number of *sites* (N) that subjected to a certain number of *surveys* (T), but allows from missing observations. In other words, not all *sites* must be visited during a single *survey* for an unbiased estimate. In their simulation study of the model they found that estimates of psi were reasonably unbiased, particularly for scenarios where sites were visited 5 or more times during a single season and where detection probabilities were greater than 30%. These restrictions work well for KLGO, where most core sites are visited more than 5 times in a summer, and where boreal toad detection probabilities are generally higher than 50% (Scherer, 2011).

This year, PRESENCE (version 9.5) was used to estimate the "actual percent of core sites with boreal toad breeding activity" (psi) for each of the last six years of the amphibian monitoring program (2010-2015). The sites included in the analysis were the 9 core sites where breeding has ever been detected (Table 1).

In order to perform an analysis of the data, detection histories (spreadsheets) were created for each site monitored, and then compiled into a table containing all sites for a single season. The number of surveys in the detection histories that I created was determined by the number of days between the first and last survey of each monitoring season. In other words, 1 day = 1 survey. Hence, the number of surveys (T) varied between years.

For each survey (day of the monitoring season), a site was either visited and breeding was detected (1), visited and breeding was not detected (0), or not visited (-). I chose this structure (1 day time interval) because in our monitoring program not all sites were monitored with the same frequency/intensity. Individual sites were only rarely visited multiple times in 1 day. In the few instances when multiple surveys of one site occurred in the single day it was due to high breeding activity and thus they were lumped into one visit with a positive detection. This structure allows for a covariate analysis of the data in the future, using time as a covariate.

No surveys (days of the monitoring season) were excluded from 2010-2015 analysis, because the first survey during each of those years occurred after the earliest detection of an eggmass in our monitoring program's record (April 21st).

After the detection histories were created, the data from each season 2010-2015 were run as a single season analysis, with a predefined model (1 group, Constant P), without any covariates. "Constant P" means that the detectability of boreal toads was assumed to be the same across sites.

The detection histories for 2010-2015 are located in T:\NRM\Amphibians\2015 Amphibians\Site Occupancy\2015 Detection Histories for Presence.xml.

Chytrid Fungus Testing

Amphibians captured during Visual Encounter Surveys were inspected for signs of *chytridiomycosis*. Symptoms were photo-documented and recorded on the VES datasheet. Additionally, adults and juveniles were opportunistically tested for the presence of the disease-causing agent, chytrid fungus (*Batrachochytrium dendrodatidis*). Following the methods outlined in SOP 4: Collecting Samples for Chytrid Fungus Testing, non-invasive skin swabs were collected from 11 adult boreal toads and 1 juvenile during the 2015 monitoring season. All samples were collected between June 16th and August 7th, and preserved in individual vials of ethanol. At the end of the monitoring field season, this year's samples, along with 8 samples that were collected in 2014, were shipped to Pisces Molecular Lab in Colorado for analysis. More information regarding the analysis procedures can be found in **Appendix B** Chytrid Fungus Test Results: qPCR assay for *B. dendrobatidis*.

Radio-tracking Study

Western toad movements and habitat use were investigated by radio-tracking two adult toads between August 7th and August 24th, 2015. Radio transmitters (Model BD-2, Holohil Systems, Ltd,; Carp, Ontario, Canada) were attached using adjustable plastic belts (Burow et al. 2012) and toads were tracked using a Telonics radio receiver and antenna at least once per week. Each time a transmittered toad was relocated, the condition of both the toad and the belt were assessed, and the toads were released in their exact capture locations. Anatomical data (including weight and sex), habitat data, and GPS coordinates of capture location were recorded on the Amphibian Movement Study Datasheet (Appendix C). All 2015 data was entered into the spreadsheet 2015 Radio Tracking Data (T:\\Resources\NRM\Amphibians\2015 Amphibians\2015 Radio Tracking\2015 Radio Tracking Data.xml), and datasheets were provided to the KLGO Museum Curator to archive. Detailed belt instructions and telemetry methods are outlined in SOP 8: Amphibian Radio-Tagging Methods.

Results

Visual Encounter Surveys

In 2015, breeding activity (defined as the presence of eggmasses, tadpoles, metamorphs, or amplexing adults) was detected at four sites. At one of those sites (DY13) only metamorphs were detected and it is suspected that they migrated up from DY14. Thus only three sites (TR01, DY14, WC04) are thought to have been actually active this year, all of which produced tadpoles that successfully reached metamorphosis. A total of 27adult boreal toads (4 of which were in amplexus), 7 egg masses, ~ 60,000 tadpoles, 4 juveniles, and ~19,000 of metamorphs were observed.

From year to year, there has been huge variation in detection of various boreal toad life stages at intensive sites (both core and non-core), both in terms of abundance and naïve site occupancy. During some years, the abundance of tadpoles and metamorphs was determined by estimating the order of magnitude (ie, "thousands"), rather than by the more precise method of ocular estimation. Thus, it is only possible to compare naïve site occupancy, as opposed to abundance, for all life stages between years. Table 4 shows the percentage of intensive sites surveyed in 2015 where life stages were detected during at least one survey. Figure 1 compares the 2015 data to percentages from previous years. Date of first observed western toad life stage for each year is shown in Table 5.

Table 4. The percentage of intensive sites surveyed in 2015 where each life stage was detected (n=9).

Year	Egg Mass	Tadpole	Metamorph	Juvenile	Adult
2015	30.0%	30.0%	40.0%	10.0%	50.0%

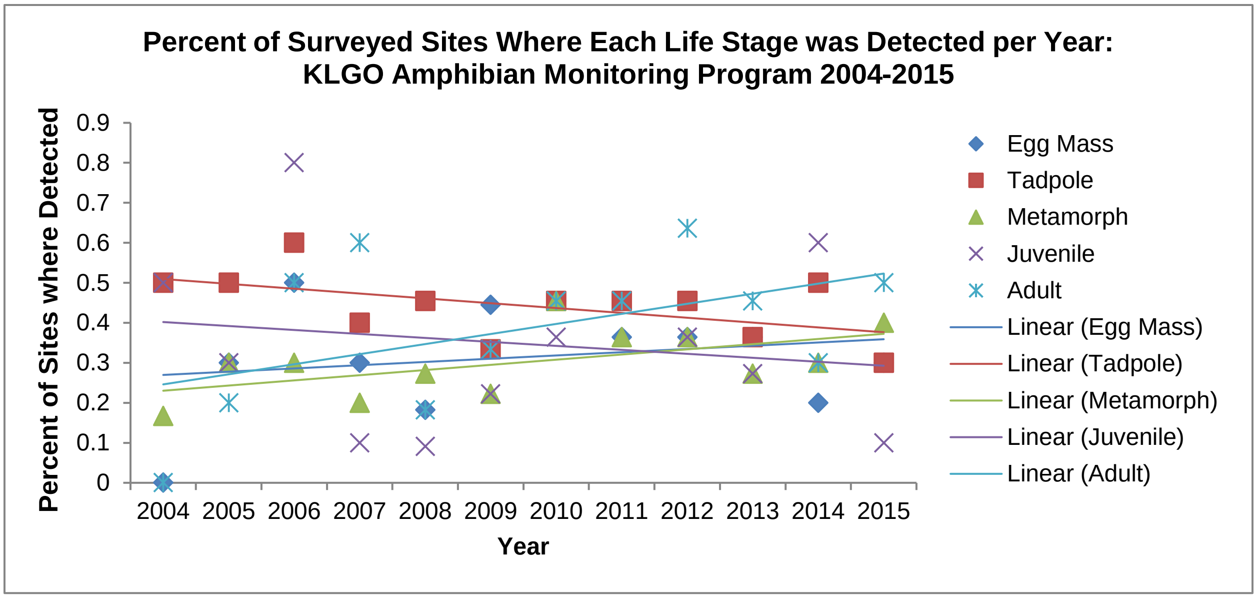


Figure 1. The percentage of intensive sites surveyed per year (2004-2015) where each life stage was detected, with linear regression. Note: no regressions had an r-squared value >0.5.

