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Amphibian Surveys at Klondike Gold Rush NHP

2016 Summary

Natural Resource Report NPS/KLGO/NRR—2018/1589

ON THE COVER

Photograph of an adult boreal toad at breeding site DY14 on June 29th, 2016 in Dyea, Alaska. Photograph courtesy of the National Park Service/ S. Muether.

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Shelby Surdyk and Stacie Evans

National Park Service
Department of Natural Resources
Klondike Gold Rush NHP
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Skagway, Alaska 99840

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Abstract

Amphibian monitoring has been conducted annually at Klondike Gold Rush National Historical Park since 2004 with the primary goal to monitor long-term changes in amphibian distribution, abundance, reproduction, and survival at core breeding sites. The Park has two confirmed amphibian species, the boreal toad *(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 2016, the monitoring season spanned for about two and a half months from May 24 to August 10. A total of 31 routine Visual Encounter Surveys were conducted at eight intensive core breeding sites in Dyea and West Creek, and five intensive non-core sites in Dyea, West Creek, Lost Lake, and the Chilkoot Trail. A total of 29 Visual Encounter Surveys were conducted at 22 extensive breeding sites in White Pass. Breeding activity was observed at four of the core sites. A total of 22 adult boreal toads (2 of which were in amplexus), 2 egg masses, ~ 82,000 tadpoles, 892 juveniles, and 918 metamorphs were observed. One adult Columbia spotted frog was discovered at White Pass at the Pumphouse Lake site (WP01) on August 10.

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Introduction

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

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

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

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

Since 2004, KLGO has monitored boreal toad occupancy and development at known breeding sites; however, effective conservation requires an understanding of habitat use across all seasons and life stages. Toads use shallow water for breeding, but depend on terrestrial habitats to feed and hibernate (Muths and Nanjappa 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 (Muths and Nanjappa 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.

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

Methods

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 2016, however, an attempt was made to visit extensive sites in Panel 1, Basin A twice in order to use repeat visit data in an occupancy analysis.

During Visual Encounter Surveys (VES), habitat and amphibian data were collected on hard copy data forms or in a field notebook and transcribed to data forms back in the office. The VES was performed by visually scanning the shoreline and all wade-able areas, counting or estimating the number of amphibians present, and recording growth and development attributes. Age class was defined according to Carstensen et al. (2003) with some minor changes as follows: metamorph- according to presence and timing at known tadpole locations; juvenile- <45mm SVL; and adult- >45mm. Counts for amplexing pairs, adults, and juveniles are “memory-less,” meaning it was assumed that the same individual was never detected twice and that there were no recaptures, as recommended by Nate Chelgren from USGS ARMI in 2005. Numbers for tadpoles and metamorphs 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 2016 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 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 2 adult boreal toads, 1 juvenile boreal toad, and 1adult Columbia spotted frog during the 2016 monitoring season. All samples were preserved in individual vials of ethanol, and stored in a refrigerator, to be sent out at a later date after more samples have been collected.

Eight core sites **(Table 1)** and five non-core sites **(Table 2)** were monitored as part of the "intensive component" of the AMP during the 2016 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. Twenty-two extensive, non-core sites **(Table 3)** in the White Pass unit were also monitored this year.

Table 1. Intensive core sites visited during the 2016 monitoring season. Coordinates are in UTM Zone8 NAD83.

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

Table 2. Intensive non-core sites visited during the 2016 monitoring season. Coordinates are in UTM Zone8 NAD83.

Site Number	Easting (meters)	Northing (meters)
West Creek 03 (WC03)	0475585	6599939
Dyea 12 (DY12)	--	--
Lost Lake (LL1)	0478471	6597508
Lost Lost Lake (LL2)	0478345	6597290
Chilkoot Trail 11 (CT11)	0480567	6598617

Table 3. Extensive sites monitored in 2016. Coordinates are in UTM Zone8 NAD83.

Site Number	Easting (meters)	Northing (meters)
White Pass 01 (WP01)	492214	6609508
White Pass 63 (WP63)	490973	6608621
White Pass 98 (WP98)	491916	6608897
White Pass 99 (WP99)	491939	6608916
White Pass 100 (WP100)	491976	6608938
White Pass 101 (WP101)	492013	6609050
White Pass 147 (WP147)	491305	6608927
White Pass 148 (WP148)	491413	6609072
White Pass 150 (WP150)	491532	6609124
White Pass 151 (WP151)	491558	6609177
White Pass 160 (WP160)	491789	6609236
White Pass 161 (WP161)	491737	6609121
White Pass 164 (WP164)	491608	6609256
White Pass 165 (WP165)	491880	6609347
White Pass 166 (WP166)	492100	6609465
White Pass 167 (WP167)	492048	6609388
White Pass 168 (WP168)	492020	6609338
White Pass 169 (WP169)	491956	6609187
White Pass 170 (WP170)	491921	6609104
White Pass 171 (WP171)	491913	6609078
White Pass 172 (WP172)	491906	6609062
White Pass 1000 (WP1000)	490776	6608446

Individual Site Descriptions

For each site included in the 2016 monitoring efforts, a general habitat description and brief summary of amphibian activity detected at the site prior to 2016 follow. Each habitat description will also include a photo of the site taken between 2004-2008, during the first 5 years of the monitoring program. 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 12 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 2016 season, and current site photos, see the "Individual Site Results" subsection, in the "Results" section of this report.

<u>Intensive Core Sites</u>

Taiya River 01 (TR01)

TR01 South Lobe on May 27th, 2007, with marked location of *Anaxyrus boreas* "larval mass" detected during a Visual Encounter Survey that day.

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 3 'lobes' (North, middle, and South) that typically maintain some water throughout the season and support extensive areas of *Equisetum* with the pond margins dominated by *Salix* and *Alnus spp*. These aquatic and riparian plants, along with a several others, increase significantly in size, density, and coverage as the summer progresses. In addition to hydrological disturbances, this site is especially prone to disturbances by people, pets, bears, and shorebirds. In previous years the old river braids used as breeding ponds have dried completely, resulting in complete desiccation of eggs and tadpoles. High water levels have also caused the river to flood and expand, ultimately overwhelming the site with turbid glacial runoff and washing away any amphibians inhabiting the site. Breeding has been detected at this site every year since the monitoring program began.

Dyea 03 (DY03)

The south pond of DY03 on May 13th, 2004, prior to road closure and restoration.

This shallow, anthropogenic wetland is frequently cited by locals as a well-known historic toad breeding area. When amphibian monitoring was first conducted there by the NPS personnel in 2004, it was directly connected to a high-traffic dirt road. 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 west of the decommissioned road. A fourth pond, on the east side of the road is the deepest (~ 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)

DY13 looking east from the Nelson Slough vehicle bridge on May 1st, 2007.

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)

DY14 looking west from the Nelson Slough vehicle bridge on June 13th, 2007.

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)

The shallow, mossy area (facing the West Creek road) in WC02 on June 9th, 2008.

