



# Amphibian Surveys at Klondike Gold Rush NHP

## *2017 Summary*

Natural Resource Report NPS/KLGO/NRR—2018/1587



**ON THE COVER**

An amphibian breeding pond on the Canadian portion of the Chilkoot Trail, June 23, 2017 (NPS/ JEN LARSEN)

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# Standard Operating Procedures (SOPs)

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## Abstract

Amphibian monitoring has been conducted annually at Klondike Gold Rush National Historical Park since 2004 with the primary goal of monitoring long-term changes in amphibian distribution, abundance, reproduction, and survival at core breeding sites. The Park has two confirmed amphibian species, the Boreal Toad (*Anaxyrus boreas*, formerly *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 2017, the monitoring season spanned about three and a half months from May 31 until September 12. A total of 45 routine Visual Encounter Surveys were conducted at eight intensive core breeding sites in Dyea and West Creek, six intensive non-core sites in Dyea, West Creek, Lost Lake, and the Chilkoot Trail, and five extensive sites on the Chilkoot Trail and in the White Pass Unit. Breeding activity was observed at five of the eight core sites. A total of 46 adult Boreal Toads were sampled, two of which were in amplexus, were observed.

## **Acknowledgments**

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We 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,” (Wilson 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 (NPS) 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 (*Batrachochytrium 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.

Two amphibian species have been confirmed in KLGO: the Boreal Toad, *Anaxyrus boreas boreas* (formerly *Bufo boreas*)<sup>1</sup>, 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 the wood frog, *Lithobates sylvatica* (formerly *Rana sylvatica*) (Lenz et al. 2002); however, its presence has not yet been confirmed.

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. Between 2001-2003, an effort was led by the Inventory and Monitoring Program (I&M) of the Southeast Alaska Network (SEAN) in the NPS to document opportunistic observations of amphibians in parks in Alaska (Anderson 2004). The project confirmed the presence of amphibians in 10 of Alaska’s 16 parks, and the presence of five species in the state (Anderson 2004). KLGO contributed 22 observation records to the I&M project, and the Park was identified as an important

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<sup>1</sup> In the 6<sup>th</sup> edition of “Scientific and Standard English names of Amphibians and Reptiles of North America, North of Mexico, with comments regarding confidence in our understanding”(Crother et al. 2008), the Society for the Study of Amphibians and Reptiles recognized two nominal subspecies Western Toad (*Anaxyrus boreas*): Boreal Toad (*A. b. boreas*), and Southern California Toad (*A. b. halophilus*). However, in the 7<sup>th</sup> ed. (Boundy et al. 2012), it was speculated that the species *A. boreas* may be a taxonomically erroneous, containing in fact a complex of distinct species. Adhering to the species account published Amphibian Declines the Conservation Status of United States Species (Lannoo 2005), Western Toads documented in KLGO as part of the Amphibian Monitoring Program have been recognized as the subspecies Boreal Toads (*A. b. boreas*), and will be referred to as such in this report.

Boreal Toad<sup>2</sup> breeding area in the I&M project report (Anderson 2004). A formalized amphibian monitoring program was established at KLGO in 2004, which began by surveying wetlands to assess the presence and absence of species (Payne 2004). The following year, KLGO partnered with the U.S. Geological Survey's (USGS) Amphibian Research and Monitoring Initiative (ARMI) to implement a mark-recapture study at Boreal Toad breeding sites in Dyea (Payne 2005). In 2006, KLGO continued the mark-recapture study and became an ARMI Apex-level monitoring site. According to the ARMI program design, apex-level monitoring sites involve population estimates, demographic studies, detailed environmental data, and long-term research (Muths et al. 2006). Due to low recapture rates, the mark-recapture study was discontinued in 2007, and KLGO was reclassified as an ARMI mid-level monitoring site (Fairchild 2007). The objective of mid-level sites are to collect data on occupancy, species richness, basic environmental data, and to document possible causes of amphibian decline (Muths et al. 2006). In 2009, KLGO drafted Standard Operating Protocols (SOPs) for the Amphibian Monitoring Program (AMP), which have guided the program from 2009 to the present. 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.

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 (Lannoo 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 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 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 (Walton, Gotthardt, and Field 2013).

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 (Lannoo 2005). 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 (Lannoo 2005). 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.

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<sup>2</sup> Reported as Western Toad (Anderson 2004)

Initiated in 2012, the aim of the radio-tracking study at KLGO is 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. Boreal Toads were not tracked in 2016 or 2017.

# Methods

## Visual Encounter Surveys

The amphibian monitoring program at KLGO is organized into intensive and extensive components in order to focus efforts appropriately given the relative abundance of amphibians. The lower Taiya River and West Creek watersheds, with their multiple Boreal 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, often only once in a season. 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). When the stratified design was first implemented in 2008, it was suggested that each site within a panel of the extensive component be 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 in the field. 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 Habitat Use of Amphibians in Northern Southeast Alaska (Carstensen 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 (Chelgren 2005). Tadpoles and metamorphs were counted using a combination of ocular estimation and individual counts, and totals represent a sum of both methods. Numbers for tadpoles and metamorphs reported in the “Results” section of this publication 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 *Hygiene SOP 3* of the Amphibian Monitoring Protocol.

During the 2017 season, chytrid swabs were not systematically collected from every amphibian captured. Rather, amphibians captured during Visual Encounter Surveys were inspected for signs of *chytridiomycosis*. Symptoms, if present, were photo-documented and recorded on the VES datasheet. Under special circumstances (such as when amphibians were detected in a new location, or at a site where no individual had been tested for chytrid previously) individuals were swabbed and tested for the presence of the disease-causing agent, chytrid fungus (*B. dendrobatidis*). Following the methods outlined in *SOP 4: Collecting Samples for Chytrid Fungus Testing*, non-invasive skin swabs were

collected from one adult Boreal Toad during the 2017 monitoring season. All samples were preserved in individual vials of ethanol, and stored in a refrigerator. The samples were sent to Pisces Molecular Lab for analysis.

Eight core sites (Table 1) and six non-core sites (Table 2) were monitored as part of the “intensive component” of the AMP during the 2017 season. In 2015, Chilkoot 11 (CT11) was considered part of the “non-intensive component.” However, due to its close proximity to other intensive core-sites, and relatively easy access, it was reclassified as part of the “intensive component” for 2016, and remained classified as an intensive site in 2017. Additionally, four extensive sites on the Chilkoot Trail (Table 3) and one extensive site in the White Pass Unit (Table 4) were surveyed in 2017.

### *Individual Site Descriptions*

For each site included in the 2017 monitoring efforts (Tables 1-4), a general habitat description and brief summary of amphibian activity detected at the site prior to 2017 follow. Due to a combination of processes (including succession, development, restoration, fluvial fluctuation, climate change, and isostatic rebound), many of the sites have changed dramatically during the 13 years of the monitoring program. Sites on the Dyea flats are particularly affected by post-glacial rebound, as the Dyea flats rise an average of 14-16 mm per year, one of the fastest rates found anywhere in the world (Larsen et al. 2005). For amphibian activity results from the 2017 season, and current site photos, see the “Individual Site Results” subsection, in the “Results” section of this report.

**Table 1.** Intensive core sites visited during the 2017 monitoring season. Coordinates are in UTM Zone8 NAD83.

<b>Site Number</b>	<b>Easting (meters)</b>	<b>Northing (meters)</b>
Taiya River 01 (TR01)	0480075	6596476
Dyea 03 (DY03)	0480267	6596890
Dyea 13 (DY13)	0479529	6595772
Dyea 14 (DY14)	0479518	6595625
Dyea 19 (DY19)	0479496	6597047
Dyea 33 (DY33)	0480408	6596190
West Creek 02 (WC02)	0479147	6598956
West Creek 04 (WC04)	0491745	6608133

**Table 2.** Intensive non-core sites visited during the 2017 monitoring season. Coordinates are in UTM Zone8 NAD83.

<b>Site Number</b>	<b>Easting (meters)</b>	<b>Northing (meters)</b>
Chilkoot Trail 11 (CT11)	0480567	6598617
Dyea 02 (DY02)	0480415	6597131
Dyea 12 (DY12)	0479723	6596120
Lost Lake (LL1)	0478471	6597508
Lost Lost Lake (LL2)	0478345	6597290
West Creek 03 (WC03)	0475585	6599939

**Table 3.** Extensive sites monitored in 2017 in the Panel 3, Basins A & B. Coordinates are in UTM Zone8 NAD83.

<b>Site Number</b>	<b>Easting (meters)</b>	<b>Northing (meters)</b>
Chilkoot Trail 01 (CT01)	0481566	6606160
Chilkoot Trail 02 (CT02)	0481054	6602154
Chilkoot Trail 03 (CT03)	0481521	6604689
Chilkoot Trail 04 (CT04)	0484131	6611732

**Table 4.** Extensive sites monitored in 2017 in Panel 4, Basin B. Coordinates are in UTM Zone8 NAD83.

<b>Site Number</b>	<b>Easting (meters)</b>	<b>Northing (meters)</b>
White Pass City 01 (WPC01)	0489273	6602639

## Intensive Core Sites

### *Taiya River 01 (TR01)*

Discovered in 2005, this productive breeding site is a wetland adjacent to the Taiya River, approximately 50 m southwest 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 three ‘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. Breeding has been detected at this site every year since the monitoring program began.

### *Dyea 03 (DY03)*

This shallow, anthropogenic wetland is frequently cited by locals as a well-known historic toad breeding area. When amphibian monitoring was first conducted at it by the NPS personnel in 2004, it was directly connected to a dirt road and frequently driven through. The 2004 report stated that despite high levels of disturbance “it had been used as a breeding area for several years.” Outside of anthropogenic impacts, DY03 is susceptible to radical changes in water depth and often floods over the 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. Breeding was not detected at the site in 2014 or 2015.

### *Dyea 13 (DY13)*

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 is considered Dyea 14 (DY14). 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. Visibility and amphibian detection are low due to dense vegetation cover. Water levels fluctuate from being entirely dry to having complete connectivity with both DY14 and DY12. Eggmasses were detected at this site in 2009, 2010, and 2014. Tadpoles probably

move freely between DY13 and DY14 during high water, and juveniles use Nelson Slough (including DY14, DY13, DY12) as a migration corridor.

#### *Dyea 14 (DY14)*

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. Bears, shorebirds, river otters, salmon and other fish share this dynamic environment. It remains the most productive breeding site currently monitored at KLGO, and breeding has been detected every year since the monitoring program began.

#### *West Creek 02 (WC02)*

Adjacent to West Creek Road, this small wetland, approximately 100 m<sup>2</sup>, 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 Boreal Toad was detected in 2009, but breeding activity has not been recorded since the construction of an adjacent road-side ditch, WC04, in 2007. Shading and cooler water temperatures make this site less suitable for breeding such that the population now using WC04 may be the same population that previously utilized WC02.

#### *West Creek 04 (WC04)*

Discovered as a breeding site in 2008 after conducting a survey of WC02, this anthropogenic pool (approximately 50 m<sup>2</sup>) 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 WC04 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 and the director of Public Works. 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. Breeding has been detected at this site every year since 2008.

*Dyea 19 (DY19)*

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 trifoliata*) and a fringe of sweet gale (*Myrica gale*). Evidence of toad breeding was observed in 2005 and 2006, but no amphibians were detected in 2007 or 2008. The site was not surveyed in 2009. Juveniles were found in high abundance in 2010 and 2011, indicating either undetected breeding activity in the area, or use of the wetland as a migration corridor. No amphibians or any lifestage were detected at the site between 2012-2015.

*Dyea 33 (DY33)*

This anthropogenic wetland, owned by the Municipality of Skagway, is subject to a high level of disturbance and is frequently used as a parking lot or gravel staging area. During periods of high water, the low-laying parking areas in the northern portion of the site flood, and form shallow ponds where Boreal Toad breeding was detected 2004-2006. 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 the “deep pond” seem unsuitable for Boreal Toad breeding habitat. No evidence of breeding was detected at DY33 in 2007 or 2008. 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. In 2010, the gravel piles were removed, and the shallow zones held water for part of the season, but no amphibians were detected. In 2014-2015, the site was used as a staging area for boulders and heavy machinery used in the Dyea Road improvement project.