Table 5. Date of first observed western toad life stage for each year of monitoring at KLGO.

Year	Egg Mass	Tadpole	Metamorph
2004	May 19	Late May	July 1
2005	April 21	May 9	July 6
2006	mid-May	May 30	July 26
2007	May 14	June 5	Aug 15
2008	May 20	June 5	July 31
2009	May 8	May 21	Aug 4
2010	May 3	May 19	June 28
2011	May 11	May 16	July 14
2012	May 14	May 21	July 30
2013	May 9	May 20	July 17
2014	–	May 22*	July 31
2015	May 14	May 20	July 13

*The first survey of the 2014 season was conducted on May 22 and tadpoles were already present at multiple sites.

Individual Site Results

For each site surveyed, a general habitat description and summary of amphibian activity follow.

<u>Intensive Core Sites</u>

Taiya River 01 (TR01)

TR01 South Lobe on June 8th, 2015

Discovered in 2005, this productive breeding site is a wetland adjacent to the Taiya River, approximately 50 m west of the National Park Service campground in Dyea. The series of three primary side channels intermittently receives flow from the Taiya River, causing water levels to fluctuate widely and rapidly throughout the summer in response to temperature and precipitation. Such a regime produces 3 ‘lobes’ (North, middle, and South) that typically maintain some water throughout the season and support extensive areas of *Equisetum* with the pond margins dominated by *Salix* and *Alnus spp*. These aquatic and riparian plants, along with a several others, increase significantly in size, density, and coverage as the summer progresses. In addition to hydrological disturbances, this site is especially prone to disturbances by people, pets, bears, and shorebirds. In previous years the old river braids used as breeding ponds have dried completely, resulting in complete desiccation of eggs and tadpoles. High water levels have also caused the river to flood and expand, ultimately overwhelming the site with turbid glacial runoff and washing away any amphibians inhabiting the site.

Unlike in previous years, this site was not subject to a major flooding event in 2015. This year, TR01 received 8 visits, 5 (63%) of which resulted in positive amphibian detection (Table 6). Evidence of breeding activity was detected, as was the successful development of metamorphs on August 28th.

Table 6. TR01 2015 VES Results

Life Stage	Count
Lone adults	4
Amplexing pairs	0
Total Adults (L+A)	4
Eggmasses	1
Tadpoles	5560
Metamorphs	85
Juveniles	0

Dyea 03 (DY03)

The South pond of DY03 on June 18th, 2015

This shallow, anthropogenic wetland is susceptible to radical changes in water depth and often floods over an old road bed or dries after breeding initiation. At average water levels, this site is composed of four distinct ponds. The northern pond has a thick layer of leaf debris along the shore, but relatively little on the bottom of the pond itself and no emergent or submergent vegetation. The center pond is the shallowest and thus the most vulnerable to water fluctuations. At high water, the north and center pond merge into one, and become water connected to the south pond, which fills a depression to the West decommissioned road. A fourth pond, on the East side of the road is the deepest at approximately 1.5 meters during high water and leaf litter, canopy cover, and depth make it less suitable breeding habitat, but it could be used as a migration area. While water levels fluctuate throughout the season, there are rarely above ground inlets or outlets to the site and the water remains stagnant and warm. In the early season and at low water levels, there are few aquatic emergent plants present. As the season progresses, plants grow and water levels rise, a larger portion of the ponds support emergent vegetation.

In 2015, this site received 9 visits, 3 (66%) of which resulted in positive amphibians detection (Table 7). Breeding activity was **not** detected at DY03.

Table 7. DY03 2015 VES Results

Life Stage	Count
Lone adults	4
Amplexing pairs	0
Total Adults (L+A)	4
Eggmasses	0
Tadpoles	0
Metamorphs	0
Juveniles	0

Dyea 13 (DY13)

DY13 looking West on June 5th

This is the section of Nelson Slough (west branch of the Taiya River) between the old Dyea town site footbridge and the vehicle bridge, south of which it is considered Dyea 14. This slow moving channel is almost entirely filled (75%) with emergent *Carex spp.* with one narrow area of open water along the center of the channel. Changes in the hydrology of Nelson Slough are related to fluctuating water levels and post-glacial rebound. The Dyea flats rise an average of 14-16 mm per year, one of the fastest rates found anywhere (Larsen et al. 2005). Water levels fluctuate from being entirely dry to having complete connectivity with DY14, and visibility and amphibian detection are low due to dense vegetation cover.

In 2015, this site received 5 visits, only 1 (20%) of which resulted in positive amphibian detection (Table 8). Evidence of breeding was *technically* detected when metamorphs were observed throughout the site on August 17th. While it is possible that eggmasses or tadpoles were simply not detected during earlier surveys, it seems far more likely that the metamorphs at DY13 migrated up from DY14.

Table 8. DY13 2015 VES Results

Life Stage	Count
Lone adults	0
Amplexing pairs	0
Total Adults (L+A)	0
Eggmasses	0
Tadpoles	0
Metamorphs	85
Juveniles	0

Dyea 14 (DY14)

DY14 looking South on June 5th, 2015

This is the section of Nelson Slough extending southwest from the vehicle bridge to its confluence with Nelson Creek on the western bank. This slow-moving channel starts out with a dense cover of *Carex spp* and has a substrate comprised of unconsolidated silt. As it nears a large bend, aquatic vegetation disappears almost entirely and a large open pool forms with a substrate consistent with the upstream portion. Moving downstream from the pool, the channel becomes increasingly covered with *Carex spp* as the average depth decreases over fine gravel. This site is part of the larger Taiya

River estuary and thus it is a slightly brackish environment. Water levels are affected by tidal movement as well as glacial melt and rainfall. During high water events, DY14 is connected with DY13 to the north and Nelson Creek to the south. Bears, shorebirds, river otters, salmon and other fish share this dynamic environment with the toad population that has been positively detected at the site every year since the monitoring program began. It remains the most productive breeding site currently monitored at KLGO.

In 2015, this site received 8 visits, 6 (75%) of which resulted in positive amphibian detection (Table 9). Evidence of breeding was detected, as was the successful development of metamorphs on July 15th.

Table 9. DY14 2015 VES Results

Life Stage	Count
Lone adults	3
Amplexing pairs	1
Total Adults (L+A)	5
Eggmasses	5
Tadpoles	54495
Metamorphs	18813
Juveniles	0

West Creek 02 (WC02)

WC02 on June 16th, 2015

Adjacent to West Creek Road, this small wetland, approximately 100 m^2, is a series of small ponds dominated by cotton grass, *Eriophoram spp*, and sedge, *Carex spp*, as well as sphagnum moss. After its initial discovery from aerial photographs in 2005, it was identified as an important breeding site in 2005, 2006, and 2007. One adult western toad was detected in 2009, but breeding activity has not been recorded since the construction of an adjacent road-side ditch, WC04, in 2008. Shading and cooler water temperatures make this site less suitable for breeding such that the population now using WC04 may be the same once utilizing WC02.

In 2015, this site received 6 visits, none of which resulted in positive amphibian detection (Table 10). Needless to say, breeding activity was **not** detected at WC02.

Table 10. WC02 2015 VES Results

Life Stage	Count
Lone adults	0
Amplexing pairs	0
Total Adults (L+A)	0
Eggmasses	0
Tadpoles	0
Metamorphs	0
Juveniles	0

West Creek 04 (WC04)

WC04 on June 16th, 2015

Discovered as a breeding site in 2008 after conducting a survey of WC02, this anthropogenic pool (approximately 50 m^2) is part of the drainage ditch adjacent to the north side of West Creek Road constructed in 2007. This site has a shoreline substrate mainly comprised of fine gravel and large rocks with grass, sedge, and rush species as the dominant vegetation. The bed surface consists of relatively thick (20-50 mm) detritus and algae that provide substantial cover for tadpoles. Unlike the other core sites, WC04 is not directly dependent on the hydrology of the Taiya River and is less prone to abrupt shifts in water levels. When first constructed, WC04 had little or no shading and consistently warm temperatures that possibly made it more suitable as breeding habitat. Over the following year, the alders at the site grew rapidly and began to shade the pool, especially the southern shoreline.

