Susitna–Watana Hydroelectric Project
(FERC No. 14241)

Wood Frog Occupancy and Habitat Use
Study Plan Section 10.18

Initial Study Report
Part A: Sections 1-6, 8-10

Prepared for

Alaska Energy Authority

SUSITNA-WATANA HYDRO

Clean, reliable energy for the next 100 years.

Prepared by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

LIST OF ACRONYMS, ABBREVIATIONS, AND DEFINITIONS

Abbreviation	Definition
ADF&G	Alaska Department of Fish and Game
AEA	Alaska Energy Authority
AICc	Akaike's Information Criteria, corrected for small sample size
AKNHP	Alaska Natural Heritage Program
APA	Alaska Power Authority
Bd	Batrachochytrium dendrobatidis, amphibian chytrid fungus
C.I.	Confidence interval
CIRWG	Cook Inlet Working Group
d	Degrees of freedom
FERC	Federal Energy Regulatory Commission
RSP	Revised Study Plan
ft	Foot, feet
GIS	Geographic Information System
GPS	Global Positioning System
ILP	Integrated Licensing Process
ISR	Initial Study Report
kph	Kilometers per hour
m	meter
min	minimum
mph	Miles per hour
n	Sample size
NHD	National Hydrography Dataset
NWI	National Wetlands Inventory
p	Detection probability
pH	A measure of the acidity or alkalinity of a solution
PLP	Pebble Limited Partnership
Project	Susitna-Watana Hydroelectric Project
qPCR	Quantitative Polymerase Chain Reaction
S.E.	Standard error
SPD	Study Plan Determination
USFWS	United States Fish and Wildlife Services
USGS	United States Geological Survey

1. INTRODUCTION

On December 14, 2012, the Alaska Energy Authority (AEA) filed its Revised Study Plan (RSP) with the Federal Energy Regulatory Commission (FERC) for the Susitna-Watana Hydroelectric Project No. 14241 (Project), which included 58 individual study plans (AEA 2012). Section 10.18 of the RSP described the Wood Frog Occupancy and Habitat Use Study. On February 1, 2013, FERC staff issued its study determination (February 1 SPD) for 44 of the 58 studies, approving 31 studies as filed and 13 with modifications. RSP Section 10.18 was one of the 31 studies approved with no modifications.

The wood frog study focused on evaluating the distribution of breeding wood frogs in those portions of the study area in the upper and middle Susitna River basin where breeding frogs could be directly or indirectly affected by Project development activities. The study is being conducted over two years, with field work scheduled in May/June each year and involving both field surveys and habitat occupancy modeling. In addition, AEA proposed to opportunistically capture and sample frogs (non-lethally) to test for the presence of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), which has been linked to amphibian declines worldwide (Olson et al. 2013).

Following the first study season, FERC's regulations for the Integrated Licensing Process (ILP) require AEA to "prepare and file with the Commission an initial study report describing its overall progress in implementing the study plan and schedule and the data collected, including an explanation of any variance from the study plan and schedule." (18 CFR 5.15(c)(1)). This Initial Study Report (ISR) on the Wood Frog Occupancy and Habitat Use Study has been prepared in accordance with FERC's ILP regulations and details AEA's status in implementing the study, as set forth in the FERC-approved RSP (referred to herein as the "Study Plan").

2. STUDY OBJECTIVES

The goal of the Wood Frog Study is to characterize the use of the Project area by breeding wood frogs to facilitate an assessment of potential impacts on wood frogs from development of the proposed Project.

The study has four objectives, as outlined in RSP Section 10.18.1:

- Review existing data on habitat use and distribution of breeding wood frogs in a broad region surrounding the study area.
- Estimate the current occupancy rate for breeding wood frogs in suitable habitats in the study area through a combination of field surveys and habitat-occupancy modeling.
- Use information on current habitat occupancy and habitat use to estimate the habitat loss and alteration expected to occur from development of the Project.
- Sample frogs opportunistically for the presence of the chytrid fungus that has been linked to amphibian population declines. (At the request of state and federal management agencies, AEA agreed to sample for the chytrid fungus to opportunistically take

advantage of planned fieldwork and thereby provide some baseline information on the occurrence of the fungus in the study area before development.)

The Wood Frog Study was planned as a two-year study. Results from the first year of work in 2013 presented here will be used to refine the study plan for the next year of study, if necessary.

3. STUDY AREA

As established by RSP Section 10.18.1, the study area includes those water bodies and suitable wetland habitats in the proposed Project area in which habitat loss, habitat alteration, and disturbance could potentially occur. The study area encompasses the reservoir inundation zone, associated areas for the dam and camp infrastructure, and the potential access-road corridors (Gold Creek, Chulitna, and Denali corridors) and material sites (Figure 3-1).

4. METHODS AND VARIANCES IN 2013

The methods for each of the Wood Frog Occupancy and Habitat Use Study components are presented in this section.

4.1. Auditory Field Surveys

AEA implemented the methods described in the Study Plan with the exception of the variances explained below (Section 4.1.1).

Because the study area is large and the calling period of breeding male frogs is short, this study did not involve a comprehensive survey of all potential frog breeding habitat present in the study area. Instead, observers surveyed for frogs in suitable habitats that were stratified into two habitat types (water bodies and wetlands). The study team used a Geographic Information System (GIS) to compile the full list of possible sampling locations (n = 148) by reviewing available information from existing GIS data layers (National Hydrography Dataset [NHD] and National Wetlands Inventory [NWI]) and by conducting additional interpretation of aerial imagery for portions of the study area for which recent imagery was available. The study team selected suitable water body and wetland habitats for frogs by (1) identifying areas with emergent vegetation; (2) removing shoreline wetland polygons adjacent to water bodies (and just including the water bodies); (3) removing locations within 250 m of another suitable location; and (4) removing sampling locations on or within 50 m of Cook Inlet Region Working Group (CIRWG) lands, for which access was not permitted in 2013. Next, the study team selected sampling locations (n = 120) by stratifying equally by area (reservoir impoundment zone, access roads and transmission corridors) and then randomly selecting equal numbers of each habitat type (water body, wetland) by area. The study team included the remaining locations (n = 28) as alternative sampling locations, if needed.

The study team conducted ground-based auditory surveys of the randomly selected water bodies and wetlands in the study area during the breeding season for frogs (May 30 to June 8). In addition to these surveys, incidental detections of wood frogs were documented during data collection efforts for other studies (mainly ground-based bird surveys), which provided additional information on the occurrence of frogs in the study area. The study team accessed survey sites by helicopter and on foot by navigating to predetermined sample sites using hand-held global positioning system (GPS) receivers. The field surveys involved listening for auditory detections of calling frogs for 5-min periods along the margins of each water body or wetland sampled to ascertain the presence or absence of wood frogs. Before the surveys began, observers trained by listening to audio files of breeding calls of male wood frogs. At small water bodies and wetlands, a single observation point was sufficient to detect the presence of frogs, but for large water bodies and wetlands, multiple observation points were needed to determine the presence of frogs. Up to four observation points were located and sampled for large water bodies and wetlands, with distances of up to 500 m (1,640 ft) between points to achieve adequate survey coverage.

Up to two (occasionally three) independent, replicate surveys were made by trained observers at each water body during the generally accepted survey times for this species in Alaska (Gotthardt 2004; PLP 2011). Due to variability in the calling frequency of male wood frogs even during the peak of the breeding season (PLP 2011), two visits were needed to detect frogs at some water bodies. The second survey at each site was conducted by a different observer who generally did not have knowledge of the survey results from the first survey. However, because this study involved the use of a “removal design” to estimate occupancy, if detected on the first survey, a second survey was not needed (i.e., that site was “removed” from further sampling; Mackenzie and Royle 2005). Surveys were conducted only under favorable weather conditions (e.g., light rain or no rain, air temperature higher than 4° C [39° F], and wind speed ≤25 kph [15 mph]). Observers spent a minimum of 5 min at each survey location listening for calling frogs, but terminated the survey early if frogs were detected.