## Intensive Non-core Sites

### *Chilkoot Trail 11 (CT11)*

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 are 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. When traveling north on the trail, after descending the first hill, Sainthly Hill, there is a spot where the trail opens up to the river bank, just after a wooden bridge. This spot is a viewpoint used by raft guides for interpretation, and affords a view of CT11. No amphibians were detected at this site during surveys in 2007, 2009, or 2011. In 2015, this site received two visits, during which two adult Boreal Toads were detected and swabbed for chytrid.

### *Dyea 02 (DY02)*

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. The southern outlet of the site is heavily shaded, and at period of high water might drain south through Toad Circle to the Chilkoot Outpost. No Boreal Toad breeding has been detected at this site. 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, and biotechs wanted to prevent the potential impact of stepping on juveniles during surveys. No amphibians were detected at the site in 2013 or 2014. A single adult Boreal Toad was detected in 2015.

### *Dyea 12 (DY12)*

This section of Nelson Creek north of the footbridge to the historic townsite 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 can reach a maximum depth of ~.8m. Evidence of toad breeding has never been detected, but lone adult toads have been seen at this site in the past. In 2015, no surveys were conducted at this site. However, based on the large number of metamorphs observed at the north end of DY13 in August of 2015, it is suspected that DY12 would also be used as a migratory corridor.

### *West Creek 03 (WC03)*

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. The majority of the water surface area, while relatively exposed in early summer, becomes increasingly enclosed with a dense cover of buckbean (*Menyanthes trifoliata*) 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 Boreal Toad breeding has been observed at this site in the past. A single adult was detected in 2005. An adult and 2 juveniles were detected in 2006. Tadpoles were found in the small complex in 2008, 2012, and 2014. No amphibians were detected in 2007, 2009, 2010, 2011, 2013, or 2015.

*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. Prior to 2016, no amphibians, nor evidence of breeding, had been detected at this site. In 2016, two juvenile Boreal Toads were detected at this site.

*Lost Lost Lake (LL2)*

Lost Lost Lake can be found by continuing down the trail at the southern end of Lost Lake. The vegetative community in and around Lost Lost Lake is similar to the lakes and ponds of Canada that support Boreal Toad, Columbia spotted frog, and wood frog populations. However, the main body of water considered LL2 has steep banks that create a deep shoreline making it potentially unfavorable for Boreal Toads. Still, a number of distinct pools lie in the meadow surrounding the north end of the lake and have much shallower water that could support breeding.

## Extensive Sites

### *Chilkoot Trail 01 (CT01) – Panel 3: Basin A*

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 reportedly filled with swamp horsetail (*Equisetum fluviatile*) and was heavily overhung with Sitka alder and black cottonwood. At the time that it was surveyed in 2017, the site was relatively deep with very little emergent vegetation. There did not appear to be any flowing water.

### *Chilkoot Trail 02 (CT02) – Panel 3: Basin A*

This large 375m<sup>2</sup> pond is located on the east side of the Chilkoot Trail before Finnegan’s Point. The pond was littered with woody detritus and emergent aquatic vegetation was absent. The terrestrial tree/shrub community bordering the pond is dominated by Sitka spruce, Sitka alder, and black cottonwood. The area is heavily shaded with closed forest on all sides.

### *Chilkoot Trail 03 (CT03) – Panel 3: Basin A*

CT03 is located on the Chilkoot Trail between Finnegan’s Point and Canyon City (south of site CT01). The 2004 KLGO amphibian monitoring report provided the following site description:

“This pond appears to be connected, during periods of high water, to the river. The turbid downstream side of the pond was occupied by several 3-5cm salmon fry. The only emergent vegetation, swamp horsetail, occurred in the shallow regions of the pool. The tree/shrub species on the shore included Sitka alder, Sitka spruce, and black cottonwood. No amphibian sign was found.”

The same site was reportedly resurveyed in 2005, 2006, 2009, and 2015. However, the two sets of coordinates reported as survey locations of CT03 in 2006 do not match each other, nor do they match the location of the CT03 wetland depicted in 2004 report maps. The wetland area surveyed in 2015 was approximately 380m north of the location of the CT03 wetland reported in 2004. In 2017, the slow moving stream north of the first wooden bridge after Finnegan’s Point campground (the bridge is labeled “NB-3Finn2Canyon.cor” in the attribute table of KLGO Chilkoot Trail Bridges GISdata file in Theme Manager) and beaver-altered wetland south of the bridge were surveyed in an attempt to locate CT03.

**Table 5.** Coordinates (UTM Zone8 NAD83) reported for the location of CT03 between 2004-2017.

Date of survey	Easting (meters)	Northing (meters)	Source of coordinate information
08July2004	0481568	6604868	Toad_Areas.shp
31May2005	0481568	6604868	Toad_Areas.shp
26June2006	0481521	6604689	2006 KLGO Amphibian Report, Appendix B
08Aug2006	0481664	6604694	2006 KLGO Amphibian Report, Appendix B
02July2009	0481521	6604689	2009 KLGO Amphibian Report, Table 3
03Aug2015	0481677	6605237	GPSmap 76CSx (15m acc.), 19June2017
19June2017	0481516	6604685	GPSmap 76CSx (15m acc.), 19June2017

*Chilkoot Trail 04 (CT04) – Panel 3: Basin B*

CT04 is located between Pleasant Camp and Sheep Camp, approximately 30 meters in elevation above the Chilkoot Trail to the east. This large 450m<sup>2</sup> pond is bordered by a *Tsuga spp.* overstory with a primarily *Vaccinium spp.* understory. Yellow pond-lily (*Nuphar polysepalum*) was the dominant aquatic vegetation. Most of the water input comes from hillside runoff, with a few outlets draining down the hillside towards the trail. CT04 appears to be ideal amphibian habitat with shallow vegetated margins along the shore and a relatively open canopy. However, amphibians have never been detected at this site.

*White Pass City 01 (WPC01) – Panel 4: Basin B*

While accompanying archeologists on a day trip to White Pass City, biotech Andrew Waldo noted the location of a marshy wetland in a clearing along the old Bracket Wagon Road that appeared to be prime amphibian habitat. The wetland, located on the east side of the Skagway River, is approximately 3km north of the AP&T hydroplant. It is comprised of an open clearing, shallow areas with muddy shorelines, sphagnum moss and emergent grasses. The site was revisited on 10Aug2017, surveyed, mapped, and assigned the name “WPC01”.

# Results

## Visual Encounter Surveys

The following sections contain amphibian activity results from the 2017 season, and current site photos. Dates of first observed Boreal Toad life stages during the 2017 monitoring season are reflected in Table 6. Field datasheets and data entries in the database have been QA/QC'd for 2017. In 2015, data from 2012, 2013, and 2014 were entered into the database. The database is now complete with data from 2007-2017, and is stored on the KLGO network at T:\NRM\Amphibians\Database\KLGOtoads v4.7F\_2010.mdb. Scanned and archived field datasheets are stored on the KLGO network at T:\Museum Curator\Archives\Processed\_Archives.

**Table 6.** Date of first observed Boreal 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 <sup>A</sup>	July 31
2015	May 14	May 20	July 13
2016	----	May 24 <sup>A</sup>	June 30
2017	----	May 31 <sup>A</sup>	Aug 8 <sup>B</sup>

<sup>A</sup> The first survey of the 2014 season was conducted on May 22, the first survey of the 2016 season was conducted May 24, and the first survey of the 2017 season was conducted on May 31. Tadpoles were already present at multiple sites for all three years.

<sup>B</sup> In 2017, no survey was conducted at WC04 (where metamorphs are typically first detected) between July 6<sup>th</sup> and Aug 8<sup>th</sup>.

### ***Individual Site Results***

For each site surveyed, a photo of the site taken during the 2017 season and summary of amphibian activity detected during the 2017 season follow.

#### Intensive Core Sites

##### *Taiya River 01 (TR01)*



Taiya River 01 (TR01) South Lobe on July 3, 2017.

We surveyed TR01 five times in 2017. The first survey was conducted on June 6<sup>th</sup>, and no amphibians were detected. During this visit, water levels were extremely low and the South Lobe, where breeding has consistently been detected in May in past years, was nearly dry. The second survey was conducted on June 15<sup>th</sup>, during which one juvenile and ten adult Boreal Toads (including one amplexing pair) were detected. Water levels were still extremely low at the site during this survey, and the pair of amplexing adults were seen on shore, not in water, in the South Lobe. During the third survey on July 3<sup>rd</sup>, five adults and one eggmass (~3200 hatchlings still clinging to the old eggmass) were observed. The fourth survey was conducted on July 27<sup>th</sup>, during which eight adults were observed. Only 19 tadpoles were observed in the South Lobe where the eggmass had been detected during the previous survey, and water levels were lower in the South Lobe than they had been on July 3<sup>rd</sup>. The final survey was conducted on August 30<sup>th</sup>, during which tadpoles (~20) and metamorphs (~20) were both observed.

*Dyea 03 (DY03)*



Dyea 03 (DY03) South Pond on June 6, 2017.

DY03 received three visits in 2017, each of which resulted in positive amphibian detection. The first survey was conducted on June 6<sup>th</sup>. A single adult toad and eggmass (~4600 eggs) were detected in the South Pond. Water levels were very low at the site during this survey, and the Middle Pond was entirely dry. During the second survey on July 12<sup>th</sup>, five adults were detected. Water levels had risen since the previous survey, but no tadpoles were detected. During the last survey on July 27<sup>th</sup>, three adults were detected. Water levels had lowered again, and no tadpoles were detected.

*Dyea 13 (DY13)*



Dyea 13 (DY13) looking North from Nelson Slough, below the vehicle bridge on August 2, 2017.

This site received three visits in 2017. The first survey was conducted on May 31<sup>st</sup>, during which one adult and two eggmasses (~4000 eggs in the first eggmass, and ~6500 eggs in the second) were detected. During the second survey on June 15<sup>th</sup>, approximately 1500 tadpoles were detected at the site. During the third survey on August 2<sup>nd</sup>, approximately 700 tadpoles were detected. During the fourth survey on August 30<sup>th</sup>, approximately 20 metamorphs were detected about 300 meters north of the vehicle bridge. They were sitting in an open spot in the sun.

*Dyea 14 (DY14)*



Dyea 14 (DY14) looking south from the north inlet on May 31, 2017.

This site was visited four times in 2017. The first survey was conducted on May 31<sup>st</sup> and approximately 10,700 tadpoles were already present at the site at that time. There were at least six distinct “larval masses” at the site, including two comprised of immobile tadpoles (Gosner Stage <25), suggesting separate clutches. Three adults were also detected during the first survey. During the second survey on June 15<sup>th</sup>, approximately 9,900 tadpoles were detected, and no distinct larval masses/clutches were observed. During the third survey on July 11<sup>th</sup>, approximately 7,300 tadpoles were detected. During the fourth survey on August 2<sup>nd</sup>, approximately 11,100 tadpoles were detected. While some tadpoles had begun to develop forelimbs by the time of the final survey (Gosner 41-42), no metamorphs were detected on shore. Natural Resource Manager, Jami Belt, observed a Boreal Toad metamorph crossing the Nelson Slough bridge, traveling east to west, on August 23<sup>rd</sup>, 2017. During the fifth survey on August 30<sup>th</sup>, approximately 1420 tadpoles were observed in the main pond. We observed thousands of metamorphs on August 30<sup>th</sup>. They were in thick grass and difficult to count and see so biotech, Andrew Waldo chose to walk in the water to keep from stepping on them. Grass was too thick to accurately estimate the number of metamorphs.

*Dyea 19 (DY19)*



Dyea 19 (DY19) looking north on August 8, 2017.

