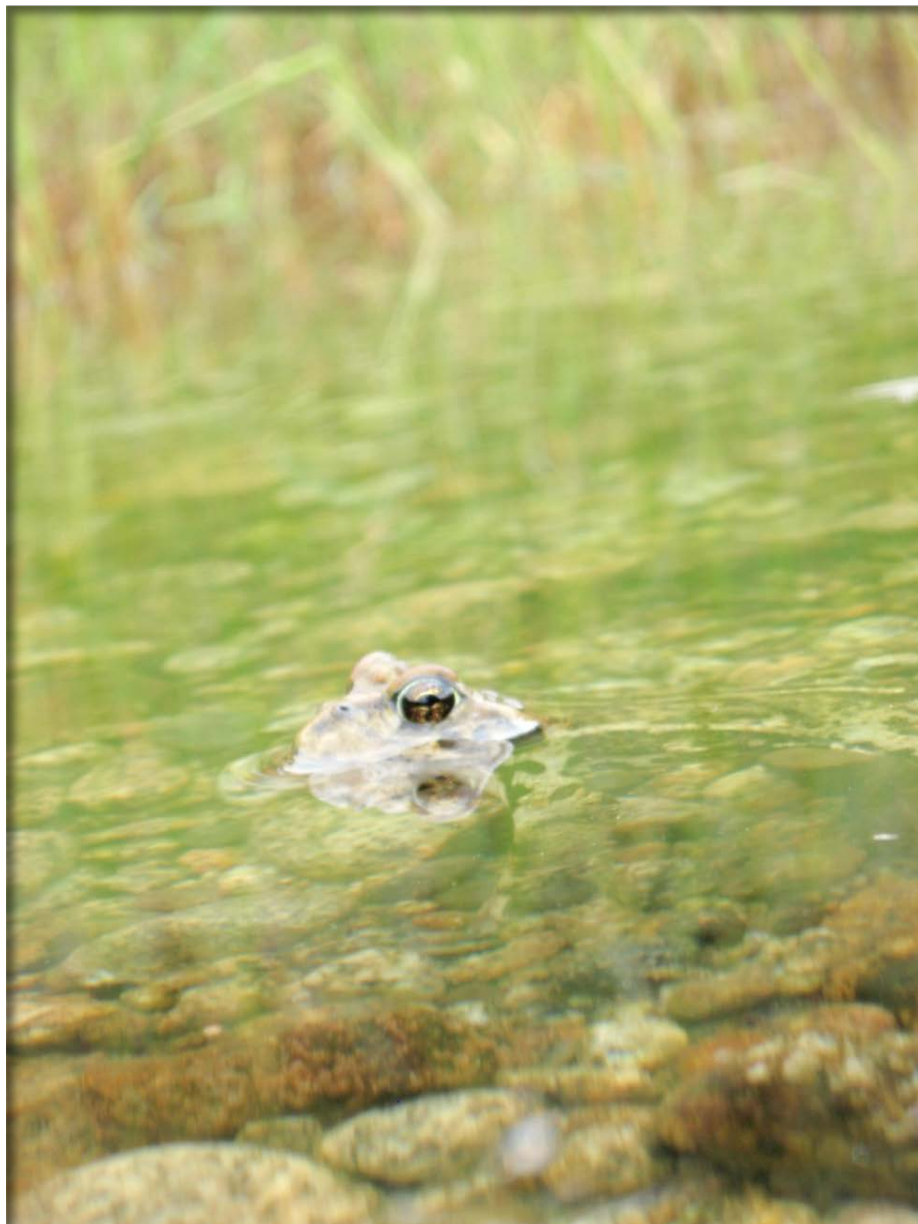




2010 Klondike Gold Rush National Historical Park Amphibian Monitoring Report

Natural Resource Report NPS/KLGO/NRDS—2014/627



ON THE COVER

Adult male *Bufo boreas* camouflaged in shallow water at core breeding site DY14 on June 15th 2010
NPS Photo

2010 Klondike Gold Rush National Historical Park Amphibian Monitoring Report

Natural Resource Report NPS/KLGO/NRDS—2014/627

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U.S. Department of the Interior
National Park Service
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Abstract

Amphibian monitoring has been conducted in Klondike Gold Rush National Historical Park (KLGO) since 2004. The 2010 monitoring season spanned a four-month period between April 26 and August 28. *Bufo boreas*, also called the Western or Boreal toad, was the only amphibian species detected. One hundred and fifty-one routine amphibian surveys were conducted at 6 core breeding sites in Dyea, three non-core sites and 5 extensive sites along the Chilkoot Trail corridor. *B. boreas* breeding activity was observed at 5 of the core sites, all of which exhibited a fully successful breeding cycle. This was highly productive in relation to the previous year's (2009) monitoring efforts, which found only 2 sites where eggs laid during spring breeding season hatched, developed in metamorphs, and migrated to their upland winter sites. Overall, 81 adult Western toads, 38 of which were in amplexus, 22 egg masses, and thousands of metamorphs were detected during the 2010 monitoring season. Additionally, egg, tadpole, and metamorph lifestages were detected at earlier dates than average in the 7 years of monitoring. Of the individual adults detected, 21 were swabbed for the presence of *Batrachochytrium dendrobatidis* (BD), and 11 of those individuals from 6 different sites tested positive for BD. For the first time since the monitoring program began dead adults were found at breeding ponds without any obvious cause of death (i.e. vehicle mortality). A necropsy of the one found at DY14 by the National Wildlife Health Center found the cause of death to be chytridiomycosis, the fungal disease caused by BD. No PIT tags were detected on any of the individuals captured.

Acknowledgements

Thank you to Biological Science Technician Heather Wetherbee for drafting the long-term monitoring protocol and also for the much needed instructions and observations. I would also like to acknowledge all of the former biotechs who initiated, grew, and contributed to the Amphibian Monitoring Program, and whose work and data is reflected in this report. Finally, thank you to David Green at the National Wildlife Health Center for analysis and insight on the health of KLGO *B. boreas* populations.

Introduction and Background

Since 2004, KLGO has maintained an amphibian detection and population monitoring program for about 4 months during the summer breeding season. Efforts to monitor amphibian populations in KLGO are part a nation-wide push in the last 20 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 over the next 3 years documented five of the 6 native species in the state's National Parks. The amphibian monitoring program was established in KLGO in 2004 and initially began by surveying wetlands previous identified by Bosworth (2000) and airborne LIDAR imagery and assessing the presence or absence of species. Since then, it has evolved from a purely inventory-based research strategy, to monitoring-based research focused on Boreal Toad (*Bufo boreas*) and the Columbia Spotted Frog (*Rana luteiventris*) populations with an intensive component in Dyea and extensive component throughout the park and surrounding areas. During the 2009 season, the program developed a draft long-term monitoring protocol specific for KLGO which was applied in 2010. Amphibian Chytrid Fungus (*Batrochochytrium dendrobatitis*), an invasive pathogenic fungus responsible for amphibian declines around the globe, was first confirmed in KLGO in 2005. Additional sampling was done in 2006, 2007, 2009, and 2010.

2010 Monitoring Objectives

The 2010 monitoring season was dedicated to implementing the long-term Amphibian Monitoring Protocol developed for KLGO in 2009 based on USGS Amphibian Research and Monitoring Initiative (ARMI) Mid-level Monitoring Protocol. Thus the focus of park resources and staff was on monitoring species composition and abundance rather conducting inventories on presence-absence data. The objectives of amphibian monitoring in Klondike Gold Rush National Historical Park are:

- Monitor long-term changes in amphibian distribution, abundance, reproduction, and survival rates at core breeding sites in Dyea
- Collect phenological and morphological data as part of the long-term monitoring effort for *B. boreas* populations at designated core breeding sites.
- Record changes and disturbances at core breeding sites, and measure water quality (Salinity, DO%, DO, pH, NO₃) for future evaluation of the effects of chemistry on site usage.
- Monitor the presence and distribution of chytrid fungus and other potential pathogens in amphibian populations within KLGO and surrounding wetlands.
- Collaborate with and provide data to other initiatives and efforts for monitoring amphibians, with an emphasis on Southeast Alaska.

In addition to the broader monitoring objectives, 2010 resources also focused on disease and abnormality detection and monitoring, as well as public outreach and awareness through Junior Ranger activities, creation of a citizen science species guide and observation form (Appendix III), and a publication and AK2day (Appendix V). This season KLGO partnered with the Taiya Inlet Watershed Council to monitor water-quality at core breeding sites, results of which are provided in Appendix VI. An amphibian oral history project was initiated during this season for collecting anecdotal evidence on changes in amphibian abundance and distribution in the Skagway and Dyea valleys. Recordings, transcribed interviews, and the Oral History Form (Appendix IV) are stored in T:\NRM\Amphibian\2010_Amphibian\Oral_History. Other achievements of the 2010 season include intensive monitoring of core breeding sites in Dyea and non-core sites around Dyea, extensive monitoring and investigation of amphibian sightings along the Chilkoot Trail Corridor, swabbing captured adult and juvenile toads for chytrid fungus presence at each site detected and submitting samples for analysis, updating the Visual Encounter Survey (VES) observation form, and submitting dead specimens to the National Wildlife Health Center for necropsy.