Habitat and environmental characteristics (e.g., size and depth of water body or wetland, substrate, presence and type of emergent aquatic vegetation, water quality [pH level, dissolved oxygen], ice cover, surrounding terrestrial vegetation, water and air temperature, precipitation, cloud cover, wind speed, time of day, beaver activity) were recorded during the field surveys for consideration in the development of a Project-specific occupancy estimation model based on the habitat characteristics of the occupied water bodies or wetlands. In addition, data from the Vegetation and Wildlife Habitat Mapping and Wetland Mapping Studies (Studies 11.5 and 11.7) and from the literature (e.g., Stevens et al. 2006; AKNHP 2008) were considered as potential model variables to characterize wood frog habitat.

4.1.1. Variances

The Study Plan (RSP Section 10.18.4.1) proposed that the potential water bodies and wetland habitats to be sampled would be identified from interpretation of aerial photos or remote-sensing imagery and from the preliminary mapping of vegetation, wildlife habitats, and wetlands. From this set of water bodies and wetlands, habitats were to be categorized as having a high or low probability of supporting breeding frogs (based on likelihood of supporting fish and presence of emergent vegetation). Lastly, the Study Plan proposed to select 10 sampling regions, two in each of the three access road corridors and four in the reservoir zone and dam and camp facilities area. In each sampling region, 12 potential water bodies or wetlands were to be selected through a stratified random process.

Several factors affected the study team's ability to institute the sampling approach described in the Study Plan: (1) current mapping of vegetation and wildlife habitats was not yet available before the 2013 field season began; (2) existing wetland information (e.g., NWI mapping) did not cover the entire study area and was not of sufficient accuracy and resolution for the study; (3) data were not available regarding the presence of fish in water bodies and wetlands before field surveys began; and (4) permission for access to CIRWG lands was not granted, precluding sampling in most of the Gold Creek corridor and parts of the Chulitna corridor and western portion of the reservoir zone. Therefore, the study team devised an alternative approach to selecting 120 sampling locations (described in Section 4.1 above) that still incorporated random selection of suitable sampling sites. This selection process fulfilled the original intent of the study plan to select sampling locations in a random manner throughout the study area.

In addition, the Study Plan (RSP Section 10.18.4.1) included the distribution of field survey times, which were originally planned for the period from approximately 1200 h to 2200 h but were conducted from approximately 0900 h to 2000 h instead because of logistical challenges. The data from acoustic monitors showed that the sampling times were appropriate for the study, as is described below in Section 5.3. The acoustic monitors provided excellent results for evaluating the times of day when frogs were calling.

As explained above, the applicable study objectives were achieved with these modified approaches.

4.2. Occupancy Modeling and Habitat Associations

AEA implemented the methods as described in the Study Plan with no variances.

Because frogs were not always detected during 5-min sampling sessions even when they were present, the study team used occupancy modeling to adjust the observed occupancy rates for non-detections (Mackenzie et al. 2002). Occupancy modeling uses resurveys of the same locations to estimate a detection rate (p) and then uses the estimated detection rate to calculate an adjusted occupancy rate estimate (Ψ). The observed ("naïve") occupancy rate of frogs in water bodies and wetlands was adjusted to account for those frogs present but not detected, thereby producing a corrected occupancy rate for the water bodies and wetlands in this study.

Occupancy modeling also allows the user to compare various models with different specifications of detectability and occupancy parameters. Because the study team used a removal design, in which locations were not revisited after frogs were detected, there was limited statistical power to estimate detectability and therefore assumed that detectability was constant for all surveys. The study team compared four covariates for occupancy: area (dam, camp, and reservoir area or road corridors), water type (wetland or water body), water depth (≤1.5 m [4.9 ft] or >1.5 m), and percent of hibernation habitat (visual estimate of the percent of herbaceous cover, low shrubs, and tall shrubs within 50 m of the shoreline). Area was included because the sample was stratified by area and the other three covariates were chosen because they were expected to be biologically important and because the analyses would only support a limited number of covariates.

The study team tested all possible combinations of these four covariates (without interactions), including an intercept-only model, for a total of 16 different models. Model calculations were run on a desktop computer using the single-season analysis format and custom model-building feature of the software program PRESENCE, Version 5.9 (Hines 2006).

These 16 models were compared using information–theoretic methods (Burnham and Anderson 2002). For each model, the study team calculated the Akaike Information Criterion corrected for small sample sizes (AICc) that compares model fit and penalizes models for the number of parameters to determine the most parsimonious model (the best fit with the fewest number of parameters). The AICc values were used to calculate the Akaike weight (ωi), which is the probability that each model is the best model in the candidate set (Burnham and Anderson 2002).

4.2.1. Variances

No variances from the methods described in the Study Plan occurred in 2013.

4.3. Acoustic Monitoring

AEA implemented the methods described in the Study Plan with no variances.

The study team used Wildlife Acoustics Song Meter SM2BAT+ platforms with SMX-II microphones to record frog calls onto 32-GB (Class 4 SDHC) data cards. The monitors were internally powered with rechargeable D-cell batteries (Imedion 9,500 mAh). Five acoustic monitors were deployed to increase accuracy in calculating the detectability of calling frogs. The monitors were deployed at a subset of water bodies and wetlands on state and federal lands known to be occupied by frogs. Although the monitors were programmed to record full-spectrum audio recordings for the first 30 min of each hour around the clock, the study team analyzed only the first 10 min of each hour. Analytical results indicated that this subsampling adequately characterized the calling activity within the hour.

The study team used the proportion of 5-min periods with frogs calling as an independent estimate of the ability to detect frogs at a given location, assuming that frogs were present. The validity of this estimate relies on several assumptions: (1) individual observers were able to detect frogs calling at least as well as the acoustic monitors; (2) the presence of observers did not lower the probability of frogs vocalizing; and (3) the locations chosen for acoustic monitoring were representative of all locations at which frogs were present. For each location surveyed, the study team determined the hour of the day the visit occurred and calculated the proportion of 5-min periods in which frog calls were heard on acoustic monitors during that hour. The study team then calculated the mean of all these proportions for each visit as a second, independent estimate of detectability.

4.3.1. Variances

No variances from the methods described in the Study Plan occurred in 2013.

4.4. Chytrid Fungus Bioassay

Sampling and laboratory assay methods for the chytrid fungus (*Bd*) were identified through consultation with U.S. Fish and Wildlife Service (USFWS) representatives in Alaska, who recommended that Tara Chestnut, an expert with the U.S. Geological Survey (USGS) in Portland, Oregon, be contacted for sampling protocols (Appendix A). Biologists wore fresh nitrile gloves and sprayed boots with a 10% bleach solution at each sampling location to prevent potential contamination among sites.

The study team captured seven frogs by hand opportunistically and swabbed the skin of the abdomen, inner thighs, and undersides of foot webbing for a total of 25 times with a sterile cotton swab, after which the frog was released unharmed. Swabs were placed in tubes that were refrigerated until all seven samples were shipped on dry ice to the USGS Microbiology laboratory in Reston, Virginia. The lab analyzed the samples using a quantitative polymerase chain reaction (qPCR) technique to test for the presence of *Bd* fungus.

4.4.1. Variances

No variances from the methods described in the Study Plan occurred in 2013.