DY19 was visited three times in 2017. The first survey was conducted on June 9<sup>th</sup>, and the second on July 11<sup>th</sup>. No amphibians were detected during either of the first two surveys. The final survey was conducted on August 8<sup>th</sup>, during which a single adult toad was detected.

*Dyea 33 (DY33)*



Dyea 33 (DY33) looking west on August 8, 2017.

DY33 received two visits in 2017; no amphibians were detected during either visit. During the first visit on July 6<sup>th</sup> the northern portion of the site was entirely dry. However, it did hold shallow standing water during the second visit on August 8<sup>th</sup>.

*West Creek 02 (WC02)*



West Creek 02 (WC02) on August 8, 2017.

WC02 received three visits in 2017; no amphibians were detected during any of the surveys.

*West Creek 04 (WC04)*



West Creek 04 (WC04) on June 1, 2017.

WC04 received four visits in 2017. During the first survey on June 1<sup>st</sup>, a single adult and approximately 250 tadpoles (Gosner Stage 26-30) were observed. During the second survey on July 6<sup>th</sup>, two juveniles, three adults, and 76 tadpoles (34-36) were detected. The final survey was conducted on August 8<sup>th</sup>, during which one juvenile and 18 metamorphs were detected.

## Intensive Non-core Sites

### *Chilkoot 11(CT11)*



Chilkoot 11 (CT11) on July 12, 2017.

CT11 received two visits in 2017. During the first survey on July 12<sup>th</sup>, a single adult toad was captured in the northeast area of the site. During the second survey on July 27<sup>th</sup>, three adults were detected.

*Dyea 02 (DY02)*



Dyea 02 (DY02) on July 27, 2017.

DY02 received two visits in 2017. During the first visit on July 6<sup>th</sup>, one adult and one juvenile Boreal Toad were detected. During the second visit on July 27<sup>th</sup>, a single adult was detected. The adult captured on July 27<sup>th</sup> was found burrowed in moss on a half-submerged log, where adults have been detected frequently in the past. The dorsum spot pattern of the adult toad captured on July 27, 2017 matched the dorsum spot pattern of an adult toad captured in the same location on June 18, 2015. Photos are stored at T:\NRM\Amphibians.

*Dyea 12 (DY12)*



Dyea 12 (DY12), looking south from the northern end of site on August 2, 2017.

DY12 received three visits in 2017. The first was conducted on May 31<sup>st</sup>, the second on August 2<sup>nd</sup>, and the 3<sup>rd</sup> on August 30<sup>th</sup>. No amphibians were detected during any survey.

*Lost Lake (LL1)*



Lost Lake (LL1) from the south end of the lake, looking north, on June 29, 2017.

LL1 was visited once in 2017. No amphibians were detected during the survey on August 16, 2017.

*Lost Lost Lake (LL2)*



Lost Lost Lake (LL2) from the north end of the lake, looking south, on June 16, 2017.

LL2 was visited once in 2017. No amphibians were detected during the survey on August 16, 2017.

*West Creek 03 – Small Complex (WC03A)*



West Creek 03 – Small Complex (WC03A) on June 29, 2017.

The Small Complex at West Creek 03 (WC03A) was visited twice in 2017. No amphibians were detected during the survey on June 29, 2017 or September 12, 2017.

*West Creek 03 – Main Complex (WC03)*



West Creek 03 – Main Complex (WC03) on June 29, 2017.

The Main Complex at West Creek 03 (WC03) was visited twice in 2017. No amphibians were detected during the survey on June 29, 2017 or September 12, 2017.

## Extensive Sites

### *Chilkoot Trail 01 (CT01) – Panel 3: Basin A*



Chilkoot Trail 01 (CT01) on June 19, 2017.

CT01 received one visit in 2017, during which no amphibians were detected. Surveyed opportunistically during the resources team hike on June 19, 2017, the area was only surveyed for 45 minutes, and the shoreline was not surveyed in its entirety. A series of small ponds north of the main pond were also surveyed as part of the site. A large wetland area in a clearing to the east was visible from the site but was not surveyed, due to time constraints.

*Chilkoot Trail 02 (CT02) – Panel 3: Basin A*



Chilkoot Trail 02 (CT02) on June 19, 2017.

CT02 received one visit in 2017, during which no amphibians were detected. Surveyed opportunistically during the resources team hike on June 19, 2017, the area was only surveyed for 45 minutes, and the shoreline was not surveyed in its entirety. We suspected that the site is in fact an extension of the Beaver Ponds (CT07).

*Chilkoot Trail 03 (CT03) – Panel 3: Basin A*



Chilkoot Trail 03 (CT03) on June 19, 2017.

CT03 received one visit in 2017, during which no amphibians were detected. Surveyed opportunistically during the resources team hike on June 19, 2017, the area was only surveyed for 45 minutes, and the shoreline was not surveyed in its entirety.

*Chilkoot Trail 04 (CT04) – Panel 3: Basin B*



Chilkoot Trail 04 (CT04) on June 20, 2017.

CT04 received one visit in 2017, during which no amphibians were detected. Surveyed opportunistically during the resources team hike on June 19, 2017, and the entire shoreline of the site was surveyed.

*White Pass City 01 (WPC01) – Panel 4: Basin B*



White Pass City 01 (WPC01) on August 10, 2017.

WPC01 was surveyed once in 2017. A single adult toad was detected, burrowed in the moss, on the western shore of the site. No evidence of breeding was detected. One week prior to the survey, archeologists observed an adult toad on the old Brackett Wagon Road, within a few hundred feet of the WPC01 site.

**Table 7.** Total numbers of Boreal Toads (at varying life stages) detected at monitoring sites in 2017.

Site Category	Site Number	Lone Adults	Adults in Amplexus	Total Adults (L+A)	Eggmasses	Tadpoles	Metamorphs	Juveniles
Intensive core sites	TR01	21	2	23	1	19	0	1
	DY03	9	0	9	1	0	0	0
	DY13	1	0	1	2	1519	0	0
	DY14	3	0	3	0	11154	0	0
	DY19	1	0	1	0	0	0	0
	DY33	0	0	0	0	0	0	0
	WC02	0	0	0	0	0	0	0
	WC04	4	0	4	0	250	18	3
Intensive non-core sites	CT11	4	0	4	0	0	0	0
	DY02	2	0	2	0	0	0	1
	DY12	0	0	0	0	0	0	0
	LL1	0	0	0	0	0	0	0
	LL2	0	0	0	0	0	0	0
	WC03A	0	0	0	0	0	0	0
	WC03	0	0	0	0	0	0	0
Extensive sites	CT01	0	0	0	0	0	0	0
	CT02	0	0	0	0	0	0	0
	CT03	0	0	0	0	0	0	0
	CT04	0	0	0	0	0	0	0
	WPC01	1	0	1	0	0	0	0
Totals	----	46	2	48	4	12942	18	5

***Chytrid testing results:***

On August 10, 2017 a Boreal Toad at the new White Pass site WPC01 was swabbed. The toad tested qPCR negative for *Batrachochytrium dendrobatidis*. This was the only Boreal Toad sampled for chytrid in 2017. Two samples from amphibians swabbed in 2016 were also submitted for analysis. A Boreal Toad that was found dead at site TR01 on June 9, 2016, tested strongly qPCR positive for the *Batrachochytrium dendrobatidis* target sequence. The other sample from a Columbia Spotted Frog at site WP01 collected on August 5, 2016 tested qPCR negative (Appendix D).

**Table 8.** Information from Pisces Molecular on chytrid samples they processed.

Source	Source ID #	Source Information	Pisces #	Sample Form/ Condition	Tested For	Total B.d. Target Copies in Original Sample
NPS-KGRNHP/JB	WD01 05AUG2016	RALU A1 WPI CT swab (on inner bag) KLGO-NPS-AMP chytrid swabs 2016(on outer bag)	144091	swab/EtOH	B. dendrobatidis	0.00E+00
NPS-KGRNHP/JB	TROI-A2 09JUNE2016	DEAD (on vial) KLGO-NPS-AMP chytrid swabs 2016(on bag)	144092	swab/EtOH	B. dendrobatidis	2.72E+06
NPS-KGRNHP/JB	WPC A1 10AUG2017	No other info given.	144093	swab/EtOH	B. dendrobatidis	0.00E+00

## 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 widespread 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 dam failed.

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.

## Amphibian Malformations

In 2017, skeletal and eye abnormalities were observed with three adult Boreal Toads, resulting in a prevalence of 6.25% (n=48) for the total area surveyed. All three abnormal adults were detected at a single breeding site (TR01), resulting in a prevalence of 13.0% (n=23) for that site. The abnormality prevalence among adult Boreal Toad at sites in KLG0 is comparable to the abnormality prevalence reported for metamorphic Wood Frogs at National Wildlife Refuges in Alaska (6.2% overall, up to 20% at individual breeding sites) (Reeves et al. 2008), but higher than background levels reported elsewhere (0-5%) (Reeves et al. 2008; Ouellet 2000).

A possible skin abnormality was observed with one juvenile Boreal Toad, resembling the skin abnormalities detected amongst juvenile Boreal Toads at KLG0 in 2014.



An adult Boreal Toad with shrunken digit (micromelia), detected at site TR01 on July 27, 2017 (Top Left) (NPS). An adult Boreal Toad with partial missing limb (ectromelia), detected at site TR01 on June 15, 2017 (Top Right) (NPS). An adult Boreal Toad with unpigmented irises, detected at site TR01 on June 15, 2017 (Bottom Left) (NPS). A juvenile Boreal Toad with possible skin abnormality, detected at site WC04 on July 6, 2017 (Bottom Right) (NPS).

## Opportunistic Amphibian Observations on Canadian Portion of the Chilkoot Trail

During the Resource Team Hike (June 19-July 24, 2017), KLGONatural Resource staff opportunistically surveyed waterbodies on Canadian portion of the Chilkoot Trail for amphibians. On June 22<sup>nd</sup>, a Columbia Spotted Frog eggmass and single adult were observed in small pond on the north end of Deep Lake. Part of the eggmass appeared to be dead and infected with watermold, and some hatchling tadpoles (Gosner Stage 21-25) were present. Tadpoles stranded onshore were washed into the pond with freshwater by KLGONatural Resource Staff. Canadian wardens at Lindeman were notified of the Columbia Spotted Frog eggmass, and they reported that they were aware of it and were considering rerouting the trail in order to protect the eggmass and future metamorphs. On June 23<sup>rd</sup>, Boreal Toad tadpoles as well as adult and juvenile Wood Frogs and Columbia Spotted Frogs were all observed in a single pond north of Bare Loon Lake. An adult Boreal Toad was observed on the Chilkoot Trail just north of Bare Loon Lake on June 23<sup>rd</sup>.

Canadian warden Rene Rivard is very interested in leading an effort to sample amphibians on the Canadian portion of the Chilkoot Trail for chytrid fungus in either the 2018 or 2019 monitoring season. Future KLGONatural Resource technicians and the Natural Resource Manager should coordinate with Parks Canada Staff to implement that project.

**Table 9.** Coordinates (NAD83, UTM Z8) for opportunistic amphibian observations on the Canadian portion of the Chilkoot Trail made by KLGONatural Resource Staff during 2017 Resource Team Hike.

Date	Easting (m)	Northing (m)	Observation Notes
22June2017	0493271	6625403	Location of Columbia Spotted Frog eggmass and adult
23June2017	0498609	6630077	Location of pond north of Bear Loon Lake where Boreal Toads, Columbia Spotted Frogs, and Wood Frogs were observed.



Pond north of Bear Loon Lake on the Canadian portion of the Chilkoot Trail where three species of amphibians were observed on June 23, 2017 (Left). Columbian Spotted Frog eggmass and adult observed north of Deep Lake on the Canadian portion of the Chilkoot Trail on June 22<sup>nd</sup> (Right).