During the first VES in 2015, it was clear that extensive brush-cutting had occurred that Spring along the West Creek road and the site was no longer shaded by alders. Concerned about the impact that future road improvements might have on the productivity of the breeding site, NPS staff met with the Municipality of Skagway's borough manager in June. The borough manager arranged for the director of Public Works to meet with two Natural Resource staff at WC02 the following week. The director agreed to avoid brush cutting along that length of road, and to limit road work in that area particularly in the fall when metamorphs disperse from the natal pond.

In 2015, this site received 7 visits, 6 (86%) of which resulted in positive amphibian detection (Table 11). Evidence of breeding was detected, as was the successful development of metamorphs on July 13th (first metamorphs of the season).

Table 11. WC04 2015 VES Results

Life Stage	Count
Lone adults	9
Amplexing pairs	1
Total Adults (L+A)	11
Eggmasses	1
Tadpoles	1242
Metamorphs	39
Juveniles	4

Dyea 19 (DY19)

DY19 on May 29th, 2015

This large bog is located behind Slide Cemetery and accessed using a conspicuous trail located along the north side of the cemetery fence. It is dominated by emergent buckbean (*Menyanthes trifoliate*) and a fringe of sweet gale (*Myrica gale*). Evidence of toad breeding was observed in 2005 and 2006, but the site was not surveyed in 2009. Juveniles were found in high abundance in 2010 and 2011, indicating undetected breeding activity in the area.

In 2015, this site received only one visit, during which no amphibians, nor evidence of breeding, was detected (Table 12).

Table 12. DY19 2015 VES Results

Life Stage	Count
Lone adults	0
Amplexing pairs	0
Total Adults (L+A)	0
Eggmasses	0
Tadpoles	0
Metamorphs	0
Juveniles	0

Dyea 33 (DY33)

DY33 on June 3rd, 2015

This anthropogenic wetland, owned by the Municipality of Skagway, becomes 2 distinct areas during high water events. A relatively deep (< 2 m), circular pond, created by excavation, lies at the southern end of the site and maintains water throughout the summer. The lack of shallow areas make it seem unsuitable for boreal toad breeding habitat. The larger portion to the north is less convex, with ephemeral water only. In 2009, gravel piles were placed in this site for road maintenance and the shallow depressions that previously supported breeding activity and egg masses were filled in. A productive breeding site in 2004, 2005 and 2006, no evidence of breeding has been detected since then. One dead adult toad, killed by a vehicle, was found at the site in 2011. Vehicles regularly move through this area and it does not appear to be suitable boreal toad breeding habitat but should continue to be periodically monitored for activity as conditions change.

In 2015, DY33 received a total of 4 visual encounter surveys, during which no amphibians, nore evidence of breeding were detected (Table 13).

Table 13. DY33 2015 VES Results

Life Stage	Count
Lone adults	0
Amplexing pairs	0
Total Adults (L+A)	0
Eggmasses	0
Tadpoles	0
Metamorphs	0
Juveniles	0

Panoramic photo of the deep pond at DY33 from July 2015.

Intensive Non-core Sites

Dyea 02 (DY02)

The North end of DY02, looking South, on June 18th. Note the moss covered log on the left where adult boreal toads have frequently been detected.

This shallow, detritus-laden wetland is dominated by *Carex*, *Equisetum*, and several species of grass in the aquatic zone, with Sitka alder and cottonwood along the edges. Water levels fluctuate from completely dry to nearly two feet of depth during high water. No physical evidence of western toad breeding has ever been observed; however, it appears to be important habitat for juveniles and adults. Juvenile toads were observed in 2005, 2006, 2009, 2010, and 2011, and lone adult toads were seen in 2007, 2010 and 2012. In previous years, site visits were sometimes limited due to high numbers of juveniles in the grass. In 2015, this site received 2 visits, 1 (50%) of which resulted in positive amphibian detection Table 14). Evidence of breeding was **not** detected this year.

Table 14. DY02 2015 VES Results

Life Stage	Count
Lone adults	1
Amplexing pairs	0
Total Adults (L+A)	1
Eggmasses	0
Tadpoles	0
Metamorphs	0
Juveniles	0

Dyea 12 (DY12)

This section of Nelson Creek north of the Nelson Slough foot bridge is a slow-moving stream filled with emergent vegetation and becomes increasingly shaded towards the northern end. In previous years, it was observed that deepest parts of the channel are centrally located and can reach a maximum depth of ~.8m. This year, however, standing water was not observed at the site. Physical evidence of toad breeding has never been observed, but lone adult toads have been seen at this site in the past. In 2015, no surveys were conducted at this site. Based on the large number of metamorphs observed at the North end of DY13 in August, it is suspected that DY12 would also be used as a migratory corridor.

West Creek 03 (WC03)

The Main Complex at WC03 on July 6th, 2015

This is an area of extensive wetlands up the West Creek drainage that can be reached by hiking approximately 2.5 km along the trail at the end of West Creek Road. The site includes a small complex (WC03-SC; approximately 150m x 70m in area) that is separated from the much larger main complex (WC03-MC; approximately 750m x 300m) by a thin band of forest to the northwest (Figure 7). The majority of the water surface area, while relatively exposed in early summer, becomes increasingly enclosed with a dense cover of buckbean (*Menyanthes trifoliate*) with the edges dominated by various grasses, sedges, alder, and willow. Such vegetative phenology makes the detection of amphibian egg and larval stages progressively difficult, especially in the vast expanse of the main complex where approximately 70-80% of the area is covered by lentic bodies of water. Evidence of western toad breeding has been observed at this site in the past. Several juveniles, an adult and tadpoles were found in the small complex in 2008, 2012, and 2014.

In 2015, only the main complex was surveyed on one occasion, and no amphibians, nor evidence of breeding, were detected.

Lost Lake (LL1)

This is a bedrock controlled lake with steep sides and very few shallow areas. The southern end is shallow with an area of buckbean similar to DY19. Amphibians have never been detected at this site. LL1 was surveyed once on June 20, 2015 and no amphibians, nor evidence of breeding, were detected.

<u>Extensive Sites</u>

Laughton Glacier Trail - Panel 4: Basin C

Located in the upper Skagway Valley, the Laughton Glacier Trail is accessed via the White Pass & Yukon Route railroad at mile 14. The three mile trail leading to the glacier is lined with many small wetlands and pools which may be suitable for amphibian habitat. Over the years, many local guide services have reported seeing Boreal Toads along the trail. One adult was captured and PIT tagged in 2005, and another was captured and swabbed for chytrid in 2012. Evidence of breeding has never been detected in the Laughton Glacier valley.

In 2015, the entire length of the Laughton Glacie trail was surveyed on June 9th, and surveyors stopped to look under every board walk along it. One pool of standing water was found within 1 mile of the trail head and the entire shoreline was searched (see Table 3 for GPS coordinates). No amphibians, nor evidence of breeding, were detected during this official survey.

Chilkoot Trail 1 (CT01) Panel 3:Basin A

CT01 looking North (right), and CT01 looking South (left) on June 27th, 2015.

This shallow pond is located on the east side of the trail between Canyon City and Finnegan's Point. In previous years this pond was filled with swamp horsetail (*Equisetum fluviatile*) and was heavily overhung with Sitka alder and black cottonwood. At the time that it was surveyed in 2015, the site was relatively deep with very little emergent vegetation. There did not appear to be any flowing water.

In addition to one VES, two woody debris transects were conducted near this site on June 27th. No amphibians, nor evidence of breeding, were detected either during the VES or woody debris transects.