5. RESULTS

Data developed in support of this study are available for download at http://gis.suhydro.org/reports/isr:

- ISR_10_18_FROG_Data_ABR.gdb/ISR_10_18_FROG_SamplingSites
- ISR_10_18_FROG_Data_ABR.gdb/ISR_10_18_FROG_IncidentalObs2013
- ISR_10_18_FROG_Data_ABR.gdb/ISR_10_18_FROG_AcousticMonitors
- ISR_10_18_FROG_Acoustic_Monitoring_Data.xlsx.

5.1. Auditory Field Surveys

The study team surveyed a total of 90 different wetlands and water bodies for the presence of wood frogs (Table 5.1-1, Figure 5.1-1). Additional water bodies and wetlands (n = 17) were visited but were excluded from the analyses for various reasons (e.g., water still frozen or insufficient water depth). Frogs were detected at 37 of the 90 locations (41.1 percent) on the first visit (Table 5.1-2) including 35 locations where frogs were heard calling and two locations where frogs were not heard but egg masses were found. The latter two locations were treated as non-detections in occupancy modeling because frogs were not detected using the normal survey method. The study team conducted a second survey visit at 50 of the 53 locations where frogs were not detected on the first visit, producing detections at 8 more locations (16.0 percent). A third visit was conducted at five of the 42 sites where frogs were not detected on the first and second visits, producing detections at two more locations (40.9 percent). Overall, frogs were heard or egg masses were observed at 47 (52.2 percent) of the 90 locations sampled (Table 5.1-3, Figure 5.1-1). Therefore, the naïve estimate of frog occupancy (assuming 100 percent detectability) was 52.2 percent.

5.2. Occupancy Modeling and Habitat Associations

5.2.1. Occupancy Modeling

The best model of frog occupancy contained only one variable: water depth. Based on the Akaike weight, this model had a 31.9 percent chance of being the best model in the candidate set (Table 5.1-3). The next three competing models contained water depth and one of the other variables, but in all cases, the 95 percent confidence interval (C.I.) contained zero, suggesting the other variables added little to the model. Once water depth was included, there was no statistical evidence that occupancy rates varied by area, by water type, or with increasing hibernation habitat.

The estimated detectability from the best model was 60.6 percent (95 percent C.I. = 34.8–81.6 percent; Table 5.2-1). The model results indicated that, if frogs were present in a pond, the study team would, on average, detect them 60.6 percent of the time with one visit, 84.5 percent of the time with two visits, and 93.9 percent of the time with three visits.

The estimated occupancy for shallow-water habitats was 36.8 percent (95 percent C.I. = 20.8–56.5 percent) and the estimated occupancy for deep-water habitats was 81.8 percent (95 percent C.I. = 44.4–96.2 percent; Table 5.2-1). As would be expected, these estimates were slightly higher than the naïve estimates of 31.0 percent and 70.8 percent, respectively. The sample included 42 shallow-water habitats (46.7 percent) and 48 deep-water habitats (53.3 percent). Assuming that this ratio is representative of the entire study area, the overall occupancy estimate is 63.4 percent (95 percent C.I. = 36.3–84.0 percent; Table 5.2-1).

5.2.2. Habitat Associations

Occupancy modeling was the primary tool to assess habitat associations with breeding male wood frogs and water depth was the most important habitat variable. Frogs were detected at a total of 13 of 42 (31.0 percent) locations with shallow water (≤1.5 m) and 34 of 48 (70.8 percent) locations with deep water (>1.5 m). The remaining habitat variables were summarized by locations where wood frogs were detected, not detected, and across all sampling locations (Table 5.2-2). The only other association of significance was dissolved oxygen, with lower levels being found where frogs were detected (although only when expressed in the units of mg/l; Table 5.2-2).

5.3. Acoustic Monitoring

Acoustic recordings from the five monitors provided a sample of 2,015 5-min intervals that were used to quantify when frogs were heard calling. Calling activity varied by date and time of day (Figure 5.3-1). The results demonstrated that the surveys were well-timed to capture the peak of calling activity in the study area; frogs were calling when the acoustic monitors were deployed on May 31 and calling activity declined by the end of the survey period on June 9 (Figure 5.3-1a). A very strong diurnal pattern of calling activity was evident. Calling activity peaked near 0100 h, then activity dropping dramatically early in the morning (0500 h) and increased throughout the remainder of the day (Figure 5.3-1b).

Based on the time-specific results from the acoustic monitors, the site visits should have had a detectability of 60.8 percent, which was essentially identical to the estimate of 60.6 percent from the occupancy modeling. This concurrence provides additional evidence that the occupancy modeling provided a reasonable estimate of detectability and indicates that occupancy rates were adjusted appropriately.

5.4. Chytrid Fungus Bioassay

The swab samples collected opportunistically from seven frogs in 2013 were sent to the USGS Reston Molecular and Environmental Microbiology Laboratory in Reston, Virginia, and tested for the presence of chytridiomycosis (*Bd*) using standard qPCR protocols (Boyle et al. 2004). All seven samples tested negative for *Bd*.

6. DISCUSSION

Amphibian populations appear to have been declining worldwide for several decades (Blaustein and Wake 1990; McCallum 2007), leading to elevated levels of concern about the conservation status of a large number of amphibian species. Although populations appear to be healthy in Alaska (Gotthardt 2004, 2005), concern has been expressed about the conservation status of wood frogs in Alaska (ADF&G 2006). Because amphibians were not included in the original Alaska Power Authority Susitna Hydroelectric Project (APA Project) environmental study program in the 1980s, information on the occurrence of wood frogs in the upper Susitna drainage was lacking and their status in the study area was unknown at the time this study began.

6.1. Distribution and Habitat Use

A review of the literature shows that wood frogs are widely distributed throughout northern North America and that, in Alaska, they occur from Southeast Alaska throughout Central Alaska to the crest of the Brooks Range (MacDonald 2010). Closer to the study area, they have been documented in Denali National Park and Preserve, near Healy, and in the lower Susitna drainage (Cook and MacDonald 2003; Anderson 2004; Gotthardt 2004, 2005; Hokit and Brown 2006).

Wood frogs were widely distributed throughout the areas sampled in 2013. It is important to recognize, however, that the study team was unable to sample the Gold Creek corridor because of the lack of permits from CIRWG landowners (thus frog distribution in that area could not be evaluated) and the higher elevations of the Denali corridor (and parts of the Chulitna corridor) were still covered in snow and ponds were only beginning to thaw at the time of the field survey. Locations at higher elevations (>2,800 ft elevation) may need to be sampled later in the year to better assess frog distribution there.

Wood frogs occurred in a variety of habitats sampled in 2013, ranging from tundra to forested wetlands (see photographs in Appendix B). Wood frogs are known to inhabit diverse vegetation communities in Alaska, including tundra, open forests, grassy meadows, and muskeg (MacDonald 2010). Not surprisingly, the habitat associations of wood frogs are diverse, so a summary of known habitat associations is presented below and related to the findings of this study.

Water-body types in the study area ranged from those having insufficient water depth to allow frog larvae to metamorphose (i.e., the ponds would dry out too early in the season) to deep water lakes. Water depth was the most important habitat factor analyzed in this study, which was consistent with the results of a similar study in southwestern Alaska, in which water depth was an important habitat factor (PLP 2011). In both studies, calling male frogs were detected more frequently in habitats with deeper water (> 1.5 m).

Water-body depth may be important because deeper water bodies retain water and often maintain more consistent water-quality characteristics during the egg and larval growth stages (Knapp et al. 2003). In Denali National Park, Hokit and Brown (2006) found that wood frogs had the highest breeding activity (defined as eggs or larvae) in sites with 51 to 75 percent of the site < 50 cm (1.6 ft) deep, but with a maximum depth of 1 to 2 m (3.3 to 6.6 ft). Differences in sampling methods, sampling times, and characterization of water body depths, however, make direct comparison with this study difficult. Water depth may be one of many factors influencing where wood frogs choose to breed, judging from the findings of Herreid and Kinney (1966), in which 96 percent of wood frog eggs and larvae died before reaching metamorphosis because of lack of fertilization, freezing, desiccation of eggs at the water surface, temperature-related abnormalities, and predation.