Survey methods

One seasonal biological technician managed the amphibian monitoring program according to the Amphibian Monitoring Protocol and Standard Operating Procedures (SOPs) for routine amphibian surveys, chytrid fungus sampling, and adult and juvenile toad measurements, which can be found at T:/NRM/Amphibians/Amphibian Monitoring Protocol. A total of 151 routine amphibian surveys of 9 intensive monitoring sites (6 core breeding sites, 3 non-core), and extensive sites at West Creek and along the Chilkoot Trail Corridor were conducted between April 26th and August 28th. No changes were made to the protocol during the 2010 monitoring season, but recommendations were made for an SOP for photodocumentation of amphibians and sites and an SOP for oral history project interviews.



Figure 1. Youth Conservation Corp Intern Kaitlyn Surdyk assisted in a VES of the Beaver Ponds on the Chilkoot Trail on August 15th. She is picture here filling out a VES form according to protocol.

Results

Routine Amphibian Surveys

Biologists conducted 152 Routine Amphibian Surveys during the 2010 monitoring season between the first survey on April 26 and the last survey on August 18. These included weekly visits to *B. boreas* core breeding sites and surveys of extensive monitoring sites. Table 1 shows dates of first observed egg masses, tadpoles, and metamorphs since the monitoring program began in 2004. Due to little snowfall at sea-level during the previous winter and an early spring, the rate of development was greater than average. Both egg masses and tadpoles were detected one week earlier than average. Metamorphs were detected at the earliest date in the 7 years of monitoring. Figure 2 compares the rate of development of *B. boreas* offspring at each of the 5 core breeding sites. Eggs laid at TR01, DY03, and WC04 reached metamorph stage (>42) by late June, nearly a month earlier than average. Metamorphs were detected at all sites by mid August.

Table 1. Date of first observed *B. boreas* life stages for each year of KLGO Amphibian Monitoring Program. Also includes mean date of first observations (n=7).

	Egg mass	Tadpole	Metamorph
2004	19-May	Late May	1-Jul
2005	21-Apr	9-May	6-Jul
2006	Mid May	30-May	26-Jul
2007	14-May	5-Jun	15-Aug
2008	20-May	5-Jun	31-Jul
2009	8-May	21-May	4-Aug
2010	3-May	19-May	28-Jun
Average	10-May	25-May	20-Jul

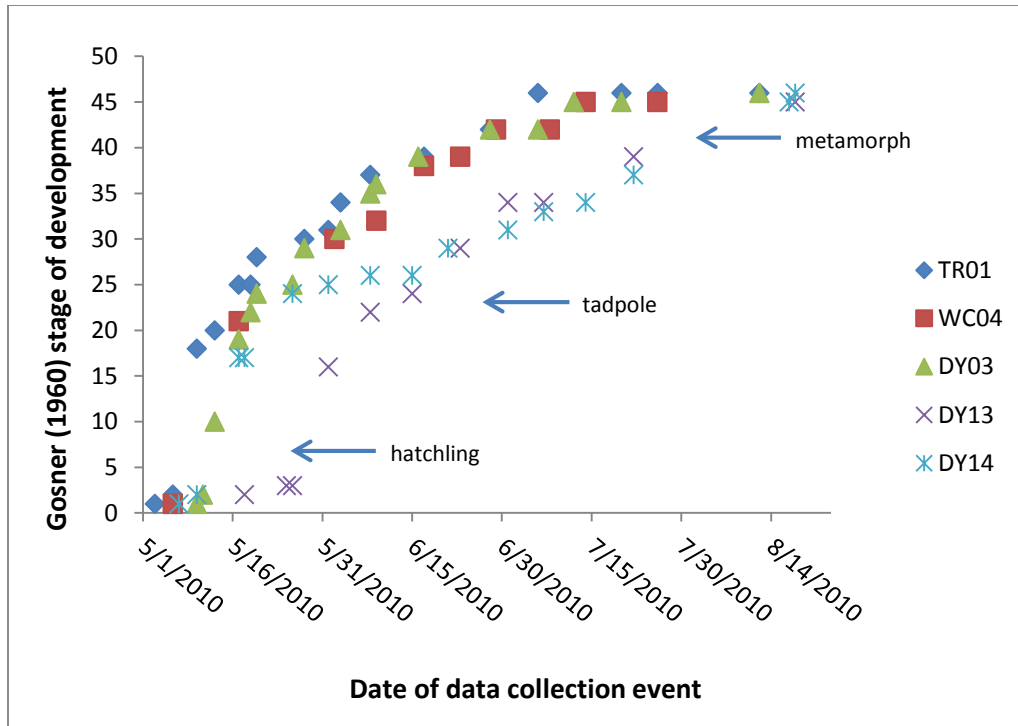


Figure 2. Developmental rate of first boreal toad egg masses laid at each core breeding site (n=5) during the summer of 2010 in KLGO.

Overall, 2010 was a very active and productive year for Boreal toads in KLGO. Lone adults were observed at breeding sites throughout the entirety of the monitoring season, as shown in Figure 3, and juveniles were observed from May 11 until the end of the season. Thirty-eight toads (19 pairs) in amplexus, 22 egg masses, 43 adults, an estimated 70,000 tadpoles, an estimated 1,800 metamorphs, and 210 juveniles were detected in total. 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. No amphibians captured were PIT tagged. Numbers for tadpoles and metamorphs are sums from the single survey counts with the greatest observations.

Amphibians were detected at 7 sites during routine amphibian surveys at intensive and extensive monitoring sites, as shown in Table 2. Five of these sites supported a fully successful breeding cycle, while two hosted amphibians but no signs of breeding were detected during the 2010 season. Table 2 also shows the changes in site *B. boreas* occupancy since 2004, reflecting changes in available habitat, such as in the case of WC02, WC04, and DY33. In 2008, maintenance of the West Creek road created a long, shallow, stagnant pond in a roadside ditch within 15m of WC02. That year toads were observed breeding in this new water body (WC04), rather than the more protected and ‘pristine’ site WC02. *B. boreas* breeding activity has not been detected at WC02 since the formation of WC04. DY33, once a very active breeding site, has been filled in with gravel for road maintenance, and thus not amphibians have been detected at the site since 2006.

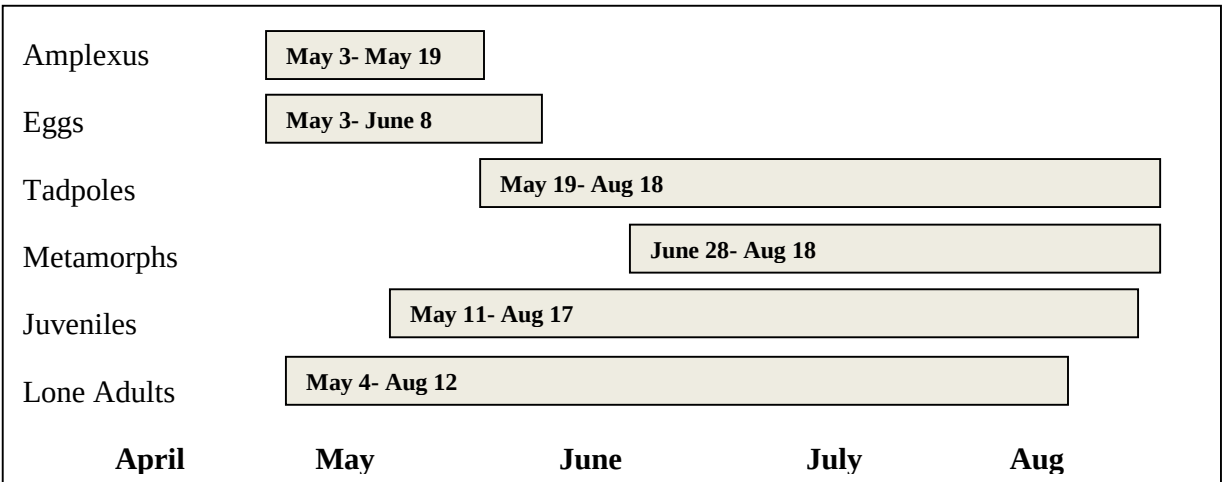


Figure 3. Boreal toad phenology date ranges observed at core sites in KLGO in 2010

Table 2. Detected site occupancy during the summer season for each life-stage from 2004-2010. "X" indicates positive detection, blank indicates no detection, and "--" indicates that the site was not surveyed.