### Anecdotal Amphibian Observations

Guides from the Skagway Hike and Float tour company, including raft guide Bob Finke, frequently reported seeing adult Boreal Toads (lone adults, never more than one adult at a time) on the Chilkoot Trail, on the north side of Saintly Hill during the months of May and June. Park trail crew staff spotted an adult Boreal Toad burrowed in a hole on the Chilkoot Trail near the Beaver Ponds on May 31<sup>st</sup>. KLG Museum Curator, Kristi Ausfresser, also sighted an adult Boreal Toad on the board walk at the Beaver Ponds on July 21<sup>st</sup>. A single adult Boreal Toad was sighted on the Laughton Glacier Trail, near the trailhead at Glacier Station, frequently throughout the summer.

**Table 10.** Anecdotal amphibian sightings in 2017, with estimated coordinates (NAD83, UTM Z8).

Date	Location	Easting (m)	Northing (m)	Observer Name
May-June 2017	Saintly Hill, Chilkoot Trail	0480740	6598421	Bob Finke
31May2017	Beaver Ponds, Chilkoot Trail	0481135	6601096	Catherine Stewart
14June2017	Glacier Station, start of the Laughton Glacier Trail	0493143	6601593	Alex Bronte Luque
21July2017	Beaver Ponds, Chilkoot Trail	0481153	6601215	Kristi Ausfresser



Adult Boreal Toad seen at the Beaver Ponds on 31 May 2017, photo by C. Stewart (Top Left). Adult Boreal Toad seen at the Beaver Ponds on 21 July 2017, photo by K. Ausfresser (Bottom Left). Adult Boreal Toad seen at the Laughton Glacier Trailhead on 14 June 2017, photo by A. Bronte Luque (Right).

## Conclusions & Recommendations

Surveys should continue in order 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. 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 Boreal Toads as well as the potential impacts of development. No toads were radio-tracked in 2017. It is recommended that the study commence when a technician is available to focus on the project in August-October and a robust sampling design has been developed.

Community observations are fundamental to understanding current and historic amphibian distributions throughout the area. In 2017, longtime Dyea resident Wayne Greenstreet reported once discovering a large number (~20) adult Boreal Toads hibernating in the well on his property in Dyea year ago. Future technicians should talk with local residents to target areas for radiobelted toads and identifying locations of hibernacula. 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 KLG0 should focus on local guide companies, school groups, and visitors. Facebook groups such as “Skagway Naturally!” (maintained by longtime Dyea resident John McDermott) should also be searched for community observations of amphibians, and it might be a good platform for sharing information and results from the Amphibian Monitoring Program at KLG0.

In 2017, a new KLG0 toad geodatabase was created and is saved at this file path:

Y:\GISData\Flora\_Fauna\_Ecology\Fauna\Amphibians\Toads\Toads.gdb. Amphibian Monitoring Protocol “SOP 6. Data Management and Annual Reporting” was updated to include guidance on GPS and GIS data management, based on the 2016 GIS Data Migration Plan. A helpful guidance document prepared by the KLG0 GIS technician in 2016 is included in Appendix C of this report, and is also available at this file path:

Y:\GIS\_Migration\_Documents\ExplanationOfDataMigration\_YDrive.pub

Two projects that should specifically be undertaken during the 2018 field season are:

- 1) A comprehensive amphibian survey and mapping effort of the Beaver Ponds (CT07) on the Chilkoot Trail should be conducted in 2018. An anecdotal report of tadpoles by Mike Tranel in 2015, and regular observations of adult Boreal Toads along the trail near the Beaver Ponds suggest that there may be undetected breeding sites in that area. At least two Visual Encounter Surveys should be conducted at the Beaver Ponds in a single season for use in future occupancy analysis.

- 2) A recent study found that Wyoming Toads (*Anaxyrus baxteri*) can be individually identified by their dorsal spot pattern with the human eye with incredibly high success (their study reported 100% of the time) (Morrison et al. 2016). Following the photo methods published by that study (Morrison

et al. 2016), photodocumentation of dorsal spot patterns of juvenile and adult toads should become a systematic step in data collection when individual Boreal Toads are captured during Visual Encounter Surveys at KLGO. Photos should be geotagged, and renamed systematically (“AmphibianSurvey\_Site\_IndivID\_Date\_ObserverName”, Ex. “Amphibian Survey\_TR01\_A3\_04June2017\_SSurdyk”). Prior to commencing photo data collection, an SOP should be developed for storing and managing digital photos and associated data. Outreach materials should also be developed for promoting citizen science submissions of Boreal Toads.

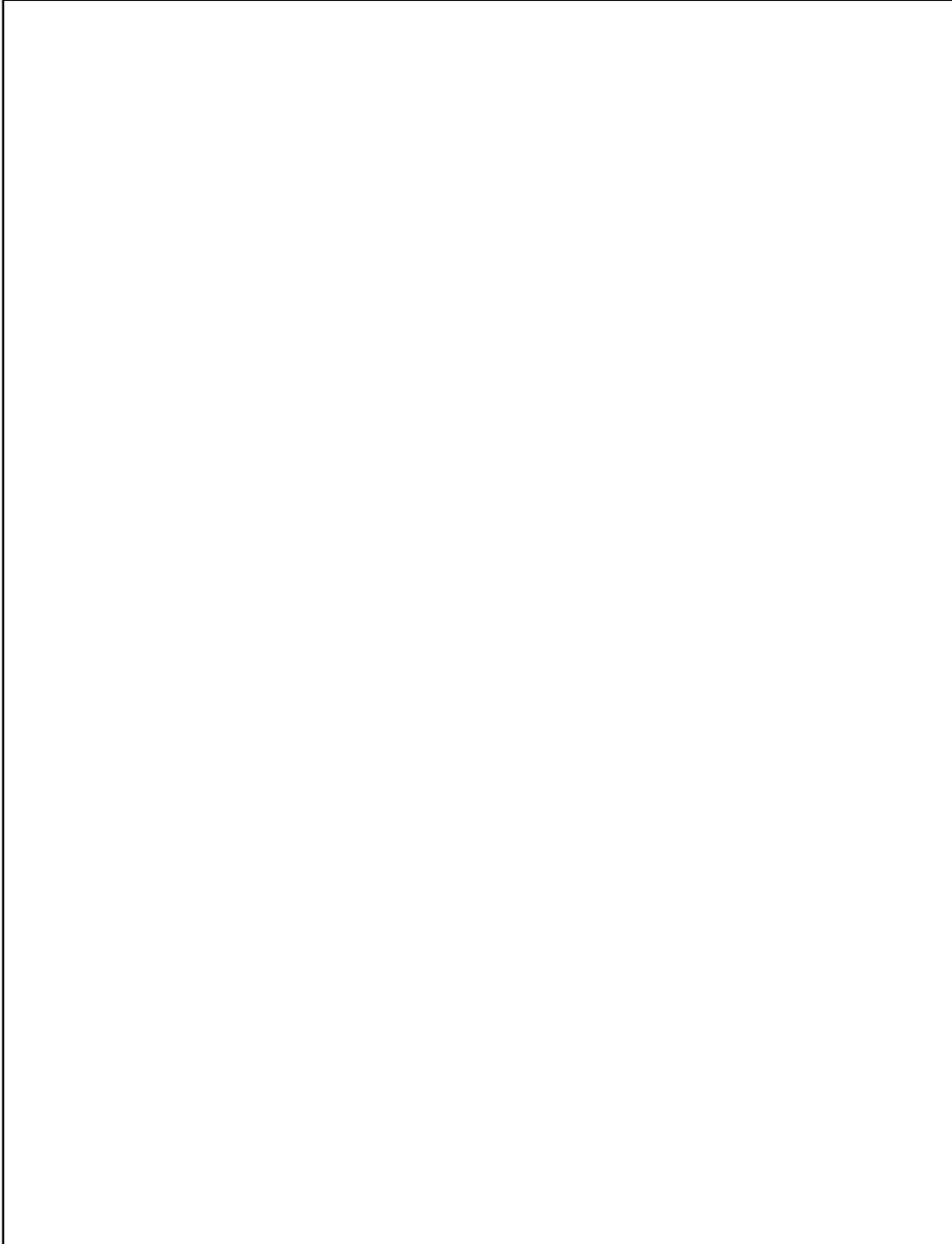
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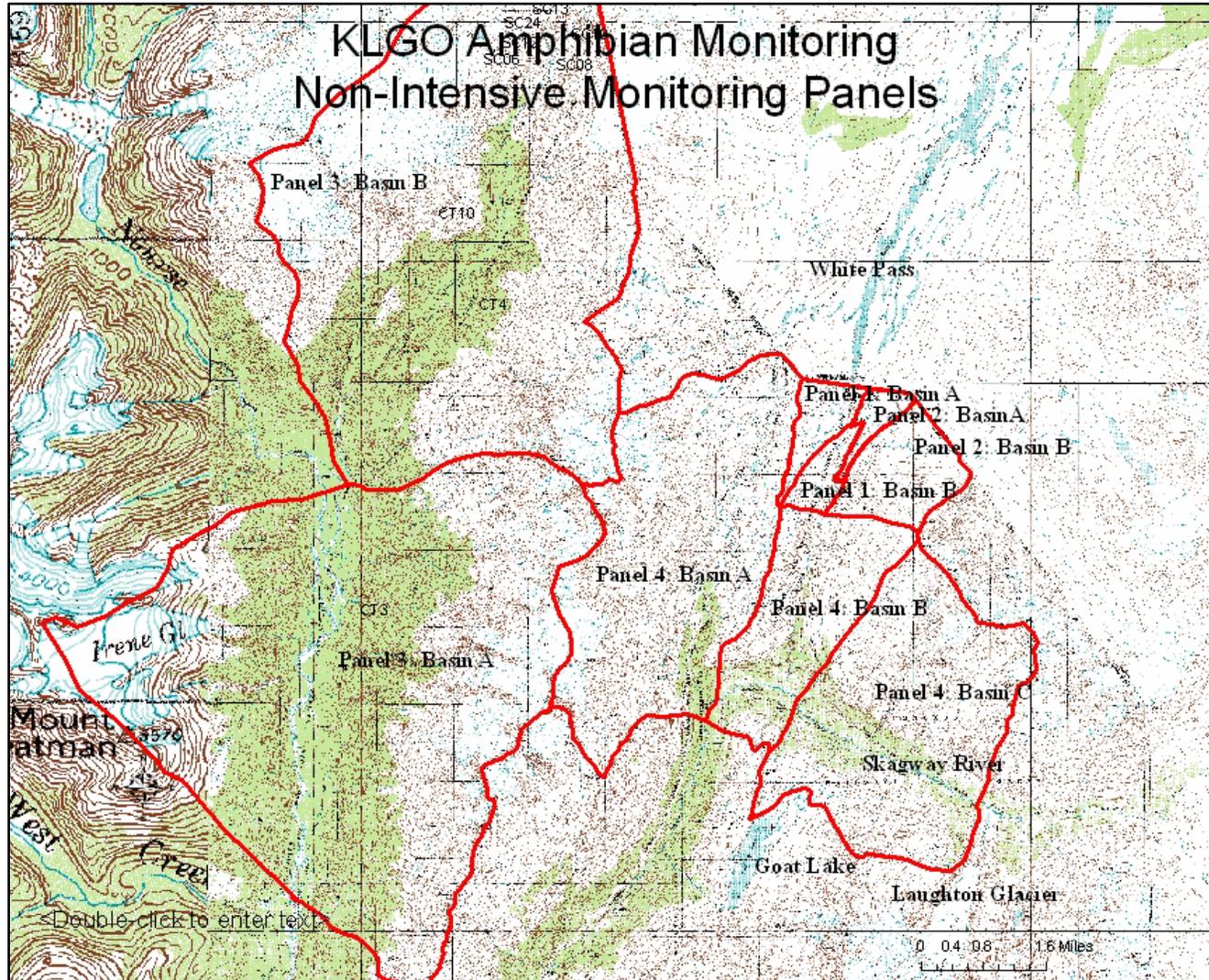
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## Appendix A. Maps

### A1. Intensive Amphibian Monitoring Sites in Dyea



A2. Non-Intensive Amphibian Monitoring Panels



## Appendix B. Routine Amphibian Survey Form

<b>Routine Amphibian Survey Form</b>		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	
<b>Amphibian Search</b>	Full? Yes No	% Shore Searched
<b>Connection</b> 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?	
<b>Comments:</b>		