Hibernation habitat (herbaceous, low shrub, and tall shrub vegetation within a 50-m radius of the shoreline) was not associated with frog detectability in this study, in contrast to the results reported by PLP (2011) in which wood frog occupancy increased as surrounding hibernation habitat increased. Increased availability of vegetation that provides suitable habitat for hibernation could be important for influencing occupancy of water bodies. The PLP (2011) study was conducted in a tundra area with much less tree cover than in the study area. Differences in habitat occupancy and vegetative cover may help to explain this difference between studies.

Emergent and aquatic vegetation in water bodies provides a substrate for frog egg masses and escape cover from aquatic predators, as well as helping to increase dissolved oxygen in the water (France 1997; Babbitt and Tanner 1998). Although the extent of emergent vegetation was not correlated with frog occupancy in this study, it provided a substrate for the egg masses observed in this study. Dissolved-oxygen levels were similar between sites occupied and not occupied by frogs and the overall level (8.53 mg/L) in this study was similar to that observed in a study in Southeast Alaska (approximately 9.0 mg/L; Carstensen et al. 2003) and was within the range of mean values from new (4.9 mg/L) and old (10.5 mg/L) beaver ponds in Alberta (Stevens et al. 2006). Increased concentrations of dissolved oxygen were thought to be important in the latter study because they were correlated with enhanced larval growth rates of wood frogs in old beaver ponds, although the authors cautioned that this may have been an artifact of landscape context (Stevens et al. 2006).

Other aspects of water quality such as pH may be important for breeding-site selection by wood frogs. A study in Quebec reported that egg mass density and hatching success were negatively correlated with pH, although hatching success was still fairly high (47 and 80 percent in ponds with pH of 4.3 and 4.7, respectively; Gascon and Planas 1986). Another study near Juneau, Alaska, measured pH levels ranging from 4.5 to 5.5 in ponds where larval wood frogs were present (Carstensen et al. 2003). New and old beaver ponds in Alberta containing wood frogs had pH levels of 7.6 and 7.8, respectively (Stevens et al. 2006). The pH values in the study were very consistent throughout the sampling locations (5.73 at occupied sites and 5.72 at unoccupied sites), within the range of other studies where wood frogs bred successfully.

Other habitat variables measured in the study did not have clear relationships with frog occupancy, including water body type (e.g., pond size, presence of beaver activity) and some aquatic habitat features (e.g., substrate). Additional sampling in the next study season will provide additional data to evaluate the importance of habitat characteristics for breeding occupancy by wood frogs.

6.2. Occupancy Modeling

Accurate habitat occupancy estimates are adjusted for the detectability of organisms in the environment. Detectability in this study (60.6 percent from the best model [water depth], 60.8 percent from the acoustic monitors [see Section 6.3 below] was high compared with the estimated detectability in another study in southwestern Alaska (26.6 percent; PLP 2011). The lower detectability in that study may have resulted from differences in habitat characteristics, survey conditions, frog densities, or the timing of the surveys. The high detectability in this study indicates a robust study design: if frogs were present in a pond, the study team would, on average, detect them 60.6 percent of the time with one visit, 84.5 percent of the time with two visits (the normal sampling protocol), and 93.9 percent of the time with three visits.

The best model of frog occupancy in this study contained only the variable water depth, with deeper water types having higher occupancy. The estimated occupancy for shallow-water habitats (36.8 percent), deep-water habitats, (81.8 percent), and all locations overall (63.4 percent) suggest a widespread distribution of frogs in the areas surveyed in 2013 (dam and camp area, reservoir inundation zone, Chulitna corridor, part of the Denali corridor). Few studies have established occupancy rates of wood frogs in Alaska. The naïve occupancy rate in Denali National Park and Preserve was estimated at 45 percent (Hokit and Brown 2006), which was generally similar to an adjusted occupancy estimate of 49.5 percent in southwest Alaska (PLP 2011), although adjustment of the Denali Park estimate would likely have resulted in a higher occupancy rate.

6.3. Acoustic Monitoring

Acoustic monitors provided a direct estimate of the detectability of calling frogs. The use of acoustic monitoring devices allowed the study team to collect a large amount of information to characterize the calling activity of breeding male wood frogs throughout the survey period and throughout all hours of the day. Frogs called throughout the survey period (May 30 to June 9) and incidental observations by other wildlife field crews noted calling frogs between May 28 and June 14, indicating that the surveys were well-timed in 2013, at least for the lower elevation locations (dam and camp area, reservoir zone, most of the Chulitna corridor). Locations at higher elevations (much of the Denali corridor), however, were still snow-covered and many water types were either frozen or just beginning to thaw during the survey period. Frog calling activity within a day showed a pattern of high calling rates throughout the late morning and afternoon, with peak calling activity occurring between 0100 h and 0200 h. The sampling times between approximately 0900 h and 2000 h mainly fell within the period of high calling activity, helping to explain the high detectability of the surveys in this study.

An additional use of the acoustic data was to calculate the detectability (60.8 percent) of frogs calling when the study team actually sampled and compare that to the estimate from occupancy modeling (60.6 percent). Concordance between these results provides strong evidence that the occupancy modeling provided a reasonable estimate of detectability and that the occupancy rates were adjusted appropriately. This concordance is key to producing meaningful habitat occupancy results for eventual use in estimating the potential habitat loss and alteration that may occur from development of the Project.

6.4. Chytrid Fungus

Bd is a chytrid fungus that causes the disease chytridiomycosis in amphibians. Since it was first discovered in amphibians in 1998, it has devastated amphibian populations around the world, including in North America (Adams et al. 2007, Olson et al. 2013). *Bd* is sometimes a non-lethal parasite and some amphibian species and some populations of susceptible species are known to survive infection. The fungus is widespread and ranges from lowland forests to cold mountain tops, and is typically associated with host mortality in high altitude environments and during winter, with greater pathogenicity at lower temperatures. *Bd* is believed to spread mainly through contact between infected frogs or with infected water.

Wood frogs have been identified as a species susceptible to infection by *Bd*, and it was first detected in Alaska in a dead wood frog found in the Kenai National Wildlife Refuge in 2002 (Reeves and Green 2006, Reeves 2008). Another positive detection of *Bd* occurred near Dyea in Southeast Alaska in 2006 and was associated with the apparent die-off of western (boreal) toads in that region (*Juneau Empire*, May 21, 2006). *Bd* was documented in boreal toads (*Bufo boreas*) and red-legged frogs (*Rana aurora*) in another study in western Canada and Southeast Alaska (Adams et al. 2007). Although *Bd* was not detected in this study, the small sample size of swabs obtained in this study is inadequate to confirm its absence unequivocally.

7. COMPLETING THE STUDY

[Section 7 appears in the Part C section of this ISR.]

8. LITERATURE CITED

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9. TABLES

Table 5.1-1. Number of Frog-survey Visits to Water Bodies and Wetlands in the 2013 Study Area.

Location	First Visit	Second Visit	Third Visit
Dam and Reservoir Area			
Water body	28	9	0
Wetland	21	9	0
Total	49	18	0
Corridors			
Water body	28	21	4
Wetland	13	11	1
Total	41	32	5
Grand Total	90	50	5

Table 5.1-2. Frog Occupancy in Shallow- and Deep-water Habitats in 2013.

	First Visit		Second Visit		Third Visit		Overall	
Location	Detected	Not Detected	Detected	Not Detected	Detected	Not Detected	Detected	Not Detected
Shallow water (<1.5 m)[a]	8	34	3	29	2	3	13	29
Deep water (>1.5 m)	29[b]	19	5	13	–	–	34	14
Total	37	53	8	42	2	3	47	43

Notes:

a 1.5 m = 4.9 ft.

b Two locations were included where egg masses were observed but no frog calls were detected.