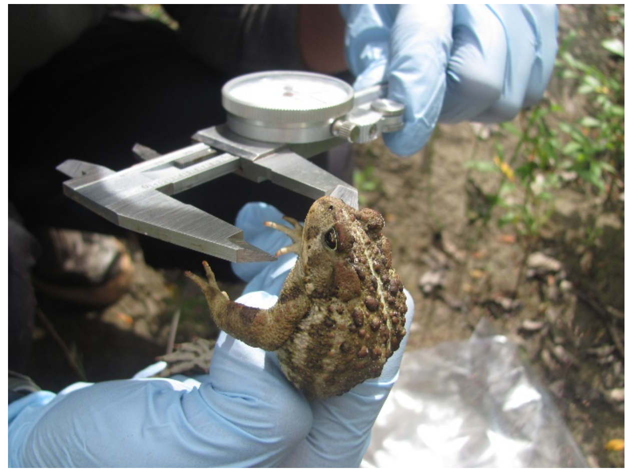
Chilkoot Trail 3 (CT03) Panel 3: Basin A

CT03 looking South (right), and evidence of beavers at CT03 (left) on June 28th, 2015.

This large wetland area South of canyon city is reminiscent of the Beaver Ponds (CT07). Located West of the trail, the turbidity of the downstream portion of this pond indicates that it is connected to the Taiya River. No amphibians were detected during our visits.

In addition to one VES, two woody debris transects were conducted near this site on June 27th. No amphibians, nor evidence of breeding, were detected either during the VES or woody debris transects.

Chilkoot Trail 11 (CT11) Panel 3: Basin A

CT11 looking West towards the Chilkoot Trail (right), and volunteer Josh De Guzman measuring one of two adult boreal toads found at CT11 (left) on June 26th, 2015.

CT11 is a wide open sand flat which is an old river braid that can receive river water flow during periods of high water. The lack of cover for much of the shore, the regular presence of spotted sandpipers , and the sand substrate on this uplift pond is reminiscent of TR01, a consistently productive breeding site in Dyea. CT11 can be reached with a short bushwhack from the raft put-in spot, 1.6 miles down the Chilkoot Trail. The bank starts to undercut downstream of the put-in spot so wading just offshore is not an option. When traveling North on the trail, after descending the first hill, Saintly Hill, there is a spot where the trail opens up to the river bank is just after a bridge. This spot is a viewpoint used by raft guides for interpretation, and affords a view of the site.

In 2015, this site received two visits. During the first visit on June 26th, two adult boreal toads were detected and swabbed for chytrid. A follow-up visit was conducted on July 31st to try and detect evidence of breeding, but no amphibians were found.

Site Occupancy Model

Tables 15, 16, and 17 present the results of the site occupancy analysis performed with PRESENCE.

Table 15. The estimated true proportion (psi) compared to the naïve estimate of core-sites used by boreal toads for breeding, 2010-2015.

Year	Naïve estimate of proportion of core-sites used by boreal toads for breeding	Estimated proportion (psi) of core-sites used by boreal toads for breeding	Stn. Error	95% confidence interval
2015	0.444	0.5235	0.1923	0.1962-0.8338
2014	0.625	0.6289	0.1722	0.2852-0.8780
2013	0.5	0.5379	0.2204	0.1700-0.8687
2012	0.5556	0.628	0.1831	0.2665-0.8870
2011	0.6667	0.6729	0.1586	0.3339-0.8941
2010	0.5556	0.5848	0.1722	0.2598-0.8497

Table 16. Estimated probability of detecting breeding by boreal toads, constant between sites but varying between years, 2010-2015.

Year	Estimated probability of detecting breeding by boreal toads	Stn. Error	95% confidence interval
2015	0.6233	0.0937	0.4309 – 0.7833
2014	0.7408	0.075	0.5707-0.8600
2013	0.8899	0.0731	0.6520- 0.9721
2012	0.7335	0.0657	0.5875- 0.8417
2011	0.8299	0.0431	0.7284- 0.8988
2010	0.7481	0.0427	0.6558- 0.8223

Table 17. Number of boreal toad breeding occupancy surveys of each site per year.

Year	DY03	DY13	DY14	DY19	DY33	TR01	WC02	WC03	WC04
2015	9	5	8	1	2	8	6	1	7
2014	6	9	11	3	0	7	5	1	8
2013	6	1	5	0	0	9	1	0	5
2012	10	6	10	1	3	14	1	1	13
2011	22	19	27	10	10	32	8	2	16
2010	25	20	21	7	5	26	7	1	20

Chytrid Fungus Testing

Of the 12 skin swab samples collected in 2015, only one tested positive for the presence of chytrid fungus, for a detection rate of **8.3%**. Of the eight samples collected in 2014, six tested positive, for a detection rate of **75.0%**. Out of the seven sample sets from KLGO analyzed by Pisces Molecular since 2005, the sample set from 2014 had the highest rate of positive chytrid fungus detection, and 2015 had the lowest (Figure 2).

Individual results from samples collected in 2014-15 (shown as total *B.d.* target copies in the original sample) are located in T:\\NRM\Amphibians\2015 Amphibians\Chytrid\2015 Results from Pisces\ JB Aug 2015 Bd qPCR S-T-U Report 8-27-15.xls. Maps showing the variation in rate of chytrid fungus detection between amphibian monitoring sites (using all Pisces results from 2005-2015) are presented in Appendix A3.

No amphibians captured in 2015 appeared to display symptoms of *chytridiomycosis*.

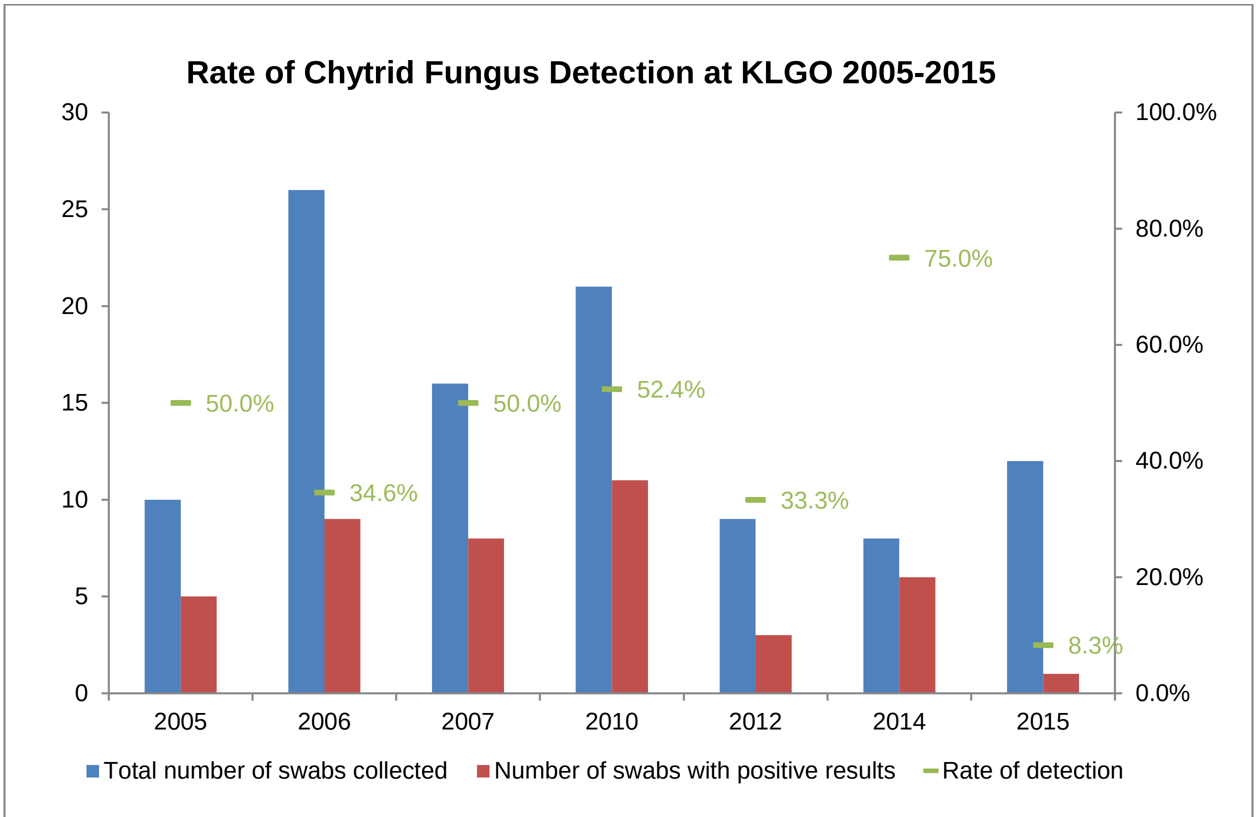


Figure 2. The total number of chytrid swab samples collected from boreal toad at KLGO has varied greatly between years (min.= 8, max.= 26), as has the rate of positive detection of chytrid fungus.