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

## Appendix C. GIS Data Migration Cheatsheet

### Adding GIS Data to the Y Drive

When editing datafiles, PLEASE work in ArcCatalog to limit errors. **FILL OUT METADATA!!**

### **PROCESSDATA**

Immediately fill out Metadata

The PROCESSDATA folder is designed to hold any file that isn't considered complete... or simply: *any file that isn't yet suitable for everyone in the park to utilize as official GIS data.*

This is the ideal location for importing files that need editing, differential correction of rover files (BACKUP, BASE, EXPORT) storing data dictionary files, xml or csv files being used to import within shapefiles/feature classes/etc and map documents (mxd). **No single file should ever be moved from PROCESSDATA to any other GIS folder without metadata.**

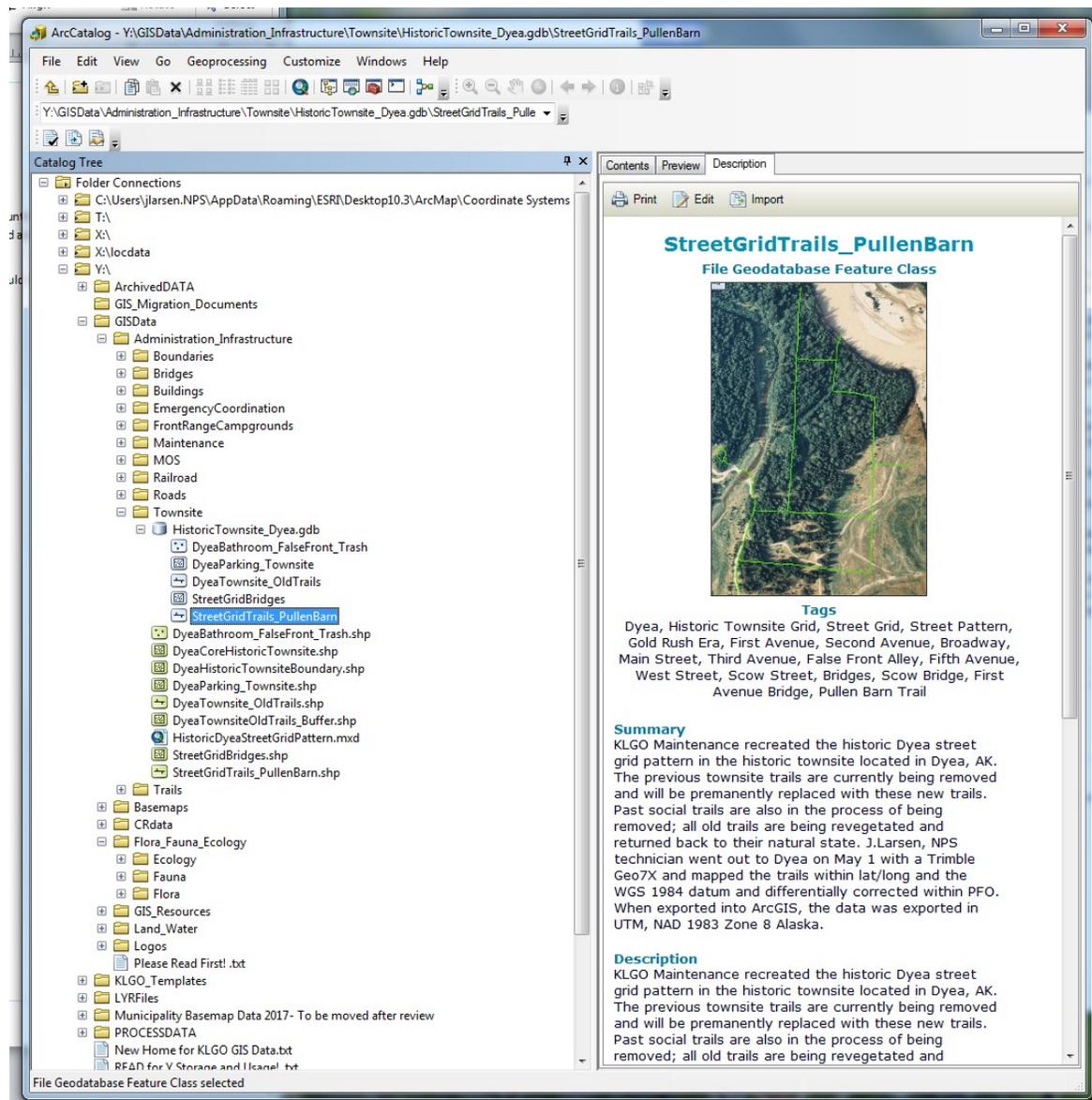


## ISData

GISData is the primary folder where all current shapefiles, file geodatabases and feature classes will reside until deemed anti- quated. Data files in this folder have already been differentially corrected, are complete with metadata and are ready for wide- spread use within KLGO.

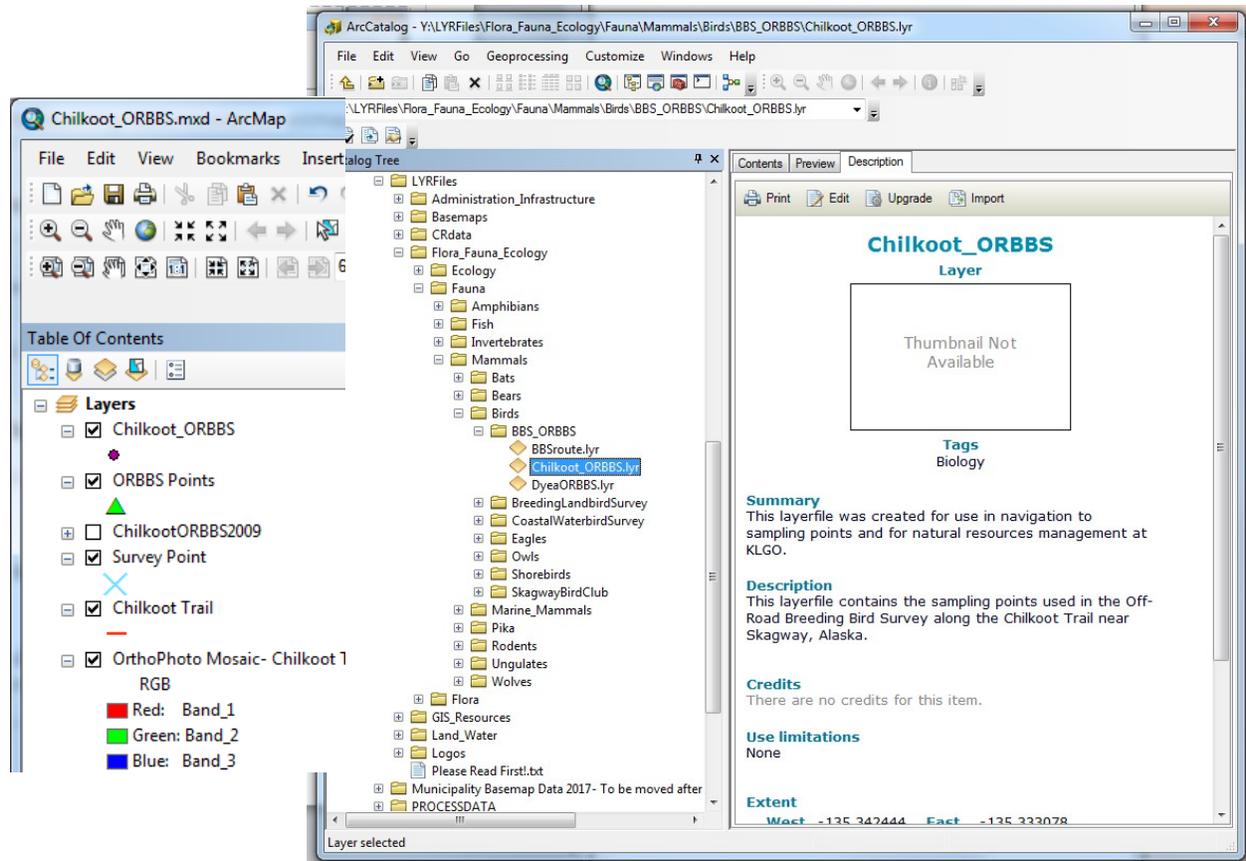
Data files that are updated each year through research and field work and are complete with metadata should be stored within GISData.

\*Once a datafile is ready for migration from the PROCESSDATA folder to the GISData folder and ready for parkwide use, please move files within ArcCatalog **only**. You can simply right click your mouse on the designated file while holding down SHIFT , drag and drop your file into its final folder within GISData. (*If you do not hold down shift while moving the file, your computer will create a duplicate by default which is not ideal. We want to limit duplicate files within the Y Drive.*)



## LYRFiles

LYRFiles is the folder designated to hold layer files only. These layer (.lyr) files represent a snapshot of a related dataset with specified symbology and other layer properties, yet do not solely hold any tabular data. They simply store the path pointing to a source dataset. The strategy behind storing layer files with completed metadata separate from shapefiles, geodatabases and the core GIS data, is to serve as an indicator to the original datasets in the event of a total data loss. Separately stored layer files can help piece back together the road to repair as opposed to having all data deleted together in the unlikely event.



## ArchivedDATA

ArchivedDATA is designed as an outlet to house any files that are considered antiquated or no longer current for park requests and day to day map making. A specific year to determine if a data file is considered antiquated has not yet been decided. If you find that a data file will not be used for current seasonal maps and work but will be useful for a long term analyzation, place within ArchivedDATA.

## KLGO\_Templates

These are KLGO templates that have been created for any park employee's use. All templates are imbedded with formal KLGO logo banners, formatted in landscape and portrait layouts and give zoomed in areas through out the park to make specific maps as easy as possible. Data to fill out the templates can be found on the Y Drive under GISData and under AKRO's Theme Manager.

# Appendix D: Chytrid Fungus Test Results

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:	9/18/17
Number of samples:	3
Type/condition of sample(s):	Swab in ethanol
Comments	

Sample Preparation:

The liquid in the swab sample was mixed by pipetting the liquid up and down repeatedly. The entire volume of the sample was then transferred into a microfuge tube. 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. 10 µg of carrier DNA was added to the lysis buffer.

Total DNA was extracted from the sample using a spin-column DNA purification procedure.

qPCR Assay:

The sample DNAs were assayed for the presence of the *Batrachochytrium dendrobatidis* ribosomal RNA Intervening Transcribed Sequence (ITS) region by 45 cycle PCR amplification using a qPCR assay developed at Pisces and an Agilent AriaMx real-time PCR instrument. The reaction master mix contained a PCR inhibitor resistant Taq polymerase (PerfeCTa Multiplex ToughMix, Quantabio) and a VIC-labeled internal positive control (IPC) (Life Technologies) 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  $4.2 \times 10^6$  to  $4.2 \times 10^0$  molecules per reaction were used to generate the standard curve.

**No DNA:** H<sub>2</sub>O 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:

Number of positive (+) samples:	1
Number of negative (-) samples:	2

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

Pisces Molecular LLC 9/25/2017

# KLGO AMP SOP1: Routine Amphibian Survey Field Methods

Amphibian Monitoring Protocol for Klondike Gold Rush National Historical Park  
Standard Operating Procedure (SOP) No. 1  
Routine Amphibian Survey Field Methods  
Version 1.0 (August 2009)

## Revision History Log

Previous Version #	Revision Date	Author	Changes Made	Reasons for Change	New Version #

## 1.1 Routine Amphibian Survey

Routine Amphibian Surveys are the main structural component of the Amphibian Monitoring Program at Klondike Gold Rush National Historical Park. Survey methods described in the SOP pertain to all field monitoring efforts at “core” and “non-core” sites within the monitoring strategy. Routine amphibian surveys include visual encounter surveys and individual measurements of adults and juveniles.