Table 5.1-3. Occupancy-model Selection Results for Presence of Wood Frogs in 2013.

Model[a]	–2*LL[b]	K[c]	AICc[d]	ΔAICc[e]	ω$_i$[f]
Ψ (Water Depth), *p* (.)[g]	162.35	3	168.63	0.00	0.348
Ψ (Water Depth, Habitat), *p* (.)	161.71	4	170.18	1.55	0.160
Ψ (Water Depth, Water Type), *p* (.)	161.97	4	170.44	1.81	0.141
Ψ (Water Depth, Area), *p* (.)	162.00	4	170.47	1.84	0.139
Ψ (Water Depth, Water Type, Habitat), *p* (.)	161.51	5	172.22	3.59	0.058
Ψ (Water Depth, Area, Habitat), *p* (.)	161.61	5	172.32	3.69	0.055
Ψ (Water Depth, Area, Water Type), *p* (.)	161.84	5	172.55	3.92	0.049
Ψ (Global), *p* (.)	161.48	6	174.49	5.86	0.019
Ψ (Habitat), *p* (.)	168.78	3	175.06	6.43	0.014
Ψ (Water Type, Habitat), *p* (.)	168.53	4	177.00	8.37	0.005
Ψ (Area, Habitat), *p* (.)	168.75	4	177.22	8.59	0.005
Ψ (Area, Water Type, Habitat), *p* (.)	168.48	5	178.87	10.24	0.002
Ψ (Area), *p* (.)	172.59	3	179.19	10.56	0.002
Ψ (.), *p* (.)	175.18	2	179.32	10.69	0.002
Ψ (Area, Water Type), *p* (.)	171.93	4	180.40	11.77	0.001
Ψ (Water Type), *p* (.)	174.81	3	181.09	12.46	0.001

Notes:

a *Ψ* = occupancy variable; *p* = detection probability; Water Depth = 1 if depth > 1.5 m (4.9 ft); Habitat = proportion of shoreline containing hibernation habitat; Water Type = water body or wetland; and Area = dam, camp, and reservoir area or road corridors.

b Negative 2 times the log-likelihood value.

c Number of estimable parameters in the approximating model.

d Akaike's Information Criterion, corrected for small sample size.

e Difference in value between the AIC_c of the current model and that of the best approximating model.

f Akaike Weight = Probability that the current model (i) is the best approximating model in the candidate set.

g *p* (.) indicates that detection probability was held constant across all locations in the model.

Table 5.2-1. Best-model Estimates of Wood Frog Occupancy and Detection Probability in 2013.

Variable	Estimate	S.E.	95% C.I.
Occupancy			
Shallow water (<1.5 m deep)[a]	0.368	0.095	0.208–0.565
Deep water (>1.5 m deep)	0.818	0.131	0.444–0.962
Overall	0.634	0.131	0.363–0.840
Detection Probability			
Intercept	0.606	0.129	0.348–0.816

Notes:

a 1.5 m = 4.9 ft.

Table 5.2-2. Habitat Characteristics of Water Bodies and Wetlands where Wood Frogs were Detected and Not Detected in 2013.

Habitat Type / Variable	Description	Wood Frog Presence[a]			*P*-value
		Detected	Not Detected	Overall	
Water Body Structure					
Water body type (%)	Big lakes (> 20 acres)	2.1	2.3	2.2	0.158[b]
	Small ponds w/o emergents	27.7	11.6	20.0	
	Small ponds w/ emergents	44.7	41.9	43.3	
	Seasonally flooded ponds	25.5	44.2	34.4	
Beaver activity (%)	No	91.3	76.7	84.3	0.157[b]
	Yes	8.7	23.3	15.7	
Aquatic Habitat Characteristics					
Emergent and submergent vegetation (%)		22.6 (4.2)	32.7 (5.2)	27.5 (3.3)	0.132
Emergent vegetation (%)	Grass	6.4	14.0	10.0	0.158[b]
	Sedge	80.9	62.8	72.2	
	None	12.8	23.3	17.8	
Substrate (%)	Boulder	4.3	2.3	3.3	0.179[b]
	Gravel	0.0	7.0	3.3	
	Mud/silt	14.9	23.3	18.9	
	Organic	80.9	67.4	74.4	
Aquatic Features					
Ice cover (%)[c]		36.7 (5.6)	26.1 (5.0)	31.7 (3.8)	0.165
Water temperature (%)[c]		7.0 (0.6)	5.7 (0.8)	6.4 (0.5)	0.175
Water depth (%)	Shallow (≤ 1.5 m)	27.7	67.4	46.7	<0.001[b]
	Deep (> 1.5 m)	72.3	32.6	53.3	
Water Quality					
Dissolved oxygen (%)[c]		64.77 (2.77)	70.63 (3.50)	67.57 (2.22)	0.193
Dissolved oxygen (mg/L)		7.96 (0.38)	9.16 (0.46)	8.53 (0.30)	0.047

Habitat Type / Variable	Description	Wood Frog Presence[a]			P-value
		Detected	Not Detected	Overall	
Specific EC[c]		0.039 (0.006)	0.040 (0.008)	0.039 (0.005)	0.950
pH[c]		5.73 (0.10)	5.72 (0.12)	5.73 (0.07)	0.932
Terrestrial Habitat within 50-m Radius					
Herbaceous (%)		18.0 (1.9)	26.4 (3.2)	22.0 (1.9)	0.029
Dwarf shrub (%)		12.7 (2.2)	11.4 (2.6)	12.1 (1.7)	0.709
Low shrub (%)		21.2 (2.1)	22.4 (2.4)	21.8 (0.6)	0.709
Tall shrub (%)		28.5 (2.3)	27.7 (3.6)	28.1 (2.1)	0.847
Trees (%)		19.0 (2.6)	12.8 (3.1)	16.4 (2.0)	0.130

Notes:

a Parenthetical values in table cells indicate 1 S.E.

b *P*-value from chi-square test (other *P*-values are from t-tests for two independent samples).

c Measured on first visit.