Radio-tracking Study

The first toad to be fitted with a transmitter this year was captured at the core breeding site DY03 during a routine VES on August 7th. Eight days later, the same individual (transmitter number: 169327; Table 18) was radio-tracked to a cluster of alders within the DY03 site, 17m from where it was originally belted. This location was recorded as a potential hibernation site, because 169327 was burrowed into the moss and completely covered by dry grass when found. The toad appeared to be in good condition, with no visible skins sores or abrasions caused by the belt. An attempt was made to radio-track 169327 again on August 24th, but the belt had slipped off. The transmitter belt was recovered 28m from the potential hibernation site.

A second toad, found at the core breeding site DY13, was fitted with a transmitter on August 17th. An attempt was made to radio-track the belted toad (transmitter number: 169329; Table 18) on August 24th, but the belt had slipped off. The transmitter belt was recovered 11m from where the toad was originally captured.

A map of radio-tracked boreal toad movements from 2015 is presented in Appendix A4.

Radio-transmitter belt attached to a toad at DY13 (left), a volunteer taking a GPS point of the relocated belt at DY13 (top right), and a volunteer revealing the location of a transmittered toad at DY03 (bottom right).

Table 18. Boreal toads radio-tracked in Dyea.

Capture Date	Location	Transmitter Serial #	Distance Tracked	Sex	Weight	Release Date	Notes
7 Aug 15	DY03	169327	45m	F	85g	24 Aug 15	Belt slipped off
17 Aug 15	DY13	169329	11m	M	50g	24 Aug 15	Belt slipped off

Discussion

Visual Encounter Surveys

Oral history accounts and anecdotal evidence suggest that boreal toads were once abundant in both the Skagway and Taiya River valleys. Elders from both communities have commented that the bottom of ponds were not visible in the spring due to all of the black egg masses and tadpoles (Kalen 2010, Albecker 2010, Fairbanks 2010). The small, isolated toad population in Dyea today may be a small fraction of a once abundant species. Similar population crashes of boreal toads were documented in Juneau, Ketchikan and Haines between 1970 and 1990 (Carstensen et al. 2003).

Anecdotal evidence suggests that amphibians in southeast Alaska may have already experienced a mass mortality event (Green 2010) and now continue to be subject to variation in breeding site occupancy and productivity due to natural metapopulation dynamics (Shmetterling 2009). A wide-spread amphibian die-off in the Dyea and Skagway valleys could have been triggered by many possible factors. The reasons for abrupt population crashes in Southeast Alaska are most likely a combination of habitat loss, climate change, increased UV-B radiation, introduction of predators (such as stocked fish in the Skagway Valley) and the pathogenic chytrid fungus. The Dyea valley also experienced a major flooding event in 2003 when the West Creek glacial moraine gave out.

Toads and metamorphs should continue to be examined for malformations or other indicators of disease and water quality testing could be conducted to assess environmental contaminants that might be responsible. Human activities may have a major impact on current boreal toad survival in KLGO as toads are killed by vehicles while crossing roads and visitors potentially spread pathogens on their footwear or trample newly metamorphosed toads. To mitigate such impacts, community and visitor education should remain an integral part of the amphibian monitoring program.

Site Occupancy Model

After correcting for imperfect detectability, boreal toad breeding occupancy at intensive core sites appears to have less variation between years than when the assuming detectability = 1. This result supports the hypothesis that while toad populations may have suffered a decline in the past, they are now relatively stable. However, the confidence intervals for the psi estimates are quite large. Scherer (2011) expressed concern that our current sample size (number of sites surveyed for occupancy) might be too small to achieve accurate estimates.

Chytrid Fungus Testing

The difference in detection rate between 2015 & 2014 is really interesting, as is the fact that 2014 had the highest rate of chytrid detection since 2005, and 2015 had the lowest of all the sets analyzed by Pisces Molecular. Here are some possible causes of or explanation for these results:

1. **Variation in chytrid prevalence between core sites**

   In 2014, most of the samples came from DY13 & DY14, which have some of the highest rates of chytrid detection (see maps in Appendix A3). In 2015, most of the samples came from WC04, which has had relatively lower rates of chytrid detection.

   The reason that DY14 has the highest prevalence of chytrid prevalence may be because it is the most productive breeding site (higher concentration of amphibians = higher risk of widespread exposure). It may also be because DY14 is the only breeding site that really has flowing water. All of the other core breeding sites are more like ephemeral ponds, and WC04 is an ephemeral pond that is virtually stagnant all summer. Kriger and Hero (2007) found that Bd in Australia is far less prevalent at ephemeral ponds than at permanent water bodies.

2. **Variation in chytrid prevalence between intensive and extensive sites**

   In 2014, all the samples came from core breeding sites. In 2015, 2 samples came from the Chilkoot. Since 2005, 9 chytrid swabs have been collected outside of core breeding sites (including the two from this year), and all have had negative results.

3. **Date of sample collection**

   Chytrid fungus may be more active, and thus more detectable, at particular times of the year. According to a recent publication by Chestnut et al (2014): "In wild amphibian populations from temperate areas, Bd infections appear to follow predictable patterns, with the highest prevalence and intensity in the cooler spring months, and decreasing prevalence in the warmer summer and autumn months, sometimes to non-detectable limits." Just by eye-balling our data, there is no obvious trend, but it might be worth formatting the data to do a little analysis.

4. **Just random variation & small sample sizes**

   But how uninteresting is that?

5. **Contamination between samples in 2014**

   Concern that 2014 samples may have become cross-contaminated comes from the fact that samples from all collection sites were stored in the same ziplock bag together over the winter, as opposed to being stored in separate bags based on breeding sites and sent off for analysis at the end of the monitoring season, as they had been in previous collection years (SOP 4). I informed Pisces Molecular (the lab that performs the analysis) that the 2014 samples had been stored in the same bag, and asked if it was worth processing them. Pisces replied that as long as the samples had been stored in a cool place, out of direct sunlight they were "fine for testing."

It is also worth noting that in 2009, chytrid swabs were sent to the Cornell Lab of Herpetology (instead of Pisces Molecular) for analysis. A girl from Haines was going to school there at the time, and offered do to the analysis for free. According to the 2010 report, all 7 samples came back negative, but "there may have been errors made during the PCR test." I worked as a biotech in 2010, and recall now that Resource Manager that year was suspicious of the 2009 results, which is why we collected swabs again in 2010. It is unknown if the RM questioned the results solely because they were all negative, or if he and Cornell student had had further communication on the subject.

If KLGO is interested in adopting a new chytrid sampling scheme in the future, the biggest challenge is the very low amphibian encounter rates in park. One way to improve encounter rates would be to ensure that the ADFG handling permit is in effect by mid-late April, and that site visits are being made daily to the most active core breeding sites (TR01, DY03, DY13, DY14, WC04) during the month of May. This year, the ADFG permit wasn't in effect until June 1st, so I wasn't able to collect Chytrid swabs before that date. Such a sampling effort may be difficult to implement if an effort at a more comprehensive occupancy study is also implemented. Site visits for occupancy analysis should take place after egg-laying has occurred, where visits for chytrid sampling should occur just before and during the egg laying period, when adults are concentrated at the breeding sites.