Site ID	2004				2005				2006				2007				2008				2009				2010			
	life stages observed				life stages observed				life stages observed				life stages observed				life stages observed				life stages observed							
	amplexus or eggs	tadpoles	metamorphs	juveniles	adults	amplexus or eggs	tadpoles	metamorphs	juveniles	adults	amplexus or eggs	tadpoles	metamorphs	juveniles	adults	amplexus or eggs	tadpoles	metamorphs	juveniles	adults	amplexus or eggs	tadpoles	metamorphs	juveniles	adults			
DY02																												
DY03		X	X			X	X		X	X		X	X	X	X	X	X				X				X			
DY12				X																								
DY13				X			X						X															
DY14		X		X		X	X				X	X	X	X	X	X	X				X	X			X			
DY19							X		X				X												X			
TR01						X	X			X	X	X	X	X	X	X	X				X	X			X			
DY33		X			X	X				X	X	X		X														
WC02							X		X	X	X	X	X	X	X					X								
WC03										X				X	X													
WC04																												

Individual site results

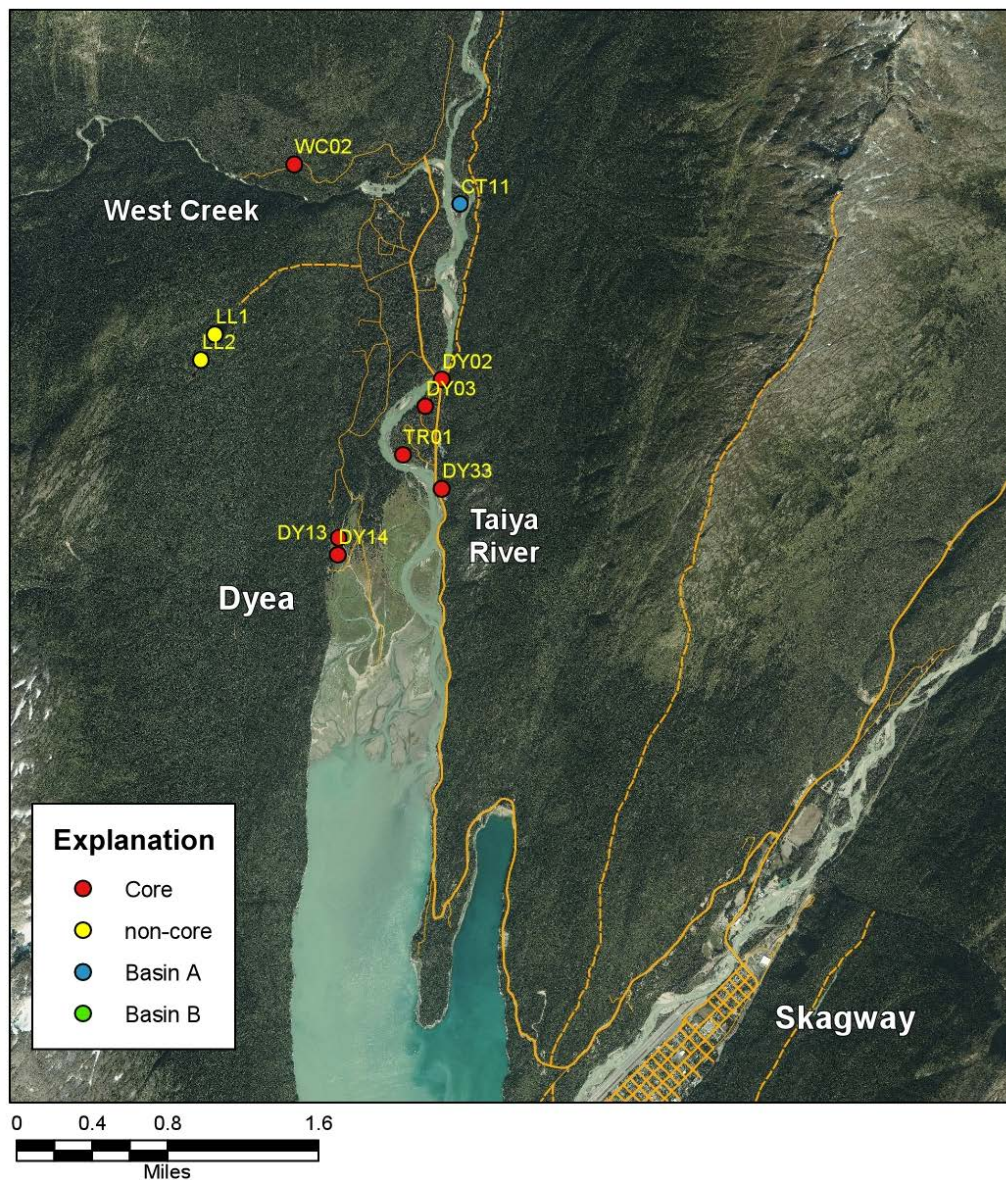


Figure 4. Location of Sample Sites in Dyea/West Creek Area

For each core breeding site and extensive site surveyed, a general habitat description for the 2010 season, summary of quantitative site data, and qualitative timeline in amphibian activity and significant morphology events are provided.



Figure 5. High water in the South lobe of TR01 on May 28th 2010

Taiya River 01 (TR01)

The productivity of this site is ever dependent of the fluctuating hydrology and morphology of the Taiya River. In some previous years the old river braids used as breeding ponds have dried completely, resulting in 100% desiccation of eggs and larva. Reversely, high water levels have also caused the river to reshape and expand, ultimately overwhelming the site with turbid glacial runoff and washing away any amphibians inhabiting the site. This year, a perfect balance of glacial melt and rainfall allowed for three distinct lobes to remain stable throughout the entire monitoring season, and resulted in the site's most productive breeding season since monitoring efforts began in 2004. To access this site, biotechs followed a path from the picnic area at the Dyea NPS campground, to the sandy embankment on the East shore of the Taiya River. Using GIS map prepared by biotech Heather Wetherbee (2009) as a guide, complete surveys included 100% shoreline search of all three lobes, each which extended approximately 150 meters.

TR01 received a total of 27 visual encounter surveys, 22 (81%) of which resulted in positive amphibian detection. The mean search duration for VES's was 41 minutes (n=26). Biotechs observed four egg masses (only two surviving to produce hatchlings and tadpoles), ~25,000 tadpoles, ~1,400 metamorphs, 6 adults in amplexus, and 10 lone adults.

Not only did this site host the earliest toad and egg mass observations of the season, but it demonstrated its most productive year since monitoring efforts began in 2004. On the morning of May 3rd, two adult toads and two egg masses were observed in South Lobe. Compared to previous years, TR01 had very low water fluctuations; the river never raised high enough to merge the three distinct lobes into a single channel or wash tadpoles downriver. However, the water did recede low enough at two points during the summer to call for minor interventions. The day after the first two

egg masses were laid, one was left exposed above water line and a portion of the egg mass was relocated to deeper water using a shovel, so as not to separate it from its original substrate. Water-levels soon returned to average depth and toads were observed breeding in the North Lobe for the first time ever, where water temperatures are consistently very low. The only egg mass laid in the North Lobe died and no larva hatched. A white water mold was observed on the egg mass. TR01 was very prone to disturbances by people, their pets, hares, otters, bears, and predation from shore birds. Despite these disturbances, the site's amphibians displayed resilience and tadpoles metamorphosed by June 28th, again the earliest observed appearance since 2004. By the end of the season, very few tadpoles remained and metamorphs had begun dispersing from the breeding pond.



Figure 6. An abundance of tadpoles were detected at TR01, pictured here at Gosner stages 30-35 on May 28th



Figure 7. High water at DY02 as seen from the Chilkoot Trail-head on June 9th, 2010

Dyea 02 (DY02)

While breeding activity has never been detected at DY02, this shallow wetland, dense with emergent vegetation appears to be an important staging ground for adult and juvenile *B. boreas*. Permission was not sought from private land owners to survey the entire area of this site as outlined in 2004, so VES were limited to the two stagnant ponds and surrounding grassy wetlands, and 20 meters south along the canopy covered outlet. At the start of the season, DY02 was completely dry, and then continued to fluctuate in temperature and depth throughout the season. The site was nearly dry on June 18th, but then refilled as water flow increased in the Taiya River.



Figure 8. Can you spot the toad? A well-camouflaged male was detected in emergent vegetation at DY02 on June 10th, 2010.

DY02 received a total of 13 visual encounter surveys, 4 (31%) of which resulted in positive amphibian detection. The mean search duration for VES's was 26 minutes (n=13). Eight adults and 1 juvenile were detected. It is likely that the same large female was detected repeatedly, as there appeared to be a type of burrow on the log in the center of the site where the adult individual would sunbathe. As many as 4 adults were observed occupying the site at once.



Figure 9. Looking North at the South pond of DY03 on May 26th, 2010

Dyea 03 (DY03)

This site is, at average water levels, composed of three distinct ponds. The Northern-most pond has a thick layer of leaf debris along the shore but relatively little leaf debris 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 expands over the decommissioned road leading into the site. While water levels did fluctuate, there were never any inlets or outlets to the site and the water remained stagnant and warm. These conditions not only supported the amphibian population detected throughout the season but a large variety of aquatic insects. Predacious diving beetles and dragonfly larva were observed hunting and feeding on tadpoles at this site.

