TESTING ENVIRONMENTAL DNA SAMPLING AND PREDICTIVE MODELING AS MEANS TO INVESTIGATE WOOD FROG (RANA SYLVATICA) DISTRIBUTION IN ALASKA AND NORTHERN CANADA

By

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Abstract

Global amphibian declines over the past 30+ years have led to a greater awareness of amphibian conservation issues. Few amphibian species occur in northern landscapes, however, and the species that do occur are widely dispersed and at the northern extent of their range. Accordingly, amphibian research is not prioritized in northern landscapes. Deficient monitoring practices have resulted in incomplete distribution knowledge that impedes the management of wood frogs (*Rana sylvatica*) in Alaska and northern Canada. I developed an environmental DNA detection assay to complement monitoring practices at the northern extent of the wood frog's range. This assay was tested to be species-specific, allowing it to be implemented in areas where wood frogs may co-occur with other amphibian species. It can detect wood frog DNA in environmental samples to a concentration of 1.83×10^{-3} pg/µL. I further demonstrate that environmental DNA occurrence data can be used to predict wood frog distribution in the Fairbanks North Star Borough. I combined environmental DNA occurrence data with environmental GIS data and analyzed the resulting dataset with machine learning algorithms to define an ecological niche for the wood frog. This niche, when extrapolated to the landscape, results in a species distribution model that attains 74% predictive accuracy. Lastly, I conducted an environmental DNA mega-transect survey along the Elliot/Dalton Highway corridor in Alaska. I combined the results of this survey with citizen science occurrence data from past and current monitoring projects to create a set of alternative occurrence data. This alternative data was combined with environmental GIS data and analyzed with machine learning algorithms to create a species distribution model that achieves 92% predictive accuracy across Alaska and the Yukon Territory, Canada. These results improve upon prior species distribution models developed for wood frogs in Alaska. They provide deeper insights into potential wood frog distribution at high latitudes and elevations in Alaska, where anecdotal observations have previously been recorded. Adoption and widespread use of an environmental DNA monitoring protocol in under-sampled regions of Alaska and northern Canada will generate larger datasets with wider geographic coverage, leading to models with even higher predictive accuracy. Alternative data, including that obtained from environmental DNA and citizen science monitoring, can boost efforts to further develop baseline knowledge of wood frog occurrence in these areas. Species distribution models generated in this research can help guide these efforts. Increasing knowledge of wood frog distribution may assist conservation managers to designate critical habitat, study climate impacts, and make more informed decisions regarding amphibians in northern landscapes.

Table of Contents

Page

List of Figures

Page

List of Tables

Page

List of Appendices

Page

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Introduction

The decline of amphibian populations at global, regional, and local scales is now a well-documented and long-standing problem (Adams et al. 2013; Stuart et al. 2004; Wake and Vredenburg 2008; Young et al. 2004). Nearly 1 in 3 (32%) amphibian species is threatened with extinction according to the International Union for Conservation of Nature (IUCN), although this number could be as high as 56% when accounting for Data Deficient and unevaluated species (IUCN 2016) (Table 1). Currently, 113 species are listed as possibly extinct. This number is far higher than any other major taxonomic group, except gastropods, assessed by the IUCN. A separate assessment estimates that approximately 200 amphibian extinctions have occurred since 1970 alone, with hundreds more to be expected over the next century (Alroy 2015).

Accurate assessments of the status of amphibians, however, are not entirely possible because relevant data are often unavailable (IUCN 2016). Almost one quarter (23%) of amphibian species are labeled Data Deficient, more than any other terrestrial vertebrate group. Data deficiency among amphibians is so high because many species are difficult to study owing to their low abundance, limited distribution, and small, isolated populations. The best estimate for percent of amphibian species threatened with extinction is 42% accounting for data deficiency.

Almost half (43%) of all amphibian species are experiencing decreasing population numbers, regardless of threat level (Stuart et al. 2004). Amphibian occupancy of breeding sites in North America declined on average by 3.7% per year between 2002 and 2011 (Adams et al. 2013). Alarmingly, declines of 2.7% per year were documented for species designated by the IUCN as Least Concern. This coincides with a larger trend in western nations, where high rates of species decline occur as a direct result of rampant development and economic growth (Rosales 2008).

Table 1 International Union for Conservation of Nature amphibian status
A summary of the global status of amphibian species, according to the IUCN (2016). The term "threatened" encompasses the CR, EN, and VU categories.

Status Category	Number
Extinct (EX)	33 (0.5%)
Extinct in the Wild (EW)	2 (0.0%)
Critically Endangered (CR)	550 (8.4%)
Possibly Extinct* (PE)	113 (1.7%)
Endangered (EN)	873 (13.3%)
Vulnerable (VU)	677 (10.3%)
Near Threatened (NT)	398 (6.0%)
Data Deficient (DD)	1,508 (22.9%)
Least Concern (LC)	2,541 (38.6%)
Total Evaluated	**6,582**

*Possibly Extinct is a subcategory of Critically Endangered that refers to species not recorded in the past century.

Amphibian biodiversity is lowest in northern latitudes (Wake and Vredenburg 2008). The arctic and subarctic regions of Alaska and Canada represent the northern extent of only a few common and widespread species in North America. As such, amphibian conservation and management are not prioritized in these landscapes. However, a recent perspective on Canadian herpetological (reptile and amphibian) conservation asserts that these common species contribute more to functional ecosystems than rare species (Lesbarrères et al. 2014). Protection of common species, they argue, serves as a proxy for the protection of intact ecosystems and the rare species that inhabit them. But knowledge of amphibian species distributions in northern Canada is limited, partly because it is a vast and sparsely populated country. This limited knowledge severely inhibits effective species management in the northern territories. Sparsely populated Interior and North Slope Alaska also lack detailed amphibian distribution knowledge.

Six species of amphibians are native to Alaska (Table 2) (MacDonald 2010). Only the wood frog (*Rana sylvatica* ITIS TSN: 775117[1]) occurs broadly throughout the interior boreal forest and coastal tundra. The wood frog has been designated by the Alaska Department of Fish and Game (2006) as a species of greatest conservation need, as have all other native amphibian species. This is despite the IUCN status of the wood frog as a species of Least Concern (IUCN 2016). This disparity reinforces the need for an increased awareness of amphibian conservation in northern landscapes, as identified by Lesbarrères et al. (2014) in Canada. Wood frog research needs in Alaska pertain to high incidence of physical abnormality (Hayden et al. 2015; Reeves et al. 2008; Reeves et al. 2013), phenological response to climate change (Benard 2015; Davenport et al. 2016), and anecdotal reports of local population declines and extirpations (Alaska Department of Fish and Game 2006; Fields and Gotthardt 2009) in the face of unabated land development (Huettmann 2014). Further, the lack of consistent and widespread monitoring efforts prevents accurate characterization of population trends. Vast areas, including the Alaska Peninsula, Yukon-Kuskokwim Delta, Seward Peninsula, and North Slope, lack the baseline occurrence data needed for a sophisticated management of species and habitat (Figure 1). Inclusion of these regions in the range extent of the wood frog in Alaska is uncertain. In particular, the North Slope is currently assumed to be unoccupied and barricaded to wood frog dispersal by the Brooks Range, yet numerous anecdotal observations are reported (Alaska Department of Fish and Game 2006; Fields and Gotthardt 2009; Hilderbrand, Larson, Torvinen personal communication).

[1] The Integrated Taxonomic Information System (ITIS) considers *Rana sylvatica* invalid, but see Yuan et al. (2016)

Table 2 Native Alaskan amphibians
A list of amphibian species considered native to Alaska. Only the wood frog occurs beyond Southeast Alaska.

Native amphibians of Alaska
Northwestern Salamander (*Ambystoma gracile*)
Long-toed Salamander (*Ambystoma macrodactylum*)
Rough-skinned Newt (*Taricha granulosa*)
Western Toad (*Anaxyrus boreas*)
Columbia Spotted Frog (*Rana luteiventris*)
Wood Frog (*Rana sylvatica*)

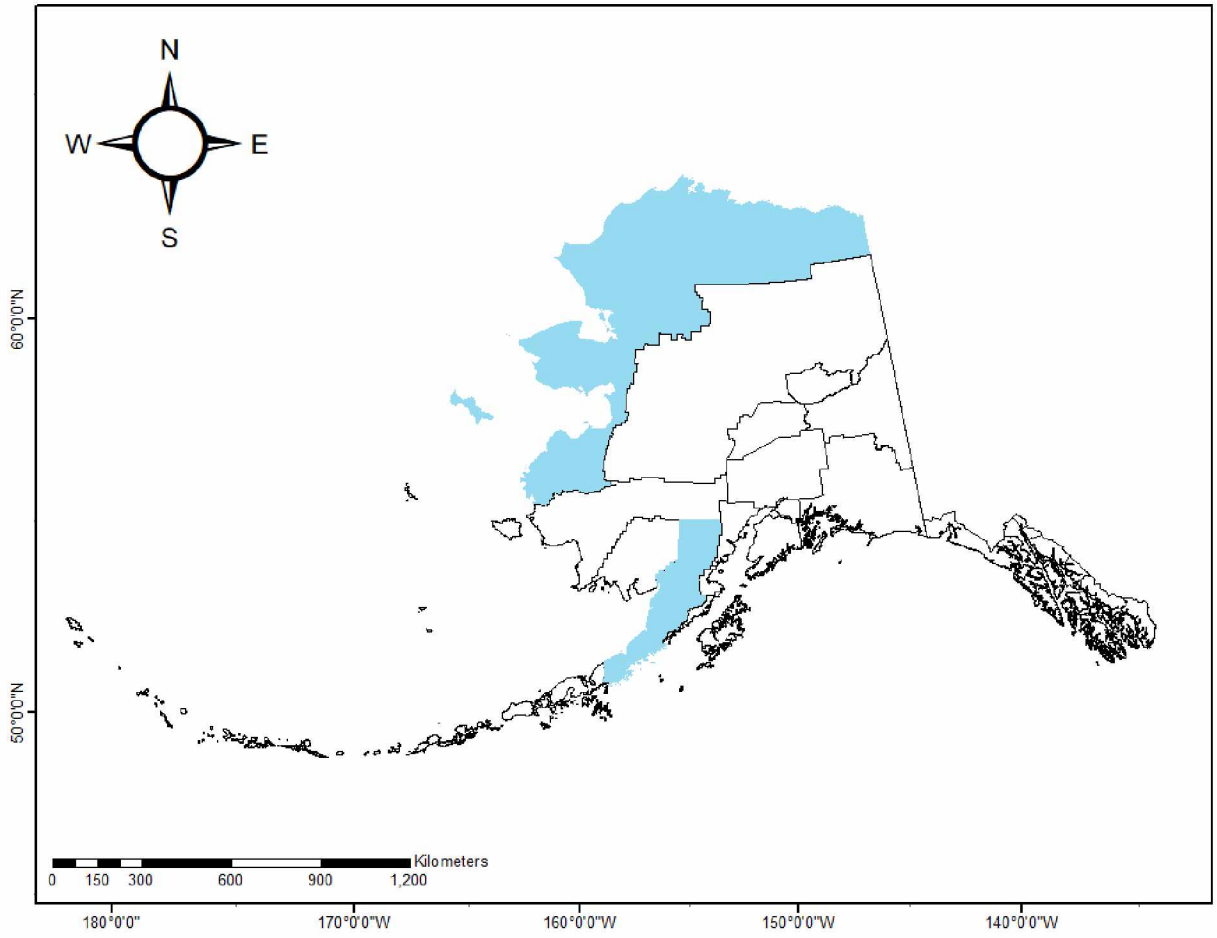

Figure 1 Alaska wood frog range uncertainties
The state of Alaska. Areas highlighted in blue are inconsistently included in various wood frog range maps. Range maps made available by the U.S. Geological Survey and the IUCN provide examples of contradictions.

The Alaska Gap Analysis Project (AK GAP) represents the first attempt to characterize wood frog distribution in Alaska using species distribution models (Aycrigg et al. 2015; Gotthardt et al. 2012). Species distribution models (SDMs) predict the occurrence of a species across a landscape using known occurrence records and computed habitat associations (i.e. ecological niche). These models provide a precise description of species

distribution (Elith et al. 2006; Jetz et al. 2012). However, the AK GAP constrained SDMs to a predetermined range for each species, something that is not firmly established for wood frogs in Alaska. AK GAP models were found to under-predict occurrence for small mammals in Alaska (Baltensperger and Huettmann 2015). This is possibly a symptom of the conservative methodologies used in the AK GAP analyses (Huettmann, personal communication).

The provision of resources needed to adequately assess and monitor wood frogs throughout Alaska and northern Canada may contribute to a more solid understanding of their occurrence in that area. Current methods for monitoring amphibians include visual, acoustic, and dip-net surveys. These surveys are time, money, and labor intensive. Additionally, they often suffer from poor detectability, requiring multiple site visits to confirm absence. This quickly becomes prohibitive in the expansive northern landscapes of Alaska and Canada. These methods are further constrained by the short breeding season in the Far North.

Environmental DNA (eDNA) detection assays represent an innovative technology for monitoring species occurrence. Environmental DNA refers to DNA molecules shed by organisms into the environment (Bohmann et al. 2014; Darling and Mahon 2011; Goldberg et al. 2015; Jerde et al. 2011; Thomsen and Willerslev 2015). The use of eDNA assays to detect rare, invasive, and cryptic species in freshwater environments is proven to be effective (Table 3). These assays, once developed, are often more sensitive and less resource intensive than most traditional monitoring techniques (Biggs et al. 2015; Olson et al. 2013; Pilliod et al. 2013). A considerable side benefit is the non-intrusive nature of the sampling technique. Environmental DNA detection is rapidly growing in use following its first implementation in 2008 (Ficetola et al. 2008) and is now applied in more complex investigations. Aquatic eDNA assays are increasingly used to measure site occupancy (Pilliod et al. 2013), monitor species abundance (Doi et al. 2017), and quantify ecosystem biodiversity (Thomsen and Willerslev 2015).

Table 3 Environmental DNA studies
A selection of projects that utilized eDNA methods for difficult-to-detect species.

Species detected	Location	Reference
Rare species		
Japanese Giant Salamander	Katsura River, Japan	Fukumoto et al. (2015)
Alabama Sturgeon, Gulf Sturgeon	Mobile River, USA	Pfleger et al. (2016)
Invasive species		
Common Carp	Lake Staring, USA	Eichmiller et al. (2014)
American Bullfrog	Multiple wetlands, France	Ficetola et al. (2008)
Pythons and Boids (multiple species)	Everglades, USA	Hunter et al. (2015)
Cryptic species		
Patch-Nosed Salamander	Georgia & North Carolina, USA	Pierson et al. (2016)
Eastern Hellbender	North Carolina, USA	Spear et al. (2015)

The application of eDNA assays to detect wood frogs has potential limitations. While eDNA assays have been used to monitor a wide taxonomic variety of organisms, most efforts are directed at fully or highly aquatic species. Adult wood frogs spend only a brief time in wetlands. They become predominately terrestrial following egg deposition, dispersing into forests. The DNA shedding rate of amphibian adults vs. eggs vs. larvae is not yet quantified, so optimal timing of eDNA surveys is uncertain. Also, the habitats surveyed in previous studies are primarily lakes (Eichmiller et al. 2014), streams (Goldberg et al. 2011), rivers (Carim et al. 2017), and oceans (O'Donnell et al. 2017). Wood frogs, however, prefer to breed in shallow and ephemeral wetlands (Alaska Department of Fish and Game 2006; Fields and Gotthardt 2009; MacDonald 2010). The conditions in these wetlands may be unfavorable for the preservation and detection of eDNA (McKee et al. 2015a). The relatively warm temperatures associated with smaller bodies of water increase rates of DNA degradation (Barnes et al. 2014). Elevated levels of turbidity in these wetlands also allow DNA to adsorb to soil particles. The extraction of DNA bound to sediment is less successful than the extraction of DNA found freely floating in water (Eichmiller et al. 2014). Low pH and high concentration of tannins and humic acids inhibit polymerase chain reaction (PCR), the process by which DNA is detected (Herder et al. 2014; McKee et al. 2015b).