Adjacent to West Creek Road, this small wetland, approximately 100 m^2, is a series of small ponds dominated by cotton grass, *Eriophoram spp*, and sedge, *Carex spp*, as well as sphagnum moss. After its initial discovery from aerial photographs in 2005, it was identified as an important breeding site in 2005, 2006, and 2007. One adult 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 once utilizing WC02.

West Creek 04 (WC04)

WC04 on June 9th, 2008.

Discovered as a breeding site in 2008 after conducting a survey of WC02, this anthropogenic pool (approximately 50 m^2) is part of the drainage ditch adjacent to the north side of West Creek Road constructed in 2007. This site has a shoreline substrate mainly comprised of fine gravel and large rocks with grass, sedge, and rush species as the dominant vegetation. The bed surface consists of relatively thick (20-50 mm) detritus and algae that provide substantial cover for tadpoles. Unlike the other core sites, WC04 is not directly dependent on the hydrology of the Taiya River and is less prone to abrupt shifts in water levels. When first constructed, WC04 had little or no shading and consistently warm temperatures that possibly made it more suitable as breeding habitat. Over the following year, the alders at the site grew rapidly and began to shade the pool, especially the southern shoreline. During the first VES in 2015, it was clear that extensive brush-cutting had occurred that spring along the West Creek road and 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.

Dyea 19 (DY19)

DY19 looking south on May 17th, 2005.

This large bog is located behind Slide Cemetery and accessed using a conspicuous trail located along the north side of the cemetery fence. It is dominated by emergent buckbean (*Menyanthes trifoliate*) and a fringe of sweet gale (*Myrica gale*). Evidence of toad breeding was observed in 2005 and 2006, but 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)

A shallow pond in the northern portion of DY33 on May 16th, 2005.

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

Dyea 02 (DY02)

The north end of DY02, looking southwest, on June 6th, 2007.

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)

DY12, looking north from the footbridge to the Dyea Historic Townsite on July 31st, 2007.

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)

The Main Complex at WC03 on June 17th, 2005.

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 trifoliate*) with the edges dominated by various grasses, sedges, alder, and willow. Such vegetative phenology makes the detection of amphibian egg and larval stages progressively difficult, especially in the vast expanse of the main complex where approximately 70-80% of the area is covered by lentic bodies of water. Evidence of 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)

The southern end of Lost Lake (LL1) on June 26th, 2007.

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.

Lost Lost Lake (LL2)

The north end of Lost Lost Lake (LL2), looking south, on June 26th, 2007.

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.

Chilkoot Trail 11 (CT11)

CT11 looking west towards the Chilkoot Trail on June 26th, 2015.

Volunteer Josh De Guzman measuring one of two adult boreal toads found at CT11 on June 26th, 2015.

CT11 is a wide open sand flat, which is an old river braid, that can receive river water flow during periods of high water. The lack of cover for much of the shore, the regular presence of spotted sandpipers, and the sand substrate on this uplift pond 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, Saintly 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.

Extensive Sites

White Pass Unit (Panel 1: Basin A)

In early August 2004, a White Pass & Yukon Route employee spotted a large amphibian sunning on a rock near White Pass (Payne, personal communication 2004). This prompted the survey of Pumphouse Lake (WP01), immediately to the south of the Pass, in which a single boreal toad adult was found the same year. Subsequent surveys in 2005, 2006, and 2007 at Pumphouse Lake did not result in any amphibian detections. However, in 2007, 9 additional lake sites were surveyed in the White Pass Railroad corridor, including sites up to 2km south of Pumphouse Lake. This additional survey effort in the White Pass area resulted in the detection of a single adult Columbia spotted frog (*Rana luteiventris*), a species whose range had previously only been documented to extend within 4 km north of the Canadian border at White Pass. Due to the detection of a Columbia spotted frog in 2007, efforts to survey the White Pass were significantly increased during the 2008 field season. In total, 163 surveys of 156 new and 7 previous delineated lake sites in the White Pass area were conducted in 2008, resulting in the sighting of 4 adult Columbia spotted frogs in 3 of the 163 lake sites visited.

Surveys of the White Pass were not conducted in 2009 or 2010. In 2011, amphibian surveys of the White Pass continued, and the annual report from that year states that 153 lake sites were surveyed, resulting in the detection of 2 adult Columbia spotted frogs. Both frogs were reportedly detected at sites near the railroad tracks where amphibians had been detected in previous years (WP01 and WP02A). However, the mapped location of WP02A in the 2011 GIS data does not match the location of WP02A as reported in 2007 and 2008. In 2012 and 2013, an additional 9 adult Columbia spotted frogs (both years, for a total of 18 individual frogs) were detected in the White Pass. The number of, or location of, lake sites surveyed in 2012 and 2013 were not reported. In 2014, amphibian surveys of the White Pass were conducted on August 3rd, focusing on areas adjacent to the railroad tracks and with previous amphibian detections. Only one Columbia spotted frog was detected in 2014 at Pumphouse Lake, but was unable to be successfully caught. No amphibian surveys of the White Pass were conducted in 2015.

In a program review of KLGO's Amphibian Monitoring Program in 2011, a recommendation was made for the park to adopt "occupancy by breeding" as the state variable for monitoring Columbia spotted frog populations in the White Pass, using the occupancy analysis software PRESENCE. It was noted in the review that the current presence-absence data previously collected by KLGO during White Pass survey efforts was "a good start" to implementing an "occupancy by breeding" component to the study. In 2005, surveys on amphibian survey on the Canadian side of the Chilkoot detected Columbia spotted frog tadpoles in ponds South of Deep Lake (CT CAN1) on July 14th, and both tadpoles and a single metamorphic Columbia Spotted frog at a separate site (CT CAN5) on July 15th. In 2016, an initial effort was made to implement the fieldwork and data collection component of the 2011 recommendation adopting the state-variable of occupancy in the White Pass for Columbia spotted frogs, and two daytrips were made to survey lake sites in the White Pass , during the period when Columbia spotted frog tadpoles or metamorphs would be present. Conducting an occupancy analysis requires repeat visits to multiple sites. Therefore, in 2016 an attempt was made during the first survey to both navigate to sites mapped previously, and to survey and map sites which had not yet been added to the Park's GIS. During the second survey, an attempt was made to both revisit sites that were monitored during the first survey, and to navigate to the sites where Columbia spotted frogs had been detected on previous years.

Results

Visual Encounter Surveys

A notable increase in juvenile boreal toad detections occurred in 2016 (Figure 1). Sites which experienced this increase include DY12, DY13, DY14, DY19, TR01, and LL1.

Table 4. 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*	July 31
2015	May 14	May 20	July 13
2016	----	May 24*	June 30

*The first survey of the 2014 season was conducted on May 22, and the first survey of the 2016 season was conducted May 24. Tadpoles were already present at multiple sites for both years.