DY03 received a total of 25 visual encounter surveys, 18 (72%) of which resulted in positive amphibian detection. the mean search duration for VES's was 31 minutes (n=25). Biotechs observed four egg masses during a single survey, ~30,000 tadpoles, 108 metamorphs, 6 adults in amplexus, and 6 lone adults. No dead or sick amphibians were observed at this site, but of the four egg masses laid only three developed into hatchlings, the first egg mass laid on May 10th died and ceased developing 10 days later. A high water event around June 3rd caused tadpoles to move into shallow puddles on the old road bed. When water levels returned to average on June 8th, many tadpoles were left stranded in the shallow depressions from bike and ATV tires and even footprint. These quickly dried up, leading to the desiccation of hundreds of tadpoles.

Dyea 13 (DY13)



Figure 10. Average water levels at DY13 on July 27th, 2010

Each successive biotech has designated the boundaries of this monitoring site slightly differently as water levels and stream morphology change to merge or detach DY12, DY13, and DY14. For the 2010 season, DY13 was considered to extend from North of the Nelson Slough vehicle bridge, to the town-site footbridge. The whole site was thick with emergent (up to 75% at high water) and submergent vegetation (75%), and water continuously flowed through it. The Northern half of this site was dry nearly the entire season. At low water the stream was only 1 meter wide, but at very high water levels was as wide as 6 meters and over 1 meter deep.



Figure 11. Thousands of hatchlings clung to grass and substrate at DY13 on June 8th, 2010

DY13 received a total of 19 visual encounter surveys, 12 (63%) of which resulted in positive amphibian detection. The mean search duration for VES's was 51 minutes (n=19). Biotechs monitored four egg masses at this site, all of which matured without desiccation or other extensive damage, and detected over 7500 tadpoles in a single survey. Overall, 218 metamorphs, 4 juveniles, 1 lone adult, and 1 dead adult were observed at this site. The only amphibians observed in the Northern half of the site were 3 juveniles, all other life stages were detected only in the Southern half of the site.

Dyea 14 (DY14)



Figure 12. At normal and high water levels, DY14 includes a large pond at its bend that is utilized for recreational purposes, such as inflatable rafting as pictured here

DY14 was one of the most dramatic and productive sites during the 2010 breeding season. Its boundaries extend south from the vehicle bridge to 10 meters North of the foot bridge to Robert Murphy's property. This site is part of the larger Taiya River estuary and thus it is a slightly brackish environment. Water levels at DY14 are affected by tidal movement as well as glacial melt and rainfall. Brown bears, black bears, great blue herons, salmon and other fish share this dynamic environment with the Boreal toad population that has been positively detected at the site every year since the monitoring program began.

DY14 received a total of 21 visual encounter surveys, 16 (76%) of which resulted in positive amphibian detection. The mean search duration for VES's was 74 minutes (n=21). Biotechs observed 12 adults in amplexus, 6 egg masses, ~30,000 tadpoles, 101 metamorphs, and 7 lone adults. Also found at the site were 2 dead juveniles and 1 dead adult. The success of the previous breeding season was evident in the number and activity of juveniles at this site. On clear, warm days they were extremely active along the shoreline of the pond. On May 26th, juveniles were observed burrowed into the muddy bank and moss in clusters of up to 15. Metamorphs at the end of the season were seen displaying similar behavior.



Figure 13. Young metamorphs at DY14 were observed clustered into piles up to 6 toads deep. This behavior helps to prevent desiccation and conserve heat.

Dyea 19 (DY19)

This site was not surveyed in 2009, but received routine amphibian surveys throughout the 2010 season. Of all the core breeding sites surveyed intensively, this site had the smallest fluctuations in water levels, and water was present from the start of the monitoring season. Its remote location, a short distance of bush-whacking from the Slide Cemetery, has also made it the least disturbed sites monitored.



Figure 14. SCA Intern Kassi Hauser surveying DY19 early in the breeding season (May 11th), before vegetation has begun to leaf out.



Figure 15. First juvenile of the season! Spotted at the edge of the DY19 wetland site on May 11th, 2010, this small *B. boreas* is likely a yearling that over wintered at the breeding pond.

DY19 received a total of 7 visual encounter surveys, 5 (71%) of which resulted in positive amphibian detection. The mean search duration for VES's was 68 minutes (n=6). No breeding activity was detected at this site in 2010, but 104 juveniles were observed overall. Juveniles which were caught and measured varied greatly in size (SVL min=19.1, max=33.9) and weight (min= <1g, max=10g). Average size and weight were 25.51mm (n=7) and 7g (n=6). This suggests that juveniles detected varied in age.

Dyea 33 (DY33)

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. DY33 now encompasses a large, deep pond in which no amphibians have ever been detected, and a gravel driveway which becomes flooded at high water. Vehicles regularly move through this area and during the 2010 season this site did not seem to be suitable toad breeding habitat.

From April 26th to June 9th DY33 received a total of 5 visual encounter surveys, 0 of which resulted in positive amphibian detection. The mean search duration for VES's was 21 minutes (n=5).



Figure 16. DY33 became flooded at high water on June 9th, but was dry for most of the 2010 breeding season.

West Creek 02 (WC02)

Due to its shaded environment, snow receded more slowly at this site than at a nearby roadside ditch, which was ice-free early in the season. While much breeding activity was detected at that nearby site, no breeding activity was detected at WC02 for the 3rd year in a row. WC02 received a total of 7 visual encounter surveys, none of which resulted in positive amphibian detection. The mean search duration for VES's was 18 minutes (n=7).



Figure 17. YCC Intern Kaitlyn Surdyk and Taiya Inlet Watershed Council Intern Alex Coberly assisted in the routine survey of WC02 on June 21st

West Creek 03 (WC03)

WC03 received just one visual encounter survey, during which no amphibians were detected. Conducting a full search of the small complex and a partial search of the large complex took a team of 5 people 105 minutes after arriving at the site.



Figure 18. One annual survey of WC03, an extensive monitoring site, was conducted with the help of KLGO interpretive and natural resource staff

West Creek 04 (WC04)

Like DY03, WC04 is an anthropogenic pond site. As essentially a road-side ditch, it receives much disturbance; dog prints, bike tire tracks, ATV tracks, and beer cans were all observed at the site in 2010. It has no inlet or outlet and water levels are dependent on initial snow melt and summer climatic conditions. Over the course of the summer it slowly decreased in size and depth.

WC04 received a total of 20 visual encounter surveys, 17 (85%) of which resulted in positive amphibian detection. the mean search duration for VES's was 31 minutes (n=20). Biotechs observed 14 adults in amplexus, 4 egg masses, 30 tadpoles in the single survey, 7 metamorphs in a single survey, and 5 lone adults.



Figure 19. Low water levels at WC04 on June 9th



Figure 20. A pair in amplexus at WC04 on May 6th

BD Detection and Swab Sampling

In 2009 Iris Holmes, a local of Haines Alaska and graduate student conducting research on genetic diversity of amphibians in the Chilkat, Chilkoot, and Taiya River valleys, offered to submit 7 BD swabs from KLGO for analysis on our behalf. Results from swab samples collected during the 2009 monitoring season received from Cornell Department of Ecological and Evolutionary Biology in Ithaca, NY all tested negative, however there may have been errors made during the PCR test. Thus, despite protocol calling for BD sampling every odd-numbered year, detection and sampling continued during the 2010 monitoring season. Of the 32 individual adult toads captured, measured, and inspected for abnormal behavior, malformations, or disease, 3 (9%) displayed symptoms associated with the BD fungal disease Chytridiomycosis. On May 18th, a male adult at DY13 (Figure 20) was captured which moved slowly, was very thin (SVL=76.8, Weight=51.0), and appeared to have a deformed or infected eye. Individual was swabbed for BD and results from PCR test by Pisces Molecular LLC came back “very strong positive”. On May 20th, a single large (SVL=91.0, Weight=72) female was captured and measured at DY02 which initially appeared dead and stiff, but began to move lethargically while being handled. PCR test results for BD presence from swab came back “negative/ too weak to be detected”. On June 1st, only one adult was observed at DY14 (Figure 21 and 22) which was very thin (SVL=75.9, Weight=46), lethargic, and had pinkish-red toes. Results from PCR test for BD presence from swab came back “very strong positive”. In total, 25 swab samples (three pre-pooled samples) from adults and juveniles at seven core breeding sites were submitted to Pisces Molecular for analysis. The report and results tables received from Pisces Molecular LLC in January 2011 are provided in Appendices I and II, and compiled swab results are shown in Table 3.



Figure 21. An apparent eye-infection or deformation was observed on this lethargic male *B. boreas* at breeding site DY13 on May 18th.



Figure 22. Protruding tail bone, taught skin on dorsal stripe, muscle deterioration in limbs, and lethargic behavior are all symptoms of the fungal disease Chytridiomycosis.



Figure 23. The toes on both the fore and hind limbs of A1, observed at DY14 on June 1st, appeared pinkish-red in color. The small male seemed to lack to motor skills to move or control the digits.

Table 3. BD swab samples and results submitted for PCR analysis during the 2010 monitoring season. “w+”= weak positive, “vw+”=very weak positive.