There is no precedent for using eDNA occurrence data to model species distribution across a landscape. One consideration is the confidence with which species presence can be inferred from the presence of eDNA. This depends, in part, on the system-specific ability for DNA to persist and be transported in the environment (Bohmann et al. 2014; Dejean et al. 2011; Goldberg et al. 2016). The strict implementation of internal control procedures is needed to prevent and monitor for human error (Darling and Mahon 2011; Goldberg et al. 2016). Effective design and validation of primers ensures species specificity (Bohmann et al. 2014; Darling and Mahon 2011; Wilcox et al. 2013). Inferring species absence from eDNA absence, on the other hand, is also influenced by many factors. The detection of eDNA may depend on how degraded the DNA is, in addition to whether it is intracellular, extracellular, absorbed, or free (Barnes et al. 2014). Conditions of the environment, including water acidity, exposure to light, temperature, salinity, turbidity, substrate type, oxygen levels, microbial communities, and the synergistic interaction of these variables, determine how quickly eDNA degrades (Barnes et al. 2014; Dejean et al. 2011). Finally, primers and assays must be designed to maximize sensitivity (Wilcox et al. 2013).

Another rapidly growing way of collecting wildlife occurrence data in North America is the implementation of citizen science monitoring programs (Domroese and Johnson 2017; Sullivan et al. 2017). Citizen

science relies on the voluntary collection of scientific data by members of the public. At least three citizen science amphibian monitoring programs have collected over 15 years of data on a national scale. The North American Amphibian Monitoring Program (NAAMP) was the first widescale attempt at recruiting citizen scientists to monitor amphibian populations in the country (https://www.pwrc.usgs.gov/naamp/). It set the precedent with a non-intrusive acoustic monitoring protocol, though it never reached the western United States and is no longer being pursued. FrogWatch USA is a similar program that has found ongoing success with the Association of Zoos and Aquariums (https://www.aza.org/frogwatch). Canada's FrogWatch program is a part of NatureWatch, a collaboration between Environment Canada and Nature Canada (https://www.naturewatch.ca/frogwatch/). Regionally, citizen science amphibian monitoring was carried out by the Alaska Department of Fish and Game's Wood Frog Monitoring Program between 2002 and 2008. Observations from this program comprise a sizable portion of occurrence records in the state, though it is no longer active (http://www.adfg.alaska.gov/index.cfm?adfg=citizenscience.woodfrog). The Alaska Herpetological Society (http://www.akherpsociety.org/citizenscience.htm) and the Alaska Native Tribal Health Consortium (https://www.leonetwork.org/en/) also maintain a few small-scale citizen science projects.

The use of citizen science methods in complex, rigorous scientific inquiry has long been constrained by perceived issues with the quality of data collected by untrained scientists (Burgess et al. 2017; Lukyanenko et al. 2016). For example, these data may be collected under varying sampling protocols, follow loose or no research design, contain sampling biases and correlations with countless variables, and/or contain false, missing, outlying or incomplete information. Such data do not meet the *a priori* assumptions needed for analysis by statistical models (Cutler et al. 2007; Elith et al. 2008; Phillips et al. 2006), leading critics to discount the data entirely. This opposition has been challenged in recent years (Crall et al. 2011; Lewandowski and Specht 2015; Lukyanenko et al. 2016), however, resulting in a rapid increase in the use of citizen science to instigate conservation action (Barnard et al. 2017; McKinley et al. 2017; Sullivan et al. 2017). Table 4 outlines some best practices in data collection, analysis, and management to consider when developing or using data from a citizen science project.

Table 4 Best practices in citizen science data management
Suggestions for best practices in data management to consider when developing or using data from a citizen science project.

Data management suggestions in citizen science
Data collection (volunteers)
• Train volunteers in proper methodology
• Use an easy to follow, standardized protocol
• Facilitate data submission (if online, provide simple user interface)
• Provide contact info for questions about protocol, data, etc.
Data analysis
• Implement quality control procedure to vet data submissions
• Consider limitations of the data and analyze accordingly
Data storage
• Use multiple means of backup
• Return data to volunteers along with a summary of results
• Create metadata according to ISO standards
• Make raw dataset and metadata openly accessible

One solution to overcoming the barriers inherent in both eDNA and citizen science datasets is the use of machine learning methods of analysis. Hundreds of machine learning algorithms have been applied to complex issues in technology, health care, banking, and other data-driven industries (Fernández-Delgado et al. 2014). These algorithms, in contrast to statistical models, excel in their ability to extract and extrapolate strong, overarching signals from imperfect datasets (Baltensperger and Huettmann 2015; Craig and Huettmann 2009; Drew et al. 2010; Mi et al. 2017). Certain algorithms, including CART, Random Forests, and TreeNet, have even been used to predict species occurrence using citizen science data containing error and bias (Bird et al. 2014; Jackson et al. 2015).

The thesis research outlined here is split into three chapters. In chapter one I describe the process by which I develop an eDNA monitoring assay for the detection of wood frogs. In chapter two I assess the use of eDNA occurrence data in predicting species distribution in the Fairbanks North Star Borough. In chapter three I demonstrate the utility of alternative occurrence data by creating a species distribution model for Alaska and the Yukon Territory from eDNA and citizen science datasets.

The University of Alaska Fairbanks Institutional Animal Care and Use Committee 871371-2 approved the use of live animals in this study (Appendix B). The Alaska Department of Fish and Game Fish Resource Permit SF2016-069 granted permission to conduct dip-net surveys and collect buccal swab samples (Appendix C). No frogs were harmed in the making of this thesis (Figure 2).

Figure 2 Wood frog in Fairbanks, AK
This photo was taken following the frog's capture, buccal sampling, and live-release.

Chapter 1

Development, validation, and evaluation of an assay for the detection of wood frogs (*Rana sylvatica*) in environmental DNA[1]

1.1 Abstract

We developed and describe a qPCR assay for the detection of wood frogs (*Rana sylvatica*) using environmental DNA (eDNA) sampling. A single primer set was designed to amplify a 115-bp region of the wood frog cytochrome *b* gene and assessed for target specificity. There was no evidence of amplification in eleven non-target species. We evaluated the utility of the primer set in qPCR assay by conducting geo-referenced eDNA field surveys in Interior Alaska. Results indicate that the assay consistently detects wood frog DNA in the environment to $1.83x10^{-3}$ pg/µL. The assay provides a complement to traditional survey methods and can be readily applied in a wider conservation and management context.

1.2 Technical Note

Wood frogs (*Rana sylvatica*) are widely distributed across North America. Northern wood frogs are a sentinel species for amphibian response to climate change and land development (Benard 2015, Davenport et al. 2016, Winter et al. 2016). A species of greatest conservation need in Alaska, increased monitoring and research efforts are needed (Fields and Gotthardt 2009). Wood frog distribution in Alaska is not well defined (Appendix A), nor are state population trends well-known (Anderson 2004, Gotthardt et al. 2014). Wood frog monitoring efforts in the north are complicated due to challenges associated with surveying large expanses of uninhabited wilderness. Further, an abbreviated aquatic breeding period limits acoustic survey opportunities.

Environmental DNA (eDNA) monitoring refers to the detection of trace macro-organismal DNA in the environment, most often water, soil, or feces (Bohmann et al. 2014). It is increasingly being used in quantitative surveys of aquatic ecosystems (Thomsen and Willerslev 2015). eDNA assays often provide improved detectability over traditional survey methods, but they also pose unique challenges including non-standardized protocols, PCR inhibition, and environmental influences on DNA degradation rates (Olson et al. 2012, Bohmann et al. 2014, Biggs

[1] Spangler, M.A., F. Huettmann, I.C. Herriott, and J.A. López. Development, validation, and evaluation of an assay for the detection of wood frogs (Rana sylvatica) in environmental DNA. Published in *Conservation Genetics Resources.* doi:10.1007/s12686-017-0881-3

et al. 2015). Further, the use of eDNA techniques for the detection of semi-aquatic species in ephemeral wetlands (e.g. wood frogs) has not been extensively assessed (McKee et al. 2015a). Conditions in ephemeral wetlands may be unfavorable for preservation and detection of DNA due to elevated temperatures, high sediment load, and high acidity contrasted with lakes and streams (Dejean et al. 2011, Barnes et al. 2014, Eichmiller et al. 2014). Here, we report the design and validation of a qPCR assay for detection of wood frogs in eDNA at the northern extent of the species' range.

We designed a species-specific primer set to target the cytochrome *b* gene of the wood frog mitochondrial genome. Sequences from the western clade of wood frogs were obtained from GenBank (PopSet 166030264, Lee-Yaw et al. 2008). The Rasy_00 primer set (Rasy_00_F: TCCTTCATCAAACAGGATCATCTA, Rasy_00_R: CCTAGTATAATGGTGAAGCCGAAT) was developed using Primer3Plus and tested for specificity in silico using NCBI Primer-BLAST (Appendix A). We tested Rasy_00 in vitro to ensure positive amplification of six high-quality wood frog genomic DNA isolates, as well as 500 mL of eDNA filtrate obtained from the aquarium of a live individual (Alaska Department of Fish & Game fish resource permit #SF2016-029) (Appendix A). No other amphibians co-occur at the northern range of the wood frog, though to assess specificity in vitro we tested Rasy_00 against genomic isolates from closely related and/or co-occurring aquatic species using the qPCR assay described herein. Rasy_00 consistently amplified wood frog DNA with 100% specificity (Table 1).

We collected 1L water samples from sixty wetlands near Fairbanks, AK throughout the breeding season to assess field performance of the eDNA assay (Appendix A). Opportunistic visual and acoustic observations were recorded at each site. Water samples (n = 155) were kept cool and dark and filtered within 24 hours of collection. We vacuum filtered water samples through 0.45µm cellulose-nitrate membranes until they became clogged (0.1-1L). Each batch of sample filtrations included a filter blank of distilled water (n = 18). Filter membranes were preserved at -80°C for less than 6 months until DNA isolation.

We isolated total genomic eDNA from filter membranes using a modified phenol-chloroform protocol (Renshaw et al. 2015; http://dx.doi.org/10.17504/protocols.io.hnfb5bn). Each batch of DNA isolations included a negative control with no filter membrane (n = 10). All pre-PCR work was conducted in a PCR-free building. DNA isolates were used as templates in a qPCR assay with the Rasy_00 primers. All qPCRs were conducted in replicate (4X) on an Applied Biosystems 7900HT Sequence Detection System. PCR conditions were as follows for 20µL reactions: 10µL 2X KAPA SYBR Universal MasterMix, 0.4µL 10µM each primer, 0.4µL 50X ROX dye, 1.25µL

100% DMSO, and 5μL template DNA (diluted 1:200, as determined by serial dilution). Thermal cycling conditions were 1x [94°C/4min], 40x [94°C/30sec, 55°C/45sec, 72°C/45sec] and a melt-curve analysis of 1x [94°C/15sec, 55°C/15sec, 94°C/15sec (2% ramp rate)]. Results were scored as the number of positive replicates. Stochastic variation among replicates was observed due to low eDNA concentrations. A relaxed interpretation (qPCR score = 1) risks false positive detection resulting from sample contamination, necessitating a cutoff score ≥2 to confidently infer species presence (Table 2). All sites with visual/acoustic detection (n = 13) had scores ≥2. Non-target amplifications resulting from primer-dimer artefacts were produced in the absence of template molecules in both negative control and unknown samples. They were excluded from the results through melt-curve analysis (Gudnason et al. 2007). A subset (n = 8) of positive samples was confirmed as wood frog DNA via Sanger sequencing (GenBank accession #MG002391-MG002398). The limit of detection for the qPCR assay was assessed on a Qubit 2.0 Fluorometer using a dilution series of DNA extracted from wood frog liver tissue (UAM:Herp:122) at $1.83x10^{-3}$ pg/μL.

Our findings suggest eDNA detection is a viable survey method for semi-aquatic species in ephemeral wetlands. The assay described here may be improved by substituting DNA template dilution with a pre-PCR column-based purification step to reduce assay variance (McKee et al. 2015b). The widespread use of this assay can provide baseline northern wood frog occurrence data for use in spatial analyses (see Chapter 2).

Table 1 Results of the in vitro Rasy_00 species specificity tests
Primer Rasy_00 species specificity, as tested in vitro using genomic DNA extracts.

Species + ITIS TSN	Amplification
Wood frog (*Rana sylvatica*, 775117)	+
Columbia spotted frog (*Rana luteiventris*, 550546)	-
American bullfrog (*Rana catesbeiana*, 775084)	-
Northern leopard frog (*Rana pipiens*, 775108)	-
Rough-skinned newt (*Taricha granulosa*, 173620)	-
Arctic Grayling (*Thymallus arcticus*, 162016)	-
Least cisco (*Coregonus sardinella*, 161938)	-
Sockeye salmon (*Oncorhynchus nerka*, 161979)	-
Northern pike (*Esox lucius*, 162139)	-
Alaska blackfish (*Dallia pectoralis*, 162159)	-
Arctic lamprey (*Lethenteron camtschaticum*, 622287)	-
Slimy sculpin (*Cottus cognatus*, 167232)	-

Table 2 Results of the wood frog field surveys
qPCR results of wood frog eDNA field surveys*.

	Successful qPCR replicates (n of 4)				
	0	**1**	**2**	**3**	**4**
Sites (n = 60)	25	19	9	3	4
Negatives (n = 28)					
Filter blanks	15	3	0	0	0
Isolate blanks	8	2	0	0	0

* Raw data available via Dryad, http://dx.doi.org/10.5061/dryad.b7g24

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Chapter 2

Application of environmental DNA-based occurrence data in modeling wood frog (*Rana sylvatica*) distribution in Interior Alaska[1]

2.1 Abstract

Knowledge of wood frog distribution in Alaska is incomplete due to insufficient baseline occurrence data. A short season of activity and difficult access to remote areas restrict implementation of consistent monitoring efforts. Detecting the presence of species in aquatic landscapes using environmental DNA (eDNA) assays is increasingly applied as a monitoring method in wildlife surveys. However, uncertainties regarding the technique's sensitivity to environmental variables and human error have thus far prevented its widespread adoption in studies of species distribution. Predictive models built on machine learning algorithms can help provide precise descriptions of species distribution using eDNA occurrence data, but they will require ground-truthing efforts to confirm accuracy in under-sampled landscapes. Here we assess the ability of wood frog eDNA occurrence data to inform species distribution models under five criteria for data use. We sampled 60 wetlands for eDNA in the Fairbanks North Star Borough during summer 2015. Samples were processed using a species-specific qPCR assay. Wood frog presence at each site was inferred from the qPCR results. This data was used to construct four different wood frog distribution models. From each model we produced a predictive distribution map encompassing the Fairbanks North Star Borough. We assess the performance of each model using available wood frog presence data. Our highest performing model achieves moderate predictive accuracy (Area Under the Curve = 0.74). Weak signals in eDNA occurrence data are important in revealing species presence at low abundance, but strict lab hygiene, quality control practices, and detailed metadata are needed to retain confidence in the results. We show a powerful new way to study wood frog distribution by combining eDNA occurrence data with machine learning techniques. Wider implementation of eDNA surveys and increased availability of high resolution GIS data will help to refine these models.

[1] Spangler, M.A., J.A. López, and F. Huettmann. Application of environmental DNA-based occurrence data in modeling wood frog (*Rana sylvatica*) distribution in Interior Alaska. Prepared for submission in *PLoS One*.

2.2 Introduction

Massive declines in amphibian populations have been documented over the past 30 years [1]. These declines range in scope from regional to global [2-4]. They have led to the stress of entire ecosystems, as measured by local extirpation and extinction of species [5]. Even common and widespread species have experienced population declines [4]. The wood frog (*Rana sylvatica*) occurs across northern North America and is the only amphibian found throughout much of Alaska [6]. The Alaska Department of Fish and Game has identified several factors that make the wood frog a vulnerable species in the state [7]. Local declines of wood frog populations have been reported, while monitoring efforts remain localized and inconsistent [8]. Similar deficiencies plague the northern provinces of Canada [9]. One implication of these inadequate monitoring efforts is poor knowledge of wood frog distribution across large regions of Alaska.