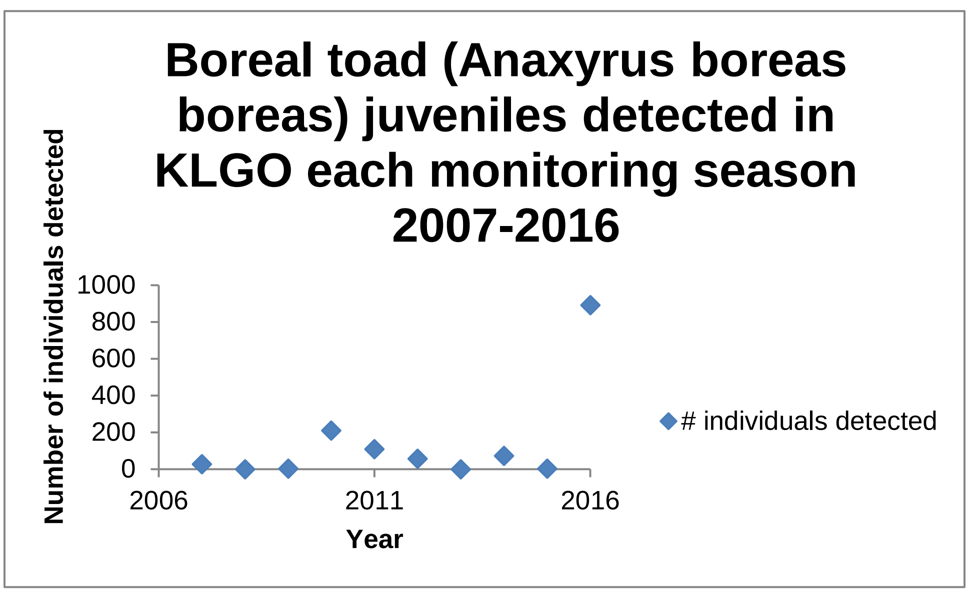

Figure 1. Number of juvenile boreal toads detected each year, from 2007-2016.

Individual Site Results

For each site surveyed, a photo of the site taken during the 2016 season and summary of amphibian activity detected during the 2016 season follow.

<u>Intensive Core Sites</u>

Taiya River 01 (TR01)

Taiya River 01 (TR01) South Lobe on July 7th, 2016.

Table 5. 2016 VES Results for Taiya River 01

Life Stage	Count
Lone adults	10
Amplexing pairs	1
Total Adults (L+A)	12
Eggmasses	2
Tadpoles	695
Metamorphs	0
Juveniles	6

This year TR01 received 4 visits, all of which resulted in positive amphibian detections. The first survey was conducted on May 24, 2016 and a single eggmass and 306 tadpoles were detected. 6 adults were detected on June 9, including one dead adult and one amplexing pair. The last survey occurred on July 27 and 3 adults and 316 tadpoles were detected.

Dyea 03 (DY03)

The North pond of Dyea 03 (DY03) on June 1st, 2016, being surveyed by two SCA interns.

Table 6. DY03 2016 VES Results for Dyea 03

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

DY03 received 3 visits during the 2016 field season. The first visit to DY03 was made on June 1st and a single adult boreal toad was detected. No amphibians were detected during the following two visits. The last visit occurred on July 27th and water levels had raised high enough to flood the old road bed. No breeding was detected at this site in 2016.

Dyea 13 (DY13)

SCA intern Kylie Mosher surveying Dyea 13 (DY13) on June 1, 2016.

Table 7. 2016 VES Results for Dyea 13

Life Stage	Count
Lone adults	1
Amplexing pairs	0
Total Adults (L+A)	1
Eggmasses	0
Tadpoles	28
Metamorphs	0
Juveniles	480

In 2016, DY13 was surveyed on June 1 and June 29. Evidence of breeding was detected with tadpoles discovered during both visits. Additionally, high numbers of juveniles were detected during each survey. One adult was discovered on the first survey.

Dyea 14 (DY14)

DY14 received 6 visits for the 2016 field season. All surveys resulted in positive amphibian detections. Evidence of breeding activity was detected, as was the successful development of metamorphs on July 11 and July 20.

Stacie Evans counting tadpoles at Dyea 14 (DY14) on May 25, 2016. A mass of tadpoles can be seen in the foreground.

Adult boreal toad found at Dyea 14 (DY14) on July 7, 2016.

Table 8. 2016 VES Results for Dyea 14

Life Stage	Count
Lone adults	3
Amplexing pairs	0
Total Adults (L+A)	3
Eggmasses	0
Tadpoles	77,900
Metamorphs	900
Juveniles	206

West Creek 02 (WC02)

WC02 received 3 visits this field season. A single adult was found in a culvert during the first survey on June 2, 2016. No other amphibians or evidence of breeding were detected.

Adult boreal toad found in a culvert at West Creek (WC02) on June 30, 2016 (circled in red).

Habitat at West Creek 02 (WC02) on July 20, 2016.

Table 9. 2016 VES Results for West Creek 02

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

West Creek 04 (WC04)

Habitat at West Creek 04 (WC04) on June 30, 2016.

Table 10. 2016 VES Results for West Creek 04

Life Stage	Count
Lone adults	2
Amplexing pairs	0
Total Adults (L+A)	2
Eggmasses	0
Tadpoles	3530
Metamorphs	18
Juveniles	2

WC04 received 3 visits this field season. The first survey was conducted on June 2, 2016. All surveys resulted in positive amphibian detections and evidence of breeding. Metamorphs were detected on June 30 and one metamorph was discovered on the last survey for the season (July 20).

Dyea 19 (DY19)

Two juvenile boreal toads in the moss at Dyea 19 (DY19) on June 8, 2016.

Table 11. 2016 VES Results for Dyea 19

Life Stage	Count
Lone adults	0
Amplexing pairs	0
Total Adults (L+A)	0
Eggmasses	0
Tadpoles	0
Metamorphs	0
Juveniles	172

In 2016, DY19 received only one visit on June 8, 2016, during which 172 juveniles were detected.

Dyea 33 (DY33)

Table 12. 2016 VES Results for Dyea 33

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

In 2016, DY 33 received only one visit on July 11, 2016, during which no amphibians, nor evidence of breeding, was detected.

Intensive Non-core Sites

Dyea 12 (DY12)

Habitat at Dyea 12 (DY12) on June 8, 2016

Table 13. 2016 VES Results for Dyea 12

Life Stage	Count
Lone adults	0
Amplexing pairs	0
Total Adults (L+A)	0
Eggmasses	0
Tadpoles	0
Metamorphs	0
Juveniles	24

In 2016, DY12 received only one visit on June 8, 2016, during which 24 juveniles were detected.

West Creek 03 (WC03)

NPS staff conducting a visual encounter survey at the Main Complex of West Creek (WC03) on June 22nd, 2016.

Table 14. 2016 VES Results for West Creek 03

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

In 2016, WC03 was visited only once, on June 22nd. During the visit, the Small Complex was surveyed completely, while only a portion of the shoreline of the large pond in the Main Complex was surveyed due to time constraints. No amphibians were detected in 2016.