10. FIGURES

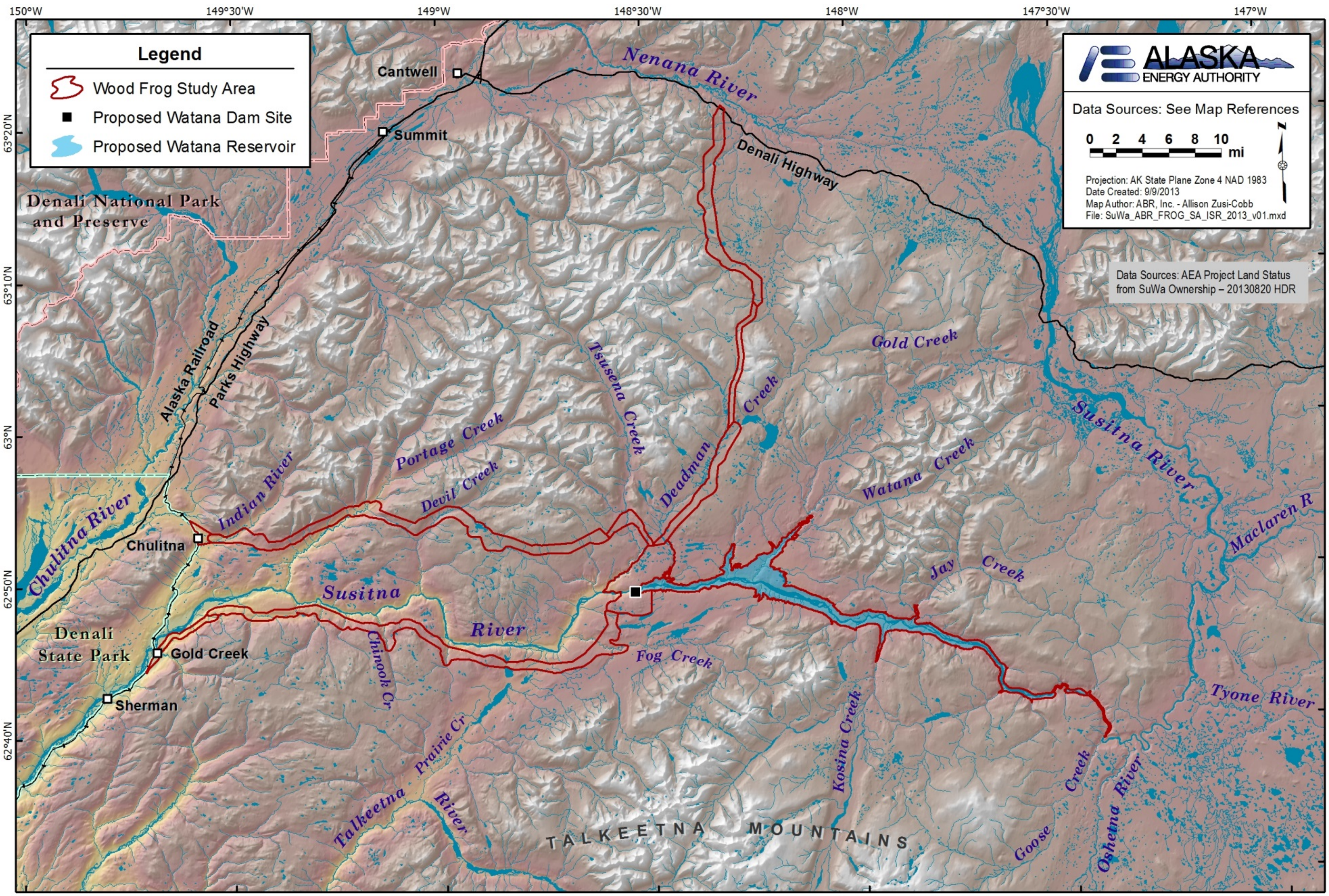

Figure 3-1. Wood Frog Study Area for the Susitna–Watana Hydroelectric Project, 2013.

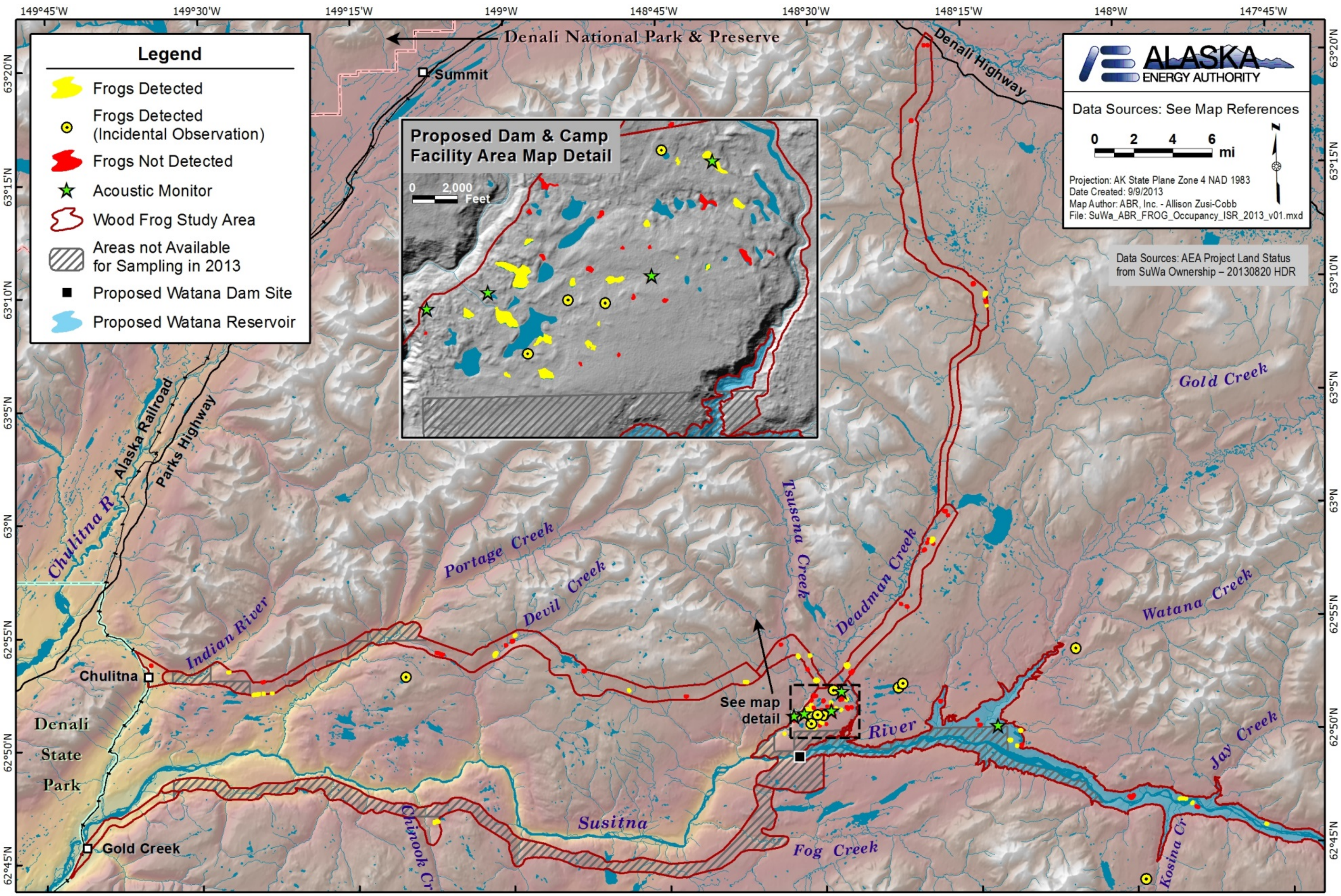

Figure 5.1-1. Locations where Wood Frogs were Detected during Auditory Surveys, plus Incidental Observations from Other Wildlife Surveys, in 2013.