Date	Site	Type	Gender	Swab ID	PCR Test Results
5/04/2010	WC04	Adult	Male	WC02May4A2	—
5/04/2010	WC04	Adult	Male	WC02May4A1	—
5/06/2010	DY14	Adult	Female	DY14May6A1	—
5/06/2010	DY14	Adult	Female	DY14May6A6	+ +
5/10/2010	WC04	Adult	Male	WC02May10A1	+ +
5/17/2010	DY14	Adult	Male	DY14May17A1	—
5/17/2010	DY14	Adult	Male	DY14May17A2	+ + +
5/17/2010	DY14	Juvenile	Unknown	DY14May17J1	—
5/18/2010	DY14	Adult	Female	DY14May18A1	+ + +
5/20/2010	DY02	Adult	Female	DY02May20A1	—
5/20/2010	TR01	Adult	Male	TR01May20A1	—
6/01/2010	DY14	Adult	Male	DY14June1A1	+ + +
6/03/2010	DY03	Adult	Male	DY03June3A1	+ + +
6/08/2010	DY14	Juvenile	Unknown	DY14June8J1	+ +
6/09/2010	DY02	Adult	Female	DY02June9A1	—
6/09/2010	DY02	Adult	Female	DY02June9A2	+ + +
6/09/2010	DY02	Adult	Male	DY02June9A3	w +
6/09/2010	DY19	Juvenile	Unknown	DY19June9J3	vw +
5/11/2010	DY19			DY19May11J1	—
5/17/2010	TR01			TR01May17A1	—
5/18/2010	DY13			DY13May18A1	+ + +

Abnormalities and Disease Observations

While the summer of 2010 saw unprecedented breeding productivity since monitoring began in 2004, it was also plagued with record incidents of adult and juvenile mortality, abnormalities among tadpoles and metamorphs, and egg mass infection and mortality. To best organize and summarize results of VES's during which abnormalities or disease symptoms were observed, this section has been subdivided into three parts: Adult and Juvenile Mortality, Tadpole and Metamorph Abnormalities, and Egg mass Infection and Mortality.

Adult and Juvenile Mortality

Two dead adults and 2 dead juveniles were observed by biotechs at core breeding sites this summer, while an additional 3 dead adults were reported by park staff and visitors on roadways, and 1 desiccated ("mummified") adult *B. boreas* found on a home porch in the spring. See Table 4.

The first dead adult found at a breeding site this year was observed and collected by biotechs Heather Wetherbee and Dawn Adams on May 18th. While conducting a routine survey, the biotechs came across the freshly-dead toad in the water approximately 30 meters south of the vehicle bridge. This was the same location where two breeding pairs were observed the previous day. The large female appeared to have a contusion or some internal bleeding on its underside, but was other-wise healthy looking. After collecting measurement and swabbing for BD, the specimen was frozen and shipped to David Green at the National Wildlife Health Center for a full diagnostic examination. The diagnostic services case update, received June 11th, reported no evidence of injuries by external, internal and/or radiographic exams. In the final report, received August 25th, the probable cause of death is determined to be an extensive infection by the pathogenic chytrid fungus. See Appendix 7 for case update and final report.

Table 4. Adult and Juvenile amphibian mortalities reported during the 2010 monitoring program.

Date Observed	Location	Type	Cause of Death	Collected
5/18/2010	DY14	Adult	Chytridiomycosis	Yes
6/01/2010	DY13	Adult	NWHC Results Pending	Yes
6/03/2010	Dyea Campground	Adult	Crushed by Vehicle	No
6/21/2010	DY14	Juvenile	NWHC Results Pending	Yes
7/14/2010	Dyea Town Site Road	Adult	Crushed by Vehicle	No
7/22/2010	DY14	Juvenile	Unknown	Yes
7/30/2010	Road South of Vehicle Bridge	Adult	Crushed by Vehicle	No

The second dead adult submitted to D. Green was found on June 1st, at breeding site DY13, 10 meters north of the vehicle bridge. It was in an advanced state of decomposition (Green estimated 2-5 days) and covered in a gelatinous water mold. Following directions of D. Green, the toad was frozen and stored. It was submitted with a second specimen on June 28th. No results have yet to be received from the NWHC.



Figure 24. Adult toad found floating in water and emergent vegetation at DY13. Specimen submitted to NWHC for a full diagnostic examination.

Submitted with the frozen adult from DY13 was the first dead juvenile to be observed since monitoring began in 2004. The specimen was floating in water 20 meters south of the vehicle bridge, near the North end of the large pond at the center of DY14. No results have yet to be received from the NWHC.

A second dead juvenile was observed at the South end of DY14 on July 22nd, and was only noticed due to the large cluster of tadpoles feeding on it. The specimen was collected and is currently stored in the Natural Resource freezer in the Mascot garage, but has not been submitted for analysis due to the advanced state of decomposition.



Figure 25. Biotechs observed a decomposing Juvenile without any obvious injury or cause of death being fed on by fellow tadpoles, caddisflies, and other insect larva

Tadpole and Metamorph Abnormalities

2010 is the first year abnormalities have been observed in *B. boreas* tadpoles and metamorphs at KLGO breeding sites. Abnormalities were recorded at DY13, DY14, and DY03, all of which are core breeding sites.

Table 5. Tadpole measurements from DY13 on July 7th 2010. Note L2 tails are 11mm shorter in length than normally developed L1.

Group ID	L1	L2
Estimated # larvae	3,000	2
Total length	30.0	19.4
Body length	13.6	13.9
Gosner stage	32	33

The first tadpole abnormalities were noted July 7th at DY13; two tadpoles (<1% of estimated tadpoles at site) were observed with incredibly short tails (Table 3). Their Gosner Stage was still larval and tail reabsorption should not have begun yet. On July 20th, 6 tadpoles (<2% of estimated tadpoles at site) were observed in the middle pond of DY03 also with usually short tails. On July 22nd during a survey of DY14, approximately 30% of tadpoles in large mass at the south end of the site were estimated to have unusually short tails.

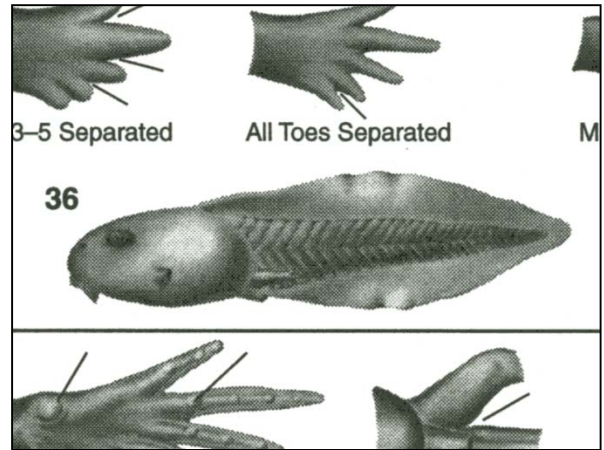
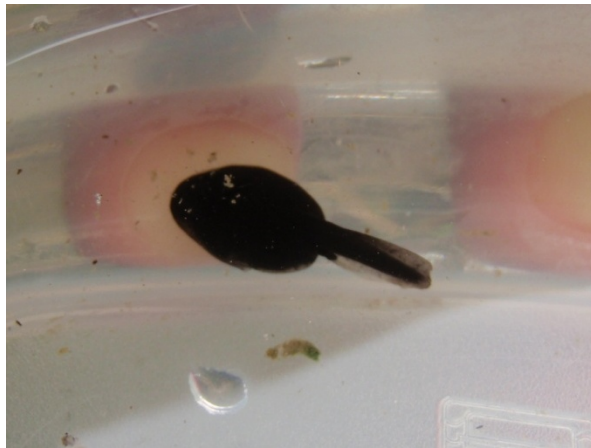


Figure 26. A tadpole with an abnormally short tail observed at DY03 July 20th as compared with a Gosner illustration of the normal development at stage 36.

On July 28th, tens of tadpoles at DY13 were observed that appeared to be sick or diseased. The “sick” tadpoles were strangely shaped (lumpy) with white spots or patches on their bellies and near their mouth parts. Biotechs collected one live tadpole (Gosner stage 39) that seemed to have some type of injury and infection around its mouth and a possible mutation. Its nostrils also looked inflamed or infected. The park has not adopted any protocol as of yet for surveying tadpole abnormalities, only the latter observations were reported to D. Green, but no specimens sent for analysis. Reports of tadpole abnormalities were not made to North American Reporting Center for Amphibian Malformations (NARCAM) or a similar program.

Metamorph abnormalities were detected at 3 core breeding sites (DY13, DY14, and DY03) which had also displayed tadpole abnormalities. On August 11th, 4 metamorphs (8%), of less than 50 detected, were observed at DY13 with disfigured bodies or abnormal limbs. On August 12th a single metamorph, of 54 observed, was captured and measured at DY03 which was missing half of its left hind leg. On August 17th, Biotechs observed a single metamorph at the North end of DY14 missing a forelimb, similar to metamorphs observed at DY13. All cases of metamorph abnormalities were submitted to NARCAM for review.



Figure 27. Biotechs observed 4 metamorphs with unsymmetrical, misshapen bodies and missing forelimbs at DY13.



Figure 28. A metamorph was captured at DY03 with an abnormal hind-limb.