Lost Lake (LL1)

In 2016, LL1 received 2 visits on June 27 and July 25, 2016. Amphibians were only detected on the first survey, during which 2 juveniles were observed.

One of two juvenile boreal toads detected at Lost Lake (LL1) on July 25th, 2016.

NPS technician Jen Larsen surveying Lost Lake (LL1) on June 27th, 2016.

Table 15. 2016 VES Results for Lost Lake

Life Stage	Count
Lone adults	0
Amplexing pairs	0
Total Adults (L+A)	0
Eggmasses	0
Tadpoles	0
Metamorphs	0
Juveniles	2

Lost Lost Lake (LL2)

NPS technician Jen Larsen and SCA intern Kylie Mosher surveying Lost Lost Lake (LL2) on June 27th, 2016.

Table 16. 2016 VES Results For Lost Lost Lake

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

Two visual encounter surveys were conducted at Lost Lost Lake on June 27th and July 25th, 2016. No amphibians were detected on either survey.

Chilkoot Trail 11 (CT11)

A volunteer surveying Chilkoot Trail 11 (CT11) on July 13, 2016.

Table 17. 2016 VES Results for Chilkoot Trail 11

Life Stage	Count
Lone adults	2
Amplexing pairs	0
Total Adults (L+A)	2
Eggmasses	0
Tadpoles	0
Metamorphs	0
Juveniles	0

In 2016, CT11 received only one visit on July 11, 2016, during which 2 adults were detected.

Extensive Sites

White Pass Unit (Panel 1: Basin A)

During two backcountry day trips on July 15th and August 5th, 2016, Visual Encounter Surveys were conducted at 22 lake sites in the White Pass Unit. For the first day trip on July 15th, technician Shelby Surdyk and volunteer C. Alex Merrell staged a vehicle on the Klondike Highway, at a pull-out south of the Summit on the U.S. side of the border, and hiked northeast from the highway towards Pumphouse Lake (WP01), which is adjacent to the White Pass & Yukon Route railway South on the U.S./Canada border. The hike from the highway to railway took about 1.5 hours. WP01 was unable to be surveyed on July 15th due to train traffic at the summit, but 16 other lake sites were surveyed, 7 of which had been mapped and surveyed in 2008, and 9 of which were newly mapped and added to the park GIS. No amphibians were detected at any of the 16 sites surveyed.

Alpine terrain in the White Pass Unit during amphibian surveys on July 15th, 2016.

Volunteer C. Alex Merrell surveying lake site WP172 on July 15th, 2016.

An adult Columbia spotted frog (Rana luteiventris), captured at White Pass 01 (WP01) on August 5th, 2016.

NPS technician Stacie Evans using a dipnet to capture a Columbia spotted at White Pass 01 (WP01) on August 5th, 2016.

For the second day trip on August 5th, technicians Shelby Surdyk and Stacie Evans staged a vehicle on the Klondike Highway North of the summit, at a large pull-out with restroom facilities on the Canadian side of the border, and hiked southeast from the highway towards Pumphouse Lake. The hike from the highway to the railway took about 1 hour, and was a far less strenuous than the route taken on July 15th. Seven of the nine lake sites that were mapped on July 15th were resurveyed on August 5th. Six lake sites (not surveyed on July 15th) that were mapped in 2008 were also surveyed on August 5th, for a total of 13 sites. One adult Columbia spotted frog (Rana luteiventris) was captured at Pumphouse Lake (WP01) and swabbed for chytrid. That was the only amphibian detected during White Pass surveys on August 5th, 2016. A map of the lake sites surveyed in the White Pass in 2016 is provided in Appendix A.

To conduct an occupancy analysis, a detection history spreadsheet was prepared for surveys of White Pass sites in 2016 (Appendix D), but has not yet been run through the occupancy software program PRESENCE.

Discussion

Visual Encounter Surveys

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

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

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

Anecdotal Amphibian Observations

In 2016, an unusually high number of incidental and anecdotal amphibian observations were reported. Mirroring the results of the Visual Encounter Surveys, park staff, Skagway and Dyea locals, and visitors alike reported a high number of juvenile boreal toads in Dyea in the spring, particularly from mid-April to early June. Many of these observations were far from known breeding sites. Juveniles were reported on private property along the Dyea Flats road, on private property along the road between the Taiya River Bridge and West Creek Bridge, and on the Lost Lake trail. A single juvenile was reported in the parking lot near DY33, and one juvenile was reportedly seen by a Skagway Hike & Float raft guide being carried by the current in the Taiya River, just south of the West Creek/ Taiya River confluence. John McDermott reported seeing juveniles throughout his garden and greenhouse in the spring and on his driveway "for the first time in decades" (Pers. Comm., 2016). Adults were reported at a few locations on the U.S. side of the Chilkoot Trail throughout the summer including: 1 adult on the trail north of Saintly Hill, 1 adult on the trail east of the metal bridge nearest the raft put-in, 1 adult on the side-trail that leads to the raft put-in, 1 adult on the north side of the wooden "toad bridge" just south of Finnegan's point, and 3 adults and 1 juvenile at the Beaver Ponds. Beginning in August, metamorphs were reported on the Dyea Flats road, particularly around the Nelson Slough Bridge, and near the Taiya River Bridge.

In addition the observations shared directly with KLGO staff in 2016, social media was "hopping" with reports of boreal toads in Dyea. Photos of adults, juveniles, and metamorphs seen in Dyea were posted on the facebook pages "Skagway Swap" and "Skagway, Naturally!," suggesting that citizen science could be a powerful tool to harness for monitoring amphibians in the future.

Photo 35. An adult boreal toad spotted at West Creek by Dirk Foss on June 4, 2016 (right). Photo courtesy of Dirk Foss.

Photo 36. A juvenile boreal toad spotted near the raft take out on the Taiya River on July 11, 2016. Photo courtesy of Tyler Geist.

Radio-tracking Study

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

Browne and Paszkowski (2010) found twenty-nine western toads in one hibernation site in Alberta, Canada. Since toads are known to hibernate communally, the disturbance of one site has the potential to severely impact the remaining population in Dyea.

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

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

No toads were radio-tracked in 2016. Locating adult toads and fixing them with radio belts is extremely time consuming. There was no technician available to dedicate time to the project this year. Furthermore, there were only enough transmitters to relocate five toads. The ability to collect more data via additional transmitters would further justify the time and resources needed for the study.

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. This might be useful for extensive sites where the probability of detecting toad activity is important given that only single surveys are conducted. The use of a dip net while surveying would also help to enhance tadpole or metamorph detection, especially at sites with poor visibility.

Radio-tracking of toads provides valuable information on upland habitat use and requirements that could be valuable in better understanding the movement patterns of boreal toads as well as the potential impacts of development. No toads were radio-tracked in 2016 due to staff shortage. It is recommended that the study commence when a technician is available to focus on the project in August-October.