Environmental DNA (eDNA) detection assays have become a standard tool complementing traditional means of conducting aquatic species inventories [10-14]. The high sensitivity of the eDNA technique lends itself well to monitoring for the presence of rare [15, 16], invasive [17-19], and cryptic [20, 21] species, including those that are ephemeral in presence. Increasingly, eDNA assays are also being used to answer complex questions in aquatic ecology. Environmental DNA assays have been applied to studies of site occupancy [19,22], species abundance [14,23], and biodiversity assessments [24,25]. The sensitivity of eDNA assays, however, imposes limitations on data interpretation. The persistence and detection of DNA is highly influenced by the life history of the species and the specific conditions of the surrounding environment [26, 27]. Transport of DNA away from its source in the environment is also poorly understood, hampering progress in the use of eDNA for conservation management [28, 29]. The spatial complexity and heterogeneity of aquatic ecosystems makes precise characterization of species distribution within these systems difficult. Spatial analyses using eDNA occurrence data have until now been limited to mapping results and delineating rough boundaries [15, 19, 30, 31]. The ability of eDNA occurrence data to inform predictive species distribution models (SDMs) has not been extensively assessed, though solutions to some of these limitations may exist in machine learning techniques.

Many machine learning algorithms have been developed to mine complex datasets [32, 33]. The group of tree-based methods including CART, Random Forests, TreeNet, and their ensembles are used to produce SDMs with predictive capabilities superior to those produced by statistical models [34, 35]. These algorithms are designed to use dozens, even hundreds, of environmental predictor variables and their interactions to identify patterns in

datasets, while remaining robust to overfitting [36, 37]. Signals extracted from complex data can be extrapolated to predict values outside of the dataset in space and time. Machine learning algorithms are non-parametric and thus do not require that data meet *a priori* assumptions [36, 37]. Incomplete datasets with missing values, outliers, and complex, non-linear relationships tend not to impose major limitations on these algorithms, making them ideal candidates for analyzing imperfect datasets [35, 38].

Wood frogs provide an opportunity to assess the utility of aquatic eDNA occurrence data in informing semi-aquatic species distribution across a landscape. In this study, we use a recently developed eDNA detection assay [39] to conduct wood frog surveys in the Fairbanks North Star Borough, Alaska. We use different criteria for establishing wood frog presence from the eDNA occurrence data to train our predictive species distribution models. These different interpretations of eDNA occurrence data reveal the complexities involved in the application of eDNA assays to detailed spatial analyses.

2.3 Methods

Environmental DNA survey

We sampled sixty wetlands covering approximately 150,000 hectares (30km x 50km) in the Fairbanks North Star Borough, Alaska for environmental DNA (Figure 1). Site selection was opportunistic, considering ease of access for repeat sampling and known wood frog breeding activity. Additionally, we sampled wetlands that are not typically considered to be suitable for wood frog use, including large lakes inhabited by predatory fish and lotic systems. This was done to test for false detection in the field. Forty sites were visited once each in May, June, July, and August of 2015 to account for variation in site occupancy throughout the breeding period. We were unable to resample ephemeral sites (n = 11) after they dried during the study. Ten additional sites were visited once only in May and another ten sites were visited once only in July due to limited accessibility. We sampled each site using a disposable vinyl glove and a previously sterilized 1-L polypropylene Nalgene bottle. Samples were collected from the surface near wetland margins without entering the water. Water samples were kept cool and dark in transit to the laboratory. We visually scanned each site for wood frogs, recording all opportunistic observations. Standardized visual encounter surveys were not conducted. Water samples were either filtered on the same day as collection or stored at 4°C and filtered the following day. We performed all filtrations in a PCR-free building using sterile equipment and work surfaces. Each water sample was vacuum filtered through a 0.45-µm cellulose-nitrate membrane (Whatman 7141). In cases where water samples contained a high sediment load, 500 mL were filtered,

the used filter membrane was replaced with a fresh one, and the remaining 500 mL were filtered. Ten samples were only partially filtered because they contained enough sediment to fully clog two filters. All equipment was soaked in a 50% bleach solution for >5 minutes, rinsed thoroughly with ultrapure deionized water to remove all residual bleach, and air-dried before reuse. Each batch of sample filtrations included a single 1-L deionized water sample (i.e. filter blank) for negative control (n = 18). Filter membranes were stored dry at -80°C until further processing.

Environmental DNA analysis

All samples were stored for less than 6 months before isolating DNA. We used a phenol-chloroform protocol [40] with minor modifications (dx.doi.org/10.17504/protocols.io.hnfb5bn). Each batch of sample isolations included a single negative control in which no filter membrane was used (n = 10). These DNA isolate blanks were used in conjunction with the filter blanks to monitor for false positive detection. Serial dilution of wood frog DNA provided qPCR standard curves and replicated assay performance. We conducted all DNA isolation and PCR preparation procedures in a PCR-free building. Positive control samples were added to the qPCR plate in a dedicated workstation. All DNA samples were amplified using a quantitative PCR (qPCR) assay with cytochrome *b* DNA primers developed for this study [39]. All filter DNA isolates, negative control samples, and standards were used in four-fold replicate eDNA assays performed on an Applied Biosystems 7900HT Sequence Detection System using the following conditions: 1X KAPA SYBR Universal MasterMix, 0.2 µM each primer, 1X ROX dye, 6.25% Dimethyl Sulfoxide (DMSO) v/v, and 5 µL template DNA (diluted 1:200) in 20 µL reactions. The thermocycler profile consisted of an initial denaturation period of 94°C for 4 minutes; 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 45 seconds; and a melt-curve analysis of denaturation at 94°C for 15 seconds, annealing at 55°C, and denaturation at 94°C for 15 seconds (2% ramp rate). Each sample was assigned a qPCR score [10] as determined by the number of successful target DNA amplifications (0-4). Replicates with stunted amplifications curves not meeting the C_t value were considered PCR inhibited.

Model development

We assigned each site a qPCR score (0-4), equal to the maximum number of replicate amplifications from a single sample at that site across all months sampled. We also calculated an average qPCR score, or the ratio of total positive amplifications to total technical qPCR replicates across all months sampled, for each site to account for sampling effort. Replicates showing evidence of PCR inhibition were removed from the analysis. We classified each site as present/absent under four different classification criteria. A site was classified as present if its qPCR score

met a cutoff threshold (1-4) for inferring species presence. These classifications and site average scores served as response variables in model development for inference from prediction [41]. Forty-two environmental GIS layers comprise the multivariate predictor dataset (Table 1). The values for each predictor were extracted to every sample site using either the Extract Multi Values to Points (raster) or the Spatial Join (shapefile) tool in ArcGIS 10.4. The resultant training dataset was uploaded into the Salford Predictive Modeler (SPM) 8.0 graphic user interface (https://www.salford-systems.com/). We performed a classification analysis for each presence/absence scenario and a regression analysis for the site average data using the TreeNet Stochastic Gradient Boosting algorithm [42]. Exploratory analyses were conducted to automate the learn rate (LEARNRATE) for each TreeNet algorithm. We grew all models to 200 trees to find the optimum solution. Model performance was internally tested using V-fold cross-validation. Default values were used for all remaining parameters in the model setup interface of the TreeNet analysis engine, following standard practice. The resultant model outputs (containing model formulae and rules) were saved as grove files (.grv) for model assessment and mapping.

Model assessment

We created a dataset consisting of a 1-km resolution lattice of points covering the entire Fairbanks North Star Borough to map the predicted species distribution. Predictor values were assigned to each lattice point using the Extract Multi Values to Points tool in ArcGIS 10.4. We used each of the predictive algorithms (groves) from the training dataset to score every point in the lattice dataset with a Relative Index of Occurrence (RIO) score. These RIO scores were mapped and interpolated using the Inverse Distance Weighted (IDW) interpolation tool in ArcGIS 10.4 [43]. We created an assessment dataset comprising 5,000 random pseudo-absence points and seventy confirmed wood frog presence points. Presence points were compiled from various open-access sources (Table 2). Presence points added to the assessment dataset were filtered for geo-referenced (WGS84) records within the Fairbanks North Star Borough and a coordinate precision of five decimal places or greater. In addition, we removed duplicate records both within and between datasets so that only unique location values were included. The RIO score for each point in the assessment dataset was acquired from the prediction surface using the Extract Values to Points tool. The performance of each model under all scenarios was assessed based on the model's ability to correctly classify points in the assessment dataset. An Area Under the Curve (AUC) score was calculated for each model to provide this metric (R 3.3.2 PresenceAbsence package).

2.4 Results

Environmental DNA

Variation in amplification success was observed both within sample replicates and across temporal replicates at the same site. Thirty-six sites had a qPCR score of 1 or greater, while only four sites had a qPCR score of 4. Accordingly, the number of sites with inferred wood frog presence for model building purposes varied from 4-36 depending on which classification criterion was followed (Table 3). Twenty-four sites displayed no evidence of wood frog eDNA. Only one site had perfect detectability (8/8 replicate amplifications). The mean site average score was 0.179 ± 0.230 (Figure 2). Five of twenty-eight negative control samples showed some indication of cross-contamination, however for all five only 1 of 4 replicates produced an amplification. We visually confirmed wood frog presence at four wetland sites.

Species distribution models

There was not enough presence data for the TreeNet algorithm to successfully discriminate between classes when the presence threshold was set at 4. Distribution maps produced from the remaining four models are shown in Figure 3. The predictive model built on regression analysis of the site average dataset had the lowest predictive accuracy (AUC = 0.52). The predictive model built on classification analysis of occurrence data with a presence threshold of 3 had the highest predictive accuracy (AUC = 0.74). Table 4 provides further metrics of performance for this model, internally assessed using V-fold cross-validation [44]. The mean RIO score for presence points in the assessment dataset was 0.4312 ± 0.03 (Figure 4). National Land Cover Database category was the most important variable in determining wood frog occurrence in the model (Table 5). The categories most closely associated with wood frog presence were low-intensity developed areas, barren lands, and deciduous forests (Figure 5).

2.5 Discussion

Environmental DNA assays inconsistently detected wood frog DNA in our study sites. Potential causes include low abundance of DNA in the environment, high incidence of PCR inhibition, and changes in site occupancy throughout the summer season. The TreeNet algorithm identified strong patterns in the eDNA occurrence dataset despite the intrasample and temporal variation within sites. Distribution maps produced from the resultant SDMs show complex habitat associations. Our highest performing model predicted confirmed wood frog presence records with moderate accuracy (AUC = 0.74), highlighting the power of machine learning algorithms to analyze small, imperfect datasets.

The incidence of false positive results warrants special consideration in studies of environmental DNA, especially when such data are used to infer species presence [28, 45]. False positive detection arises when eDNA is detected at a site where the species itself is not present, either via human error or allochthonous DNA. It can also result from contamination in the lab. The positive amplification in a single replicate among five negative control samples indicates that contamination may be contributing to the false positive detection of wood frogs in this study under the most relaxed interpretation of the data. The incidence of false positive results in a dataset can cause overprediction in an SDM built from such occurrence data. Evidence for overprediction is shown in the classification model with the presence threshold of 1 (AUC = 0.55). These findings reinforce the importance of including rigorous negative control experiments in all eDNA-based studies. Extensive documentation and accessibility of protocols and data following metadata standards and minimum reporting guidelines boosts confidence in transparency and repeatability of positive results [45]. A considerable benefit of implementing eDNA into species surveys is its sensitivity in sampling rare, cryptic, and invasive species in low abundance [15-21]. Weak signals derived from low concentration eDNA play a key role in this improved detectability. False positive detections in negative control samples are often unavoidable due to the sensitivity of eDNA assays, however. These errors do not strongly impact results if they remain infrequent and low quantity [45].

On the other hand, stringent interpretation of eDNA data risks false negative results. Only four presence points remained when the presence threshold was set at 4, while 32 sites with evidence of wood frog eDNA (qPCR score 1-3) were inferred as species absence. A predictive model for this scenario could not be constructed due to the TreeNet algorithm's inability to extract a signal from so few presence points. Opportunistic visual confirmation was also recorded at four sites, highlighting the impracticality of a high presence threshold in this study. Environmental DNA is usually highly degraded and occurs in low abundance. There will almost always be inconsistency between qPCR replicates [45]. Some of the inconsistencies resulting in few high qPCR scores in this study may be attributed to the semi-aquatic nature of wood frogs and their preference for ephemeral wetlands. Suboptimal conditions for the preservation and detection of eDNA in ephemeral wetlands have similarly impacted previous eDNA studies [11, 46]. The evidence suggests that setting such a high standard for inferring species presence from eDNA data risks false negative results and poor assay detectability. Models derived from these interpretations of eDNA data are likely to under-predict species occurrence. Stringent interpretation of eDNA data when modeling species distribution should be reserved for only the most conservative analyses.

Species distribution maps generated from the predictive models exhibit high spatial heterogeneity. Higher resolution predictor variables may improve model predictive accuracy by refining these clusters. The complex nature of the models suggests that wood frog occurrence may be most influenced by microhabitat factors, but that the species is generally widespread across the Fairbanks North Star Borough. All models predict a strong association between wood frog occurrence and landcover type. The positive association of wood frog presence with low intensity developed areas and deciduous forests is to be expected based on known wood frog natural history [6-8]. Unexpectedly, a strong negative association is shown for the evergreen forests, while woody wetlands have only a slightly positive association. These relationships may reflect a negative sampling bias caused by the poor conditions for DNA detection found within these habitat types [11, 46, 47], landcover misclassification [48], or an unknown interaction between predictor variables. Predicted wood frog occurrence is also determined by precipitation amount in the late spring/early summer months and percent slope on the landscape. Excessive precipitation (>6.5 mm April, >18 mm May, >37 mm June) is negatively associated with wood frog presence. Slope greater than 6% is positively associated with wood frog presence. These predictors in concert indicate suitability for wood frog breeding habitat in the study area. Site-specific variables, such as presence of aquatic predators, water pH, turbidity, oxygen levels, and conductivity likely play a key role but are difficult to extrapolate for prediction. Remote regions in the study area are under-sampled and will require ground-truthing efforts to confirm and improve model accuracy.

2.6 Conclusion

We demonstrate the utility of environmental DNA data to inform semi-aquatic species distribution models built on machine learning algorithms. Our findings suggest wood frog distribution across Fairbanks North Star Borough is ubiquitous and occurrence is likely determined at a microhabitat scale. Our top-performing model had only moderate predictive accuracy (AUC = 0.74). We conclude that the availability of higher resolution GIS predictor layers and a wider implementation of wood frog eDNA surveys leading to a larger dataset will result in improved species distribution models and estimates with higher predictive accuracy. The use of environmental DNA data in fine-scale spatial analyses will entail many complexities. The interpretation of these data can have major influence on the final analysis. Future studies will benefit from testing all scenarios, as factors specific to each study system will influence the outcome. The role for environmental DNA data in these studies is best seen as a complement to, not a replacement of, traditional monitoring methods. Nevertheless, eDNA is likely to progress as a powerful tool in landscape ecology, resulting in more effective conservation management.