Radio-tracking Study

The protection of habitat and prevention of disease are both essential factors in conserving the remaining western toad populations in Dyea and Skagway. However, very little is known about habitat requirements of the toads outside of the short spring breeding season. Knowledge of habitat use is especially important for successful management in Dyea as development and changes to the area may impact the populations during non-breeding times when their presence is often undetected.

Results from the radio-tracking study suggest that toads are dependent on a variety of habitat features. All toads were initially captured in grassy or open areas in close proximity to known breeding sites. They maintained small summer home ranges and often remained in nearly the same location for several weeks. While no toads in 2015 were tracked far from breeding sites, in previous years the toads traveled away from wetland breeding sites and up into forested areas as fall approached. Previous studies found that female toads tend to travel further from breeding ponds and utilize terrestrial habitats more than males (Schmetterling et al. 2008, Bartelt et al 2004).

Browne and Paszkowski (2010) found twenty-nine western toads in one hibernation site in Alberta, Canada. Since toads are known to hibernate communally, the disturbance of one site has the potential to severely impact the remaining population in Dyea. In 2015, adult toads were still active when tracking concluded in August, and no hibernation sites were identified.

Results from the tracking study also indicate that habitat fragmentation can have a significant impact on western toads. In 2014, one toad was found dead on the vehicle bridge and another was last observed traveling toward the Dyea road. In 2015, one radio-tracked toad was captured within 50 ft of a road, and when relocated, the belt (which had fallen off) was even closer to the road.

Because they are so slow moving, toads may be more susceptible to vehicle collision. The multiple habitat requirements of western toads (shallow breeding ponds and upland hibernation sites) make the impacts of fragmentation more severe. Proposed development in the Dyea and West Creek areas would likely further impact the toad population.

Conclusions & Recommendations

Surveys should continue to monitor long-term changes in amphibian distribution, abundance, reproduction, and survival. Monitoring should begin at the start of the breeding season in April when toads can be found in amplexus and eggs are present. Egg masses can often be more easily detected than mobile tadpoles and an early start to monitoring could provide more accurate detections of breeding activity. This might be useful for extensive sites where the probability of detecting toad activity is important given that only single surveys are conducted. The use of a dip net while surveying would also help to enhance tadpole or metamorph detection, especially at sites with poor visibility.

Radio-tracking of toads provides valuable information on upland habitat use and requirements that could be valuable in better understanding the movement patterns of western toads as well as the potential impacts of development. As toads were still active in late September when the study ended, tracking should continue into October if possible in order to locate potential hibernation sites.

Community observations are fundamental to understanding current and historic amphibian distributions throughout the area. Education efforts are also essential in reducing the spread of chytrid or other diseases and mitigating human impacts at sensitive breeding sites. Increased outreach efforts at KLGO should focus on local guide companies, school groups, and visitors.

It would be good to reach out to Josh Reams (president of AK herpetological society), or Sanjay Pyare (herpetologist who helped establish the monitoring program at KLGO), or someone from ARMI, to see what they think of our results and sampling design. It has been a few years since KLGO has had any communication with ARMI regarding the design or results of the AMP.

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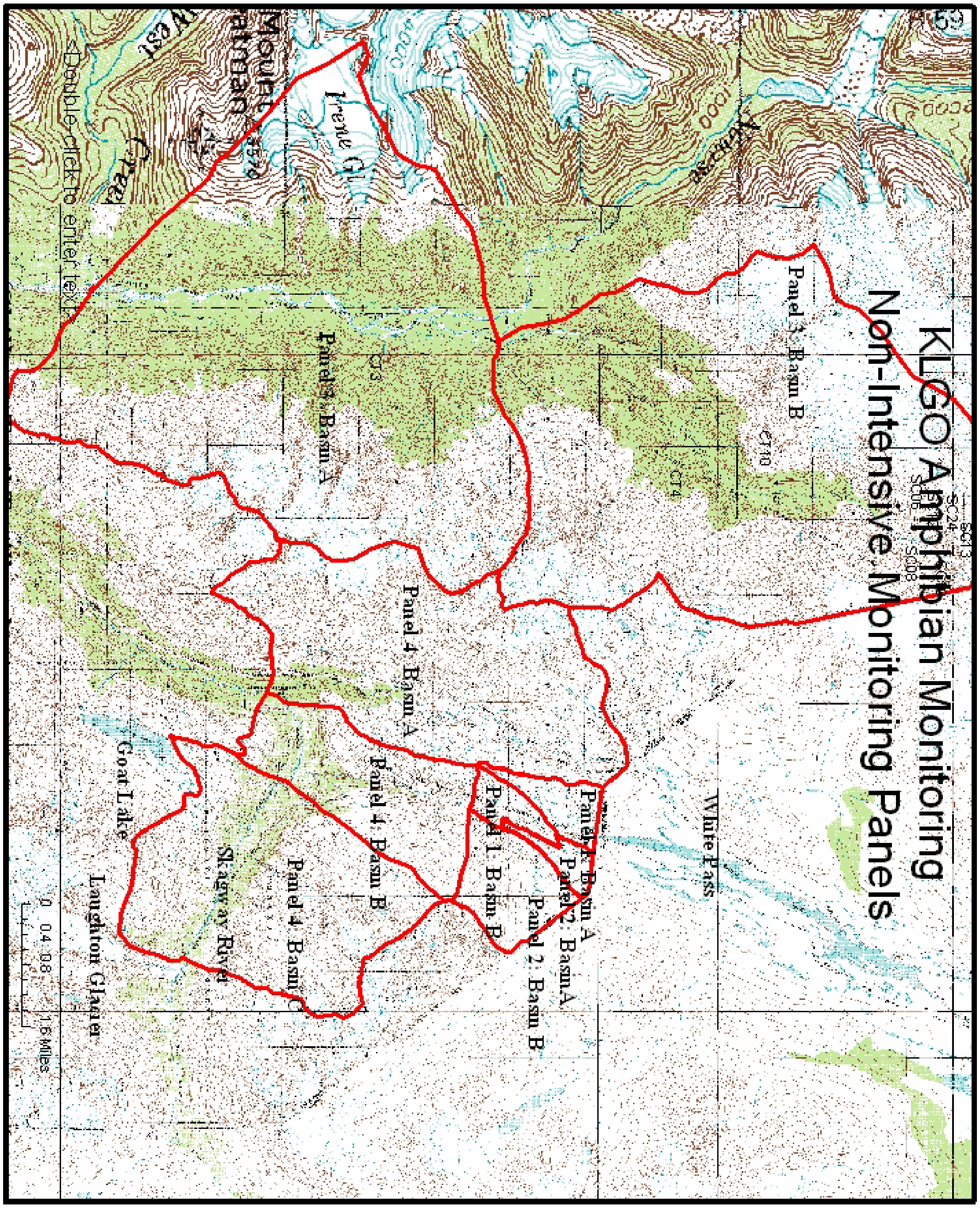
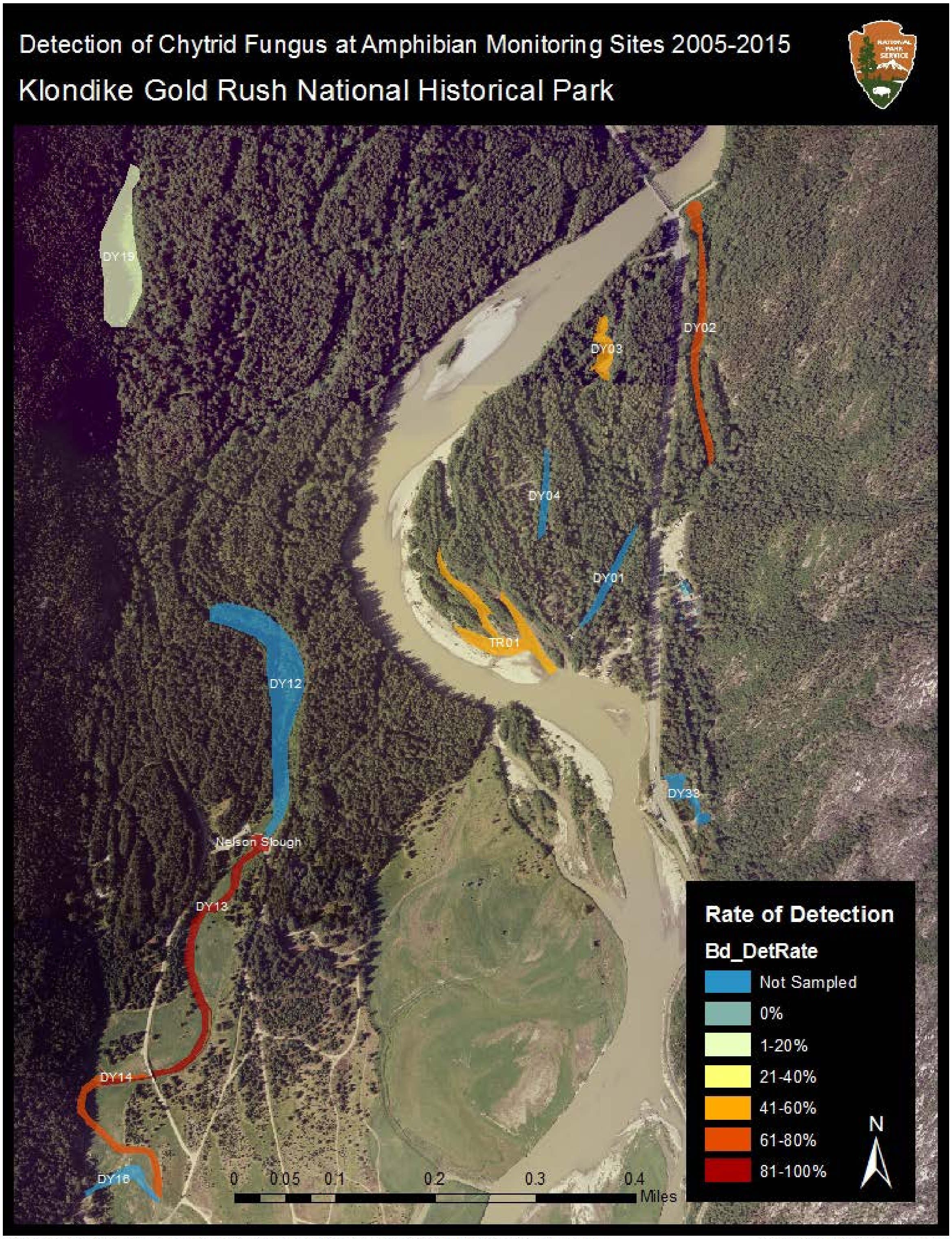
Appendix A. Maps