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

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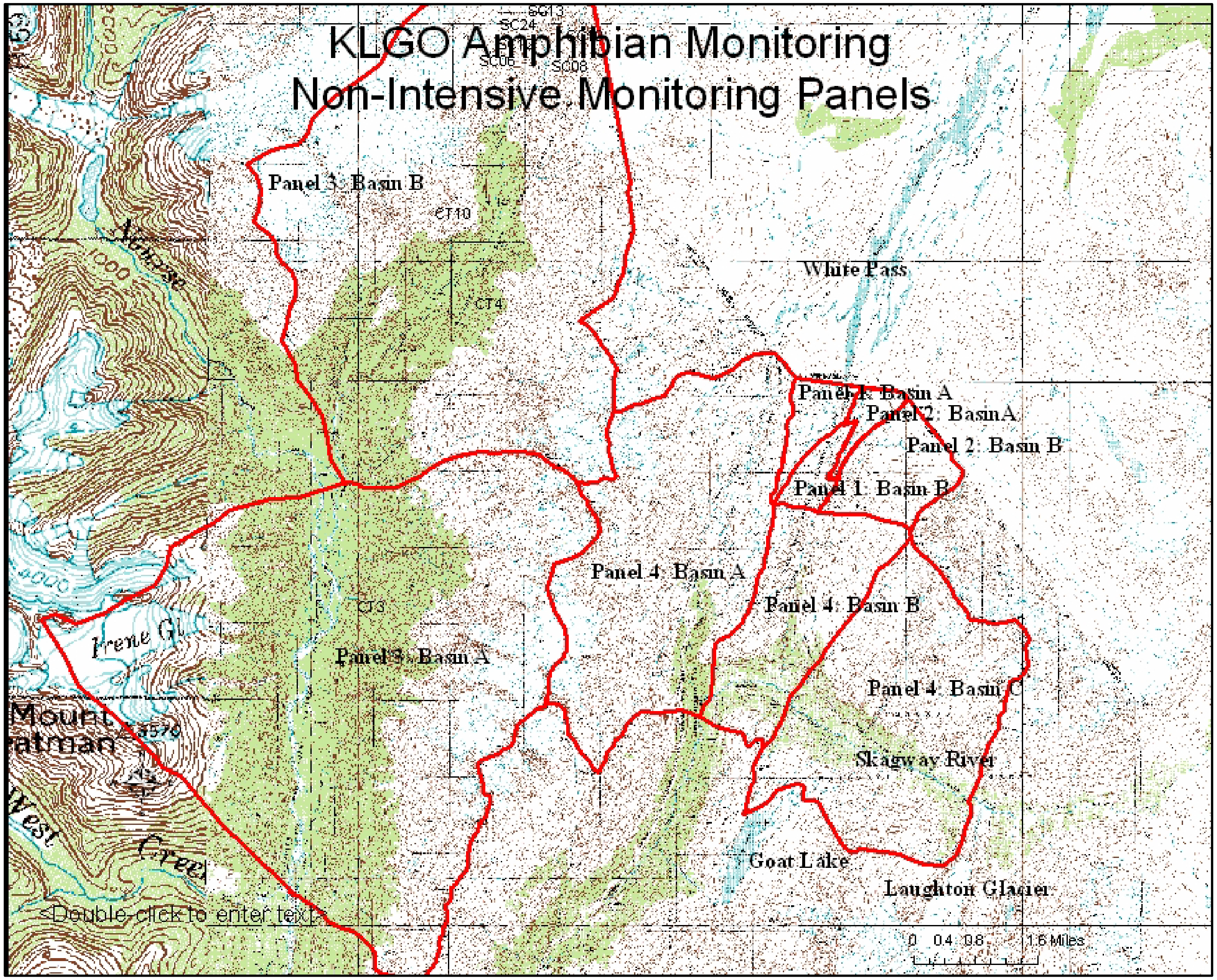
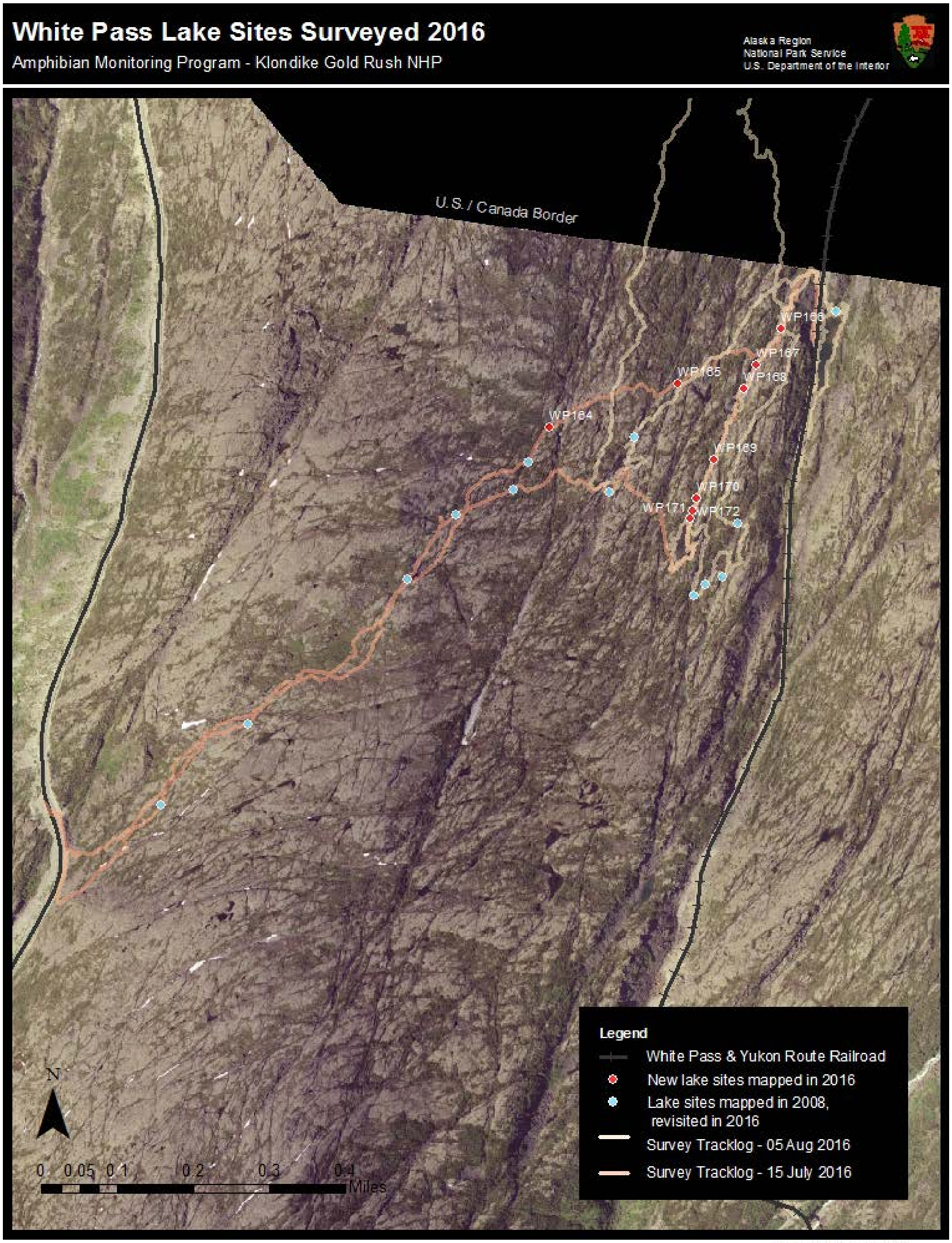
Appendix A. Maps