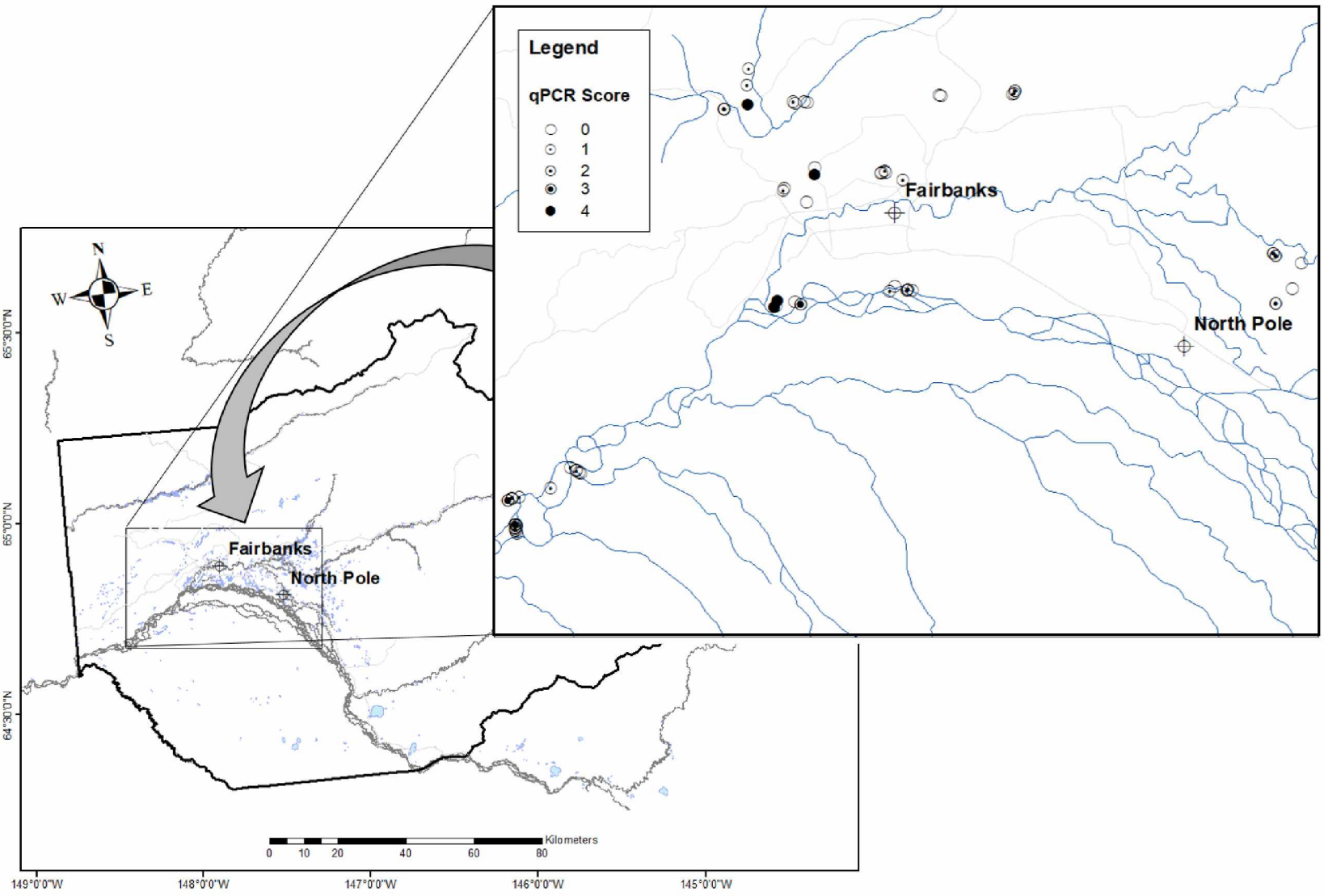

Figure 1 Sample site map
Depiction of the eDNA survey sampling locations within the Fairbanks North Star Borough. Fairbanks and North Pole, AK are shown for reference.

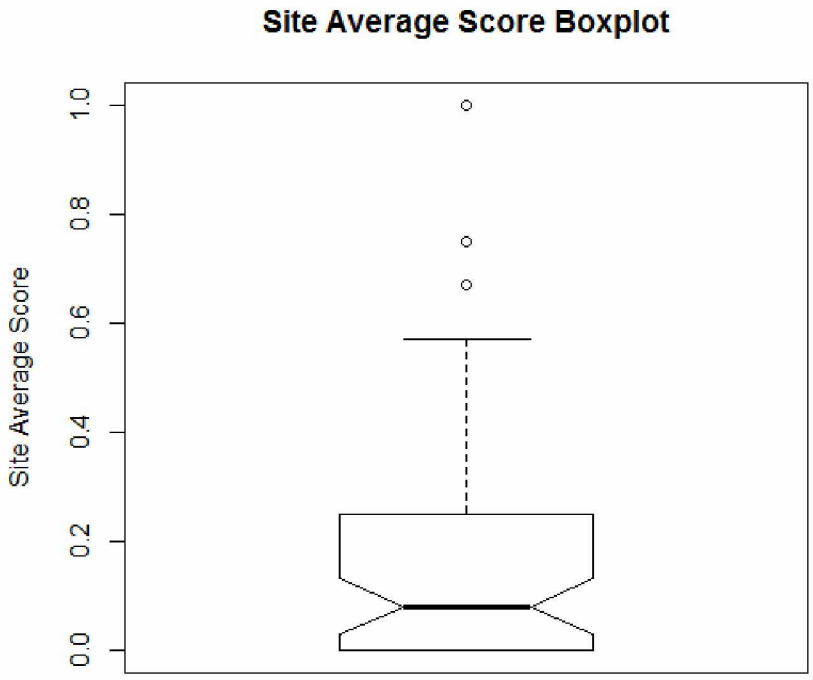

Figure 2 Site average score plot
A box plot of site average scores for each of the sample sites. Site average is defined by the ratio of positive amplifications to total qPCR replicates *across all months sampled*.

Notches indicate 95% confidence
Mean = 17.9%
Upper quartile = 25.00%
Lower quartile = 0.00%

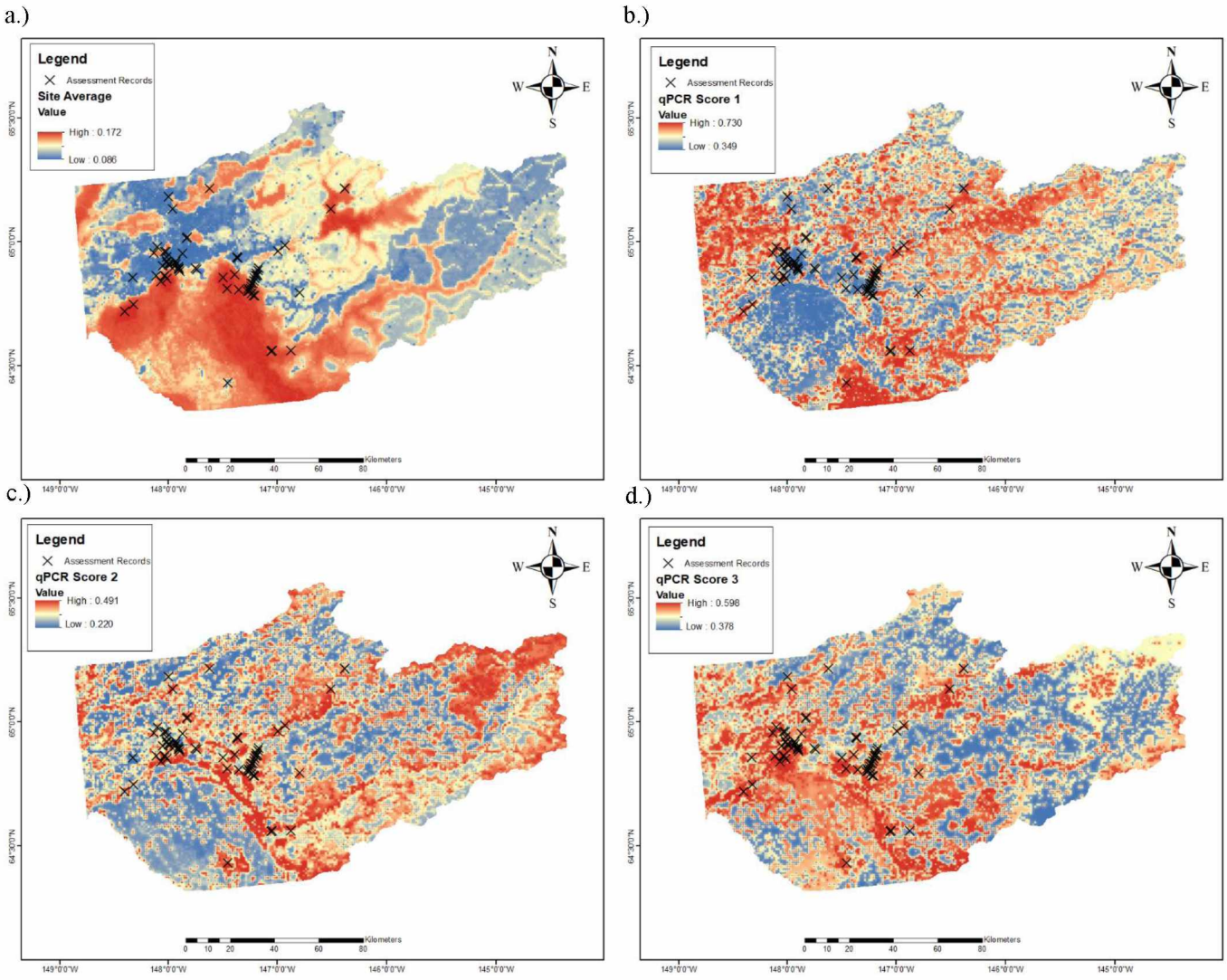

Figure 3 Predictive distribution maps

a.) regression analysis of the site average scores (AUC = 0.52)

b.) classification analysis of the qPCR scores with a presence threshold of 1 (AUC = 0.55)

c.) classification analysis of the qPCR scores with a presence threshold of 2 (AUC = 0.62)

d.) classification analysis of the qPCR scores with a presence threshold of 3 (AUC = 0.74)

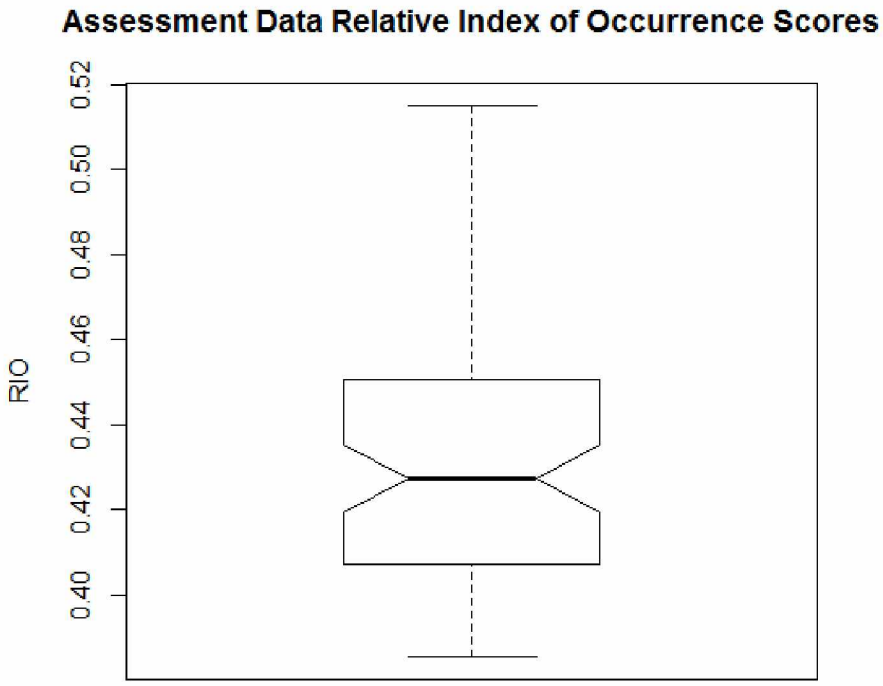

Figure 4 Distribution of RIO scores
A box plot of RIO scores for the confirmed presence points in the assessment dataset, as predicted by the top performing model (AUC = 0.74).

Notches indicate 95% confidence intervals
Mean = 0.43
Maximum = 0.52
Upper quartile = 0.45
Lower quartile = 0.41
Minimum = 0.39

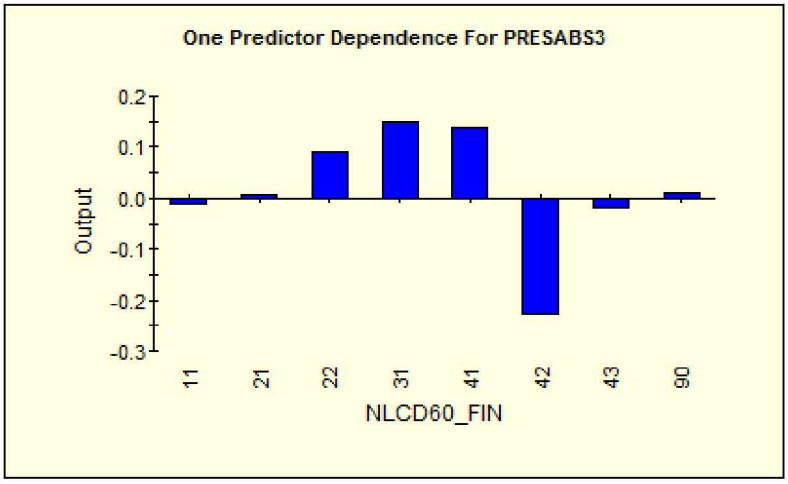

Figure 5 Top predictor variables
The top predictor variable for the model with the highest predictive accuracy is NLCD landcover classification. Positive associations are indicated by output values greater than zero, while those less than zero represent negative associations.

11 = open water
21 = developed, open space
22 = developed, low intensity
31 = barren land
41 = deciduous forest
42 = evergreen forest
43 = mixed forest
90 = woody wetlands

Table 1 Predictor variable list

A list of environmental predictor variables, data type (continuous or categorical), format (raster or shapefile), and their original source. Updated from original dataset compiled by Baltensperger et al. 2013 [38].

Predictor (resolution)	Type	Format*	Source
Proximity to lotic systems (60m)	Continuous	Raster	AK Gap Analysis Project [49]
Proximity to lentic systems (60m)	Continuous	Raster	AK Gap Analysis Project [49]
Proximity to permafrost (60m)	Continuous	Raster	AK Gap Analysis Project [49]
USGS hydrologic unit code (HUC) (polygon)	Categorical	Shapefile	AK State Geospatial Data Clearinghouse [49]
Relative surface water inundation (polygon)	Categorical	Shapefile	Zona *et al.* 2016 [50]
Census block in year 2010 (polygon)	Both†	Shapefile	Fairbanks North Star Borough
Distance to infrastructure (60m)	Continuous	Raster	AK Gap Analysis Project [49]
Distance to roads (650m)	Continuous	Raster	Fairbanks North Star Borough
Distance to resource extraction site (1km)	Continuous	Raster	AK State Geospatial Data Clearinghouse
Distance to EPA FRS sites (1km)	Continuous	Raster	von Hippel *et al.* 2016 [51]
Distance to ADEC sites (1km)	Continuous	Raster	AK State Geospatial Data Clearinghouse
Land ownership (polygon)	Categorical	Shapefile	Alaska Generalized Land Status 2016 [49]
NLCD landcover classification (60m)	Categorical	Raster	AK Gap Analysis Project [49]
Aspect (60m)	Continuous	Raster	AK Gap Analysis Project [49]
Elevation (60m)	Continuous	Raster	AK Gap Analysis Project [49]
Slope (60m)	Continuous	Raster	AK Gap Analysis Project [49]
Ruggedness (60m)	Continuous	Raster	AK Gap Analysis Project [49]
Average monthly precipitation 1-12 (60m)	Continuous	Raster	AK Gap Analysis Project [49]
Average monthly temperature 1-12 (60m)	Continuous	Raster	AK Gap Analysis Project [49]
Average growing season length (3km)	Continuous	Raster	UAF Scenarios Network for AK + Arctic Planning [49]

* ESRI GIS data format

† The census block predictor is a container file for 45 additional socio-economic predictors.

Table 2 Assessment dataset sources

Openly-accessible databases from which confirmed wood frog presence records were drawn. Presence records were used to assess model performance.

Database Name	URL	Selected Reference
Alaska Center for Conservation Science	http://accs.uaa.alaska.edu	Aycrigg et al. 2015 [52]
Alaska Gap Analysis Project	http://akgap.uaa.alaska.edu	Gotthardt et al. 2014 [49]
AmphibiaWeb	http://amphibiaweb.org	Pyron & Wiens 2011 [53]
Global Biodiversity Information Facility	https://www.gbif.org/	Edwards 2004 [54]
iNaturalist	http://www.inaturalist.org/	Michonneau & Paulay 2015 [55]
University of Alaska Museum ARCTOS	https://arctos.database.museum/home.cfm#UAM	Tessler et al. 2014 [56]
USGS Biodiversity Information Serving Our Nation (BISON)	https://bison.usgs.gov/#home	Hampton et al 2013 [57]
VertNET	http://vertnet.org/	Constable et al. 2010 [58]

Table 3 eDNA survey results
Results of the eDNA surveys. A qPCR score of 0 indicates species absence. Scores 1-4 are thresholds under four different criteria for establishing wood frog presence at a site. Note that the presence categories are inclusive.