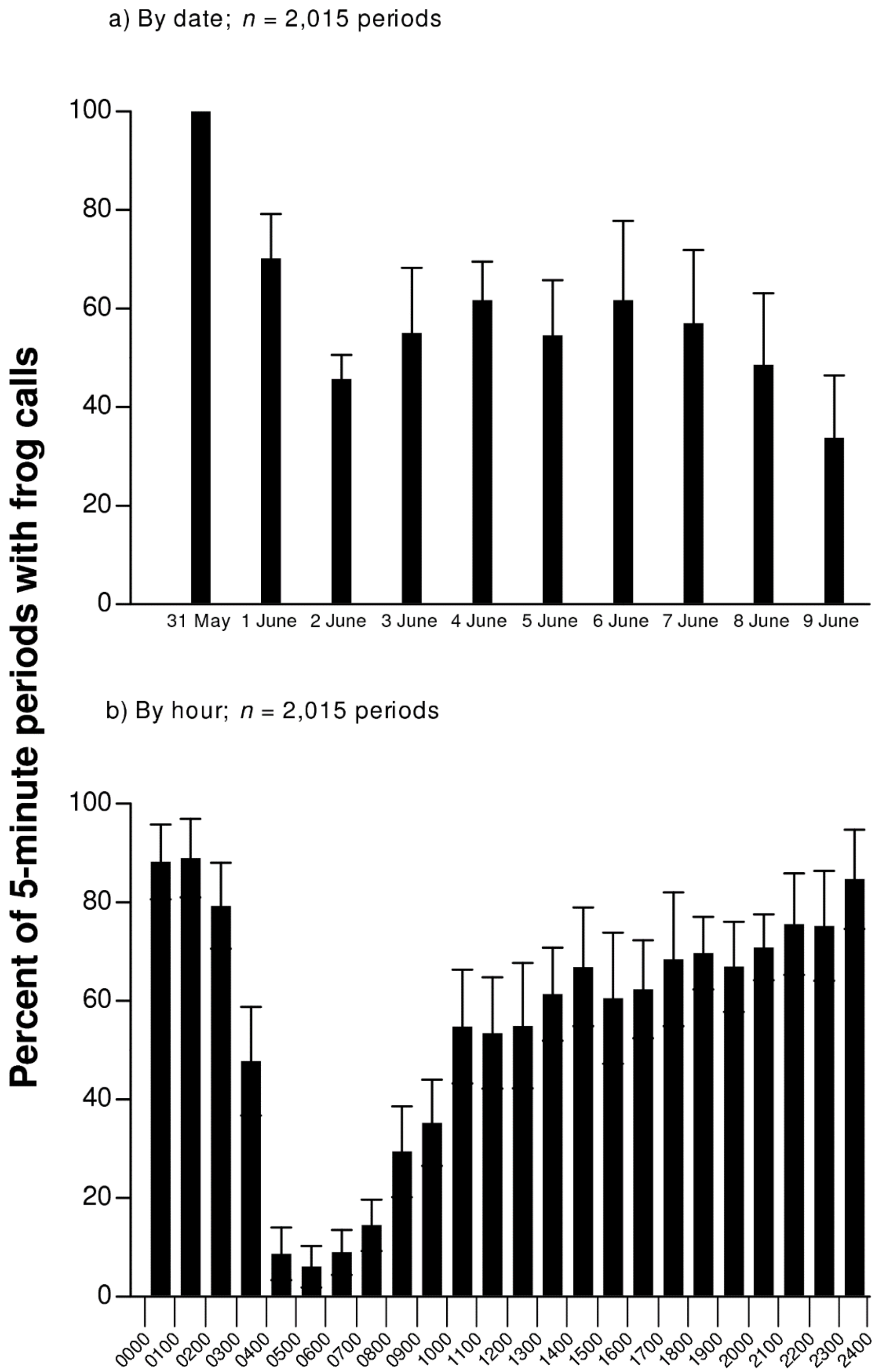

Figure 5.3-1. Wood Frog Calling Activity by Date and Hour in 2013 (error bars depict 1 S.E.).

PART A - APPENDIX A: RECORDS OF CONSULTATION WITH USFWS AND USGS REGARDING SAMPLING PROTOCOL AND ANALYTICAL METHOD FOR AMPHIBIAN CHYTRID FUNGUS.

SUSITNA-WATANA HYDROELECTRIC PROJECT

MEETING RECORD

AEA Team Member		Other Party	
Name:	*Todd Mabee*	**Name:**	*Tara Chestnut*
Organization:	*ABR, Inc.; Forest Grove, OR*	**Organization:**	*USGS, Portland, and Oregon State University, Corvallis*
Study Area:	*Project area (RSP Section 10.18)*	**Phone Number:**	
Date:	*26 April 2013*	**Time:**	*12:00 PM*
Meeting held by: [x] AEA Team [ ] Other Party			

Others at Meeting: None.

Subject: Sampling protocol and analytical method for amphibian chytrid fungus (Bd).

Discussion:

Swabbing: Tara demonstrated how to take swab samples for Bd from a wood frog.

Lab work: Two labs in the country do QPCR, the analysis she recommends (as opposed to straight PCR). Labs are at Pullman, WA (WSU) and a USGS lab in Reston, VA. Tara has used both and recommends the USGS lab if timelines are important. Cost is ~$25/sample.

No. of samples: Ideally, Tara recommended sampling one "population" from each of the SuWa survey areas, hence four "populations." Four ponds @ 15 frogs/pond, with all four ponds within approximately a 5-mi radius. She suggested this effort is what is needed to detect Bd if it has only a 5% prevalence. (This level of effort may not be achievable using opportunistic sampling during acoustic surveys, however.)

Timing: Tara described wood frogs as "explosive breeders that may call for a few days up to a few weeks," which could easily be missed with acoustic surveys. She recommended also doing egg mass surveys. Frogs deposit eggs *en masse* in one location. She recommended contacting Dave Tessler with ADF&G for more information on survey timing.

Action Items:

Todd will finish ordering the equipment needed for swabbing and Tara will loan some if necessary (some suppliers were completely sold out of the swabs needed, but other suitable swabs were obtained.) Todd followed up after meeting with questions about the use of gloves during swabbing and decontamination of boots between survey sites.

SUSITNA-WATANA
HYDROELECTRIC PROJECT

EMAIL RECORD

From: Chestnut, Tara chestnut@usgs.gov
Date: 4/30/13
To: Todd Mabee tmabee@abrinc.com

Hi Todd,
You bet!

The individual tubes are problematic for the DNA extraction so I would recommend against using them. How about you place the order and I send you with some of my extra swabs and you can replace them when you get back? I'll be in PDX next on May 10. Is that too late?

Just use gloves. It was wrong of me to introduce the possibility of not using them. I use the cheapest nitrile gloves available through Fisher and wet them with native water before handling animals. Some folks use double gloves when it's rainy or if they get sweaty hands so it's easier to change them. Change gloves between each adult or metamorphic animal but you can use the same pair for larvae if you choose to swab them.

I decontaminate between sites. It's not as hard as it seems, especially if you use rubber hip waders rather than fancy boot/wader combinations. In fact the refuges prohibit felt soled boots and gortex waders because of the difficulty with decontamination. Complete drying also kills Bd but it's important to remember that you are not just decontaminating against Bd. Compared to Didymo, whirling disease, etc. Bd is nothing! In the backcountry, I use a spray bottle filled with bleach water and just spray gear when I finish sampling a site and let it dry without rinsing. It's really important to get the soil and dirt off of gear between sites.

I hope this helps!

Cheers,
Tara

---------- Forwarded message ----------
From: Todd Mabee <tmabee@abrinc.com>
Date: Mon, Apr 29, 2013 at 9:16 AM
Subject: Bd swabs & misc questions
To: "Chestnut, Tara" <chestnut@usgs.gov>
Cc: "Todd J. Mabee" <tmabee@abrinc.com>

Good morning Tara,

Thanks so much for making the time to educate me about Bd sampling in AK last week, this really helped!

I contacted MW&E and they have sold out of the MW113 but they do have the MW100 (identical swab with individual tubes). Guess I'll get these unless you recommend something different?

I reviewed my notes and had a few remaining questions and/or just wanted to double check some things we discussed.

Gloves: what type, brand, etc do you recommend? Can you send me a website please?

If we only use our bare hands, do you recommend any sort of cleaning (hand sanitizer) between ponds?

Do you recommend any sort of decontamination for our boots between the wetlands? This may be very difficult in the field (especially if we are hiking between wetlands) but maybe something we do at the end of the day? Open to ideas. Below is what Meg said they did -

"As far as decontamination - we did this between every site using a 10% bleach solution followed by clean water rinse to clean our boots and nets and any other gear that went in the water."

Appreciate the help!

Best,
Todd

---------- Forwarded message ----------
From: Perdue, Margaret <margaret_perdue@fws.gov>
Date: Sun, Apr 7, 2013 at 4:33 PM
Subject: Re: Bd sampling protocol
To: Todd Mabee <tmabee@abrinc.com>

Hi Todd ---

I would say Mari Reeves (mari_reeves@fws.gov) is the species expert for AK and that Tara Chestnut (chestnut@usgs.gov) is the Bd expert and has done most of her work on wood frogs, much of it up here.