A1. Intensive Amphibian Monitoring Sites in Dyea

A2. Non-Intensive Amphibian Monitoring Panels



A3. KLGO Chytrid Distribution Maps 2005-2015



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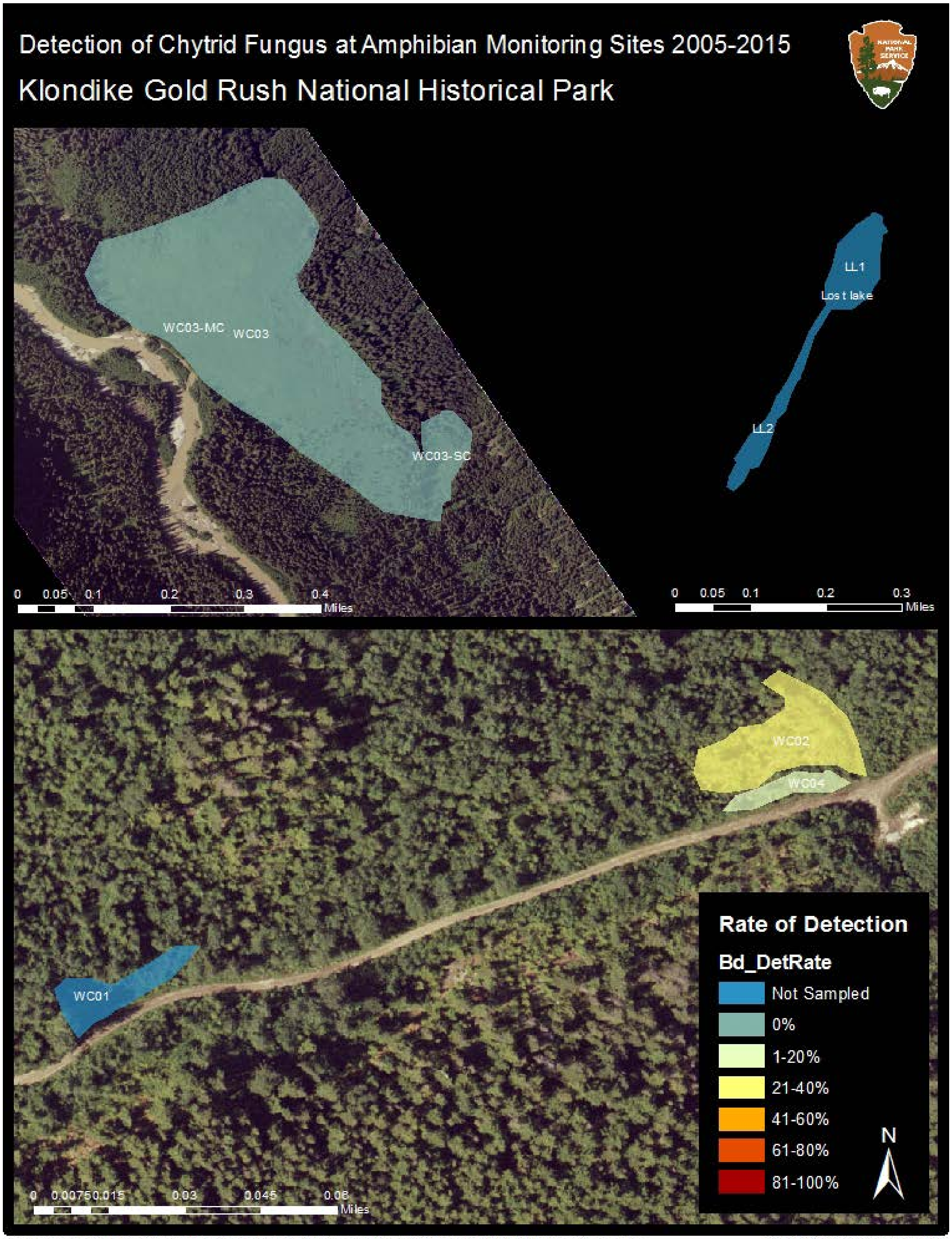
Shelby Surdyk, August 31, 2015