Egg mass Infection and Mortality

Not all of the egg masses detected during the 2010 monitoring season survived to produce hatchlings. A variety of biotic and abiotic factors are reported to damage or act as stressors on *B. boreas* egg masses including UV-B radiation, non-optimal temperatures, changes in pH, pollution, physical disturbances, and water-borne pathogens such as water-molds or fungi (Kiesecker, 1995). The reason for detected egg mass mortality in KLGO is not known, but white eggs were detected on each of the egg masses shortly before or at the same time that it was determined that the egg masses were no longer developing. In the Oregon Cascades, *B. boreas* egg masses have seen 50-100% mortality due to a pathogenic fungus, *Saprolegnia ferax* (Kiesecker, 1995). White eggs were observed on egg masses DY03, TR01, and DY13. Two of the 4 egg masses laid at WC04 died without developing into hatchlings.

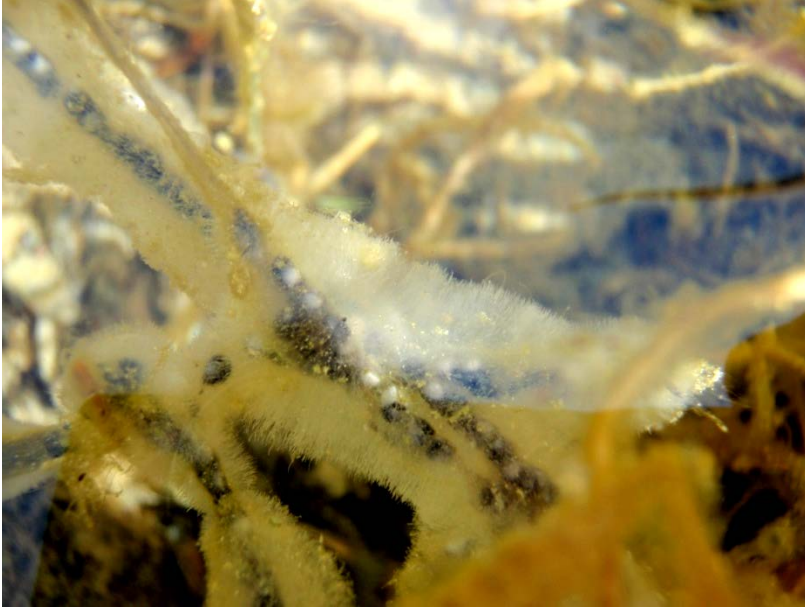


Figure 29. Dead egg mass infected with white water mold in the North Lobe of TR01

Conclusions

Discussion

Both the Skagway and Dyea River valleys once teemed with toads (Kalen 2010). Each spring, hundreds of drowsy amphibians thawed in the increasing daylight and made their way to warm, shallow breeding ponds. Elders from both communities have commented that bottoms of ponds were not visible in the spring, for all the freshly-laid, jelly-strings of egg masses (Kalen 2010, Albecker 2010, Fairbanks 2010).

Since 2004, KLGO has studied a small isolated group of amphibians in the Dyea valley whose population dynamics hardly resemble the proliferate toads of 50 years ago. In all seven years of monitoring, the breeding data results of the 2010 monitoring season are the first to indicate a population not in precipitous decline. Instead they have presented a more dramatic picture of the survival of a resilient, remnant population which may have already experienced a mass mortality event (Green 2010) and continues to be subject to variation in breeding site occupancy and breeding 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 causes. Anthropogenic development of critical breeding habitat, extensive harassment of amphibians during breeding season, climate change, increased UV-B radiation, introduction of predators (such as stocked fish in the Skagway valley) or the pathogenic BD fungus, are all suspected causes (Green 2010, Albecker 2010, Kalen 2010).

Tim Shields, herpetologist for the Takshanuk Watershed Council in Haines, AK (13 miles away) has reported a “ripping good year for tadpoles” in the Chilkat Valley. Though isolated from toads in the Chilkoot Valley, previous years of monitoring amphibian populations in the Chilkat had indicated a similar decline as was thought to be taking place in Dyea (Shields 2010). The simultaneous productivity of neighboring populations suggests synchronized, cyclical breeding patterns and not permanent precipitous decline.

Dyea’s remnant population, which has remained relatively stable since monitoring efforts began in 2004, faces continuous hardship from the presence of BD and may be facing new threats from spreading parasites and pathogenic water-molds. While abnormalities and symptoms of disease observed in each Western toad life-stage were at or below base-line (0-5%) abnormalities, it is important to note that no effort beyond Routine Amphibian Surveys was taken to survey or monitor reported abnormalities. All abnormality encounters were incidental.

Recommendations

Results from the 2010 monitoring season, review of recent scientific publications, and communication with amphibian disease expert David Green from the NWHC have led to several recommendations for improving KLGOs long-term monitoring strategy. These recommendations do not expand the scope or scale of the monitoring program, so much as increase the value and application of data collected and reported. Each recommendation is listed below with a justification for the proposal.

Genetic bottle-neck study or histological examinations of museum specimens

Despite the positive detection of BD on adult and juvenile toads for the last 4 years, *B. boreas* populations do not appear to be headed towards extirpation from the pathogenic fungus. If there is a trend in population decline, evidence from the last 7 years of monitoring suggests that it is gradual rather than abrupt. According to anecdotal evidence of historical abundance, populations are very low and distribution greatly reduced. Based on this information, Green has hypothesized that *B. boreas* population in the Dyea valley have already experienced a mass mortality event, as was observed in Colorado after the introduction of the fungal disease chytridiomycosis. This would predict that the toad population now being monitored during breeding season at a handful of core breeding sites is a remnant population of survivors that are resilient towards the disease. Two potential studies could be done to test the validity of this hypothesis and prediction. Genetic material in the form of tadpole tissue samples collected by Iris Holmes in 2008 could be used to determine whether the current toad population in Dyea has experienced bottle-neck population growth, as they would after an extreme mortality event. The second study would be a histological examination of museum specimens from the Skagway, Chilkoot, and Chilkat valleys to approximate the year BD was introduced to amphibians in the region.

Standard Operating Protocol for amphibian and site photodocumentation

Photography is a critical form of data collection in nearly all studies of biological diversity. However, photos can also become a burden to a long-term project if not taken, labeled, and organized in a valuable manner. For this reason, KLGO should adopt an SOP for amphibian and site photodocumentation. This should include procedures for taking photos in the field, and how and where to save them post-field. Repeat photography from designated locations of breeding sites could be valuable in monitoring long-term changes of the sites. The University of Wyoming's 2010 toad monitoring and reintroduction program report describes the successful pilot study of a toad photographic identification software developed by Tom Morrison from Dartmouth. This software has potential to be a valuable mark-recapture tool for long-term population studies of *B. boreas* as well as Wyoming Toad. KLGO should adopt the photodocumentation protocol developed by the Wyoming Natural Diversity Database for individual toad captures. Each photograph associated with a captured individual should also be geotagged and incorporated into the larger Amphibian GIS project.

Breeding site water quality monitoring and Standard Operating Protocol

During the 2010 monitoring season, KLGO partnered with the Taiya Inlet Watershed Council to collect water quality data at *B. boreas* core breeding sites. This data, collected long-term, could be valuable to understanding long-term changes in *B. boreas* abundance and distribution. In Colorado, episodic acidification may have made amphibians more prone to BD infection and contributed to the precipitous decline of toad populations in the state (Garcia, 2006). A Standard Operating Protocol for use of water testing equipment should be added to the Amphibian Monitoring Protocol, and water quality tests conducted as part of routine visual encounter surveys. Another condition that could be tested for at core breeding sites is the presence of BD in pond water. Results from 2010 chytrid swab samples found that not all amphibians captured at a single breeding site were infected with BD. This may suggest that BD does not survive for long without a host at the core breeding sites or that the rate of disease transmission through pond-water is low. Studies have found that the aquatic zoospores

produced by BD could survive in lake water for up to 7 weeks without a host (Johnson, 2003) and in sterile, moist river sand for up to 3 months (Johnson, 2005). A study in 2008 found that virulence of BD and prevalence of BD infection may depend on abiotic environmental factors and climatic suitability (Rodder, 2008). BD can be detected in sediments and water using PCR detection techniques. KLGO should consider funding a study utilizing the techniques as described by Kirchtein (2007).

Female body condition and survivorship as part of long-term monitoring efforts

The effect of climate change on breeding start dates for amphibians with seasonal breeding cycles (generally meaning high elevation or high latitude populations) has been extensively studied and reported in the academic literature (Blaustein, 2001; Corn, 2003; Beebee, 1995; Reading, 1998; Reading, 2006). The only evidence of breeding detected within the park boundaries of KLGO has been *B. boreas* populations in Dyea and West Creek and their start date of breeding (earliest detection of egg masses or adults in amplexus) has been reported by the amphibian monitoring program since 2004. Another way that the effect of climate change on toad populations has been measured is through analyzing the decline of female toads as a result of warmer temperatures (Reading, 2006). Morphological data such as Sex, SVL, Gape, Fore-arm length, and weight has been collected from captured individuals as part of the monitoring program since 2005, but has not been provided or summarized in annual reports. Female pre-spawning and post-spawning body condition index (BCI), based on protocol developed by Reading (2006), should be summarized in future annual reports. Between year comparisons of BCI and female survivorship could also be monitored through mark-recapture data. Photographic identification software is a noninvasive method for tracking individual toads over-time.