I have been fortunate to work with both of them and have been doing the field work and sampling on a wood frog study in the Kenai for the last couple years that Mari started and Tara helped with the Bd work. I have provided some information below from the methods used on that project.

As far as capture we were largely sampling for Bd in adults that we captured by hand (no nets) but for much of our work we were using dipnets to capture tadpoles for Gosner staging and metamorphs still in the water. The nets we used we got through Jonah's aquarium: http://jonahsaquarium.com - there is a link on their page for dipnets and when you go to it the 'perfect dipnet' comes up and that is what we used.

As far as decontamination - we did this between every site using a 10% bleach solution followed by clean water rinse to clean our boots and nets and any other gear that went in the water.

I hope this helps and if you have other questions I can try to answer them or you could try to contact them directly.

This is the method we used in a recent study for sampling for Batrachochytrium dendrobaditis (Bd):

Swabbing consists of 25 strokes on their ventral patch, legs and thighs, and between their toes. The swab samples will be preserved dry in 1.7 ml sterile microcentrifuge tubes.

At the start of our study we were putting swabs in EtOH but Tara Chestnut who has done a lot of work with Bd and is definitely the best resource I know for information on this had us switch to allowing swabs to air dry…

"Based on one too many leaky samples and mistrust of airlines I'm not preserving swabs in any liquid any more, just letting them air dry and storing @ room temp, see Hyatt et al. 2007. http://www.jcu.edu.au/school/phtm/PHTM/frogs/papers/hyatt-2007.pdf)"

She also confirmed the method and gave a suggestion for numbers of animals though we were sampling adults opportunistically for our work and did not reach these numbers in our study…

"Yes, I've been doing 25 strokes. As for numbers, my goal is to collect 15 swabs per pond and 60 swabs per population, although the way I'm defining a population is kind of subjective. I'm assuming some level of mixing within basins with a high density of small wetlands and not accounting for barriers such as roads or fine scale population dynamics. I've been avoiding brand new metamorphs since there's some suggestion that it can take a week or two for Bd to colonize the tissue but if that's what you are encountering I will happily accept them."

Here is info on the type of swab we used based on Tara's suggestion:

We use Medical Wire fine tip swabs, cat # MW113 and place them into 1.5 snap cap microcentrifuge tubes after air drying. I order from Advantage Bundling http://www.advantagebundlingsp.com/mwe_dryswab.pdf The benefit of this over the tubed swabs is that you don't have to change tubes for the DNA extraction and risk losing any material that may be left in the tube.

On Thu, Apr 4, 2013 at 8:43 AM, Todd Mabee <tmabee@abrinc.com> wrote:

Good morning Meg,

I'm working with Brian Lawhead on the Su-Watana project and will be heading up the Wood frog study. Brian told me you were the species expert for AK, so I wanted to introduce myself and ask you a few questions. I've been lucky enough to have been involved with some headwater amphibian research in OR on Torrent Salamanders and also some work on pond-breeders (mainly Northwestern salamanders). I'm also heavily involved with all our avian and bat studies, so I get to do a lot of interesting work!

Brian forwarded some correspondence between you & Lori Verbrugge on the occurrence of Bd in Alaska and where it has been tested for in AK. As I believe you know, we plan on opportunistically sampling for Bd during our field work this year. Would you be able to provide your sampling protocol for our use? Also, are there any special decontamination issues that we should be thinking about coming up from OR (my location)? Any tips you have on capture of frogs (nets of particular size) would also be appreciated.

Thank you for your help and feel free to let me know if you have comments or questions about our study.

Best regards,
Todd

Todd J. Mabee
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From: Lori_Verbrugge@fws.gov
Sent: Tuesday, June 12, 2012 8:41 AM
To: lawhead@abrinc.com
Cc: BMcGregor@aidea.org; Michael_Buntjer@fws.gov; Jennifer_Spegon@fws.gov
Subject: Fw: Meg's answers to chytrid questions

Hi Brian,

Meg has responded to our preliminary questions about wood frogs, chytrid fungus and project development - please see below.

Please don't hesitate to follow up with her (or her contacts) if you have more questions!

----- Forwarded by Lori Verbrugge/R7/FWS/DOI on 06/12/2012 08:36 AM -----

Margaret Perdue/R7/FWS/DOI
06/11/2012 09:10 PM
To Lori Verbrugge/R7/FWS/DOI@FWS
Subject Re: Fw: Meg's contact info

Hey Lori ---

Yes chytrid has been found infecting frogs in Alaska. We have had positive results for a number of frogs down here in the Kenai — 17 sites last year had frogs that came back positive for *Batrachochytrium dendrobatidis* (Bd) the species of chytrid fungus that causes the disease chytridiomycosis. There is also a USGS person / doctoral student, Tara Chestnut, who is doing her dissertation on its distribution and has found it elsewhere up here (not sure exactly where, Tara doesn't want to give out too much info until she completes her research) and I also believe another researcher found it in Denali NP.

As far as how it might be spread and whether a project like Su-Watana could be a potential means of spread is one of the big questions but it certainly seems possible that the associated traffic to an area that comes with development of any sort at least raises the possibility for increased incidence.

Mari tested for it down here and in a couple of the other refuges where she did the amphibian survey work and she found it down here then (2006) but not in Innoko or Tetlin (the other places where she tested) leading to speculation that road proximity, like with the malformations, could be a factor.

I hope that helps. I can put you in touch with Tara and Bill Battaglin, the researcher at USGS who is coordinating the analysis of the samples we are sending them from here — they would be more up on the latest, greatest hypotheses concerning disease spread.

Meg Perdue, Biologist
U. S. Fish & Wildlife Service
Environmental Contaminants Program
Anchorage Field Office
605 W. 4th Ave., Rm G-61
Anchorage, AK 99501
phone: 907-271-6647
fax: 907-271-2786
margaret_perdue@fws.gov

Lori Verbrugge/R7/FWS/DOI
06/07/2012 03:09 PM
To Margaret Perdue/R7/FWS/DOI@FWS
Subject Fw: Meg's contact info

Hi Meg,

Just a heads up - we were talking about wood frogs in a Su-Watana meeting yesterday, and a question came up about wood frogs. Someone at ADF&G had suggested that AEA's study in the project area should include testing for the chytrid fungus (hope I'm spelling that right!) No one at the meeting knew much about wood frogs, so I offered you up as a potential expert that might know the answers. Specifically, they were wondering,

1. Has the chytrid fungus ever been found on a frog in Alaska?

2. Is there any potential link between the proposed project (Su-Watana dam) and chytrid in wood frogs? If there is no way that the project could impact the incidence, virulence etc., then they don't have to study it just because we'd like the data for other reasons....

Just a heads up that questions like these may be coming your way. Any thoughts at this point?

Thanks,

Lori Verbrugge, PhD
Contaminants Biologist
US Fish and Wildlife Service
605 W 4th Avenue, Room G-61
Anchorage, AK 99501
Phone: (907) 271-2785
FAX: (907) 271-2786
lori_verbrugge@fws.gov

PART A - APPENDIX B: PHOTOGRAPHS FROM FIELD SURVEYS IN 2013.

Example of Water Body at which Wood Frogs were Detected in the Chulitna Access Corridor in 2013.

Example of Water Body in the Reservoir Inundation Zone at which Wood Frogs were Detected in 2013.

Example of Water Body with Emergent Vegetation at which Wood Frogs were Detected in 2013.

Wood Frog Egg Mass, 2013.

Acoustic Monitoring Device used to Supplement Auditory Surveys in 2013.