A1. Intensive Amphibian Monitoring Sites in Dyea

A2. Non-Intensive Amphibian Monitoring Panels



A3. White Pass Lake Sites Surveyed in 2016



Shelby Surdyk, August 2016

Appendix B. Amphibian Movement Datasheet

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

Appendix C. Routine Amphibian Survey Form

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

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

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

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

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

Comments:

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

Egg Masses and Larvae

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

Recaptures

Individual ID	Tag #	Swab ID

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 lying 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	Time at Site 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 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	

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 "lt 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.

<table>
<tr><td>Habitat</td><td colspan="2">Water level high average low dry</td></tr>
<tr><td>Clarity clear stained</td><td>Turbidity clear cloudy</td><td>iron floc absent present severe</td></tr>
<tr><td>Sheen none organic petro</td><td colspan="2">Pictures? Yes No Picture #'s:</td></tr>
<tr><td>%Shallow</td><td colspan="2">% surface area w/ emergent vegetation</td></tr>
<tr><td colspan="2">% bottom cover by submergent veg</td><td>% surface area w/ floating vegetation</td></tr>
<tr><td colspan="2">% shoreline covered by snow</td><td>% surface area covered by snow</td></tr>
<tr><td>Water Temp</td><td>loc'n</td><td>Temp Logger deployed collected</td></tr>
<tr><td>Water Temp</td><td>loc'n</td><td>Under water? Yes No Light?</td></tr>
<tr><td>Water Temp</td><td>loc'n</td><td>Temp Time</td></tr>
<tr><td>Water Temp</td><td>loc'n</td><td rowspan="3"></td></tr>
<tr><td>Water Temp</td><td>loc'n</td></tr>
<tr><td>Water Temp</td><td>loc'n</td></tr>
</table>

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.

Photo SOP1-1. Iron flock 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).

Amphibian Search	Full? Yes No	% Shore Searched
Connection inlet outlet both neither		Recent disturbances?
% bottom visible	Fish Present? Yes No	
Fish Species		Other Predators?
Survey Method visual hand ID boards traps net sweep	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.

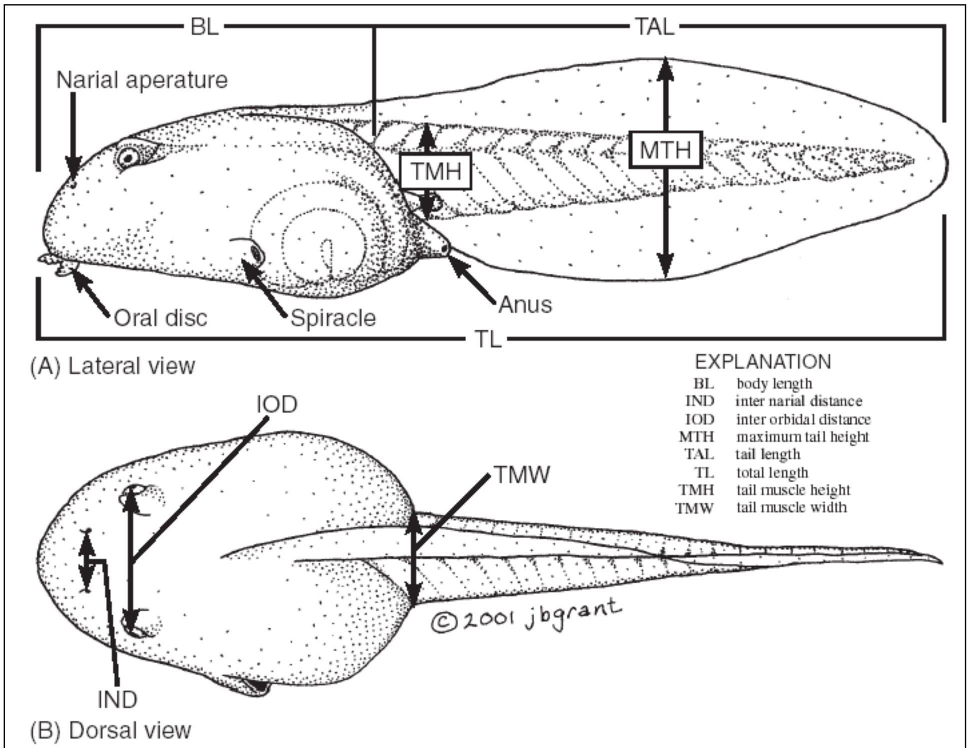


Figure 28. Body morphology of a tadpole.

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 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)

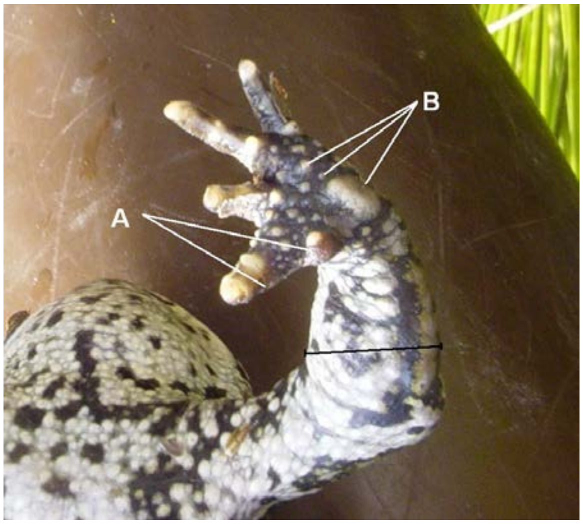


Photo SOP1-2 Tubercles (B, light in color) are present on all toads. Darkened nuptial pads (A), effectively calluses, are present only on males and are most developed during the breeding season.

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”.

Photo SOP1-3. 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.

Chelgren, N. (2005). Some Thoughts on Toad Monitoring. B. Kevin Payne, KLGO. Skagway, Alaska: 10.

Hocker, K., T. Schwarz (2003). Streamwalker's Companion. Juneau, Discovery Southeast.

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 bucked 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.

Berger, L., A. D. Hyatt, et al. (2005). "Life cycle stages of the amphibian chytrid Batrachochytrium dendrobatidis." Diseases of Aquatic Organisms 68(1): 51-63.