	Presence Threshold				
	qPCR score 0	qPCR score ≥ 1	qPCR score ≥ 2	qPCR score ≥ 3	qPCR score 4
Number of sites (n = 60)	24	36	16	7	4
Negative controls (n = 28)	23	5	0	0	0

Table 4 Confusion matrix
A confusion matrix of the test data (internal v-fold cross validation) and performance metrics for the model with the highest predictive accuracy.

Model accuracy = 62.23%
Model specificity = 67.31%
Model sensitivity = 57.14%
Model precision = 63.61%

		Predicted Class: Absence	Predicted Class: Presence
Actual Class	Absence	35	17
	Presence	22	30

Table 5 Relative variable importance as determined by TreeNet
A list of relative variable importance for the model with the highest predictive accuracy (AUC = 0.74).

Variable	Score
NLCD landcover classification	100.00
Mean precipitation – June	52.85
Mean precipitation – April	37.80
Slope	37.07
Mean precipitation – May	30.88
Mean precipitation – November	30.53
Mean temperature – September	28.92
Census tract	23.01
Proximity to lentic systems	22.87
Ruggedness	19.96

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Chapter 3

A reassessment of wood frog (*Rana sylvatica*) distribution in Alaska and northern Canada based on environmental DNA and citizen science[1]

3.1 Abstract

Alaska has designated the wood frog (*Rana sylvatica*) as a species of greatest conservation need, in part due to limited and inconsistent monitoring efforts across the state. The distribution of this species across northern limits of its range remains unclear. Here we aim to quantitatively model the distribution of wood frogs in Alaska and the Yukon Territory of Canada. We provide a species distribution model derived from mining alternative sources of data, including environmental DNA and citizen science occurrence data. Further, we report on the first known implementation of environmental DNA monitoring for wood frogs. We collected 171 water samples from wetlands along the Elliot/Dalton Highway corridor. Samples were processed and analyzed using a species-specific assay. Environmental DNA survey results were combined with citizen science data on the occurrence of wood frogs. This dataset was used with 104 open-access environmental geographic information system layers to produce a predictive species distribution model based on the Random Forests machine learning ensemble algorithm. The environmental DNA survey detected wood frog DNA in 31 wetland sites along the Dalton Highway corridor, including four sites north of the known range limit for the species. Our predictive model shows that suitable wood frog habitat is more widely distributed across Alaska and the Yukon Territory than previously documented.

3.2 Introduction

The growing awareness of global amphibian declines over the past 30+ years (Alroy 2015; Stuart et al. 2004; Wake and Vredenburg 2008) has brought attention to how little is understood about the conservation status of local amphibian populations. Nearly one-fourth (23.7%) of amphibian species are classified as Data Deficient by the International Union for Conservation of Nature (IUCN), more than any other terrestrial vertebrate taxa (IUCN 2016). There remain large gaps in knowledge of even our most widespread and common species, which are also experiencing decline (Adams et al. 2013). The wood frog (*Rana sylvatica*) occurs throughout the eastern United

[1] Spangler, M.A., J.A. López, and F. Huettmann. A reassessment of wood frog (*Rana sylvatica*) distribution in Alaska and northern Canada based on environmental DNA and citizen science. Prepared for submission in *Biological Conservation.*

States, much of Canada, and into Alaska (Figure 1). Numerous threats to the health of wood frog populations in Alaska have been identified by the Department of Fish and Game (Alaska Department of Fish and Game 2006; Fields and Gotthardt 2009). Research priorities identified by the agency pertain to the spread of disease, high incidence of physical abnormality, increasing land development, impact of permafrost thaw on vernal pools, and local response to climate change, including extirpation and shifts in range and phenology. The wood frog is the only amphibian found in the interior and northern regions of the Yukon Territory and Alaska. As such, knowledge of arctic and subarctic wood frog distribution and population trends are vital to understand amphibian response to local climate change, land management, and related issues (Benard 2015; Davenport et al. 2016; Lesbarrères et al. 2014; Winter et al. 2016). But remote areas in Alaska and northern Canada suffer from deficient monitoring practices, leading to incomplete knowledge of wood frog distribution and trends (Lesbarrères et al. 2014). Specifically, areas north of the Brooks Range in Alaska are under-sampled for amphibians. Anecdotal observations of wood frogs from those areas are reported (Fields and Gotthardt 2009), but attempts to document indigenous knowledge of amphibians in the region are not known.

The most complete understanding of northern wood frog distribution comes from the Alaska Gap Analysis Project (AK GAP). This massive undertaking provided Alaska with species distribution models (SDMs) for nearly all its terrestrial vertebrate species (Gotthardt et al. 2014). AK GAP SDMs were derived, in part, from inductive models built with the Maximum Entropy algorithm (MaxEnt). It is the first model-based approach at describing wood frog distribution in Alaska. No such efforts are known for the Yukon Territory of Canada. The Alaska GAP serves as the current standard against which to compare future SDMs in the state. The project also created an opportunity to build on its success by providing access to all data and models for further refinement. Occurrence records used in AK GAP analyses were sourced from a relatively small number of large databases due to the scale of the project (347 species). AK GAP SDMs are also constrained to a predetermined species range, something that is not firmly established for wood frogs in Alaska. Further, the MaxEnt algorithm used in their predictive models can underperform when occurrence records are unevenly distributed across the species range, as is the case of the wood frog. No comprehensive assessment of wood frog distribution in arctic and subarctic North America exists that uses all available data and the most recent, powerful SDM methods.

Environmental DNA (eDNA) assays are rapidly becoming a common conservation management tool in aquatic ecology and elsewhere (Bohmann et al. 2014; Goldberg et al. 2015; Thomsen and Willerslev 2015).

Applications are diverse and range from monitoring the spread of invasive species (Eichmiller et al. 2014; Ficetola et al. 2008; Hunter et al. 2015) to describing ecosystem biodiversity (Thomsen and Willerslev 2015). Benefits of this technique over more traditional methods include substantial reductions in time, labor, and monetary resources needed to carry out species surveys in the field and improved species detectability on a landscape scale (Biggs et al. 2015; Olson et al. 2013; Pilliod et al. 2013). The application of eDNA assays is expanding beyond qualitative research into quantitative analyses as detection technology and our understanding of DNA's persistence in the environment progresses. Environmental DNA assays are increasingly used to compute species abundance and habitat occupancy (Doi et al. 2017; Pilliod et al. 2013). Previous work has shown that eDNA occurrence data can be used to model small-scale wood frog distribution in Interior Alaska (see Chapter 2).

Citizen science represents another non-traditional method of monitoring wildlife populations. Some of the most successful citizen science programs focused on North American wildlife involve amphibians. The North American Amphibian Monitoring Program (NAAMP), FrogWatch USA, and FrogWatch Canada are three examples of national, long-term (15+ years) citizen science monitoring programs that have resulted in extensive datasets available for full-scale analysis. NAAMP was discontinued in 2015 after being coordinated by the United States Geological Survey (USGS) for 18 years (https://www.pwrc.usgs.gov/naamp/). The closure of NAAMP ended an important government-sponsored long-term monitoring program and put the future storage and accessibility of the dataset at risk. FrogWatch USA is a similarly run program, coordinated by the Association of Zoos and Aquariums (https://www.aza.org/frogwatch). The program began soliciting observations in Alaska with the establishment of a regional chapter in Fairbanks in 2016. Canada's FrogWatch program is part of the larger NatureWatch national volunteer monitoring program hosted by Nature Canada and Environment Canada (https://www.naturewatch.ca/frogwatch/). Observations are accepted for all provinces and territories, though efforts are focused in the south. Many other local and statewide programs have used these projects as a model, including the Alaska Wood Frog Monitoring Program (http://www.adfg.alaska.gov/index.cfm?adfg=citizenscience.woodfrog). The AK Wood Frog Monitoring Program comprises a sizeable portion of wood frog occurrence data in the state (approx. 23%), with data accessible from 2002 – 2008. Other sources of citizen science amphibian occurrence data come from the Alaska Native Tribal Health Consortium's Local Environment Observer Network (https://www.leonetwork.org/en/) and the online web application iNaturalist (https://www.inaturalist.org/). Rapidly growing acceptance of citizen science data (Crall et al. 2011; Lewandowski and Specht 2015; Lukyanenko et al.

2016) reveals the importance of citizen science projects not only to education/outreach but also to scientific research (Barnard et al. 2017; McKinley et al. 2017; Sullivan et al. 2017).

Here we provide an assessment of northern wood frog distribution using two alternative sources of data intended to complement traditional monitoring efforts. Consideration of such data is integral to understanding spatial patterns and population trends of species, especially in under-sampled areas. We arrive at a more complete understanding of wood frog distribution in Alaska and the Yukon Territory of Canada by analyzing these alternative datasets with an advanced machine learning ensemble algorithm.

3.3 Methods

Environmental DNA sample collection

We conducted an 800-km transect survey between Fairbanks and Prudhoe Bay, AK, along the Elliott-Dalton Highway corridor. Hereafter, we refer to a landscape-level transect that spans multiple ecosystems as a mega-transect (Baltensperger and Huettmann 2015; Cohn 2008). This mega-transect was selected to provide a latitudinal gradient between the forested interior of Alaska through the Brooks Range and into the Arctic Coastal Plain. We collected 171 samples from wetlands accessible within 1-km of the roadway. An additional 25 samples were collected from areas of special interest across Alaska including the Stikine River corridor, Gates of the Arctic National Park, Utqiaġvik, and along the Upper Kuskokwim River. Samples were collected immediately following the wood frog breeding season, during embryo and larval development from mid-May to mid-June. We used disposable vinyl gloves and a sterilized polypropylene Nalgene grab bottle to collect a 1-L water sample from each site. Samples were obtained from the surface and we avoided physically entering the water prior to sample collection to prevent site contamination. We searched for wood frog presence via visual encounter and dip-net survey following eDNA sample collection. Captured individuals were live-released immediately upon positive identification (University of Alaska Fairbanks Institutional Animal Care and Use Committee 871371-2 [Appendix B]; Alaska Department of Fish and Game Fish Resource Permit SF2016-069 [Appendix C]). Water samples were kept cool and dark and vacuum filtered through 0.45µm cellulose-nitrate filter membranes (Whatman #7141) the same day. This pore size captures most cellular and absorbed eDNA and clogs less rapidly than smaller pore sizes. We filtered samples with high sediment load until water would no longer pass through the filter member. Total volume filtered was recorded in these instances. We filtered 1-L distilled water for every nine samples to detect field contamination (n = 19). Filter membranes were stored dark in Longmire's solution (Longmire et al. 1997; Renshaw

et al. 2015). We soaked all equipment in a 50% bleach solution for >5 minutes to prevent cross-contamination and the potential spread of disease between sites. We thoroughly rinsed equipment with distilled water following the bleach soak and air dried everything before reuse. Filter membranes were stored at -80°C in a PCR-free building after the end of field sampling.

Environmental DNA sample processing

All filter membranes were processed in the lab within six months of sample collection. Pre-PCR procedures were carried out in a biosafety cabinet workstation with positive air pressure, HEPA filtration, and ultraviolet sterilization. We cut filter membranes in half using disposable blades and isolated total genomic DNA from one half of each membrane following a modified phenol-chloroform protocol (Renshaw et al. 2015) (dx.doi.org/10.17504/protocols.io.hnfb5bn). We substituted Qiagen ATL lysis buffer from the protocol with Longmire's solution obtained from the sample tubes that housed the filter membranes. The remaining half of each membrane was archived for future use at the University of Alaska Museum (UAM:Env:1 – UAM:Env:197). In each batch of sample isolations (n = 9), we processed a sample tube with fresh Longmire's solution and no filter membrane to test for false positive detection.

We performed a quantitative PCR (qPCR) assay on all unknown sample and negative control isolates. Samples, including qPCR standards obtained from serial dilutions of wood frog DNA, were run in four replicates on an Applied Biosystems 7900HT Sequence Detection System. Each qPCR sample reaction contained 1X KAPA SYBR Universal MasterMix, 0.2 µM Rasy_00 primers (Spangler et al. 2017), 1X ROX dye, and 5 µL template DNA (diluted 1:100) in 20 µL reactions. Cycling conditions for all reactions consisted of an initial denaturation period of 94°C for 4 minutes; 50 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 45 seconds; and a melt-curve analysis of denaturation at 94°C for 15 seconds, annealing at 55°C, and denaturation at 94°C for 15 seconds (2% ramp rate). All samples that amplified target DNA in 1 or more replicates were interpreted as a wood frog occurrence. PCR inhibition was determined by stunted amplification curves that did not meet the cycle threshold (C_t). Samples displaying PCR inhibition were processed with DNEasy PowerClean Pro Cleanup Kit (MoBio) and OneStep PCR Inhibitor Removal Kit (Zymo Research) and reanalyzed. Any sample replicates still showing PCR inhibition following these procedures were considered negative results.

Citizen science occurrence data collection

We obtained wood frog occurrence records in Alaska and Yukon from the online, open-access databases of four citizen science monitoring programs. Table 1 outlines the protocol followed, type of observation collected, and data validation procedures implemented by all four programs. Occurrence data were filtered for geo-referenced records within Alaska and the Yukon Territory. The Alaska Wood Frog Monitoring Program (n = 523), FrogWatch USA (n = 21), iNaturalist (n = 44), and Environment Canada's FrogWatch (n = 1) provided a total of 589 wood frog presence-only records within the study area. We further filtered records for unique coordinate values with a precision of five decimal places or higher. The resultant dataset contained 226 records used in the subsequent analysis.

Species distribution model training

Environmental DNA presence points were added to the citizen science presence-only records to create a training dataset of 257 wood frog occurrence records. The training dataset was overlaid on a set of 104 GIS environmental raster layers (Sriram and Huettmann 2017) in ArcGIS 10.4. We extracted values from each layer to every point in the training dataset using the Extract Multi Values to Points tool. The resultant dataset was used to perform a classification analysis with species presence as the response variable in Salford Predictive Modeler (SPM) 8.0. Models were constructed using the Random Forests ensemble analysis engine. Exploratory analyses were conducted to optimize the number of predictors used in bootstrap aggregation (RFNPREDS = 7). We grew models to 200 trees and selected the optimal output based on the Area Under the Curve (AUC). Model performance was internally tested using out-of-bag data. All other parameters in the model setup interface were kept at their default settings. Model results were saved as grove files to assess performance using an outside dataset.

Species distribution model assessment

We created a regular point lattice of 5-km pixel resolution to extrapolate the model predictions across Alaska and the Yukon Territory. Values for each of the 104 predictors were assigned to the lattice dataset in ArcGIS 10.4 using the Extract Multi Values to Points tool. We scored the lattice dataset in SPM 8.0 using the model grove. Each lattice point was given a Relative Index of Occurrence (RIO) score (Baltensperger et al. 2013) in relation to the environmental condition, as computed by the model algorithm. We interpolated RIO values across the study area using the Inverse Distance Weighting (IDW) interpolation tool in ArcGIS 10.4. We obtained 888 confirmed wood frog presence records from databases sourcing original research, museum specimens, and primary literature (Table

2). In addition, we created a random set of 5,000 points evenly dispersed across Alaska and the Yukon Territory using the Create Random Point*s* tool in ArcGIS 10.4. We removed random points from distant Bering Sea islands, leaving 4,738 pseudo-absence records spanning the study area at a sampling density of approximately 0.01 records per 5-km cell. This research-grade dataset (not to be confused with Research Grade data in iNaturalist) was used to assess model performance. RIO values were extracted from the prediction surface to each point using the Extract Values to Points tool. An AUC score was calculated for each model in the R 3.2.2 statistical software, PresenceAbsence package (Freeman and Moisen 2008). We also generated a binary presence-absence map from the prediction surface to aid in model interpretation. The presence threshold was set to capture 95% of the confirmed wood frog presence data.

3.4 Results

Environmental DNA survey

The eDNA assay amplified wood frog DNA in 31 of 224 total samples (Figure 2). Wood frog DNA was not detected in any of the negative controls (n = 28). We detected target amplification in a single replicate from three samples originating north of the Brooks Range. A sample collected from a nearby fourth site had two replicate amplifications. The furthest north DNA detection was at N 69.5691 W 148.6047, near the Sagwon Hills on the Arctic Coastal Plain. We confirmed wood frog presence at seventeen eDNA sampling sites via visual detection and dipnet surveys. The furthest north confirmed sighting occurred at N 67.4531 W 150.0637, near the settlement of Wiseman.