Amphibian oral history project SOP and historic distribution map

Anecdotal evidence for the past presence of toad populations at various locations in the Skagway and Taiya river watersheds has been collected and reported by the amphibian monitoring program since it began in 2004. During the 2010 monitoring season, this qualitative form of data collection was formalized in an amphibian oral history project. Two interviews were conducted and transcribed in 2010, describing the interviewees personal observations of historic abundance and distribution of amphibian species in Skagway and Dyea. An SOP should be written for using the Oral History Form and conducting interviews that produce meaningful data. GPS points should be estimated for each observation and an associated historic distribution map be generated based on those points.

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Appendix 1: Chytrid Fungus Test Results

Chytrid Fungus Test Results PCR assay for *B. dendrobatidis*

Test samples:

Organization:	National Park Service - Klondike Gold Rush National Historical Park
Received From:	Dave Schirokauer
Received Date:	8/30/10
Number of samples:	21
Type/condition of sample(s):	Swabs in ethanol (swabs in more than 2mls of ethanol were treated as pre-pooled samples)
Need to be pooled?	No

Sample Preparation:

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

For each pre-pooled swab sample the liquid in the tube was mixed by pipetting the liquid up and down repeatedly. Then the entire volume from each pooled tube was transferred into a clean 15 ml screw-capped centrifuge tube. After spinning the tubes at 1000 x G for 3 minutes, all of the supernatant, except 0.5 ml, was drawn off and discarded. The remaining 0.5 ml was transferred into clean microfuge tubes. The tubes were spun in a microcentrifuge at ~16,000 x G for 3 minutes. Next, the supernatant was drawn off and discarded. Lysis buffer was added to the tubes and any pellet present was resuspended by vortexing. 10 µg of carrier DNA was added to the lysis buffer.

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

PCR assay:

The sample DNA(s) prepared was/were assayed for the presence of the *Batrachochytrium dendrobatidis* ribosomal RNA Intervening Transcribed Sequence (ITS) region by 45 cycle single-round PCR amplification using an assay developed by Seanna Annis and modified for greater specificity and sensitivity at Pisces.

Each PCR run included the following controls:

- Positive DNA: DNA prepared from a laboratory culture of *B. dendrobatidis*, Strain JEL 270, kindly provided by Joyce Longcore. This sample was previously demonstrated to be positive by PCR. The signal from this sample is the standard for a very strong positive (+++) signal.
- Negative DNA: DNA prepared from an uninfected amphibian. This sample has been previously demonstrated as negative (-) by PCR.
- No DNA: H₂O in place of template DNA. This reaction remains uncapped during addition of sample DNA to the test reactions, and serves as a control to detect contaminating DNA in the PCR reagents or carryover of positive DNA during reaction set-up.

PCR Results for the *B. dendrobatidis* ribosomal RNA ITS region:

Number of positive (+) samples:	11
Number of negative (-) samples:	10

Individual sample results are shown on the following page(s).

- Scoring: +++ = very strong positive signal
- ++ = strong positive signal
- + = positive signal
- w+ = weak positive signal
- = no signal/below limit of detection

Individual sample PCR test results

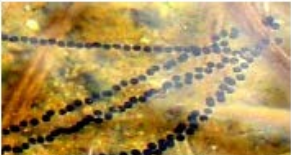
Source	Source ID #	Source Information	Piscines #	Sample Form/Condition	Tested For	Results	Comments
NPS-KGRNHP/DS	WC02May4A1	2010 Amphibian Monitoring (on bag)	103594	swab/EtOH	B. dendrobatidis	-	Source ID from sticker label
NPS-KGRNHP/DS	WC02May4A2	2010 Amphibian Monitoring (on bag)	103595	swab/EtOH	B. dendrobatidis	-	Source ID from sticker label
NPS-KGRNHP/DS	DY14May6A1	2010 Amphibian Monitoring (on bag)	103596	swab/EtOH	B. dendrobatidis	-	Source ID from sticker label
NPS-KGRNHP/DS	DY14May6A6	2010 Amphibian Monitoring (on bag)	103597	swab/EtOH	B. dendrobatidis	++	Source ID from sticker label
NPS-KGRNHP/DS	WC02May10A1	2010 Amphibian Monitoring (on bag)	103598	swab/EtOH	B. dendrobatidis	++	Source ID from sticker label. Retested positive.
NPS-KGRNHP/DS	DY14May17A1	2010 Amphibian Monitoring (on bag)	103599	swab/EtOH	B. dendrobatidis	-	Source ID from sticker label
NPS-KGRNHP/DS	DY14May17A2	2010 Amphibian Monitoring (on bag)	103600	swab/EtOH	B. dendrobatidis	+++	Source ID from sticker label
NPS-KGRNHP/DS	DY14May17J1	2010 Amphibian Monitoring (on bag)	103601	swab/EtOH	B. dendrobatidis	-	Source ID from sticker label
NPS-KGRNHP/DS	DY14May18A1	MAY 18 2010 (on tube) 2010 Amphibian Monitoring (on bag)	103602	swab/EtOH	B. dendrobatidis	+++	Source ID from sticker label
NPS-KGRNHP/DS	DY02May20A1	5/20/10 DY02 A1 (on tube) 2010 Amphibian Monitoring (on bag)	103603	swab/EtOH	B. dendrobatidis	-	Source ID from sticker label
NPS-KGRNHP/DS	TR01May20A1	2010 Amphibian Monitoring (on bag)	103604	swab/EtOH	B. dendrobatidis	-	Source ID from sticker label; label over previous writing
NPS-KGRNHP/DS	DY14June1A1	6/1/2010 DY14 A1 KLGO (on tube) 2010 Amphibian Monitoring (on bag)	103605	swab/EtOH	B. dendrobatidis	+++	Source ID from sticker label
NPS-KGRNHP/DS	DY03June3A1	6/3/2010 A1 DY03 KLGO-Skagway (on tube) 2010 Amphibian Monitoring (on bag)	103606	swab/EtOH	B. dendrobatidis	+++	Source ID from sticker label
NPS-KGRNHP/DS	DY14June8J1	6/8/2010 DY14 J1 (on tube) 2010 Amphibian Monitoring (on bag)	103607	swab/EtOH	B. dendrobatidis	++	Source ID from sticker label
NPS-KGRNHP/DS	DY02June9A1	6/9/2010 A1 DY02 KLGO-Skagway (on tube) 2010 Amphibian Monitoring (on bag)	103608	swab/EtOH	B. dendrobatidis	-	Source ID from sticker label
NPS-KGRNHP/DS	DY02June9A2	6/9/2010 A2 DY02 KLGO-Skagway (on tube) 2010 Amphibian Monitoring (on bag)	103609	swab/EtOH	B. dendrobatidis	+++	Source ID from sticker label. Retested positive.

Appendix 2: Individual Sample PCR Test Results

Individual sample PCR test results

Source	Source ID #	Source Information	Piscines #	Sample Form/Condition	Tested For	Results	Comments
NPS-KGRNHP/DS	DY02June9A3	KLGO-Skagway (on tube) 2010 Amphibian Monitoring (on bag)	103610	swab/EtOH	B. dendrobatidis	w+	Source ID from sticker label; label over previous writing. Retested positive.
NPS-KGRNHP/DS	DY19June9J3	06/09/2010 J3 DY19 KLGO-Skagway (on tube) 2010 Amphibian Monitoring (on bag)	103611	swab/EtOH	B. dendrobatidis	vw+	Source ID from sticker label. Retested positive.
NPS-KGRNHP/DS	DY19May11J1	2010 Amphibian Monitoring (on bag)	103612	prepooled swabs/EtOH	B. dendrobatidis	-	Source ID from sticker label; 1 swab
NPS-KGRNHP/DS	TR01May17A1	TR01 MAY 17 (on tube) 2010 Amphibian Monitoring (on bag)	103613	prepooled swabs/EtOH	B. dendrobatidis	-	Source ID from sticker label; 1 swab
NPS-KGRNHP/DS	DY13May18A1	DY13 A1 MAY 18 2010 (on tube) 2010 Amphibian Monitoring (on bag)	103614	prepooled swabs/EtOH	B. dendrobatidis	+++	Source ID from sticker label; 1 swab

Appendix 3: Two-sided note-card amphibian observation form and species guide

Amphibian Species Guide:		
		
Western Toad (<i>Bufo boreas</i>)		
		
Columbia Spotted Frog (<i>Rana luteiventris</i>)		
		
Wood Frog (<i>Rana sylvatica</i>)		

Name:		Phone:
Address:		
Date of Observation:		
Amphibian Species:		# seen:
Description of location:		
Description of observation:		
Please return completed sheets to NPS Visitor Center on 2nd and Broadway, or mail to: Department of Natural Resources Klondike Gold Rush NHP P.O. Box 517 Skagway, AK 99840. Questions? Call: (907)983-9240		

Appendix 4: Oral history interview form

A large, empty rectangular box with a thin black border, intended for the oral history interview form. It occupies the majority of the page below the title.