Chelgren, N. (2005). Some Thoughts on Toad Monitoring. B. Kevin Payne, KLGO. Skagway, Alaska: 10.

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.

Weldon, C., L. H. Du Preez, et al. (2004). "Origin of the Amphibian Chytrid Fungus." Emerging Infectious Diseases 10(12): 2100-2105.

Wood, J. (2008). Isopropyl alcohol as disinfectant. L. Fairchild. Skagway, Alaska.

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 (KLGO) (Adams 2007). Samples were collected again in 2006 and 2007 to better understand the presence and distribution of the fungus in KLGO western toad populations.

4.2 Collection Objectives

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

1. Monitor the presence and spread of *Batrachochytrium dendrobatidis (BD)* infection (chytridiomycosis) in KLGO western toad populations.
2. Detect changes in the distribution of chytrid fungus in infected breeding sites (core sites) in KLGO.
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.

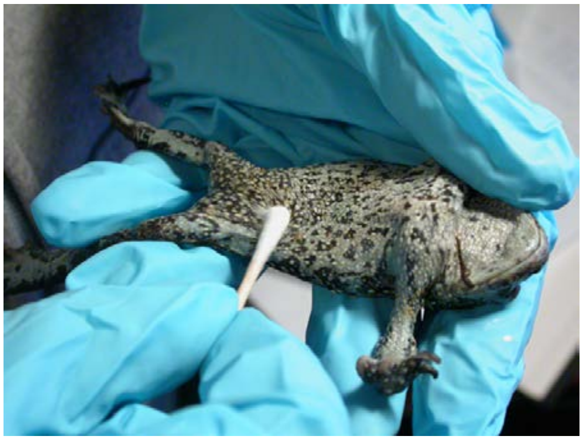


Photo SOP4-1. Swabbing the ventral surface of the amphibian

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.

Photo SOP4-2. 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.

Photo SOP4-3. 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.

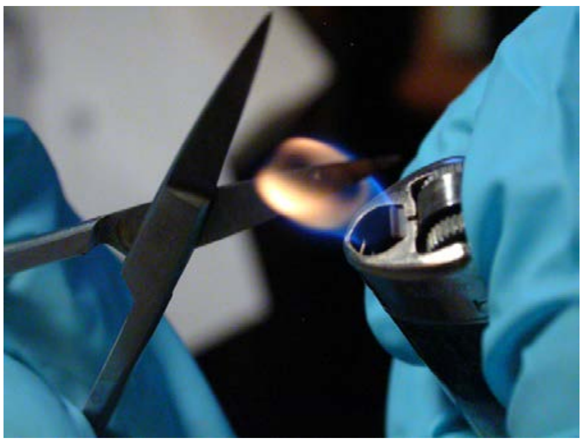


Photo SOP4-4. Passing scissor blades through flame to destroy residual DNA.

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

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KLGO AMP SOP8: Routine Amphibian Survey Field Methods

Aquatic Amphibian Monitoring Protocol for Klondike Gold Rush National Historical Park
Standard Operating Procedure (SOP) No. 8
Amphibian Radio Tagging Methods
Version 1.0 (April 2012)

Revision History Log

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

8.1 Boreal Toad Movement Study in Klondike Gold Rush National Historical Park

The effective conservation of amphibians requires an understanding of core habitat usage, a challenge for animals with distinct life stages that use distinct habitats. Boreal Toads for example, use shallow pools for breeding but use terrestrial habitats to feed and hibernate.

Klondike Gold Rush National Historical Park (KLGO) has been monitoring the breeding and recruitment success of toads at 7 core wetlands in Dyea since 2005. However, the park lacks information on the toads upland habitat use. Outside of their brief breeding period in spring and early summer, adults toad are very difficult to detect as they spend most of the time in upland habitats.

Boreal Toads are known to use communal hibernation sites. Browne and Paszkowski (2010) found twenty-nine Western Toads in one hibernation site in Alberta, Canada. Due to the small population size of Boreal Toads in KLGO, the disruption of one hibernation site could have a significant impact on the species survival in this area. Increasing use levels, proposed infrastructure development and enhanced road maintenance in Dyea means KLGO needs information on the toads timing and use of upland habitats in Dyea.

Information on the spatial behavior and movement patterns of individuals is necessary for a complete understanding of species' ecology. For most amphibian species, patterns of movement and habitat use are poorly known, especially in habitat away from breeding sites. One of the most valuable techniques for gaining information on the microhabitat use, home range and movement of a species is remotely tracking individuals in the field. The most commonly used method to track amphibians is radio telemetry.

Objectives of the Radio Tracking Study at KLGO:

1. Provide information about use and selection of upland habitat by Boreal Toads
2. Provide locations of key habitat elements (hibernacula and additional breeding locations) which are required to facilitate the conservation of the species
3. Use the data on Boreal Toad upland habitat use to inform the planning process for any infrastructure development or enhancement proposed for Dyea.

Western toad movements and habitat use will be investigated by radio tagging 5- 9 adult toads through their active period (June through September), after breeding activity (amplexus and egg lying occurs in April and May) has concluded. Radio transmitters (Model BD-2, Holohil Systems, Ltd,; Carp, Ontario, Canada) will be attached using plastic belts (Bartlett and Peterson 2000) and tracked using a Telonics radio receiver and antenna at regular intervals to check the location of tagged toads. Toad relocations will become part of the parks GIS.

8.2 Capture Methods

Adult Boreal Toads should be captured by hand or dip net. See SOP No.7 Amphibian Handling Guidelines for specific handling methods. This SOP details the guidelines in place for the Amphibian Research and Monitoring Initiative (ARMI), as assembled by the USGS National Wildlife Health Center (NWHC).

Adult amphibians are most easily located in or around breeding ponds from April into August. Special care should be used to avoid disrupting breeding activities. Adult toads in amplexus or females laying eggs should not be disrupted. Previous radio tag studies performed on Boreal Toads found minimal impacts on toad behavior (Bartelt and Peterson 2000, Burrow et al. 2012) and toads with radio tags have even been observed in amplexus (Bartelt and Peterson 2000, Browne and Paszkowski 2010). Even so, as an extra precaution, breeding season disruptions should be avoided. Breeding activity (amplexus and egg laying) at KLGO begins in early April and lasts through May, with rare occurrences in early June. In order to avoid disruption, radio transmitter attachment should not take place until mid-June.

8.3 Pre-transmitter Attachment Assessments

Before transmitter application, basic physical data should be collected to determine if the individual toad is well suited for radio tag application. Previous studies on amphibian radio tagging suggest that harness and transmitter weight should not exceed 5% of toad body weight (B.C. Environment Animal Tracking Guidelines, Bartelt and Peterson 2000, Bull 2000). The maximum transmitter plus belt weight is 2.0 grams. Therefore, the toad must weigh a minimum of 40 grams to be considered for transmitter application.

The following data should be collected for each captured toad: weight, snout vent length (SVL), sex, approximate age, and scanned for PIT tag. See SOP No. 1: Routine Amphibian Survey Field Methods for specific methods.