Species distribution model

Internal assessment using out-of-bag data for the Random Forests model was very favorable (AUC = 0.99), indicating high reproducibility of the algorithm and homogenous data. The model also displayed high predictive accuracy in classifying the research-grade dataset (AUC = 0.92). Human footprint and human influence index were the two most important predictors in this model (Table 3). Slope, annual potential evapotranspiration, and mean temperature in the months of April, May, and August were the most important physical and climate predictors. This multivariate predictor set most accurately explains wood frog occurrence in the study area. A distribution map of the model RIO values (Figure 2) shows large swaths of predicted hotspots of wood frog occurrence in red (high RIO). Areas of relative absence (low RIO) are shown in blue. The derived presence-absence classification map is shown in

Figure 3. The presence threshold in this map was set at RIO = 0.041, thus capturing the top 95% of the research-grade presence data (Figure 4).

3.5 Discussion

Here we combine eDNA and citizen science techniques to inform species distribution models. The Random Forests machine learning ensemble algorithm provides a powerful tool to mine such alternative data and extrapolate signals with high predictive accuracy. Combined with a mega-transect approach, this framework provides a powerful template for spatial analyses in vast, under-sampled areas (Baltensperger and Huettmann 2015; Cohn 2008). The limitations of eDNA occurrence data yet to be overcome relate to factors influencing the persistence, transport, and detectability of DNA in the environment (Bohmann et al. 2014; Goldberg et al. 2016). Limitations surrounding the use of citizen science data are perhaps more easily overcome. They traditionally include a perceived lack of proper project design and data quality, though current research is turning the trend (Burgess et al. 2017; Crall et al. 2011; Lewandowski and Specht 2015; McKinley et al. 2017). Guidelines for best practices in citizen science data management can help inform appropriate use of such data (see Introduction, Table 4).

A sampling bias towards populated areas is an intrinsic constraint evidenced in both our training and assessment datasets. This, in part, can be explained in the citizen science dataset by a tendency for volunteers to monitor wetlands near their residence. It also likely accounts for the importance of human footprint and human influence index (http://sedac.ciesin.columbia.edu/data/collection/wildareas-v2) in the algorithm. Machine learning ensemble algorithms are appropriate methods of analysis for spatially biased datasets, as they are not constrained to datasets that conform to *a priori* assumptions and goodness-of-fit tests (Cutler et al. 2007; Drew et al. 2010; Elith et al. 2008; Phillips et al. 2006). Pattern recognition techniques employed in these algorithms can, to an extent, sift through outliers, biases, and data gaps to extract underlying signals (Craig and Huettmann 2009). Previous work has shown that spatial biases can have minimal impact on the predictive accuracy of models built on machine learning algorithms (Kadmon et al. 2004). Accordingly, we demonstrate here that machine learning ensemble algorithms are robust to the biases in alternative datasets and ultimately classify research-grade occurrence data with high predictive accuracy.

In this study, the Random Forests SDM is presented in two different formats. A detailed examination of our RIO distribution map (Figure 2) reveals widespread hotspots of predicted wood frog occurrence throughout Southcentral and Interior Alaska. Outlying hotspots appear in the Yukon-Kuskokwim Delta, near Iliamna Lake,

around Wrangell St. Elias National Park, in the Arctic Coastal Plain near Sagwon, and from Utqiagvik east to Teshekpuk Lake in Alaska. In the Yukon Territory of Canada, hotspots occur on the Old Crow River basin, north through Vuntut National Park, throughout Kluane National Park, and generally following river corridors throughout central portions of the territory. We found certain hotspots to be more isolated than others. Landscape connectivity and habitat complementation are important for amphibians, especially during juvenile dispersal (Cushman 2006). Specifically, wood frogs have complex habitat requirements and relatively high dispersal rates for amphibians (Baldwin et al. 2006). Even so, individual migrations seldom exceed 500 m. Little gene flow is observed beyond 1,000 m and there is no evidence for metapopulation structure in the species (Berven and Grudzien 1990). The consequences arising from a patchy distribution of wood frog habitat will require further study. Distantly isolated hotspots in the high Arctic and on oceanic islands represent suitable wood frog habitat, but not necessarily wood frog occupancy. Presence in these areas is predicated on a successful colonization event. Further research on consistency of wood frog occupancy across the landscape is needed.

A more simplified view of predicted wood frog distribution across the study area is perhaps gained by analyzing the derived presence-absence classification map (Figure 3). Biases are lessened in this interpretation of the model. All presence pixels are represented equally, regardless of their relative score. This simplification comes with an inherent loss of information, but allows for a comparison with existing models. Our binary distribution map (AUC = 0.92) can be contrasted with that of the Alaska Gap Analysis Project (AUC = 0.78). AK GAP is the most recent and complete attempt at mapping wood frog distribution in Alaska using advanced modeling methods (http://akgap.uaa.alaska.edu/species-data/wood-frog-annual-distribution/#content). Our derived classification map, specified to encapsulate more of the outlying points in the assessment dataset, implies a more widespread distribution of arctic and subarctic wood frog habitat. This coincides with predictive SDMs generated with Random Forests for small mammals in Alaska, which also show an explicit underprediction in AK GAP models (Baltensperger and Huettmann 2015). We believe the evidence shows AK GAP models systemically under-predict species occurrence, in part due to conservative methodology. Specifically, our model predicts more widespread wood frog occurrence at high elevation (>600m) and north of the Brooks Range. This finding is in line with anecdotal observations of wood frogs as described by the Alaska Center for Conservation Science and compiled by the corresponding author (Fields and Gotthardt 2009; Hilderbrand, Larson, Torvinen, personal communication). Ground-truthing efforts, using our model as a guide, carry the potential to confirm wood frog presence in parts of

Alaska and Yukon far outside their currently defined range. Environmental DNA sampling is a resource-friendly complement to traditional monitoring efforts and may be the tool best equipped to rapidly survey under-sampled areas in Alaska and the Yukon.

The intent behind this study was to show how citizen science and eDNA occurrence data can be used to supplement knowledge of species distribution where research-grade data are lacking. The first implementation of wood frog eDNA assays in Alaska provides a new option for expanding survey efforts in the region. We recommend a wider adoption, revival, and continuation of citizen science projects to address concerns over deficient wood frog monitoring efforts in the Far North. Data derived from such projects are dismissed by critics on claims of low quality and poor research design. However, benefits derived from implementing both methodologies far exceed shortcomings in the data. They are far less resource-intensive than traditional monitoring techniques and more easily applied on large spatial and temporal scales. Additionally, we show the power of advanced data mining and machine learning technology to extract useful information from imperfect datasets. We stress that by considering all lines of evidence a more complete understanding of species distribution is achieved. We openly provide digital access to all material used in this study, delivering a truly transparent and repeatable template for further use and refinement.

3.6 Conclusion

The expanding use of environmental DNA detection assays and the growing consideration of citizen science data in rigorous scientific research provide alternative avenues for answering complex questions on a landscape scale. This is increasingly pertinent to modern conservation management and policy. The implementation of these survey techniques to regularly monitor wood frog populations and distribution, especially in remote areas, has high potential to facilitate conservation decision-making. Evidence of amphibian population declines in the arctic and subarctic may be more thoroughly investigated using environmental DNA and/or citizen science monitoring, adding a complement to the lack of research efforts thus far. We suggest that these alternative occurrence data and associated techniques also be more widely adopted to address similar issues in a conservation context.

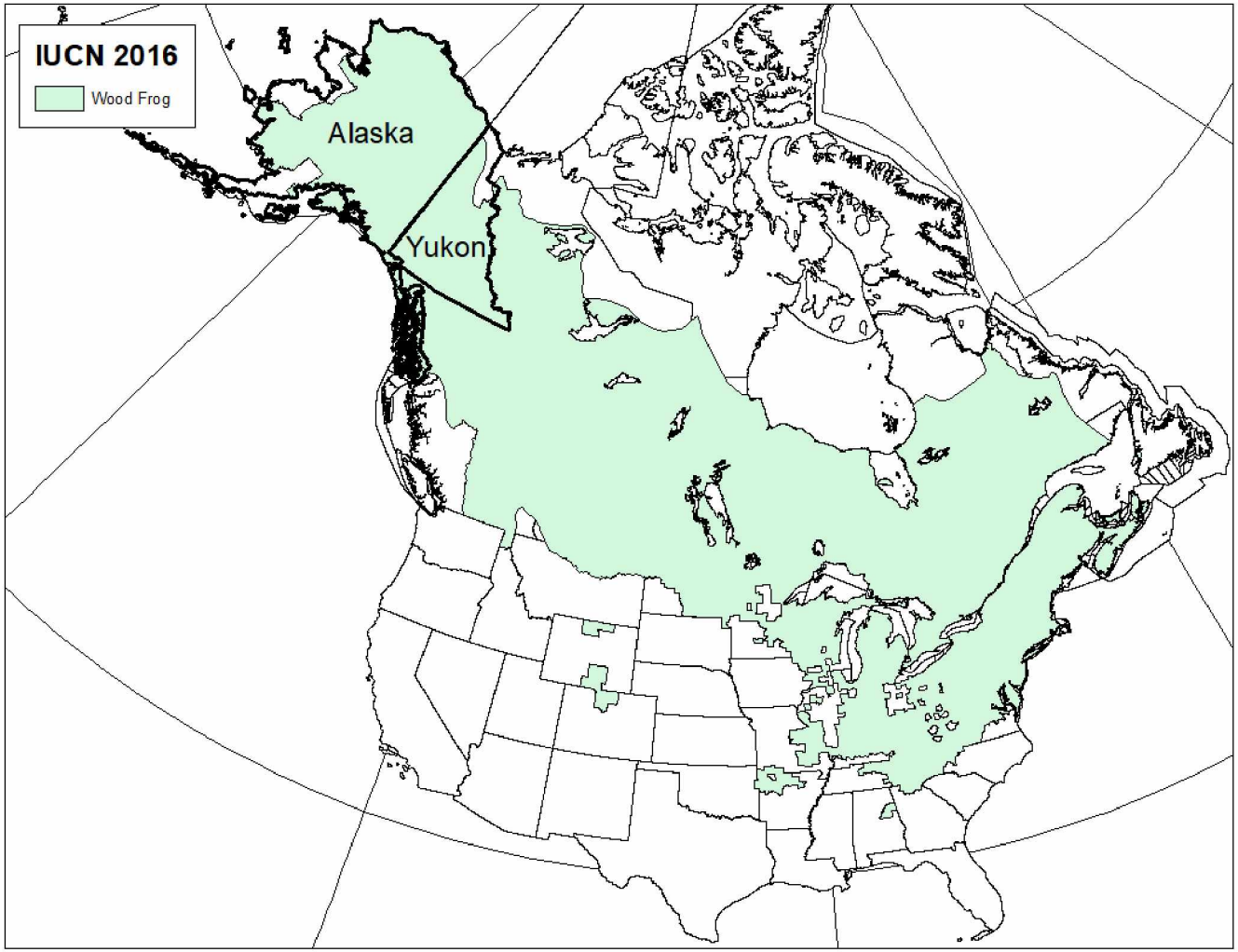

Figure 1 Wood frog range
Range of the wood frog in North America, as defined by the International Union for the Conservation of Nature (IUCN). The U.S. state of Alaska and Canada's Yukon Territory comprise the study area.

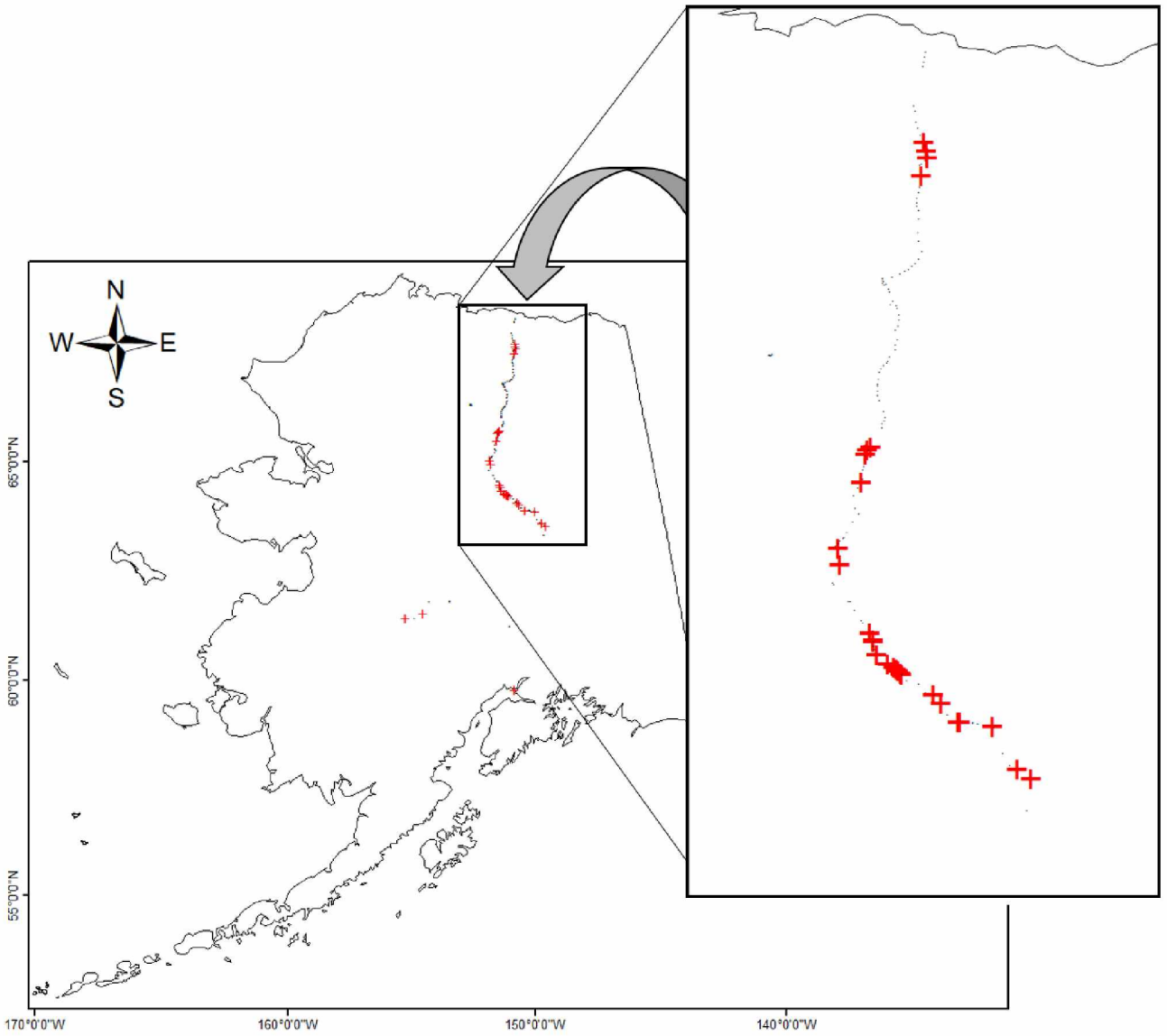

Figure 2 Environmental DNA survey results
Thirty-one sites showing positive amplification of target wood frog DNA are identified with a (+). Negative sites are indicated by (.).