Appendix 5: AK2Day Amphibian Bulletin



NPS Photo by Alex Coberly. Hundreds of tiny toads, some with tails still remaining, rush to seek shelter in shallow water.

It's an exciting year for toads at Klondike Gold Rush NHP! On Monday, June 28th Natural Resource staff observed the earliest documented toad metamorphs since monitoring began in 2004. Over 600 of these toadlets appeared about one month earlier than average in massive clusters along pond shores in Dyea at a site that has suffered complete mortality of tadpoles in previous years. Coupled with record numbers of observed juveniles, this seems to be a highly productive year for our park's boreal toads. Such breeding success is a hopeful sign considering current rapid amphibian declines in Southeast Alaska, which reflect a larger, global decline trend.

Western toads, also called boreal toads, were historically widespread in the western United States and Canada, but have suffered extreme population loss due in part to a fatal fungal disease, *Chytridiomycosis*. In Colorado, western toad populations suffered an 80% decline over the course of several weeks in 1999 due to Chytrid. Department of Natural Resources has confirmed this disease in the park and has been closely monitoring the prevalence of the fungal infections since 2005. For the first time since monitoring efforts began, 2 adult toads and 1 juvenile were found at breeding site this summer which suffered no apparent injuries or wounds. Only one of the deceased amphibians was diagnosed as a victim of the disease by the USGS National Wildlife Health Center; results on the other two are pending.



Swabbing a juvenile's stomach to detect Chytrid fungus.



A healthy female laying eggs in Dyea.

Treating this disease in wild populations is extremely tricky; not all strains of the fungus are deadly, and many are helpful in the environment. Because Chytrid fungus digests carotene, without it our planet would be 6 feet deep in insect exoskeletons. But this situation is not hopeless, there are many things that we, as park employees, can do to help take care of our toads and prevent the further spread of *Chytridiomycosis*. First, watch your step when out in Dyea or giving tours in the town site, as juveniles and metamorphs will occasionally sunbathe in paths of high grass. Encourage visitors to stay away from breeding ponds; Chytrid could be transported between wetlands on our shoes and other surfaces. Finally, report any toad sightings to the Natural Resources office so that we can monitor our toads as closely as possible. Thank you for helping protect and conserve our amphibian friends, a valuable park resource!

Appendix 6: Water Quality Results

Location	Date	Time	Temp (°C)	Salinity (ppt)	DO% (sat)	DO (mg/l)	pH (units)	NO ₃ (mV)
TR01 (south)	6/28/2010	11:10 AM	16.13	0.06	106.50	8.66	8.92	167.80
	6/28/2010	11:13 AM	16.28	0.05	93.00	7.49	9.03	167.60
	6/28/2010	11:15 AM	15.89	0.05	81.80	6.98	8.69	166.60
	6/28/2010	11:17 AM	15.91	0.05	79.90	6.60	8.75	170.60
Average			16.05	0.05	90.30	7.43	8.85	168.15
TR01 (middle)	6/28/2010	11:23 AM	8.50	0.04	45.60	4.46	7.77	163.40
	6/28/2010	11:25 AM	9.62	0.04	56.30	5.36	7.63	165.60
	6/28/2010	11:26 AM	9.72	0.04	55.40	5.25	7.61	166.30
Average			9.28	0.04	52.43	5.02	7.67	165.10
DY03 (south)	6/28/2010	11:41 AM	15.87	0.02	98.90	8.18	7.88	157.30
	6/28/2010	11:43 AM	15.92	0.02	97.50	8.05	7.85	155.40
	6/28/2010	11:44 AM	15.76	0.02	99.90	8.27	7.92	153.80
Average			15.85	0.02	98.77	8.17	7.88	155.50
DY03 (middle)	6/28/2010	11:53 AM	15.31	0.02	98.00	8.20	8.47	157.60
	6/28/2010	11:54 AM	15.34	0.02	96.60	8.08	8.28	155.50
	6/28/2010	11:55 AM	15.27	0.02	96.20	8.06	8.17	153.90
Average			15.31	0.02	96.93	8.11	8.31	155.67
DY19	6/28/2010	12:27 PM	13.70	0.03	21.70	1.89	7.40	156.40
	6/28/2010	12:28 PM	13.50	0.03	77.80	6.77	7.81	152.20
	6/28/2010	12:28 PM	13.39	0.03	37.00	3.22	7.23	152.70
			13.53	0.03	45.50	3.96	7.48	153.77

Appendix 7: NWHC Diagnostic report



NATIONAL WILDLIFE HEALTH CENTER

6006 Schroeder Road
Madison, Wisconsin 53711-6223
608-270-2400 (FAX 608-270-2415)

DIAGNOSTIC SERVICES CASE REPORT

Final Report

8/17/2010

CASE: 23089

EPIZOO:

Legal ☐ INV#:

Declassified ☐

Submitter:

Heather Wetherbee
Klondike Gold Rush Nat'l Historical Park
PO Box 517
3rd and Broadway
Skagway, AK 99840
Date Submitted: 5/28/2010

Specimen description/identification/Location:

ACC	SPECIES	SPECIMEN TYPE	BAND NUMBER	SUBMITTER'S ID	COUNTY	STATE
001	Toad, Western	CARCASS	Site# DY14		Skagway-Hoonah-Angoon	AK

DIAGNOSIS

1. Chytridiomycosis
2. Kidney parasites: Tubular mesonephritis probably due to myxozoan infection by *Sphaerotheca ohlmacheri*
3. Lung parasites: Verminous pneumonitis due to *Rhabdias* sp. in both lungs
4. Stomach parasites: Mild verminous gastropathy due to *Oswaldocruzia* sp.
5. Intestinal parasites: Mild verminous enteropathy due to *Cosmocercella* sp.

Comment:

8/17/2010

The immediate cause of death in this well-nourished, gravid adult female Western Toad is attributed to widespread infection of the skin by the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* ("Bd"). This chytrid infection (chytridiomycosis) was detected histologically in the ventral skin and toe webs. Due to autolysis (decomposition) and sloughing of portions of the skin, the severity of the chytrid infection was difficult to access, but it probably was sufficient to have been the immediate cause of death of the toad.

Bacterial cultures of the liver and oviduct produced moderate numbers of the bacterium, *Pseudomonas* sp. This is a ubiquitous bacterium of freshwater ecosystems and is among the first bacteria to invade internal organs after the death of aquatic animals. Hence, the bacteria were considered postmortem invaders and probably were not present in the organs prior to the toad's death. Special bacterial cultures on feces from the cloaca for *Salmonella* spp. were negative.

Virus cultures on tissues from this toad have been challenging and are still in progress. Inoculation of cell cultures with ground tissues from this toad have repeatedly resulted in the death of nearly all cells in the culture. The precise cause of the cell death has not been determined, but polymerase chain reaction (PCR) tests on the dead culture cells have repeatedly been negative for ranaviruses. It is suspected that toxins produced by *Pseudomonas* sp. in the viscera are complicating the virus cultures. A supplemental pathology report will be submitted if any viruses are isolated from this toad.

This toad had several parasites in its lungs, stomach, intestine, and mesonephroi ("kidneys"). Each genus and species of nematode (Diagnoses # 3 - 5, above) were present in very low numbers, so it is unlikely the worms caused illness in this toad or contributed to its death. Mesonephroi ("kidneys") of this toad contained mild to moderate numbers of the myxozoan parasite, *Sphaerotheca* (formerly *Leptotheca*) *ohlmacheri*. This parasite is widespread in amphibians in the US and Canada, but disease and mortality have not been attributed to it. It is considered unlikely this renal parasite caused illness or contributed to the death of the toad.

In summary, the probable cause of death in this well-nourished, gravid Western Toad was extensive infection by the pathogenic chytrid fungus (chytridiomycosis). Although virus cultures have been inconclusive to date, in our experience it is rare for viral infections to be detected in adult frogs and toads. The myxozoan and nematodal parasites in this toad were considered incidental findings.

CASE: 23069
EPIZOO:
Legal ☐ **INV#:**
Declassified ☐

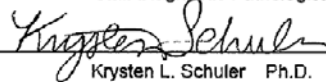
Final Report

8/17/2010



David Earl Green DVM, DACVP
Staff Diagnostic Pathologist

If you have questions regarding this case, contact:



Krysten L. Schuler Ph.D.

Wildlife Disease Specialist

Phone: 608-270-2447 E-Mail: kschuler@usgs.gov

Diagnostic findings may not be used for publication without the pathologist's knowledge and consent.

Copies To:

SHELBY SURDYK

Klondike Gold Rush Nat'l Historical Park, PO Box 517, 3rd and Broadway, Skagway, AK 99840

The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

NPS 461/123946, February 2014

National Park Service
U.S. Department of the Interior



Natural Resource Stewardship and Science

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Fort Collins, CO 80525

www.nature.nps.gov