All radio tagged toads should be tested for chytrid fungus (*Batrachochytrium dendrobatidis)*. See SOP No. 4: Collecting Samples for Chytrid Fungus Testing.

8.4 Radio Transmitter Attachment

There are several methods for attaching radio transmitters to amphibians including Velcro waist belts (Guscio et al. 2007), ball and beaded chain belts (Rathbun and Murphey 1996), soft surgical grade polyethylene tubing (Bartelt and Peterson 2000, Browne and Paszkowski 2010), and flexible PVC tubing (Burow et al. 2012). After much research, an adjustable belt made of copper wire and PVC tubing was selected for use in the KLGO study. The primary reasoning being that it has been specifically tested on Boreal Toads and has minimal adverse impacts, is inexpensive, can be precisely fitted in the field, is light weight (0.01g), and will break off with time if the transmitter fails and cannot be recovered.

Transmitter model BD-2 (Holohil Systems Ltd., Carp, Ontario, Canada) was specifically designed for Boreal Toads, weighs 1.9 g each with a battery life of 100 days. Transmitters should be attached using a waist belt outlined by Burow et al. 2012, A Fully Adjustable Transmitter Belt for Ranids and Bufonids:

> The waist belt is made from two sizes of flexible tubing and a length of thin (0.28 mm diameter), copper wire. Holohil Systems imbedded a 1 mm inside diameter (ID) plastic tube within the epoxy of the transmitter. The length of copper wire, approximately equal to the circumference of the animal's waist, is passed through this tube. Two lengths of dark gray, flexible PVC tubing (PVC 105–18, 1.0668 mm outside diameter (OD); Alphawire, Elizabeth, New Jersey), each equal to about one-third the animal's waist circumference, are slipped over the ends of the copper wire (one length on either side of the transmitter). We secured these lengths of PVC tubing against the transmitter by bending the ends of the copper wire over their ends (Fig. 1). The belt is secured around the animal's waist by passing the ends of the PVC tubing into a length of silicone tubing (1/16" ID and 1/8" OD; Cole Parmer Instrument Company, Vernon Hills, Illinois), cut to approximately one-half the circumference of the animal's waist (Fig. 2). The belt can be adjusted for fit by changing the amount of PVC tubing extending inside the silicone tubing. Because the OD of the PVC tubing was very close to the ID of the silicone tubing, friction held the belt together (i.e., similar to a Chinese finger trap).

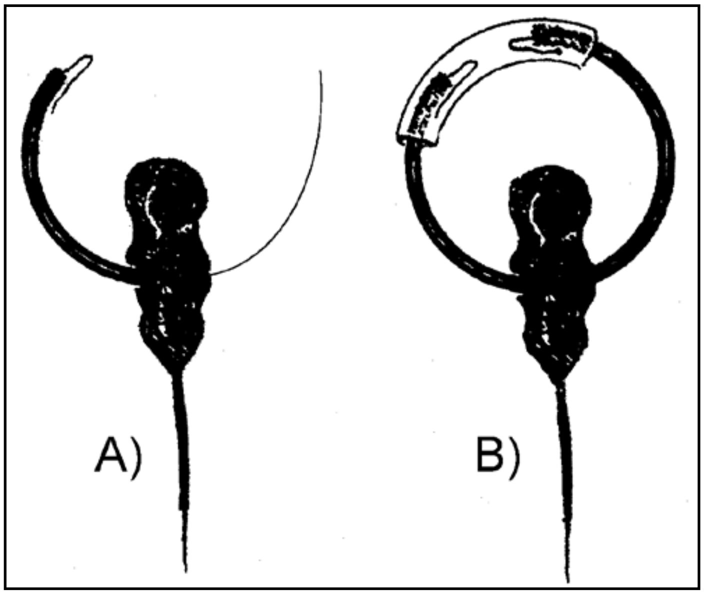

Photo SOP8-1. Details of this belt design. A) Belt showing copper wire extending through one length of PVC tubing and 1 mm tube imbedded within the transmitter epoxy. The copper wire is bent over an end of the PVC tubing. B) Finished belt affixed to a radio transmitter. Both lengths of PVC tubing are held firmly against the transmitter by the copper wire. A length of silicone tubing fits snugly over each end of the PVC tubing and allows for easy and fine adjustments around the waist of a frog or toad. (A slight angle to this drawing makes the silicone tubing appear slightly off center.)

Photo SOP8-2. Finished belt attached to a Boreal Toad. The length of silicone tubing, visible in the middle of the belt, makes the belt fully adjustable.

8.5 Radio Telemetry Procedures

The following telemetry instructions are adopted from Advanced Telemetry Systems:

Basic Radio Tracking (homing) Techniques

Complex triangulation methods may be used, but homing is the basic skill used in tracking. The objective is to visually locate the animal and record habitat and other biological data.

With your receiver, antenna and cable, and headphones, first to tune the receiver to the frequency for the transmitter you want to track.

Set the receiver's volume to a comfortable level. Set the gain control (which controls the sensitivity of the receiver) to the "full" gain position. As you track toward the animal, the gain should be reduced to the lowest level that allows you to hear the signal; adjust the receiver's gain control as required. Avoid changing the volume level if possible.

Hold the antenna in the vertical plane, use a slow sweeping motion of the antenna around you 360 degrees. Use the speaker, or better, a set of headphones, to listen for your transmitters.

The ability to sense changes in signal volume (amplitude; the volume of the transmitter's beeps), is one to practice. Try closing your eyes as you sweep the antenna in a circle and try to listen for the changes in volume.

Try to determine which direction the animal is located; as the volume of the beeping increases, you are getting closer. You'll continue to move closer to the animal while continuing to slowly sweep the antenna in smaller "slices" of a circle.

As the signal becomes stronger and directionality more difficult to discern, receiver gain can be reduced in order to decrease its sensitivity. If you need very low sensitivity, disconnect the receiver from the antenna, increase the gain, and then you can move back and forth in search of the transmitter. You’ll soon find your target.

Radio tagged toads should be located every 7-10 days. After animals are visually located, mark location with GPS unit and collect habitat data. Check for skin sores around the belt attachment area, check belt fit, and remove any debris from the belt. Track animals for 90 days and remove transmitter unit.

8.6 References

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8.7 Vendor Addresses

Holohil Systems Ltd.
112 John Cavanagh Road, Carp, Ontario K0A 1L0
Phone: (613) 839 0676 Fax: (613) 839 0675

Telonics
932 East Impala Avenue, Mesa, Arizona 85204
Phone: (602) 892 4444 Fax: (602) 892 9139

Advanced Telemetry Systems, Inc.
470 First Ave. N., Isanti, MN 55040
Phone: (763) 444 9267 Fax: (763) 444 9384

Skagway True Value Hardware
400 Broadway Street, Skagway, AK 99840
Phone: (907) 983 2233

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NPS 461/142427, February 2018

National Park Service
U.S. Department of the Interior

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