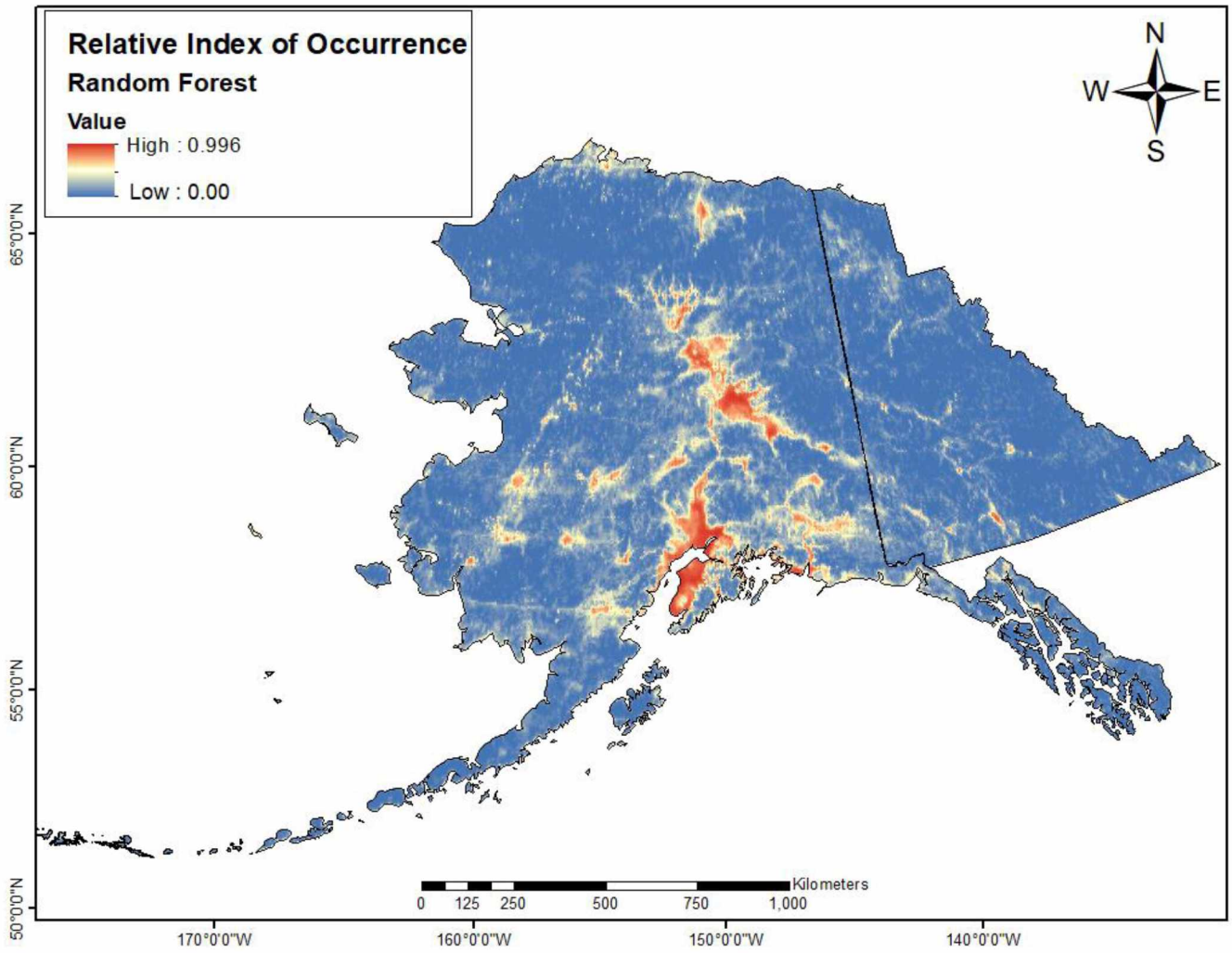

Figure 3 Predicted RIO distribution map
Predicted distribution of wood frog Relative Index of Occurrence across Alaska and Yukon Territory.

Classification accuracy = 95.40% Specificity = 95.40% Sensitivity = 95.33% Precision = 52.92%

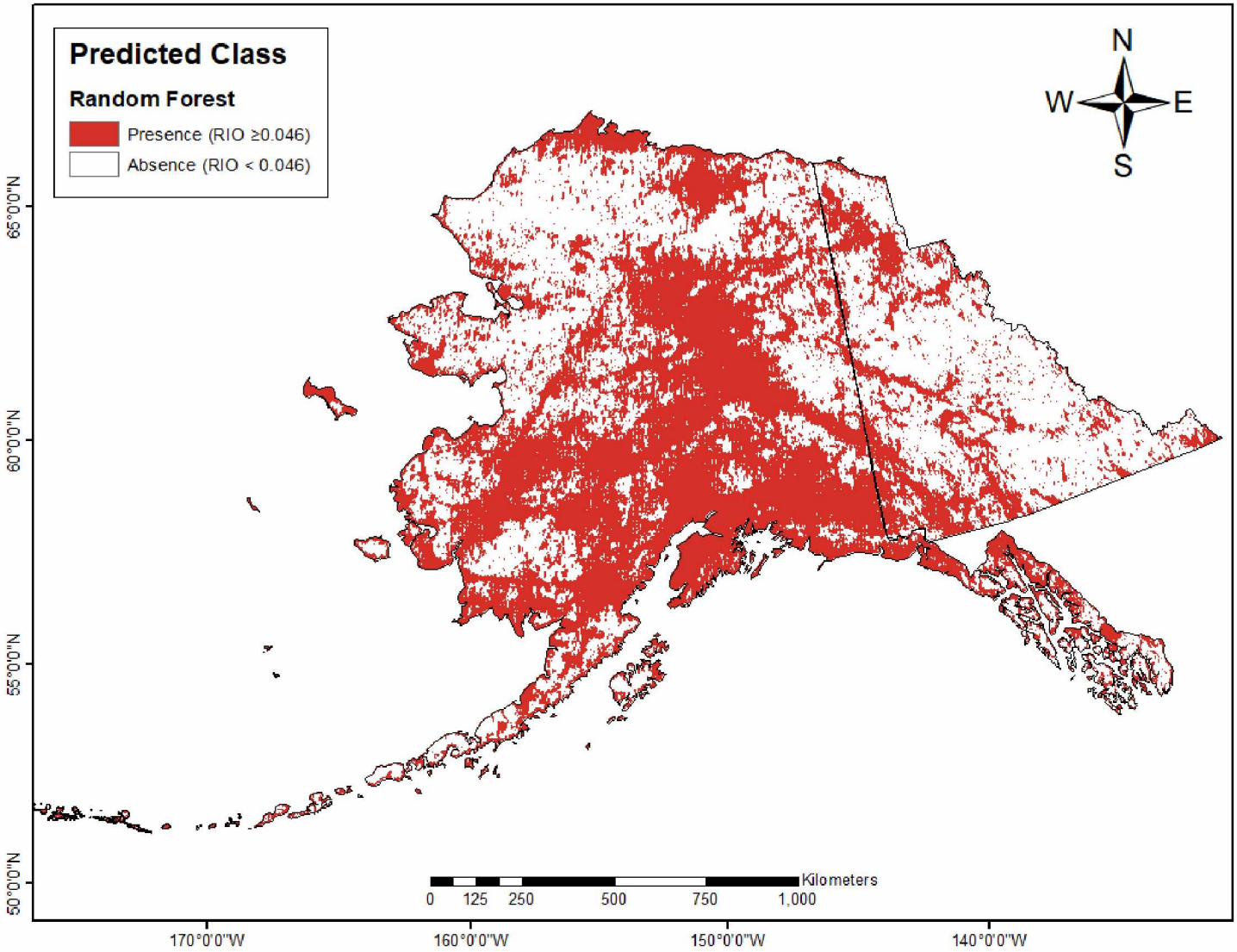

Figure 4 Predicted presence-absence distribution map
Predicted classification of wood frog occurrence, derived from Random Forests model used to produce Figure 2. Pixels with RIO values ≥ 0.0410 are classified as presence.

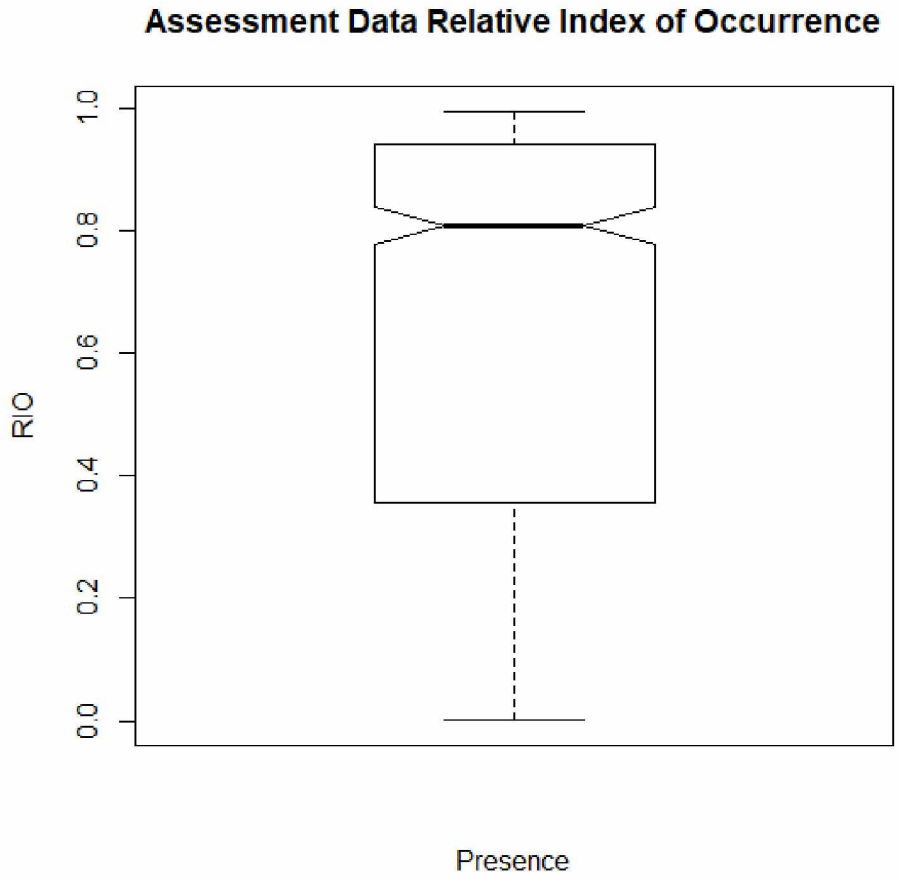

Figure 5 Presence data RIO
Distribution of relative index of occurrence (RIO) scores for the model assessment dataset, extract from the model prediction surface.

Notches indicate 95% confidence
Maximum = 0.99
3rd quartile = 0.94
Median = 0.81
Mean = 0.66
1st quartile = 0.36
Minimum = 0.00

Table 1 Citizen science databases
Occurrence records were sourced from the following databases to assemble the "alternative" training dataset. Protocol, type of observations collected, and data validation procedures are described.

Program	Protocol*	Observation	Data Validation
Alaska wood frog monitoring program	Standardized Incidental	Acoustic Visual	Volunteer trained online in survey methods Data screened by program managers
FrogWatch (Environment Canada)	Standardized Incidental	Acoustic Visual	None described
FrogWatch USA	Standardized	Acoustic	Volunteers trained annually by regional chapter coordinators Submissions by new volunteers screened by regional chapter coordinators Suspicious records flagged by online user community for review by program coordinators
iNaturalist	Incidental	Acoustic or visual	Observations are classified as "Research Grade" when they contain: date, georeferenced location, photo and/or audio, community-agreed, species-level identification

*The Alaska wood frog monitoring program, FrogWatch USA, and Environment Canada's FrogWatch all utilize a similar standardized acoustic monitoring protocol, viewable online (https://www.aza.org/frogwatch-monitoring-protocols).

Table 2 Assessment dataset
A list of open-access data sources comprising the research-grade assessment dataset for Alaska and the Yukon Territory.

Database Name	URL	Reference
Alaska Center for Conservation Science Amphibian Database	http://accs.uaa.alaska.edu	(Aycrigg et al. 2015)
Alaska Gap Analysis Project	http://akgap.uaa.alaska.edu	(Gotthardt et al. 2014)
AmphibiaWeb	http://amphibiaweb.org	(Pyron and Wiens 2011)
US Fish and Wildlife Service	http://datadryad.org/	(Reeves et al. 2010; Reeves et al. 2013)
Global Biodiversity Information Facility	https://www.gbif.org/	(Edwards 2004)
UAM ARCTOS	https://arctos.database.museum/home.cfm#UAM	(Tessler et al. 2014)
USGS Biodiversity Information Serving Our Nation (BISON)	https://bison.usgs.gov/#home	(Hampton et al. 2013)
VertNET	http://vertnet.org/	(Constable et al. 2010)

Table 3 Relative variable importance as determined by Random Forests
List of the ten most important variables in classifying wood frog presence/absence using the Random Forests ensemble algorithm. The model was built using the eDNA and citizen science alternative dataset.

Variable	Score
Human Influence Index	100.00
Human Footprint	91.40
Slope	57.26
May mean temperature	56.75
April mean temperature	54.01
Population count	53.55
Night light pollution	51.91
August mean temperature	43.83
Population density	37.82
Annual average potential evapo-transpiration	31.08

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Conclusion

Development of the wood frog eDNA assay follows a larger trend in aquatic ecology to monitor species using this novel technique (Bohmann et al. 2014; Goldberg et al. 2015; Thomsen and Willerslev 2015). This assay, developed and applied to wood frogs in Alaska, can also be used as a generic study template to monitor the species throughout the western portion of its range, including most of Canada. It was tested and proven to be species-specific; thus, it can be used to monitor wood frogs in ecosystems where multiple amphibian species co-occur. The assay detected DNA at concentrations as low as 1.83×10^{-3} pg/μL in this study, providing high sensitivity that resulted in a detectability greater than that of a visual encounter survey. Further validation of this assay, and others like it, will come from comparing the detectability of multiple methods to determine the best approach to species monitoring (Biggs et al. 2015; Haynes et al. 2013; Pilliod et al. 2013).

The use of eDNA occurrence data for semi-aquatic species can be used in predictive species distribution models. SDMs developed here were found to predict wood frog occurrence in the Fairbanks North Star Borough with moderate accuracy (AUC = 0.74). I speculate that fine-scale landscape patterns have considerable influence on wood frogs in that study area. Accordingly, wood frog occurrence in the borough is probably best determined by microhabitat factors. The availability of relevant environmental GIS layers at high resolution, combined with expansive eDNA datasets, provides a novel approach to create and improve amphibian distribution models using machine learning algorithms. This, for example, may allow conservation managers to reconsider how critical habitat is designated for rare species and endangered landscapes. However, a standardized set of best-practice protocols are still needed to account for biases, transparency, and repeatability in eDNA data (Bohmann et al. 2014; Goldberg et al. 2016). This is especially true for samples collected from warm, turbid, and high-acidity wetlands that may be intrinsically problematic for eDNA preservation and detection (Barnes et al. 2014; Dejean et al. 2011; Herder et al. 2014; McKee et al. 2015b).

Species distribution models built on machine learning algorithms and trained with eDNA and citizen science occurrence data are shown to predict research-grade observations in Alaska and the Yukon Territory with high accuracy (AUC = 0.92). This model corrects for errors and outperforms previous wood frog distribution models (Gotthardt et al. 2012), despite clear biases in the training dataset. Acceptance of these data prompt a reinterpretation of wood frog distribution in the arctic and subarctic. We must now consider that suitable wood frog habitat is more widely dispersed at high latitudes and altitudes than previously documented, acknowledging the credibility of

anecdotal observations. Evidence from eDNA surveys confirms that wood frog DNA can be detected in wetlands on the Arctic Coastal Plain, beyond the recorded range of the species. It follows either that wood frogs occur in low abundance north of the Brooks Range or DNA can travel vast distances in the environment. Follow-up surveys of wood frog occurrence in areas highlighted by the SDM can confirm or deny their presence in those area. This knowledge will help improve management of wood frogs and their habitats in the Far North. Further investigations of DNA transport in the environment, especially between closed systems, are also warranted. Continued dismissal of alternative data in favor of data that adhere to generic research design principles, particularly where such research-grade data are deficient, is irresponsible and may inhibit conservation management.

The research highlighted in this thesis is intended to be of practical value to land managers, research scientists, and conservation decision makers. I encourage the adoption and continued refinement of the eDNA detection assay for wood frogs across Alaska and Canada. Likewise, the species distribution models are an attempt to restart the process of learning more about wood frogs at their northern range extent. The models require extensive ground-truthing efforts for further improvement. Citizen science efforts started as part of this thesis research will continue to complement our knowledge of wood frog population trends at the local scale.

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Appendix A Electronic supplementary material for Chapter 1

Online Resource 1
For the most complete archive of wood frog specimens collected in Alaska, see the University of Alaska Museum of the North's database at http://arctos.database.museum/archive/wood_frog.
The Alaska Center for Conservation Science maintains the most complete database of wood frog occurrence in Alaska at http://accs.uaa.alaska.edu/zoology/amphibian-database/wood-frog/.