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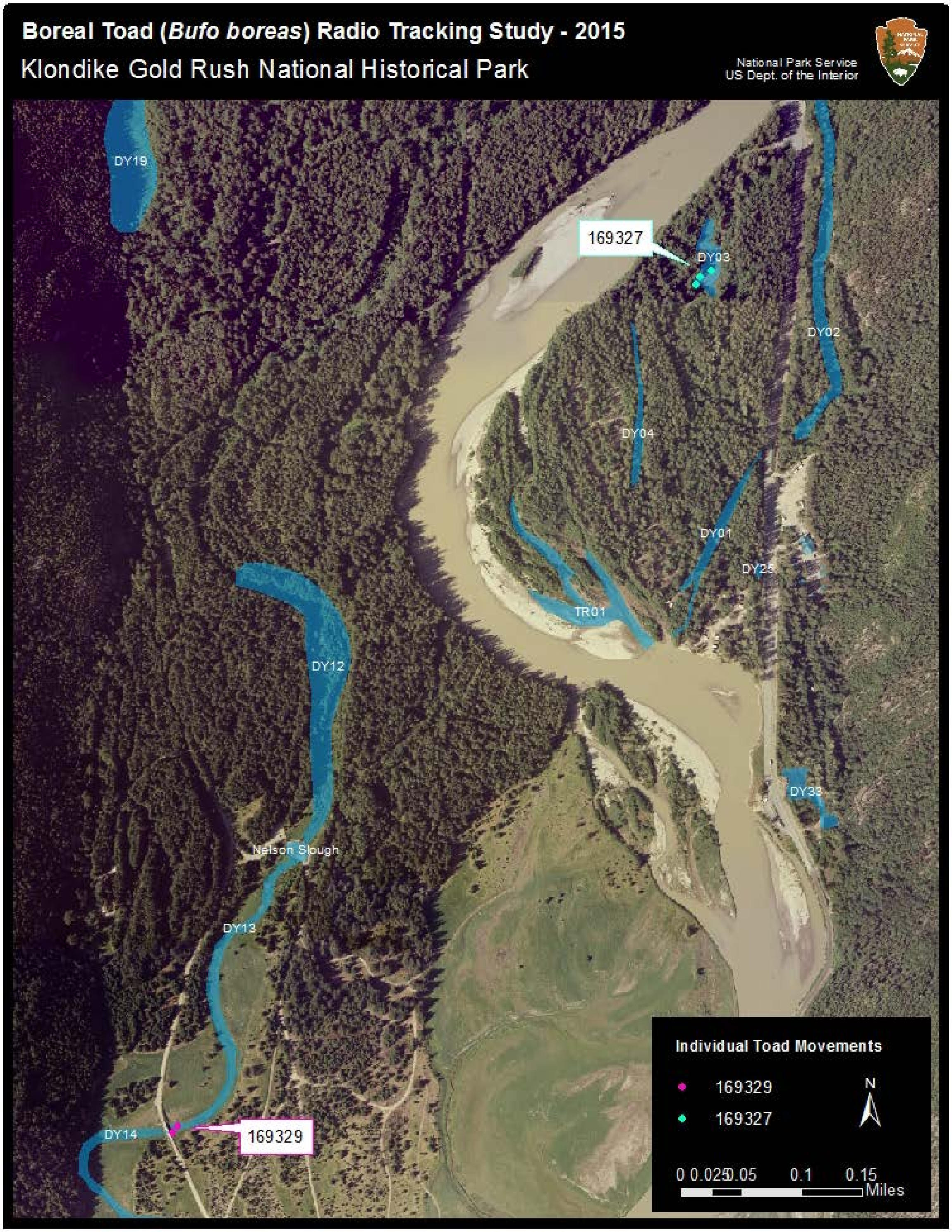
Shelby Surdyk, August 31, 2015

A4. Results of Radio-Tracking Study 2015



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Shelby Surdyk, August 2015

Appendix B. Pisces Molecular Results 2015

Chytrid Fungus Test Results

qPCR assay for B. dendrobatidis

Test samples:

Organization	National Park Service – Klondike Gold Rush National Historical Park
Received From:	Jami Belt
Received Date:	8/14/15
Number of samples:	20
Type/condition of sample(s)	Swab in EtOH
Comments	–

Sample preparation:

The liquid in the skin swab sample was mixed by pipetting the liquid up and down repeatedly. The entire volume was then transferred into individual microfuge tubes. The tube was spun in a microcentrifuge at -16,000 x G for 3 minutes. Next, the supernatant was drawn off and discarded. Lysis buffer was added to the tube and any pellet present was resuspended by vortexing. 10µg of carrier DNA was added to the lysis buffer.

Total DNA was extracted from all samples using a spin-column DNA purification procedure.

qPCR Assay:

The sample DNAs were assayed for the presence of the Batrachochytrium dendrobatidas ribosomal RNA Intervening Transcribed Sequence (ITS) region by 45 cycle PCR amplification using a qPCR assay developed at Pisces and a Stratagene MX4000 real-time PCR instrument. The reaction master mix contains ROX as passive reference dye for normalizing variations in individual reaction total volumes, and a VIC-labeled internal positive control (IPC) (Life Technologies TaqMan® Exogenous Internal Positive Control, catalog #4308323) to detect PCR inhibition. The detection sensitivity of this assay is three target sequence molecules (approximately 0.02 zoospore equivalents).

Each PCR run included the following controls:

Positive DNA: DNA prepared from a plasmid constructed at Pisces containing the B. dendrobatidis ribosomal RNA Intervening Transcribed Sequence (ITS) region. Serial ten-fold dilutions of this plasmid DNA from 4.2 x 10^6 to 4.2 x 10^0 molecules per reaction were used to generate the standard curve.

No DNA: H2O in place of template DNA. This reaction remains uncapped during addition of sample DNA to the test reactions, and serves as a control to detect contaminating DNA in the PCR reagents or carryover of positive DNA during reaction set-up.

Summary qPCR Results for the B. dendrobatidis ribosomal RNA ITS region:

Parameter	Result
Number of positive (+) samples:	7
Number of negative (-) samples:	13

Results are shown as total B. d. target copies in the original sample in the attached excel spreadsheet file.

Appendix C. Amphibian Movement Datasheet

Amphibian Movement Study Datasheet	
Observer(s):	
Date:	
Start Time:	
End Time:	
Animal Identification Information	
Animal ID (Transmitter Serial #) :	
Radio Frequency:	
Attachment Date:	Total Days:
Weight (g):	Sex: Male Female Unknown
Belt/skin Condition: (Skin sores present?)	
Chytrid Swab?	
Location	
GPS Coordinates:	
Last Location:	
Habitat Pick one that best describes the animals location	
Large Woody Debris Human Made Structure Rock Formation Moss Bare Ground Other ______________________	Cut Bank/Soil Leaf Litter Grass Shrubs Water
% Covered When Located: (0% = fully visible, 100% = not visible at all)	
Closest Known Breeding Site:	
Other Toads Present?: Yes No	How many?
Possible Hibernation Site? : Yes No	

Appendix D. Routine Amphibian Survey Form

Routine Amphibian Survey Form		Observer(s)
Date	Time Start	Time at Site Arrive:
Pond ID	Time End	Leave:

Air Temp.	Ripples none some significant
Precip dry lt rain heavy rain snow/sleet	Wind calm light mod heavy
Clouds clear partially cloudy overcast	Surface Glare none some significant
Precip last 2 days? none, fog, light rain, medium rain, heavy rain, snow	

Habitat	Water level high average low dry	
Clarity clear stained	Turbidity clear cloudy	iron floc absent present severe
Sheen none organic petro	Pictures? Yes No	Picture #'s:
%Shallow	% surface area w/ emergent vegetation	
% bottom cover by submergent veg	% surface area w/ floating vegetation	
% shoreline covered by snow	% surface area covered by snow	

Water Temp	loc'n	Temp Logger deployed collected
Water Temp	loc'n	Under water? Yes No Light?
Water Temp	loc'n	Temp Time
Water Temp	loc'n	
Water Temp	loc'n	
Water Temp	loc'n	

Amphibian Search	Full? Yes No	% Shore Searched	
Connection inlet outlet both neither		Recent disturbances?	
% bottom visible	Fish Present? Yes No		
Fish Species		Other Predators?	
Survey Method visual hand ID boards traps net sweep			
Amphibian Species Seen (if any seen, complete back of form also)		Where?	

Comments:

Location within Site:							
Individual ID (e.g. A1,J2)							
SVL (mm)							
Gape (mm)							
Forearm (mm)							
Weight (g)							
Nuptial pads?							
Calling?							
Tag? (Y/N)							
Swab?(Y/N)							

Egg Masses and Larvae

Location within Site:							
Individual ID (EM1, L1)							
Estimated # in egg mass							
Estimated total # larvae							
Tot. Length							
Body Length							
Gosner stage							

Recaptures

Individual ID	Tag #	Swab ID

NPS 461/145404, May 2018

National Park Service
U.S. Department of the Interior

Natural Resource Stewardship and Science
1201 Oakridge Drive, Suite 150
Fort Collins, CO 80525

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