### 1.1.1 Time Commitment

Time commitment for each monitoring site survey varies, depending on the breeding activity at the site, the number of adult toads to be captured, chytrid fungus sampling schedules, and the site location. Approximate time commitments for each activity of a routine amphibian survey:

Equipment preparation: 30 minutes

Driving to Dyea monitoring sites from office: 30-40 minutes

Walking to sites from vehicle parking: 5-10 minutes

Equipment disinfecting between sites: 10-20 minutes

Amphibian survey at site: 1-2 hours

Driving to office from Dyea monitoring sites: 20-25 minutes

Data entry and editing: 30-60 minutes

Data management: 60-90 minutes

Typically four to five core site visits can be completed in a nine or ten-hour monitoring day. Core sampling can be completed by one person, but may be easier with two people if there are many individuals to be captured. For extensive non-core monitoring sites, extra time is needed for travel to

the sites. Chilkoot Trail sites can be logistically divided into two monitoring expeditions. A two-day, one-night trip is suitable to cover the five monitoring sites between the trailhead and Canyon City. A longer three-day, two-night trip may be necessary to visit sites between Pleasant Camp and Chilkoot Pass. See Appendix C for site descriptions and maps.

Equipment should be assembled into backpacks and vehicles for the day. An equipment list is included in SOP 2: Field Preparation and Equipment. Navigation to the survey site can be accomplished using site maps (Appendix B). Upon arriving at each monitoring site, data recording begins using the Routine Amphibian Survey Form (Figure 1). If data is being collected with the PDA, the appropriate Pendragon Form is used (See SOP 9: Using Pendragon Forms).

**1.1.2 Seasonal monitoring schedule**

Core sites are visited once a week from late April to early May, or until toads start gathering at the sites for breeding. Additional searches specifically targeting adult toads are made once the period of breeding and egg laying starts, at sites with prior records of breeding. Core monitoring sites are visited at least twice-weekly, and daily if needed during the egg-laying period because toads will be concentrated at the ponds during this time. Ideally two or more full visual encounter searches occur at each core site per week, in each of the egg period, larval period, and metamorphosis period for western toads (Chelgren 2005). When several weeks have passed without new detection of adult or juvenile toads, monitoring can be reduced to once-weekly visits to the sites, with continued monitoring of larvae development. Monitoring activities end for the season when new toadlets start leaving the breeding ponds for upland forest sites.

Non-core sites within the high intensity stratum are visited at least twice in the season, at times when detection is predicted to be higher such as time of breeding or metamorphosis. Late June is an appropriate time to visit sites within the Chilkoot Trail Monitoring Panel and early July can be a suitable time to search sites monitoring panels in the White Pass area.

**1.2 Survey Procedures**

**1.2.2 Visual Encounter Survey**

Upon arrival at the site, a Routine Amphibian Survey is initiated. All Routine Amphibian Survey data is recorded on the Routine Amphibian Survey Form (Appendix 1) and is organized into the following categories:

**General site information:** observer, date, arrival time, start time, end time, and pond ID.

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

Fields in the general site information category are defined as follows (see Routine Amphibian Survey Field Reference Sheet):

Observer(s)- the three-letter initials of individuals conducting the survey at the site

Date- date the survey is being conducted. Use MM-DD-YY format (e.g. 4/09/09 for April 9 of 2009)

Pond ID- the site number, name (e.g. "Dyea3), or 4-digit code (DY03) assigned to the pond site. If the site was not pre-assigned a number, document it as a new site and assign it a four-digit code based.

Time Start- start time of Visual Encounter Survey

Time End- end time of Visual Encounter Survey. Time between start and end is meant to represent time spent searching and net sweeping the site for amphibians, excluding time spent taking habit, weather, or amphibian measurements and recording data. Make a note or adjust end time to account for other activities. Record in 24-hour format (e.g. 16:35 for 4:35 pm)

Time at Site Arrive: time of arrival at the monitoring site recorded in 24-hour format (e.g. 16:35 for 4:35pm)

Time at Site Leave: time of departure from the monitoring site

**Weather: Air temperature, precipitation, wind, cloud cover, surface glare, and ripples are recorded.**

Air Temp.	Ripples	none	some	significant
Precip dry It 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

Fields in the weather category are defined as follows:

Air Temp- air temperature in the shade, approximately 1 meter off the ground, in Celcius

Ripples- circle "none" if the surface of the water is perfectly flat and smooth. Circle "some" if there is some disturbance in the surface, but there is still good visibility beneath the water. Circle "significant" if visibility below the surface is significantly affected by ripples or surface disturbance.

Precip- circle "dry" if no precipitation falls during all or most of the survey. Circle "It rain" if there is light rain falling during all or most of the survey. Circle "heavy rain" if a heavy rain is falling during all or most of the survey. Circle "snow/sleet" if snow or sleet is falling during all or most of the survey.

Wind- circle "calm" if there is no wind. Circle "light" if the wind is 0-10 knots (0-12mph, 0-19kph, Beaufort 1-3). Circle "mod" when the wind speed is 10-20 knots (13-24mph, 20-38kph, Beaufort 4-5), circle "heavy" if the wind speed is over 20 knots (>24mph, >38kph, >Beaufort 5).

Clouds- circle “clear” when cloud cover is 0-35%, “partially cloudy” when cloud cover is 35-70%, “overcast” when cloud cover is >70%

Surface Glare- circle “none” if there is no visible glare off the surface of the water, “some” if there is noticeable glare but still some visibility below the surface, or “significant” if below-surface visibility is significantly affected by glare. Polarized glasses should be used during field surveys and when recording surface glare data.

Precip last 2 days?- circle the condition most dominant in the past two days. Selected precipitation rating should represent conditions for a minimum of ½ day during the past two days.

**Habitat information:** Water level, clarity, turbidity, iron floc, sheen, vegetation, and water temperatures are recorded.

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		

Fields in the Habitat Information category are defined as follows:

Water level- circle the best description of the water level relative to the water level at the site throughout the season. For an initial rating, refer to pictures from the previous year

Clarity- select the true color of the water, represented by the materials dissolved in the water and not by pond floor material

Turbidity- how cloudy the water is, represented by the mixture of dissolved or suspended sediments in the water column

Iron floc- iron flocculent is formed when highly mineralized, iron-rich groundwater surfaces, comes into contact with air and oxidizes, forming an orange scum that coats rocks and

vegetation (Hocker 2003). Record the presence of rusty orange to red color in the water or on the bottom of the pond.



Iron floc forms a rusty-orange colored scum on rocks and vegetation in wetlands.

Sheen- sheen is a shiny, rainbow-colored smear across the surface of stagnant water. To test whether the sheen is organic or inorganic, stir through it with a finger or a stick. If it swirls, it is likely inorganic and petroleum-based, such as oil or gas. If it breaks apart into plates, it's most likely organic in origin (Hocker 2003). Note whether or not sheen is detected on the surface of the water and whether the source is petroleum or organic.

Pictures- Indicate if pictures are taken of the site and record photo numbers assigned to the image by the digital camera. Do not include pictures taken of individual amphibians in this field.

%Shallow- record the percentage of the site area that is covered in water less than 0.5 meters deep

%Shoreline w/ emergent vegetation- record the percentage of the site area that is covered by emergent vegetation

%Bottom covered by submergent vegetation- record the percentage of the site area that is covered by submerged vegetation

%Shoreline covered by snow- record the percentage of the shoreline that is covered by snow

%Surface area covered by snow- record the percentage of the pond and site surface area that is covered by snow

Water temperature- collect water temperatures in different locations of the pond site.  
Submerge thermometer until temperature reading stabilizes and record the number in Celsius.

Location- describe the location of water temperature measurements taken at the site in relation to general compass direction and the depth (e.g. NW edge of pond, at surface)

Amphibian Search

The entire perimeter of the wetland is scanned for embryos and larvae by having one person wading and the other walking along the shoreline. Polarized sunglasses must be worn to reduce glare and increase visibility below the surface of the water. Early in the season, eggs and tadpoles can be very small and difficult to see. Care is taken to closely examine the path ahead to ensure eggs and larvae are not trampled. If moveable cover items are present, they can be moved to see if larvae are hiding beneath them. Vegetated areas and other habitats likely to harbor embryos and larvae should also be searched. Record each species-stage detected and total numbers of each life-stage of each species encountered are recorded on the datasheet. If toadlets (metamorphs) or adult amphibians are found, they are captured using a dipnet and are placed in individual containers if there are too many to keep track of at the site (see SOP No. 7: Amphibian handling guidelines).

<b>Amphibian Search</b>				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				hand ID	boards	traps	net sweep
Amphibian Species Seen (if any seen, complete back of form also)						Where?	

Fields in the amphibian search data are defined as follows:

Full?- indicates whether the entire length of the shoreline is searched (yes or no)

%Shore searched- percentage of shoreline searched

Connection- indicates whether the water body is visibly connected to the water source (inlet) or outlet. Bubbles in the substrate may indicate underground inlets.

Recent disturbances?- note any human disturbances such as trash, foot prints, vehicle tracks, natural disturbances such as flooding, landslides, windfall, or any significant changes to the pond/site structure

%Bottom visible- percent of the bottom up to 2m depth that is visible, excluding areas that are covered with vegetation, obscured by stained or turbid water, or other obstructed by anything

Fish present?- were any fish observed during the survey?

Fish species- record fish species observed, if known

Other predators?- note signs or presence of other predators. Common predators are predaceous diving beetles, damselfly or dragonfly nymphs, other aquatic beetles and insects, domestic dogs, coyotes, foxes, weasels, minks, martens, badgers, black bears, owls, magpies, ravens, crows, jays, shrikes, mallards, and spotted sandpipers.

Survey method- circle methods used to search for amphibians at the site during the survey

Amphibian Species Seen- For each species record the first two letters of the scientific genus and species names (e.g. BUBO for Bufo boreas)

Where?- describe where amphibians were generally found at the site (e.g. E shore of pond in emergent veg <1m water depth)

Egg Masses and Larvae

If egg masses are distinct, they are counted. If egg masses cannot be counted, their number is estimated based on the average size of isolated egg masses. For larvae, ocular estimation is used to determine total numbers (e.g., a crew member counts all the larvae found within a small area and then counts the number of those size areas which have the same density). Median body length of larvae is determined by measuring the largest, smallest, and a few in the middle range (Appendix B. Body Measurement of Tadpoles). Median Gosner stage development should be determined with the least amount of disturbance to larvae or larval mass (Appendix C. Gosner Developmental Stages). Care is taken to search the area in a manner that prevents double-counting individuals.

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

If egg masses and/or larvae are encountered, data is recorded based on the following definitions:

Location within site- description of where egg masses and larvae were found at the survey site

Individual ID- assigned alpha-numerical identification number for the day and site of capture (e.g. EM1 for the first egg mass identified at the site that day, or L3 for the third larvae measured).

Estimated # in egg mass- estimate number of individual eggs within an egg mass

Estimated total # of larvae- visually estimate total number of tadpoles, counting by 10's or 100's

Tot. Length- average total length of tadpoles at the site. Measure the length from the tip of the snout, not including any fleshy projections from the oral disk, to the end of the tail.