Online Resource 2
The following values were used with the Primer3Plus web application to create the Rasy_00 primers (default values were used for all other parameters):

Primer3Plus parameter values	
Product Size Ranges:	80-120
Primer Size Min:	18
Primer Size Max:	26
Primer Tm Min:	50.0
Primer Tm Max:	60.0
Max Tm Difference:	1.0
Primer GC% Max:	40

Online Resource 3
In silico test results for Rasy_00 primer specificity (NCBI Primer-BLAST). All results are shown for species with ≤ 3 mismatches per primer.

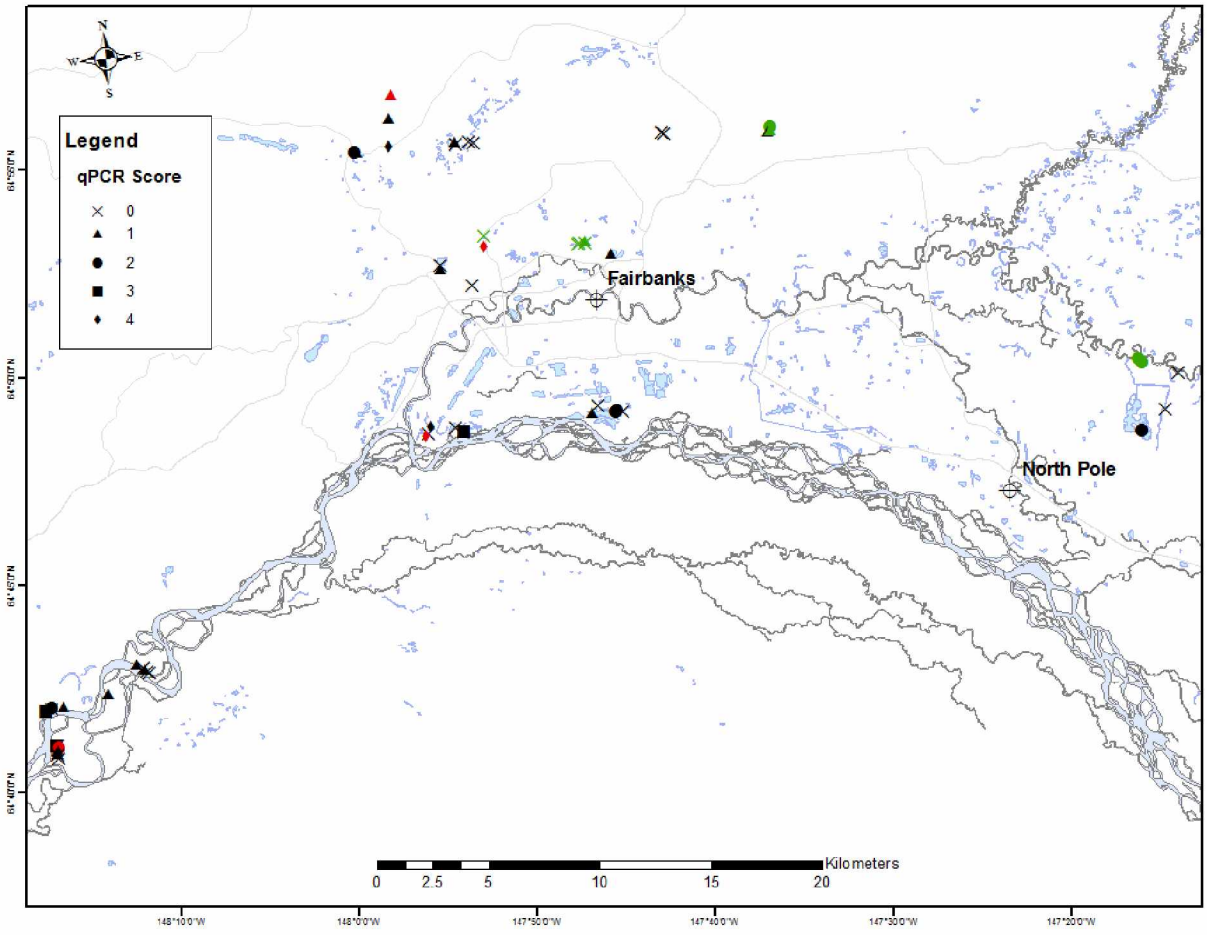
Species + ITIS TSN	**Rasy_00_F mismatches**	**Rasy_00_R mismatches**
Rana sylvatica (western clade), 775117	0	0
Rana sylvatica (eastern clade), 775117	0	1
Rana septentrionalis, 775112	2	1
Rana clamitans, 775087	2, 3	2
Rana grylio, 775091	2	2
Rana okaloosae, 775103	3	2
Rana virgatipes, 775123	3	2

Online Resource 4
Standard PCR conditions for preliminary screening of primer development. Use of the Rasy_00 primers in areas with co-occurring amphibian species not tested in **Table 1**, especially those listed in **Online Resource 3**, will necessitate further preliminary screening.
25µL reactions:
5µL 5X Buffer (Green GoTaq® Flexi), 0.5µL 40mM dNTP's, 2.5µL 25mM $MgCl_2$, 2.5µL 100X Bovine serum albumin (BSA), 1.25µL 100% Dimethyl Sulfoxide (DMSO), 1.0µL 10µM each primer, 2µL template DNA, and 0.15µL 5U/µL *Taq* polymerase (GoTaq® Flexi)
Thermocycler profile:
1x(94°C/2min), 35x(94°C/30sec, 55°C/45sec, 72°C/45sec), 1x(72°C/5min)

Appendix A (cont.) Electronic supplementary material for Chapter 1

Online Resource 5

A map of the 60 sampling locations in Interior AK, geographic projection in Albers (meters). Green symbols indicate sites with acoustic confirmation of wood frogs. Red symbols indicate visual confirmation. Background layers (ROAD_MAJOR_FNSB and WATER_BODIES_POLYGONS) were obtained from the Fairbanks North Star Borough GIS (http://gis.co.fairbanks.ak.us/website/fnsbgis/viewer.htm). Sample site coordinates are available via Dryad.



Appendix B University of Alaska Fairbanks Institutional Animal Care and Use Committee approval letter

(907) 474-7800
(907) 474-5993 fax
uaf-iacuc@alaska.edu
www.uaf.edu/iacuc

Institutional Animal Care and Use Committee
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

March 22, 2016

To: Falk Huettmann
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [871371-2] Environmental DNA sampling and spatial modeling as means to investigate wood frog range extent in northern Alaska

The IACUC reviewed and approved the Response/Follow-Up referenced above by Designated Member Review.

Received:	March 13, 2016
Approval Date:	March 22, 2016
Initial Approval Date:	March 22, 2016
Expiration Date:	March 22, 2017

This action is included on the April 14, 2016 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*

Appendix B (cont.) University of Alaska Fairbanks Institutional Animal Care and Use Committee approval letter

(The following information is also available in a printable format in the IRBNet Forms and Templates)

HOW DO I REPORT CONCERNS ABOUT ANIMALS IN A UAF RESEARCH FACILITY?

- All "live" animal concerns related to care and use should be reported to the IACUC
- Email: uaf-iacuc@alaska.edu Phone: 474-7800
- Report form: www.uaf.edu/iacuc/report-concerns/
- IACUC Committee Members: www.uaf.edu/iacuc/iacuc-info/
- Additional information: www.uaf.edu/ori/responsible-conduct/research-misconduct/ and www.uaf.edu/ori/responsible-conduct/conflict-of-interest/

WHAT SHOULD I DO IF AN ACCIDENT OR INCIDENT OCCURS IN AN UAF ANIMAL FACILITY?

- **For all immediate *human* emergencies call 911** or UAF Dispatch at 474-7721 for less immediate emergencies.
- If you have **suffered an animal bite or other injury**, complete an "Accident/Incident Investigation form" (personal injury) form available at www.uaf.edu/safety/incidentreport-2012.pdf.
- If an accident such as a **chemical spill** occurs, contact the Environmental Health, Safety, and Risk Management (EHS&RM) Supervisor at 474-5617 or the Hazmat Coordinator at 474-7889.

WHO DO I CONTACT IF I FIND A DEAD, INJURED, OR DISTRESSED ANIMAL IN A UAF RESEARCH FACILITY?

- During regular business hours, immediately contact facility staff and/or Veterinary Services Staff at 474-7020.
- After hours or on weekends, immediately contact facility staff and/or Veterinary Services Staff using the contact numbers posted on the "Emergency Contact Information" in the facility or call UAF Dispatch at 474-7721.
- Contact the IACUC at 474-7800 or uaf-iacuc@alaska.edu if an "Emergency Contact Information" sign is NOT posted in the facility.
- Contact the IACUC if you are not satisfied with the response from Vet Services.

HOW DO I REPORT ANY CONCERNS REGARDING WORK HAZARDS OR ANY GENERAL UNSAFE CONDITIONS?

- Complete an "Unsafe Condition Reporting Program" form, available at the EHS&RM website: www.uaf.edu/safety/unsafe-condition/

WHERE CAN I OBTAIN GENERAL OCCUPATIONAL SAFETY INFORMATION?

- www.uaf.edu/iacuc/occupational-health/

Appendix C Alaska Department of Fish and Game Fish Resource Permit

STATE OF ALASKA
DEPARTMENT OF FISH AND GAME

333 Raspberry Road
ANCHORAGE, ALASKA 99518

Permit No. **SF2016-069**

Expires: **9/1/2016**

FISH RESOURCE PERMIT
(For Scientific/Collection Purposes)

This permit authorizes: **Mark A. Spangler**
(whose signature is required on page 2 for permit validation)

Of

University of Alaska Fairbanks
Box 756100, Fairbanks, AK 99775-6100
(402) 873-2223 maspangler@alaska.edu

to conduct the following activities from **May 1, 2016** to **September 1, 2016** in accordance with AS 16.05.930 and AS 16.05.340(b).

Purpose: To investigate wood frog presence along the Dalton Highway

Location: Ephemeral and shallow water wetlands in the Dalton Highway Corridor between Fairbanks and Deadhorse

Species: Wood frog

Method of Capture: Dip net, hand

Final Disposition: Any number of wood frogs may be captured, identified, and released in the target study area.
≤5 individuals at each collection site may be non-lethally swabbed for genetic samples prior to release.
≤2 individuals of each unknown species may be killed and saved for later identification.
All unintended mortalities must be recorded and returned to the capture site.

COLLECTION REPORT DUE October 1, 2016 and **RESEARCH REPORT DUE February 28, 2017**; see **Stipulations #2** and **#3** for more information. Data from such reports are considered public information. Reports must be submitted to the Alaska Department of Fish and Game, Division of Sport Fish-HQ, 333 Raspberry Rd, Anchorage, AK 99518, attention: Scott Ayers (267-2517; dfg.dsf.permitcoordinator@alaska.gov). A report is required whether or not collecting activities were undertaken.

GENERAL CONDITIONS, EXCEPTIONS, AND RESTRICTIONS

1. This permit must be carried by person(s) specified during approved activities who shall show it on request to persons authorized to enforce Alaska's fish and game laws. This permit is nontransferable and will be revoked or renewal denied by the Commissioner of Fish and Game if the permittee violates any of its conditions, exceptions, or restrictions. No redelegation of authority may be allowed under this permit unless specifically noted.
2. No specimens taken under authority hereof may be sold, bartered, or consumed. All specimens must be deposited in a public museum or a public scientific or educational institution unless otherwise stated herein. Subpermittees shall not retain possession of live animals or other specimens.
3. The permittee shall keep records of all activities conducted under authority of this permit, available for inspection at all reasonable hours upon request of any authorized state enforcement officer.
4. Permits will not be renewed until detailed reports, as specified in the Stipulations section, have been received by the department.
5. UNLESS SPECIFICALLY STATED HEREIN, this permit does not authorize the exportation of specimens or the taking of specimens outside of existing regulations.

Scott D. Ayers
Permit Coordinator
Division of Sport Fish

Sheila A Cameron for
Director
Division of Sport Fish

1 MARCH 2016
Date

SF2016-069 ***continued (page 2 of 2)***

Authorized Personnel: The following persons may perform collecting activities under terms of this permit:

Falk Huettmann, Mark Spangler

Employees and volunteers under the direct supervision of, and in the presence of, one of the authorized personnel listed above may participate in collecting activities under terms of this permit.

Permit Stipulations:

1) The local Area Management Biologist (AMB) must be contacted for final authorization **prior** to you engaging in any collecting activities. *The time/date of this contact must be included in your collections report (using the "data submission form" furnished by ADF&G).* AMBs have the right to specify methods for collecting, as well as limiting the collections of any species by number, time, and location.
April Behr (459-7362; april.behr@alaska.gov) – Yukon River (Fairbanks)
Brendan Scanlon (459-7268 or 460-7567; brendan.scanlon @alaska.gov) – Northwest/Arctic (Fairbanks)
Klaus Wuttig (459-7344; klaus.wuttig@alaska.gov) – Tanana River (Fairbanks)

2) **A report of collecting activities, referencing this fish resource permit, must be submitted within 30 days after the expiration of this permit.** The report, (using a data submission form furnished by ADF&G), shall include all species, numbers, dates, locations of collection (datum/GPS coordinates in the decimal degrees format (dd.ddddd)), and disposition, and if applicable, sex, age, and breeding condition, and lengths and weights of fish handled. It must also include the date/time the local biologist was contacted for final authorization to carry out collecting activities.

3) **A report of research activities, referencing this fish resource permit, must be submitted within 6 months after the expiration of this permit.** This report should present the research conducted in a format similar to a scientific paper including the following: introduction (objective of the study plan and hypothesis), methods, and results. The report is intended to show that the specimens were used in a scientific method, and allows for the evaluation of potential cumulative effects from multiple projects in the same area. A report is required whether or not collecting activities were undertaken.

4) An instance of >10% unintended collecting mortality requires sampling at a site to cease and the AMB contacted.

5) **The following rules must be followed for all indigenous amphibian collections:** 1) all boots and collecting gear must be washed and disinfected between sites by with a 5% bleach solution; 2) Gore-Tex boots and waders are not permitted as they are too difficult to disinfect; 3) permission from the land owner must be obtained before collections may occur on non-state property; and 4) Single use gloves or single use plastic bags must be used when handling animals that will be released alive.

6) If new anadromous fish species *or* previously undocumented life stages of anadromous fish are found in permitted streams, rivers, and lakes, the permit holder will work closely with ADF&G to see that information is included in the database for the *Catalog of Waters Important for Spawning, Rearing or Migration of Anadromous Fishes.* Anadromous fish include *Oncorhynchus spp.*, Arctic char, Dolly Varden, sheefish, smelts, lamprey, whitefish, and sturgeon. Please direct questions to **J Johnson** (907-267-2337; j.johnson@alaska.gov).

7) Contact **Tammy Davis** with the ADF&G Invasive Species Program (907-465-6183 or 1-877-INVASIV), and the nearest AMB (**Stipulation #1**) within 24 hours should you find any species suspected to be a **non-native species** during your sampling. If possible the organism should be killed, preserved by freezing or placing into 90% alcohol, and taken to the nearest ADF&G office. Please take a photo of the organism, as well as a photo of the organism in the environment in which it was observed, and note the location with a GPS or by describing it on a map with landmarks.

8) A copy of this permit, including any amendments, must be made available at all field collection sites and project sites for inspection upon request by a representative of the department or a law enforcement officer.

9) Issuance of this permit does not absolve the permittee from securing any other required state, federal, or local permits, including securing permissions to trespass on controlled lands.

10) Failure to comply with the conditions of this permit will result in the loss of future permitting privileges.

11) PERMIT VALIDATION requires permittee's signature agreeing to abide by permit conditions before beginning collecting activities:

_____
Signature of Permittee

ecc: Klaus Wuttig, Division of Sport Fish, Fairbanks
Brandy Baker, Division of Sport Fish, Delta Junction
April Behr, Division of Sport Fish, Fairbanks
Brendan Scanlon, Division of Sport Fish, Fairbanks
Bonnie Borba, Division of Commercial Fisheries, Fairbanks
Jim Menard, Division of Commercial Fisheries, Nome
Audra Brase, Division of Habitat, Fairbanks
Michelle Morris, Commercial Fisheries Permit Coordinator, Juneau
Colonel Bear, Alaska Wildlife Troopers
Captain Leath, Alaska Wildlife Troopers Northern Detachment