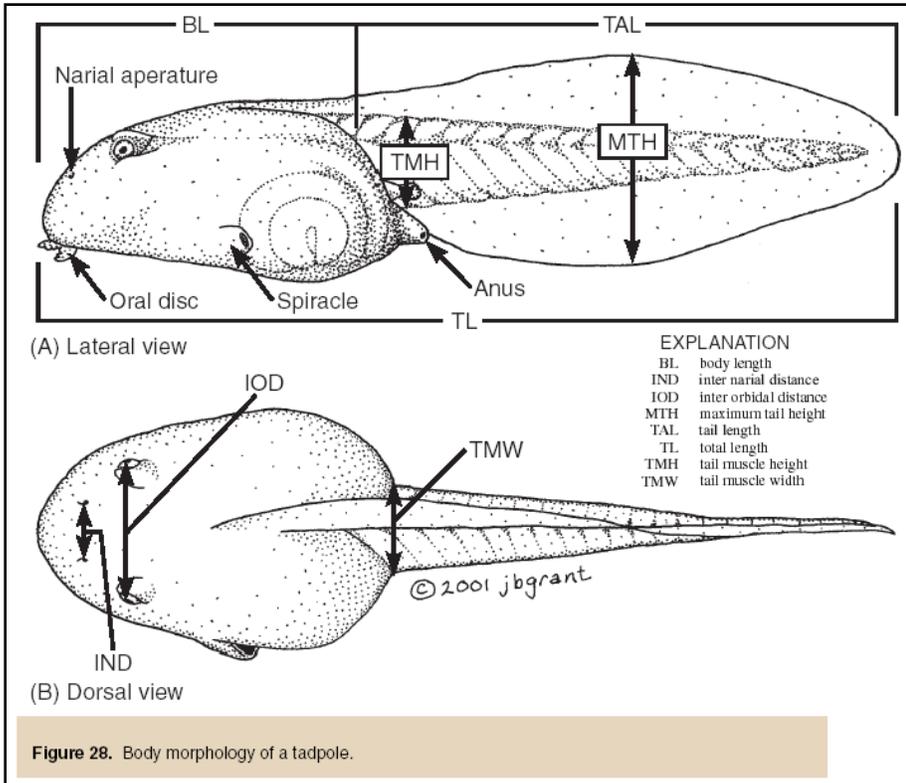


Figure SOP1-1 Body length (BL) and Total Length (TAL) measurement locations for tadpoles.

**Body Length-** average body length of tadpoles at the site. Measure the length from the tip of the snout, not including any fleshy projections from the oral disk, to the junction of the posterior body wall with the axis of the tail myotomes (Figure SOP1-1). The axis is defined by an imaginary line connecting the apices of the tail myotemes (Altig 2005). Measure 6 tadpoles at the site to calculate an average length and length range.

**Gosner stage-** record the average Gosner stage of larvae at the site

### 1.2.3 Adult and Juvenile toad measurements.

Capturing adult and juvenile toads is conducted according to the handling considerations and instructions outlined in SOP 7: Amphibian Handling Guidelines. A net may be used to facilitate capture at monitoring sites.

Measurements are collected and recorded for each captured adult amphibian: SVL, Gape, Forearm, Weight, Nuptial pads, Calling, Location within site, and Individual ID (Appendix 2. Field Reference Sheet)

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)							

If juvenile or adult toads are captured during the Visual Encounter Survey, measurement data is recorded according to the following definitions:

Location within site- general area where the amphibian was found at the site (e.g. SE shore of pond in submerged veg <0.5 m water depth)

Individual ID- assigned alpha-numerical identification number for the day and site of capture (e.g. A1 for adult 1 observed and/or captured at the site that day, or J3 for the juvenile observed and/or captured at the site that day)

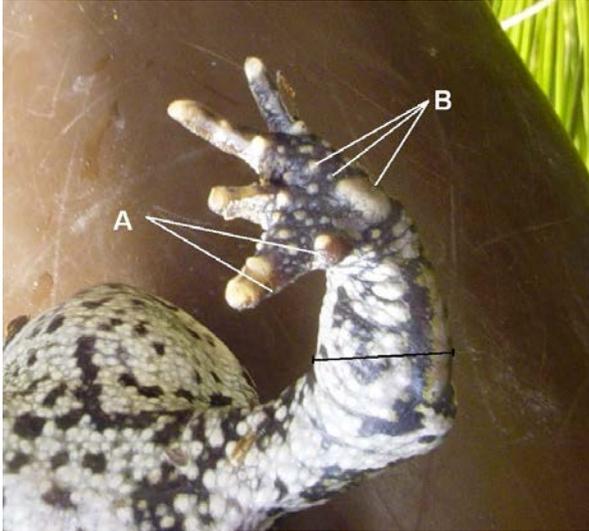
SVL (mm)- snout-to-vent length of the amphibian

Gape (mm)- head width at the point of jaw articulation

Forearm (mm)- length of the right forearm from the end of the bent elbow (including the elbow) to the tip of the longest toe. Straighten the limb gently so the elbow is at 90 degrees before measuring.

Weight (g)- weight of toad after subtracting empty weight of the bag

Nuptial pads?- note the presence or absence of darkened nuptial pads on the thumbs and first 2 fingers (Figure SOP1-2)



Tubercles (B, light in color) are present on all toads. Darkened nuptial pads (A), effectively calluses, are present only on males and are most developed during the breeding season.

Calling?- note whether a chirp-like release call is made by the individual when handled

Tag?- Is a PIT tag detected? Any adult toad >45 mm SVL that is encountered in the study area is scanned for an existing PIT tag. If a PIT tag is identified, the toad is considered a “recapture”.



PIT tag reader scanning an adult toad for a tag number.

Swab?- was a swab taken to test for chytrid fungus? If sampling is being conducted for chytrid fungus (*Batrachochytrium dendrobatidis*), a swab is collected for each adult amphibian and the swab number is recorded (see SOP No.4 Chytrid Fungus Testing Protocol).

During Visual Encounter Surveys, amphibian pairs in amplexus should not be separated, but should be captured after they separate naturally. Sites of known amplexing pairs are frequented throughout the day to check on their status. Amphibians are released as close to the spot of capture as possible. While conducting adult and juvenile measurements, it is helpful to keep individuals in plastic containers (see SOP 7: Amphibian Handling Guidelines) to avoid recapture and repeat sampling until the site survey is completed.

#### **1.2.4 Completion of Routine Amphibian Surveys**

Comments and notes pertaining to the habitat, amphibians, data collected or general activities are recorded on the amphibian survey form or in a separate field notebook. At the end of each site survey the “end time” and “site leave time” must be recorded.

### **1.3 Cleaning Field Equipment**

All field equipment that has come in contact with stream water, including all footwear, must be cleaned and disinfected between any sites that are not “water-connected”, or that amphibians don’t freely move between. At the end of each sampling day all field equipment must be thoroughly cleaned and sterilized. Procedures for cleaning field equipment exposed to stream water during sampling are found in SOP No.3: Hygiene Protocol.

### **References**

- Altig, R. (2006). "Comments on the descriptions and evaluations of tadpole mouthpart anomalies." *Herpetological Conservation and Biology* 2(1): 1-4.
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# KLGO AMP SOP3: Routine Amphibian Survey Field Methods

Amphibian Monitoring Protocol for Klondike Gold Rush National Historical Park  
Standard Operating Procedure (SOP) No. 3  
Hygiene Protocol for Control of Disease Transmission between Amphibian Study Sites  
Version 1.0 (May 2009)

## Revision History Log

Previous Version #	Revision Date	Author	Changes Made	Reasons for Change	New Version #

### 3.1 General Principles and Background

Conducting fieldwork at amphibian breeding sites requires a code of practice to minimize the spread of disease and parasites between sites that are not “water-connected”, or that amphibians don’t freely move between. Observations of diseased and parasite-infected amphibians are frequently reported from breeding sites throughout the world and contribute to the decline and extinction of amphibian populations and species.

Amphibian pathogens can be carried in a variety of ways between habitats, including on the hands, footwear, and equipment of fieldworkers. Of special concern in Southeast Alaska and British Columbia is the spread of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), which has been documented and confirmed in western toad (*Anaxyrus boreas*) populations in Klondike Gold Rush National Historical Park (KLGO). Subsequently, handling of amphibians during field studies should be done in a manner that does not significantly increase their risks of exposure to the fungus above those normally experienced in the absence of handling (Berger 2005).

#### 3.1.1 *Batrachochytrium dendrobatidis* fungus

The fungus *Batrachochytrium dendrobatidis* (BD), also called chytrid fungus, is a disease agent responsible for chytridiomycosis in amphibians. Although thought to have been spread throughout the world in the last century, its extent and current distribution are unclear (Adams 2007). The disease was originally described in 1998 from observations of dead, dying, and deformed frogs and is one of the main factors attributed to the loss of amphibian biodiversity worldwide. Dispersal of the fungus is assumed to be via infected frogs, contaminated water, or an unknown host (Weldon 2007, Adams 2007). Amphibians exposed to the fungus may die soon after their skin is infected and can quickly spread through an area, causing a rapid collapse of the entire population at the site.

The amphibian chytrid fungus is extremely sensitive to temperatures above 29 deg. C and will die at 32 deg. C. *B. dendrobatidis* will not grow on human skin and complete drying will kill the chytrid fungus (Berger 2005). The greatest risk of transmission is when amphibians are placed together in contact or in the same container or in containers reused for holding amphibians without disinfection between uses (Chelgren 2005). See SOP No. 4: Collecting samples for *Batrachochytrium dendrobatidis* (BD) testing.

### **3.2 Handling Amphibians**

Amphibians can be handled using bare hands as long as the handler washes their hands between amphibians in water to which the animals would normally be exposed. This will ensure that the risks to frogs of exposure are not increased above environmental levels (Berger 2005). If no water is available for washing hands between amphibians, the handler should wear unused disposable gloves and change them between water-connected sites. Amphibians do not appear to show signs of stress after handling; however, unnecessary handling should be avoided and amphibians should be handled and released as quickly as possible at the site from which they were captured (Chelgren 2005). See SOP No. 7: Amphibian Handling Guidelines.

#### **3.2.1 Handling considerations for larvae**

Tadpoles normally share water and placing them in a common container does not increase their rates of physical contact. They can therefore be held in groups in containers, as long as all members of the group are from the same site. Tadpoles should not be held with batches of tadpoles collected from other sites in the same or different water bodies (Berger 2005).

### **3.3 Field Hygiene Procedures {Livo, 2005 #106}**

The following equipment is required for hygiene procedures:

- Plastic bucket with handle, for sterilizing and holding cleaning gear
- Chlorine bleach (6% concentration of sodium hypochlorite)
- Stiff scrub brushes with handles, 2 (1 for sterilization, and 1 for removing soil)
- Rubber dishwashing gloves
- Spray bottle
- Isopropyl alcohol, in bottle or individual wipes

The following procedure should be conducted between any sites that are not “water-connected”, or that amphibians don’t freely move between. The procedure applies to all equipment that may have touched water or amphibians at the site. This includes, but is not limited to: waders, shoes, boots, dip nets, rulers and other instruments, specimen bags, and containers. All sterilizing is done well away from streams or ponds, preferably on gravel or asphalt surfaces where the chlorine solution will not move directly into water bodies.

1. Before leaving the pond site, use site water to wash off mud, dirt, vegetation, and other detritus attached to the equipment by shaking, rinsing, and hand picking
2. Away from the site, fill a five-gallon bucket with two gallons of clear water (can be from a natural water source or from a spigot)
3. Add 12 capfuls (6 Tablespoons or 1/3 cup) of bleach to create a 1% concentration
4. Stir the water/bleach mixture with a scrub brush to mix
5. Clean off any remaining mud, dirt, vegetation, or detritus from equipment that was missed earlier
6. Dip instruments, equipment, and shoes in the solution and scrub. Shake off and let dry in the sun.
7. Either dip and scrub waders in the solution or lay waders on the ground and pour solution over them while scrubbing.
8. Use spray bottle with the same solution concentration to apply solution where needed.
9. Sterilize brushes in solution.
10. Dispose of solution on asphalt, cement or hard roadbed, well away from other water bodies.
11. When possible, allow all gear and equipment to dry completely before reuse at the next site. UV light will quickly break down any residual bleach.

### ***3.3.1 Field hygiene considerations***

Using multiple pairs of boots and waders, such as one pair for each site, is the easiest and safest way to prevent spreading disease from footwear.

A covered, sealed bucket of bleach solution may be stored in or near the Dyea Ranger Station for convenience if the bucket is clearly labeled and if residents and employees using the station are notified.

When it is impractical to disinfect field equipment using bleach solution, isopropyl alcohol (70%) may be used for small items such as scales, calipers, plastic bags, and thermometers. Another option is to let equipment dry completely (at least 3 hours) to kill the chytrid fungus.

If conducting chytrid fungus testing by collecting swabs from individual amphibians, it is important to note that Isopropyl alcohol does not destroy DNA of the chytrid fungus. DNA testing is used to detect presence or absence of the fungus during laboratory analysis, so dipping utensils in isopropyl alcohol is not sufficient to decontaminate them for this purpose (Wood 2008). However, metal utensils may be ignited or held under a flame to destroy the DNA, as long as there are no large chunks of tissue remaining. See SOP No.4: Collecting Samples for BD testing.

### 3.4 References

- Adams, M. J., Stephanie Galvan, David Reinitz, Rebecca A. Cole, Sanjay Pyare, Meg Hahr, Purnima Govindarajulu (2007). "Incidence of the Fungus *Batrachochytrium dendrobatitis* in Amphibian Populations along the Northwest Coast of North America." *Herpetological Review* 38(4): 2.
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- Morgan, J. A. T., V. T. Vredenburg, et al. (2007). "Population genetics of the frog-killing fungus *Batrachochytrium dendrobatidis*." *Proceedings of the National Academy of Sciences* 104(34): 13845-13850.
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# KLGO AMP SOP4: Collecting Samples for BD Testing

Amphibian Monitoring Protocol for Klondike Gold Rush National Historical Park  
Standard Operating Procedure (SOP) No. 4  
Collecting Samples for *Batrachochytrium dendrobatidis* (BD) testing  
Version 1.0 (April 2009)

## Revision History Log

Previous Version #	Revision Date	Author	Changes Made	Reasons for Change	New Version #

### 4.1 Chytrid fungus in Klondike Gold Rush National Historical Park

The fungus *Batrachochytrium dendrobatidis* (BD), also called chytrid fungus, is a disease agent responsible for chytridiomycosis in amphibians. The disease was originally described in 1998 from observations of dead, dying, and deformed frogs and is one of the main factors attributed to the loss of amphibian biodiversity worldwide (Adams 2007).

Although thought to have been spread throughout the world in just the last century, its extent and current distribution are unclear (Weldon 2004). Dispersal of the fungus is assumed to be through infected frogs, contaminated water, or an unknown host (Morgan 2007). Amphibians exposed to the fungus may die soon after their skin is infected and can quickly spread the fungus through an area, causing a rapid collapse of the entire population at the site. The chytrid fungus colonizes keratinized skin cells in individual adult amphibians. Often the fungus is concentrated in patches on the ventral surface near the groin and on the webbing between the toes of the hind legs (Poulter 2009), but few observable symptoms are exhibited in the individual until the final stages of infection and near death. Symptoms may include deformities and anomalies in tadpole mouthparts (Altig 2006).

Chytridiomycosis in amphibians can be diagnosed through several methods. Histological examination of skin tissue through a microscope is a widely used technique for detecting BD in skin scrapes and toe clips from amphibians (Weldon 2006). Recently-developed quantitative (real-time) polymerase chain reaction (PCR) assays provide a more sensitive and specific test to detect the presence of BD zoospores (Olsen 2004). Studies have shown that the PCR assay can be almost twice as likely to detect BD as histology and is sensitive to a single zoospore (Kriger 2006). However, presence of BD zoospores does not equate to a diagnosis of infection or chytridiomycosis (Smith 2007). Samples collected in KLGO are analyzed by Pisces Molecular LLC using PCR assays. Results from the data are used to monitor the presence of chytrid fungus in KLGO western toad populations.

Sampling for chytrid fungus in 2005 confirmed its presence in western toads (*Anaxyrus boreas*) in Klondike Gold Rush National Historical Park (KLG0) (Adams 2007). Samples were collected again in 2006 and 2007 to better understand the presence and distribution of the fungus in KLG0 western toad populations.

#### **4.2 Collection Objectives**

The objectives of collecting samples to monitor for the presence of *Batrachochytrium dendrobatidis* in KLG0 are:

1. Monitor the presence and spread of *Batrachochytrium dendrobatidis* (*BD*) infection (chytridiomycosis) in KLG0 western toad populations.
2. Detect changes in the distribution of chytrid fungus in infected breeding sites (core sites) in KLG0.
3. Contribute to chytrid studies that are studying the relationships between geographic distribution, climate gradients, population genetics, and human disturbance in relation to population declines of native amphibians in Southeast Alaska.

#### **4.3 Collection Frequency**

Sample collection and testing of western toads is conducted every other year, in odd-numbered years. Recaptured individuals that have PIT tags are opportunistically tested every year.

#### **4.4 Equipment Preparation**

Skin swab collection kits are ordered from Pisces Molecular LLC and consist of 2 ml vials of 70% ethanol and cotton swabs. Sampling kits are stored in the Resources office mini-fridge in the Mascot building if any are left from previous seasons.

Pisces Molecular LLC  
2200 Central Avenue, Suite F  
Boulder, CO 80301  
303-546-9033  
303-546-9400 fax  
jwood@pisces-molecular.com

## 4.5 Collection Procedures

The following field equipment is required for collecting samples:

- Skin swab collection kits
- Disposable gloves, many pairs
- Plastic containers and bags, to hold individuals while sampling
- Labels or laboratory tape
- Alcohol-resistant permanent marking pen
- Plastic storage box, to transport samples

### 4.5.1 Obtaining Skin Swab Samples

Obtain the sample before doing other procedures with the animal (e.g. weighing, checking PIT tags, and measuring)

Using fresh gloves hold the animal in one hand. Gently but firmly swab, with the cotton swab, the ventral surface 25 times. For large animals, scrape the ventral surface 20 times and the feet and webbing 5 times. Place the swab, cotton side down, in a vial. Secure the lid and place in a rack or other container so that the tube remains upright.



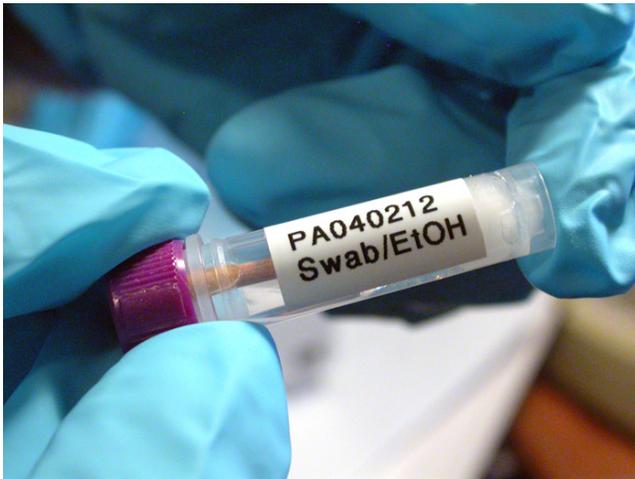
Swabbing the ventral surface of the amphibian

Label each sample vial with the site name (e.g. DY03), date (DDMMYY), 4-letter species code (e.g. BUBO for *Anaxyrus boreas*), 1 letter describing lifestage ('A' for adult, 'J' for juvenile, 'M' for metamorph, 'L' for larva), followed by a number identifying the individual (A1 for adult number one). The full label for an adult western toad captured at Dyea site 3 on June 5, 2007 would read: DY0305JUN07A1.



Insert swab into tube with the sample at the bottom.

Use an alcohol-resistant permanent marking pen and store samples in plastic bags by groups to minimize damage to data interpretation if a sample should leak and cause contamination of other samples. Samples from the same geographic location should be grouped in the same bag. After collecting a sample, vials should remain upright to prevent leakage. Leakage from one tube with BD may get on other tubes and result in contamination of samples.



Example of label on tube.

Do not place sample information inside the tube. It can be difficult to extricate, may contaminate other samples through handling, and as paper may contain bleaching agents, may inhibit detection of the target DNA.

### Collecting Samples for Chytrid fungus testing

1. Obtain the sample before doing other procedures with the animal
2. Using fresh gloves, hold the animal in one hand
3. Gently but firmly swab, with the cotton swab, the ventral surface 25 times
4. For large individuals, swab the ventral surface 20 times and the feet and webbing 5 times
5. Place the swab, cotton side down, in a vial
6. Secure the lid and place in a container so tube remains upright
7. Label each sample vial with the site number, date, and individual ID (eg. DY03Jul2J1)

### **4.5.2 Obtaining Skin Tissue Samples**

Other skin tissues such as samples of ventral skin from dead animals may also be collected for PCR testing.

Required additional equipment:

- Small, fine, metal scissors
- Alcohol wipes

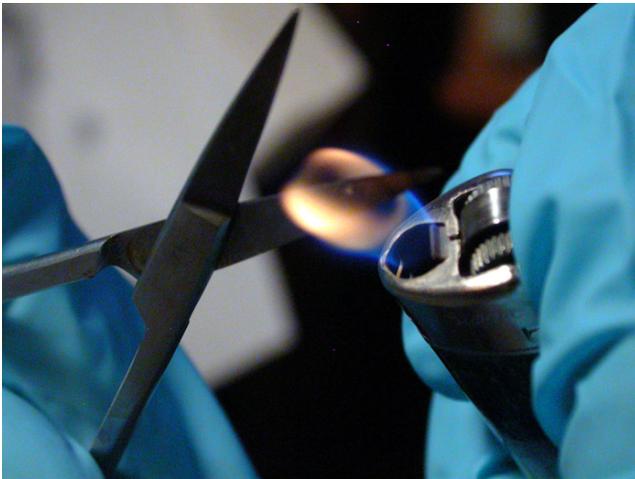
#### 4.5.2.1 Procedures for obtaining other skin tissue samples

Following hygiene procedures as for other sample collection, use fine scissors to obtain the tissue and place each sample in a 2ml vial containing 70% alcohol.

#### 4.6 Equipment cleaning and hygiene procedures (Livo 2005)

Animals should be collected with clean, decontaminated equipment, individually handled with fresh disposable gloves, and placed in individual containers prior to obtaining the samples. Do not place multiple animals in the same container prior to sampling. In this situation, a single infected animal could infect others, and PCR tests could have inflated numbers of positive test results. See SOP No. 3: Hygiene Protocol for Control of Disease Transmission between Amphibian Study Sites.

Although using Isopropyl alcohol to clean equipment and hands will prevent the spread of live chytrid fungus between individuals, it will not prevent contamination of the lab samples with chytrid fungus DNA. Small, metal utensils, such as scissors, may be wiped with isopropyl alcohol and passed through a flame to destroy residual DNA. This procedure must be done between each individual.



Passing scissor blades through flame to destroy residual DNA.

Equipment to be used on multiple individuals can be cleaned and bleached for reuse, but the equipment must be rinsed well to remove any residual bleach and be allowed to dry completely prior to reuse. Even parts per million of bleach in, on, or around a sample could possibly destroy all the chytrid fungus DNA in a sample over the course of a few weeks. Designate a plastic bag for the disposal of gloves and other materials (for example, alcohol wipes) to minimize the possibility of contamination.

Sample collection and preservation is only the first step in collecting accurate and useful data. Preventing contamination is the next step. Because of its extraordinary signal amplification, the lab test for BD is very sensitive to contamination. The BD assay has a demonstrated sensitivity of less than 0.1 zoospore. Therefore, all sample collection and subsequent handling procedures should be done to minimize contamination risks.

#### 4.7 Transporting samples for lab analysis

Samples are shipped in a sturdy box or mailing tube, but never in a soft-sided envelope, and are returned to Pisces Molecular for analysis:

Pisces Molecular  
2200 Central Avenue  
Suite F  
Boulder, CO 80301

Another possibility for sample analysis is the lab at Cornell University's Department of Ecological and Evolutionary Biology. Arrangements need to be made with lab staff there before sending samples:

Angela Stevenson, or current lab manager  
Cornell University Dept.of Biology  
E145 Corson Hall  
Ithaca, NY 14853

#### 4.8 References

- Adams, M. J., Stephanie Galvan, David Reinitz, Rebecca A. Cole, Sanjay Pyare, Meg Hahr, Purnima Govindarajulu (2007). "Incidence of the Fungus *Batrachochytrium dendrobatitis* in Amphibian Populations along the Northwest Coast of North America." *Herpetological Review* 38(4): 2.
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The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

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